Australian Government
Department of Health and Aged Care
Australian Industrial Chemicals Introduction Scheme

Graphene

Assessment statement (CA09624)

6 February 2023

Final

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AICIS assessment statement

Chemical in this assessment

Name	CAS registry number
Graphene	1034343-98-0

Reason for the assessment

An application for an assessment certificate under section 31 of the *Industrial Chemicals Act 2019* (the Act).

Certificate Application Type

Health and environment focus

According to information submitted by the applicant and criteria in the Industrial Chemicals (General) Rules 2019 and the Industrial Chemicals Categorisation Guidelines, this introduction is in the **assessed** category. The reason is that this introduction has **medium to high** indicative risk for **human health** and **the environment** because:

- the chemical is a non-soluble solid
- the chemical consists of particles in an unbound state or as an aggregate or agglomerate, at least 50% (by number size distribution) of which have at least one external dimension in the nanoscale of 1-100 nm
- the introduction of the nanoscale portion of the chemical is not incidental to the introduction of the non-nanoscale portion of the chemical

Defined scope of assessment

The chemical has been assessed as manufactured in Australia at up to 10 tonnes per year

- for reformulation into industrial diesel fuels at a concentration of up to 0.005% for use only by workers in a professional setting
- for reformulation into industrial coatings for heating, ventilation and air conditioning (HVAC) and mining equipment, lubricants and coolants at a concentration of up to 2% for use only by workers in a professional setting
- as a component in batteries at a concentration of up to 60% for use by workers or consumers
- with no direct release to natural water ways, municipal water supplies, or municipal sewerage systems

Summary of assessment

Summary of introduction, use and end use

The assessed chemical will be manufactured in Australia from a process that uses microwave energy to crack methane gas into carbon and hydrogen. Two forms of the assessed chemical (ML-100 and XE) will be manufactured. Following manufacture, the assessed chemical will be filtered, collected and manually transferred into a kiln for further refinement. The assessed chemical will be separated based on quality and manually packaged into sealable 60 L drums or in sealable zip locked bags that are further packaged in sealable 80 L plastic tubs. Drums and plastic tubs are stored at the applicant's warehouse and then distributed to reformulation sites by road, rail, sea and air.

The assessed chemical in the neat powder form will be incorporated into various end use products during reformulation. Industrial diesel fuels will contain the assessed chemical at up to 0.005% concentration. Industrial coatings for HVAC and mining equipment, lubricants and coolants will contain the assessed chemical at up to 2% concentration. Batteries will contain the assessed chemical at up to 60% concentration.

Diesel fuels, coatings, lubricants and coolants containing the assessed chemical are for use only by workers in professional settings and batteries containing the assessed chemical will be for use by workers or consumers.

Human health

Summary of health hazards

No toxicological data were provided for the assessed chemical. The assessed chemical is a two-dimensional nanomaterial with one dimension in the nanoscale (1-100 nm). For insoluble nanomaterials, the inhalation route is generally considered as the main route of exposure for potential systemic toxicity.

Based on the limited analogue data available on various dimensions of graphene particles, the assessed chemical:

- is likely to be of low acute oral toxicity;
- acute inhalation toxicity cannot be ruled out (LC50 > 1.99 mg/L)
- is likely to be non-irritating to skin and eyes;
- is unlikely to be a skin sensitiser; and
- is likely to be non-genotoxic.

Based on the available repeated dose inhalation toxicity studies for graphene analogues, the assessed chemical may have the potential to cause lung toxicity (e.g. inflammation and microgranulomas), if inhaled. However, given that the toxicity can be dependent on a number of factors including lateral size of the particles, number of layers and surface chemistry, there remains uncertainty as to the potential lung toxicity of the assessed chemical.

Health hazard classification

As only limited analogue toxicity data were provided, the assessed chemical cannot be classified according to the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS, UNECE 2017), as adopted for industrial chemicals in Australia.

Summary of health risk

Public

When introduced and used in the proposed manner, it is unlikely that the public will be exposed to the assessed chemical. The public may come into contact with solid materials containing the assessed chemical (such as batteries or coated substrates). However, as the assessed chemical will be encapsulated in an article or within a solid matrix on the substrate, it is not expected to be available for exposure. Therefore, no risks are identified for public health during this assessment that require specific risk management measures, if the assessed chemical is introduced and used in accordance with the terms of the assessment certificate.

Workers

Given the lack of toxicity data and uncertainty regarding systemic health effects from inhalation of insoluble nanoscale particles, workers may experience adverse health effects if exposed to the assessed chemical during its manufacture, reformulation and some end uses (if used by spray application). Control measures (see **means for managing risks** section) are required to manage the risk to workers.

Environment

Summary of environmental hazard characteristics

The assessed chemical is an inorganic substance, therefore the determination of whether it meets the PBT criteria is not applicable.

Environmental hazard classification

There is currently no global consensus as to whether the aquatic hazard of nanomaterials can be classified according to the *Harmonised System of Classification and Labelling of Chemicals* (GHS, UNECE 2017). Hence, the aquatic hazards of the assessed chemical have not been classified for this assessment. Nevertheless, it is noted that ecotoxicity data evaluated for this assessment does show that graphene similar to the assessed chemical adversely affects aquatic life under certain exposure conditions.

Summary of environmental risk

The assessed chemical will be manufactured in Australia and used as a component of thermal coatings and batteries and as an additive in coolants, lubricants, and diesel fuels in professional settings. The manufacture and assessed end uses are not expected to result in the release of the assessed chemical to the environment.

If the assessed chemical is released, it is expected to be long-lived in the environment. The assessed chemical may accumulate within the digestive tracts of organisms, and it is not certain if it can cross digestive membranes. The assessed chemical is not expected to be a biomagnification concern. If released, the assessed chemical may cause adverse effects to aquatic organisms and impact the development of aquatic organisms.

Based on its assessed use patterns and lack of environmental release, it is expected that the environmental risk from the introduction of the assessed chemical can be managed within existing frameworks.

Means for managing risk

Assessment Certificate

The assessment certificate includes a defined scope of assessment (see **defined scope of assessment** section) and the following specific requirements to provide information:

- if the assessed chemical is introduced with parameters significantly outside those stated in the assessment statement, specifically particle size and size distribution, surface functionalisation, surface area, layer number, or purity.

Workers

Information relating to safe introduction and use

- The information in this statement should be used by a person conducting a business or undertaking (PCBU) at a workplace (such as an employer) to determine the appropriate controls under the relevant jurisdiction Work Health and Safety laws.

- The following control measures should be implemented to manage the risk arising from exposure during manufacturing, reformulation and end use (if used by spray application):

    - Use of engineering controls such as
        - Enclosed, automated processes where possible
        - Local exhaust ventilation fitted with high-efficiency particulate air (HEPA) filter

    - Use of safe work practices to
        - Avoid generation of dusts, aerosols or mists
        - Avoid inhalation of dusts, aerosols or mists
        - Avoid contact with skin and eyes

    - Workers should wear the following personal protective equipment (PPE)
        - Appropriate respiratory protection (such as a P2 respirator) if inhalation exposure may occur

- Spray applications should be carried out in accordance with the Safe Work Australia Code of Practice for *Spray Painting and Powder Coating* (SWA, 2020) or relevant State or Territory Code of Practice.

- A copy of the SDS should be easily accessible to employees.

Environment

Information relating to safe introduction and use

- The packaging of industrial coolants, lubricants, diesel fuels and thermal coatings containing the assessed chemical should be labelled “Dispose of appropriately. Do not pour down drains or sinks.”

Conclusions

The conclusions of this assessment are based on the information described in this assessment statement.

Considering the proposed means of managing risks, the Executive Director is satisfied that when the chemical is introduced and used in accordance with the terms of the assessment certificate the human health and environment risks can be managed within existing risk management frameworks. This is provided that all requirements are met under environmental and workplace health and safety and poisons legislation as adopted by the relevant state or territory and the proposed means of managing the risks identified during this assessment are implemented.

Note: Obligations to report additional information about hazards under section 100 of the *Industrial Chemicals Act 2019* (the Act) apply.

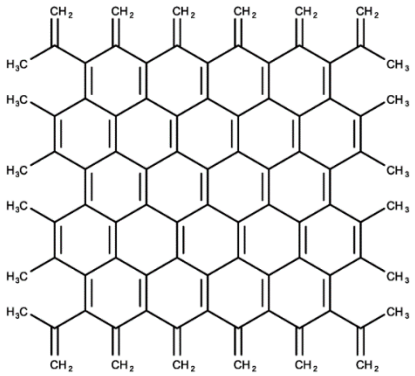
Supporting information

Chemical identity

The assessed chemical is a two-dimensional nanomaterial with a degree of purity of ~98%.

Chemical name	Graphene
CAS No.	1034343-98-0
Trade names	Two different physical forms of the assessed chemical will be introduced under the following trade names: • ML-100 (powder) • XE (powder)

Representative structural formula:



Molecular formula	Unspecified
Molecular weight (g/mol)	Unspecified

Relevant physical and chemical properties

Physical form	Black, solid powder containing two dimensional flakes
Density*	2259 kg/m^3
Water solubility*	< 3 mg/L
Ionisable in the environment?	No
Particle size distribution (in ethanol)	ML-100: d10 = 200 nm, d50 = 220 nm, d90 = 300 nm XE: d10 = 400 nm, d50 = 500 nm, d90 = 650 nm
Particle size	ML-100: X: 71 ± 18.25 nm, Y: 51 ± 12.83 nm, Z: ~6.46 nm‡

	XE: X: 68 ± 42.45 nm, Y: 53 ± 37.62 nm, Z: ~6.46 nm‡
Layer number	ML-100: 8-25 layers XE: 4-25 layers
Specific surface area	ML-100: 71.31 m^2/g XE: 219.62 m^2/g
Crystallinity (X-ray diffraction)^	ML-100: intense peak at 2θ = 25.82°, 2nd peak at 2θ = 43° XE: intense peak at 2θ = 25.96°, 2nd peak at 2θ = 42.72°
Zeta potential (pH dependent)	ML-100: 4.58 -16.80 mV XE: 12.00 -17.90 mV
Self-heating properties*	Not self-heating
Layer ignition temperature*	360 °C
Ignition sensitivity*	Minimum ignition energy > 1,000 mJ Minimum ignition temperature > 1000 °C
Dustiness*	Inhalable fraction: 6473 mg/kg Thoracic fraction: 1267 mg/kg Respirable fraction: 151 mg/kg
Oxygen level	ML-100: 2.09% XE: 1.95%
Surface functionalisation	None

* Based on graphene analogues
‡ Z dimension is calculated from the formula Z = (n -1)*0.37 nm, where n is the number of graphene layers specified by the applicant and 0.37 nm is the interspatial distance (Koh et al. 2011)
^ Peaks are corresponding to the peaks reported in a published journal article (Lin et al. 2014)

Human exposure

Workers

Transport and storage

Transport and storage workers may come into contact with the assessed chemical in the neat powder form only in the event of accidental rupture of containers. The applicant states that exposures are likely to be minimised through the use of personal protective equipment (PPE) including protective clothing, chemical resistant gloves, safety glasses and appropriate respiratory protection such as a particle filter device (filter type P2) for workers handling or disposing the chemical.

Manufacture

During manufacture, dermal, ocular and inhalation exposure of workers to the assessed chemical at up to 100% concentration may occur during transfer, quality control analysis, packaging, and cleaning and maintenance of equipment. The applicant states that exposure

is expected to be minimised through the use of enclosed and automated systems where possible, and these include adequate ventilation and appropriate PPE for workers including protective clothing, impervious gloves, safety glasses and appropriate respiratory protection such as a particle filter device (with filter type P2) if inhalation exposure may occur.

Reformulation

Typically, reformulation processes may incorporate blending operations that are highly automated and occur in an enclosed/contained environment, followed by automated filling using sealed delivery systems into containers or articles (e.g. batteries). Dermal, ocular and inhalation (if dusts, aerosols or mists are formed) exposure of workers to the assessed chemical at up to 100% concentration may occur during transfer, blending, quality control analysis, packaging, and cleaning and maintenance of equipment. The applicant states that exposure is expected to be minimised through the use of enclosed and automated systems, adequate ventilation and PPE including protective clothing, impervious gloves, safety glasses and appropriate respiratory protection such as a particle filter device (with filter type P2) if inhalation exposure may occur.

Professional end use – coatings

Dermal, ocular and inhalation exposure to the assessed chemical (at up to 2% concentration) may occur during mixing, manual transfer of the coating to spraying equipment, during application and also during equipment cleaning and maintenance. The applicant states that exposure during application of coatings is expected to be minimised through adequate ventilation and the use of appropriate PPE (such as protective clothing, impervious gloves, safety glasses and appropriate respiratory protection). Once the coating has cured and dried, the assessed chemical will be bound in a solid matrix and not available for exposure.

Professional end use – diesel fuels, lubricants and coolants

Dermal and ocular exposure to the assessed chemical (at up to 2% concentration) may occur during the use of industrial diesel fuels, lubricants and coolants containing the chemical in professional settings. Inhalation exposure is not expected given the estimated very low vapour pressure of the assessed chemical and the liquid form of end use products. The applicant states that exposure is expected to be minimised through the use of PPE (including protective clothing, impervious gloves and safety glasses).

Professional end use – batteries

Occupational exposure to end use batteries containing the assessed chemical is not expected as the chemical will be encapsulated in the article and not available for exposure.

Public

Diesel fuels, coatings, lubricants and coolants containing the assessed chemical are for use only by workers in professional settings and public exposure to these end use products are not expected. The public may come into contact with solid materials containing the assessed chemical (such as batteries or coated substrates). However, as the assessed chemical will be encapsulated in an article or within a solid matrix on the substrate, it is not expected to be available for exposure.

Health hazard information

For the purpose of this hazard assessment 'assessed chemical' refers only to the physical form of graphene particles described within the parameters indicated in the relevant physical and chemical properties section. All other physical forms of graphene will be described as 'graphene analogues'.

No toxicological data for the assessed chemical were provided. The applicant submitted studies on graphene analogues which have been used to estimate the toxicity of the assessed chemical.

Acute toxicity

Oral

In an acute oral toxicity study (OECD TG 420), five female Wistar rats were administered graphene analogue 1 in Arachis oil BP at a dose of 300 mg/kg bw. No mortalities were observed. Black faeces were observed on the first three days after exposure, which were not observed during the remaining study period. No abnormalities were noted at necropsy. The LD50 was determined to be greater than 300 mg/kg bw (maximum attainable concentration). Based on the available information, the assessed chemical is likely to be of low acute oral toxicity.

Inhalation

In an acute inhalation toxicity study (OECD TG 436), Sprague Dawley (SD) rats (n = 3/sex/dose) were exposed (nose-only) to an aerosol of graphene analogue 2 at a measured concentration of 0.878 mg/L or 1.99 mg/L for 4 hours and observed for 14 days. For each dose, the aerosolised chemical comprised of particles with a median mass aerodynamic diameter (MMAD) of 3.5–5.3 µm and 3.9–4.2 µm, respectively. No mortalities were observed at concentrations up to 1.99 mg/L. As 1.99 mg/L was the maximum feasible aerosol concentration determined in the study, it cannot be confirmed that LC50 would fall between 1–5 mg/L, which is the range the chemical requires classification for Acute Toxicity (Inhalation) – Category 4 (Harmful if inhaled) according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS, United Nations 2017). Based on the available information, acute inhalation toxicity of the assessed chemical cannot be ruled out.

Corrosion/Irritation

Skin irritation

Graphene analogue 2 was determined to not be irritating to the skin in an *in vivo* skin irritation test (OECD TG 404) after a single 4-hour application to the intact skin of three male albino rabbits under occlusive conditions. No clinical signs of irritation or mortalities were observed.

Graphene analogue 3 (layer number: 4; lateral dimension distribution: 50-600 nm; lateral dimension: 171 ± 147 nm; carbon content: 94.93 ± 0.28%; impurity: 0.074 mg/L iron) was reported to be not irritating after being tested in an *in vitro* skin irritation test using the SkinEthic™ reconstructed human epidermis tissue model (OECD TG 439) (Fusco et al. 2020).

Based on the available data on two graphene analogues, the assessed chemical is likely to be not irritating to the skin.

Eye irritation

In an *in vitro* eye irritation test using the reconstructed Human EpiOcular™ Cornea-like Epithelial Model (OECD TG 492), graphene analogue 4 was tested to determine whether it requires no classification for eye irritation or requires classification for serious eye damage. The relative mean tissue viability obtained after 6 hours of treatment with the test item compared to the negative control tissues was 162.6% (greater than 60%). Therefore, under the conditions of this study and according to the test guideline, the graphene analogue requires no classification for eye irritation according to the GHS criteria.

Based on the available data on a graphene analogue, the assessed chemical is likely to be not irritating to eyes.

Sensitisation

Skin sensitisation

In an *in vivo* skin sensitisation study (OECD TG 406) using the Buehler method, 20 male Hartley guinea pigs were induced by topical administration with 0.25 g of graphene analogue 2 in 0.9% saline and then challenged by topical administration with 0.25 g of graphene analogue 1 in 0.9% saline. There were no mortalities or test substance-related clinical observations. None of the test substance-treated animals showed skin reactions during both the induction and challenge phases.

Based on the available information on a graphene analogue, the assessed chemical is unlikely to be a skin sensitiser.

Repeat dose toxicity

Inhalation

In a 28-day repeated dose inhalation toxicity study with a 90-day recovery period (OECD TG 412), rats were exposed (nose-only) to an aerosol of graphene analogue 5 (average lateral dimension: < 2 µm; surface area: 750 m^2/g; density: 0.2 g/mL; average thickness of aggregates: 20 – 30 layers [size in nm not provided]) at measured concentrations of 0.12, 0.47 and 1.88 mg/m^3 for 5 days/week 6 hours/day. No significant toxicological effects were observed. Graphene was mostly deposited in lung macrophages with some deposition in lung epithelial cells. Translocation of graphene to lung lymph nodes was observed. No adverse lung pathology (no lung epithelial cell proliferation, no inflammatory cell migration to the alveolar space, and no fibroblast proliferation after 90-day recovery period) was reported in exposed animals within all treated groups following recovery. This finding was supported by an absence of any significant increases in inflammatory cells, inflammatory biomarkers or cytokines in the broncho-alveolar fluid or lung tissue lysate in all treatment groups when compared to control animals. Furthermore, no oxidative stress markers (hydrogen peroxide, glutathione and malondialdehyde) were elevated indicating that graphene had no effect on oxidative stress at the concentrations tested. The No Observed Adverse Effect Concentration (NOAEC) was established as greater than 1.88 mg/m^3 in this study, based on no toxicological effects in rats up to the highest dose tested (Kim et al. 2016).

In a 5-day repeated dose inhalation toxicity study with a 24-day recovery period (non-guideline study), rats were exposed (head-nose) to an aerosol of graphene analogue 6 (particle size distribution (SEM) primary structure: ≤ 10,000 nm diameter, flakes; nano pore size: 9 nm, 100 nm, 40,000 nm; purity: approximately 85%) at measured concentrations of 0.54, 3.05 and 10.1

mg/m^3 for 6 hours/day. At 3.05 and 10.1 mg/m^3, the graphene analogue induced a concentration-related inflammatory response based on increases in lymphocytes, polymorphonuclear neutrophils and cytokines in broncho-alveolar lavage fluid. Microgranulomas were also observed in the lungs. No clinical signs of toxicity were observed and body weight changes were comparable to control animals. No toxicologically relevant changes were observed regarding haematology and protein levels (α2-macroglobulin and haptoglobin). There were no other effects reported in other organs. A NOAEC for graphene analogue 6 was not reported in this study (Ma-Hock et al. 2013).

Based on the findings of the two repeated dose inhalation toxicity studies for graphene analogues, the assessed chemical may have the potential for lung toxicity (e.g. inflammation and microgranulomas) at exposure concentrations of 3.05 mg/m^3 or above with even short term exposures. However, given only short term inhalation toxicity studies are available and toxicity can be dependent on a number of factors including lateral size of a particle, number of layers and surface chemistry, there remains uncertainty as to the potential lung toxicity of the assessed chemical.

Genotoxicity

In an *in vitro* mammalian cell gene mutation test (OECD TG 476) with Chinese hamster V79 cells at the Hypoxanthine-Guanine Phosphoribosyl Transferase (HPRT) locus, graphene analogue 1 was negative in the presence or absence of metabolic activation.

In an *in vitro* mammalian chromosome aberration test (OECD TG 473) in human peripheral blood lymphocytes, graphene analogue 2 was negative in the presence or absence of metabolic activation. However, the study authors noted that there was no evidence that the test substance was able to enter the cells.

A combined *in vivo* mammalian erythrocyte micronucleus test (OECD TG 474) and alkaline comet assay (OECD TG 489) were conducted using cells from the bone marrow and lungs of rats (5 per sex/group) that repeatedly exposed (nose-only) to an aerosol of graphene analogue 2 for 4 hours/day for 3 days, at measured concentrations of 0.55, 1.00 and 1.92 mg/L (maximum respirable particle dose). No significant increases in polychromatic erythrocytes in the micronuclei of bone marrow or induction of DNA damage in the lung were observed. Under the conditions of the study, the test substance was considered to be non-clastogenic and non-aneugenic.

In a comet assay (OECD TG not specified) using cells from the lungs of rats repeatedly exposed to an aerosol of graphene analogue 5 (average lateral dimension: < 2 µm; surface area: 750 m^2/g; density: 0.2 g/mL; average thickness of aggregates: 20 – 30 layers [particle size in nm not provided]) for 28 days at up to 1.88 mg/m^3, no DNA damage was detected at 1-day post-exposure and at 28-day post exposure. Furthermore, the 28-day repeated dose inhalation toxicity study also showed that there were no increases in inflammatory cytokines or hydrogen peroxide release, both known to mediate oxidative stress and be associated with DNA damage (Kim et al. 2016).

Overall, based on the available information on graphene analogues, the assessed chemical is likely to be non-genotoxic.

Environmental exposure

The assessed chemical will be manufactured in Australia and used as an additive in industrial coolants, lubricants, diesel fuels, and as a component of thermal coatings and batteries.

The assessed chemical is manufactured by extracting carbon from methane in a closed process and subsequently manually transferred to an enclosed kilning process to remove impurities. The powdered chemical is packaged and transferred to the formulation sites.

The formulation process for coatings, coolants, lubricants, and diesel fuels involves the manual addition of the assessed chemical into the respective products using a high shear mixer. Once mixing is complete the formulated product is transferred into a secondary container for storage prior to use. Release is not expected from formulation processes and any spills and cleaning materials are to be collected and disposed in line with local government regulations.

Coatings, coolants, lubricants, and diesel fuels containing the assessed chemical will only be available to professional users and will not be available to the public for DIY use. Any waste product or spills occurring during use of these products are expected to be collected for appropriate disposal.

Blended lubricants and coolants containing the assessed chemical will be used within closed systems in machinery. Manual filling by professional workers is expected to occur during maintenance activities. The used fluids are expected to be collected and disposed of through waste management contractors for recycling, re-refining, or disposal under local government regulations.

Fuels containing the assessed chemical will be used in diesel machinery, including trucks and mining equipment. Releases of the chemical during fuelling processes are expected to be minimal due to existing controls on fuel releases. The fuel containing the assessed chemical will be combusted during use and the assessed chemical is expected to transform into carbon dioxide, carbon monoxide and soot. As such, no release of the assessed chemical to the environment is expected during use in diesel products.

The coating products containing the assessed chemical will be applied by professional workers to air conditioner units and mining equipment via a spray method. Appropriate containment measures will be used to limit overspray and incidental releases. Once the coating has been cured, the assessed chemical will be immobilised within the cured matrix. The coating containing the assessed chemical will be disposed to landfill with the article it is applied to at the end of its useful life.

During battery manufacture, the assessed chemical is manually mixed into a slurry and spread onto a thin sheet where it dries. These sheets are cut to size and utilised in battery assembly. These processes take place in a controlled laboratory environment and no releases to the environment are expected. Wastes and accidental spills are to be collected and disposed in line with local government regulations. The assessed chemical will be contained within the battery and no release is expected during battery use. Batteries containing the assessed chemical will be broadly available to the public and are expected to be recycled or disposed of to landfill, in line with existing battery disposal processes.

Environmental fate

Dispersion and dissipation in aquatic environments

The fate of graphene and other carbon-based nanomaterials in the aquatic environment is complex and subject to on-going research. In waters, carbon-based nanomaterials appear to agglomerate as predicted by colloidal theory (DVLO theory) (Su et al. 2017). As such, the agglomeration of nanomaterials is influenced by the attractive (van der Waals) forces and the repulsive (electric double layer) forces. Therefore, the key properties of carbon-based nanomaterials that determine their behaviour in water are particle size, surface morphology,

and surface potential. The medium that the nanomaterials are present in, and the concentration of nanomaterials will also influence the behaviour of graphene nanomaterials in waters.

Graphene with reduced oxygen content is also observed to have a greater propensity to agglomerate compared to graphene substances, such as graphene oxide, that have higher oxygen content and surface charge density (He et al. 2017). The effective charge on graphene nanoparticles (i.e. the zeta potential) is used to assess their tendency to form stable dispersions in water. The zeta potential values for the assessed chemical (ML100: 4.58 → -16.8 mV, XE: 12 → -17.9 mV) indicate that the notified chemical will have low stability in an aqueous dispersion in the pH range relevant for aquatic environments (pH 4 – 9). As such, the assessed chemical is expected to agglomerate. This is further supported by tests conducted on the assessed chemical which found that both forms of graphene aggregated readily in distilled water to form agglomerates with D10 particle size greater than 1100 nm. Therefore, if exposed to the environment the assessed chemical is expected to agglomerate into larger particles.

Water chemistry has a significant impact on the surface charge of graphene. Natural organic matter is reported to have a stabilising effect on the suspension of graphene in water, while increased ionic strength of the water has been determined to increase the sedimentation rate of graphene (Su et al. 2017). As such, sedimentation rates of graphene vary depending on the type of environmental water it is present in. In one experiment using environmentally relevant few layered graphene (FLG) concentrations (4 µg/L), the sedimentation half-lives for FLG suspensions were found to be 1.6 days in sea water, 2.7–3.0 days in STP influent and effluent, and 8.7–13.9 days in surface waters (rivers and lakes) (Su et al. 2017). The graphene used in this study has a zeta potential range of -11.0 → -31.1 mV, which indicates a similar level of instability as the assessed chemical. Correspondingly, it can be assumed that the chemical will have similar behaviour in the environment. Therefore, the assessed chemical is not expected to stay within suspension in environmental waters if released to waterways and is expected to sediment out and be incorporated into river sediments.

The behaviour of graphene materials in soils and porous media remains an area of active research and has predominately been investigated using graphene oxide. Factors such as the particle size of the porous media, the presence of cations, and the surface potential of the graphene may influence the mobility of graphene through porous media (He et al. 2017). An increase in ionic strength of a test solution causes graphene oxide to be less mobile through porous media, attributed to reductions in the repulsive electric double layer forces between graphene oxide and other particles. Additionally, the consistency of the porous media impacts the transport of graphene substances. Graphene oxide has been observed to more readily transport through media consisting of large quartz sand than through smaller quartz sand particles (He et al. 2017). Transport of graphene oxide is also significantly inhibited through soils and clays. As the assessed chemical has a lower surface potential than graphene oxide, it can be expected that the assessed chemical will more readily interact with soil and sediment particles and therefore have lower mobility through porous media than graphene oxide.

Overall, the assessed chemical may have some dispersibility in water, but over time the dispersed particles in environmental waters are expected to agglomerate and deposit with other suspended materials onto sediments. The assessed chemical is not expected to significantly transport through sediments into ground waters or other compartments.

Degradation

Graphene consists of nanosheets, nanoplates and nanoparticles of elemental carbon and is considered to be chemically stable (Arvidsson et al. 2013).

A supplied ready biodegradation screening test, performed similar to OECD TG 301D (closed bottle), found 0% degradation according to oxygen demand after 28 days. This indicates that the assessed chemical may not be degradable by typical STP inoculum.

Abiotic degradation of the assessed chemical is expected to be slow. Strong oxidants coupled with acidic conditions are required to functionalise graphene and initiate biodegradation pathways (Marcano et al. 2010), but certain naturally occurring enzymes can reportedly biodegrade graphene (Liu et al. 2015). The assessed chemical is, therefore, likely to be very long-lived in the environment similar to other materials based on elemental carbon (such as graphite and carbon black).

Bioaccumulation

Uptake of graphene nanoparticles in biota has been investigated for aquatic invertebrates and for fish (Dong et al. 2018; Guo et al. 2013; Lu et al. 2017). While graphene is observed to accumulate within the gut of test organisms, excretion is generally rapid with feeding and/or in the presence of natural organic matter. Graphene with small lateral dimensions (20–70 nm) may be of higher concern due to its ability to cross the gut membranes in fish. The assessed chemical has lateral dimensions within the 50–70 nm range, however, it is not expected to be a bioaccumulation concern due to expected rapid agglomeration into larger particles.

In one exposure study, adult zebrafish (*Danio rerio*) were exposed to various concentrations of C^{14}-radiolabelled FLG (Lu et al. 2017). A small FLG (S-FLG; lateral size 20–70 nm; 3 layers thickness) and a larger FLG (L-FLG; lateral size 300–700 nm; 4 layers thickness) were used. Uptake concentrations within the fish were found to be dependent on the exposure concentrations, and peak accumulation was observed to occur after 48 hours of exposure. At a concentration of 250 µg/L, the L-FLG had a higher peak body burden than the S-FLG (48 µg/g dw and 0.29 µg/g dw respectively). The inclusion of natural organic matter in the test solution increased the uptake of both graphene materials (2-fold for L-FLG, 16-fold for S-FLG). The L-FLG was found to accumulate within the digestive tract of the fish, with some minor accumulation on the gills. The S-FLG was found in the guts and the liver of the fish, indicating that the S-FLG was able to pass through the walls of the digestive tract. Depuration of L-FLG was rapid, with 95% excretion after 4 hours in clean water. After 120 hours of depuration, no L-FLG was present within the gut tract of the fish. In contrast, only 30% of S-FLG was able to be excreted after 4 hours of depuration. No further S-FLG was excreted up to 72 hours of depuration. The S-FLG used in this study has comparable lateral dimensions to the assessed chemical, although a portion of the assessed chemical's morphologies have a much higher thickness (20-25 layers). The assessed chemical may have the potential to cross intestinal membranes, however the higher thickness of the assessed chemical could lower the likelihood of this occurring.

In another exposure study, uptake by neonatal (< 1 day old) *Daphnia magna* was investigated through exposure to C^{14}-radiolabelled graphene (Guo et al. 2013). The graphene used in this study was a mixture of FLG with lateral dimensions of 300 nm, and FLG with lateral dimension of 2000 nm. All FLG was approximately 4 layers thick. Uptake concentrations in daphnia were dependant on the exposure concentrations. The peak body burden of 8 µg/mg dw was observed after 24 hours exposure to a graphene concentration of 250 µg/L. Uptake of the graphene was observed to occur solely within the digestive tract of the daphnia. Depuration of the graphene was affected by the initial exposure concentration and the depuration medium. No depuration of graphene was observed in daphnia exposed to 50 µg/L after 24 hours in clean water, while after 24 hours, 46% and 64% of the graphene was excreted during depuration for the daphnia exposed to 100 and 250 µg/L respectively. Depuration rates were increased in the presence of humic acid, and when the daphnia was fed algae during depuration. Daphnia that were fed algae during depuration had cleared all graphene from their

digestive tract after 10 hours. As such, accumulation of the assessed chemical in invertebrates is not expected to be a concern under environmental conditions, where organic matter is expected to be present, and feeding is expected to occur.

Another uptake experiment demonstrated that accumulation of graphene in higher level organisms can occur from consumption of graphene-contaminated biota (Dong et al. 2018). While higher body burdens were observed after uptake through diet, when compared to body burdens from exposure to graphene suspensions, levels of accumulation were not indicative of a biomagnification concern for *Daphnia magna* or zebrafish.

Predicted environmental concentration (PEC)

The predicted environmental concentration (PEC) has not been calculated as release of the assessed chemical to the aquatic environment will be negligible based on the assessed use patterns.

Environmental effects

As a nanoscale chemical, the assessed chemical will have unique physical and chemical properties, compared to non-nanoscale chemicals, resulting in different toxic effects. Carbon nanomaterials also differ in their toxicity based on their shape and surface characteristics.

In general, harmful effects from graphene may occur as a result of surface interactions and physical effects between graphene and biota. These effects include cell damage caused by direct penetration of graphene to cells and nutrient deficiency caused by accumulation of graphene within the guts of biota or the pores of fish embryos.

Effects on Aquatic Life

Acute toxicity

The following acute toxicity endpoints, using graphene similar to the assessed chemical, are available in the public domain or were supplied by the applicant for the specific assessed chemical for fish toxicity:

Taxon	Endpoint	Method
Fish	96h LC50 > 100 mg/L	*Gobiocypris rarus* (Rare minnow) OECD TG 203 Semi-static conditions Nominal concentration based on loading rate
Invertebrate	48h LC50 > 16 mg/L	*Daphnia magna* (water flea) Non-standard test Static conditions Nominal concentration
Algae	96h EC50 = 62 mg/L	*Chlorella pyrenoidesa* (green algae) Growth inhibition Non-standard test Static conditions Nominal concentration

In the supplied acute fish toxicity study detailed above, a graphene was used that had typical lateral size of 2.5 µm x 4.6 µm, much larger than the assessed chemical. A suspension containing the graphene was filtered through a 0.45 µm polyether sulfonate filter and the filtrate was used as the test solution. As graphene is insoluble in water, any graphene particles present in the suspension were likely removed during filtration. It is unlikely that the fish were exposed to graphene throughout the test period.

In the acute invertebrate toxicity study detailed above, FLG with larger lateral dimensions than the assessed chemical held in suspension was found to have 40% mortality to neonatal (< 1 day old) *Daphnia magna* at the highest test concentration of 16 mg/L (Fan et al. 2016). As the potential for adverse effects increases with smaller particle size, the assessed chemical may cause similar effects at similar or lower exposure concentrations.

The algal toxicity study tested graphene-oxide, reduced graphene-oxide and multi-layer graphene (Zhao et al. 2017). While the multi-layer graphene (MLG) used in the study has a larger lateral particle size than the assessed chemical, it also has a low surface oxygen content and is considered the most relevant for this assessment. The toxicity caused by MLG was determined to be due to algal cell membrane damage induced by oxidative stress, physical cell penetration, and extraction of cell contents by the graphene particles. Nutrient depletion was also indicated to play a role in the observed toxic effects. As the assessed chemical has a larger BET surface area and smaller lateral dimensions than the MLG used in this study, the assessed chemical may be expected to more readily interact with the surfaces of algae particles, potentially causing similar effects at lower concentrations.

Chronic toxicity

The following chronic and reproductive endpoints using graphene similar to the assessed chemical were available in the public domain or supplied by the applicant:

Taxon	Endpoint	Method
Fish (embryo acute toxicity)	96h LOEC = 0.005 mg/L	*Danio rerio* (zebrafish) Mortality Non-standard test Semi-static conditions Nominal concentration
Invertebrates (chronic)	21d NOEC = 0.1 mg/L	*Daphnia magna* (water flea) OECD TG 211 Daphnia size, brood time, brood number Semi-static conditions Nominal concentration
Amphibian (larvae)	12d NOEC = 1 mg/L	*Xenopus laevis* (African clawed frog) ISO 21427–1 Larvae growth Semi-static conditions Nominal concentration

The effects of graphene on the survivability and development of zebrafish embryos were investigated using pristine graphene (PG; single layer thickness, 170–390 nm lateral) (Manjunatha et al. 2018). All embryos exposed to PG at concentrations of 30 µg/L or higher died within 2 hours of exposure. Various developmental effects were observed for embryos exposed to PG at concentrations as low as 5 µg/L. The PG used in this study is smaller than the assessed chemical (in terms of thickness and lateral size), and therefore may more readily accumulate within the chorions of the embryos and cause development damage. As such, this represents a conservative estimate of fish embryo acute toxicity.

A *daphnia magna* reproductive toxicity test, performed according to OECD TG 211, showed that FLG with larger lateral dimensions to the assessed chemical can inhibit reproduction at concentrations above 0.1 mg/L (Fan et al. 2016). Exposure to graphene caused reduced size in daphnia offspring and reduced numbers of offspring. As the FLG accumulated within the digestive tract of the daphnia, it was believed that the changes in reproduction may have been caused by malnutrition resulting from reduced digestive efficiency. The FLG used in this assessment is larger than the assessed graphene, and therefore may accumulate more readily in the organism than the assessed chemical. As such, this represents a conservative estimate of daphnia reproductive toxicity.

Similarly, MLG (2–20 layers, 1.2–5.4 µm lateral size) was found to inhibit the growth of *Xenopus laevis* (African clawed frog) larvae (Muzi et al. 2016). The MLG was found to accumulate within the digestive tract and on the gills of the larvae, potentially inhibiting nutrient intake. No mortality was observed up to the highest test concentration of 50 mg/L. The assessed chemical consists of smaller particles than the MLG used in this study. As body burdens of graphene are observed to increase as particle size decreases (see Bioaccumulation section), the assessed chemical may cause similar effects at similar or lower exposure concentrations.

Effects on terrestrial Life

The following measured lethal concentration (LC50) endpoint, using a form of graphene similar to the assessed chemical, was supplied by the applicant:

Taxon	Endpoint	Method
Earthworm	14d LC50 ≥ 1001 mg/L	*Eisenia fetida* (Earthworm) Mortality OECD TG 207 Artificial soil Nominal concentration

The supplied earthworm study report did not specify the particle size of the tested graphene. However, the supplier and batch number matched the graphene used in the acute fish toxicity study (see effects on aquatic life; acute toxicity) suggesting that the test used graphene with a typical lateral size of 2.5 µm x 4.6 µm.

Predicted no-effect concentration (PNEC)

A predicted no-effect concentration (PNEC) of 0.5 µg/L was calculated for the assessed chemical in the aquatic environment. This value was derived using the most sensitive chronic endpoint value, which is for zebrafish larval malformation (5 µg/L). An assessment factor of 10 was applied to this endpoint as chronic or reproductive toxicity data were available for at least three trophic levels and was expected to have considered the most sensitive cases (EPHC 2009).

Categorisation of environmental hazard

The assessed chemical is an inorganic substance, and therefore classification according to PBT criteria is not appropriate.

Environmental risk characterisation

The assessed chemical has end uses in coolants, lubricants, diesel fuels, and as a component of thermal coatings and batteries. For diesel fuels, the assessed chemical is expected to be combusted during use. For all other uses, the assessed chemical will share the fate of the product or article. As such, it will be collected and recycled or disposed to landfill at the end of the products useful life. As there will be no consumer or do-it-yourself use, release of the assessed chemical to the environment is not expected.

If released to the environment, agglomeration and sedimentation is expected to minimise the concentrations of the assessed chemical in aquatic compartments. Rates of agglomeration will be dependent on the ionic strength and presence of organic matter of the receiving waters. The assessed chemical is not expected to be mobile through soils and sediments. Agglomeration and sedimentation processes are expected to reduce the bioavailability of the assessed chemical.

The assessed chemical has potential to accumulate within the digestive tracts and on the gills of aquatic organisms. The potential of the assessed chemical to cross digestive membranes is uncertain. The assessed chemical also has potential to cause cell damage to aquatic algae. Low concentrations of the assessed chemical may have effects on the development of organisms when exposure occurs during key developmental stages.

A Risk Quotient (PEC/PNEC) for the aquatic compartment has not been calculated. However, release of the assessed chemical to the aquatic environment will be negligible based on its

assessed use patterns. Therefore, based on the limited exposure from the assessed use patterns, the environmental risk from the assessed chemical can likely be managed.

This current assessment may require revisions if information becomes available that indicates that the bioaccumulation potential and toxic effects of graphene and other carbon-based nanomaterials are greater than outlined in this assessment.

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