Australian Government
Department of Health
Australian Industrial Chemicals Introduction Scheme

Graphene

Assessment statement (CA09526)

30 May 2022

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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

The assessed chemical is a non-soluble solid containing particle sizes in the nanoscale and its introduction is a specified class of introduction (Section 7, subsection 3(c) of the Rules). In accordance with Section 28, table item 3 of the Rules, the indicative human health risk for the proposed introduction is medium to high. In accordance with Section 29, table item 3 of the Rules, the indicative environment risk for the proposed introduction is medium to high.

Defined scope of assessment

The chemical has been assessed:

- as manufactured or imported at less than or equal to 1 tonne/annum in volume and as a liquid dispersion at less than or equal to 15% concentration; and
    - for use in research and development as a liquid dispersion at less than or equal to 15% concentration;
    - for a non-consumer end use (not to be available to the general public) in automotive, computing and photovoltaics industries as a component in heat transfer fluids at less than or equal to 10% concentration;
    - for a do-it-yourself end use as a component in heat transfer fluids in
        - automobiles at less than or equal to 10% concentration, or
        - in photovoltaic batteries or computers at less than or equal to 2% concentration.

Summary of assessment

Summary of introduction, use and end use

Based on the information provided by the applicant, the assessed chemical will be manufactured in Australia as a liquid dispersion containing the chemical at up to 15% concentration from imported graphite powder, using a continual liquid phase exfoliation process. The resulting liquid dispersion will be used for research and development (R&D) or reformulation into heat transfer fluids.

The assessed chemical will be reformulated into heat transfer fluids at up to 10% concentration. Reformulation processes will likely vary depending on the nature of the formulated products. End use products containing the assessed chemical at various concentrations (up to 10%) will be in packaging appropriate for the use of the products.

Heat transfer fluids containing the assessed chemical at up to 10% concentration will be used in automotive, computing and photovoltaics industries and will be used by professionals.

Heat transfer fluids containing the assessed chemical will also be available to do-it-yourself (DIY) users in up to 25 L containers for use in automobiles (at up to 10% concentration) and in photovoltaic batteries or computers (at up to 2% concentration).

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 (< 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, the assessed chemical:

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

Summary of health risk

Public

When introduced and used in the proposed manner, inhalation of nanoscale particles to DIY users from manual addition of heat transfer fluids containing the assessed chemical to automobiles (at up to 10% concentration), and photovoltaic batteries or computers (at up to 2% concentration) is not expected due to end use products' characteristics as stated by the applicant. Only infrequent incidental dermal and ocular exposure to the assessed chemical in liquid dispersion is expected. 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 health effects if exposed to the assessed chemical during its manufacture, reformulation and R&D activities. Control measures (see **means for managing risk** 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). 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 introduced as an additive for use in heat transfer fluids, following manufacturing in Australia. This end use may result in the release of the assessed chemical to sewers and surface waters. In these compartments, the assessed chemical is expected to sediment out depending on the specific water conditions.

The assessed chemical is expected to be long-lived in the environment. The assessed chemical may accumulate within the digestive tracts of organisms but is not expected to cross digestive membranes. The assessed chemical is not expected to have a biomagnification concern. The assessed chemical may cause adverse effects to aquatic organisms and impact the development of aquatic organisms.

Based on its assessed use pattern and predicted environmental concentration, the assessed introduction is unlikely to cause environmental risks.

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.

Research and Development (R&D)

Information relating to safe introduction and use

- The following control measures could be implemented to manage the risk arising from exposure to the assessed chemical at up to 15% in liquid dispersion during R&D activities:
    - Use of engineering controls such as
        - Enclosed, automated processes where possible
    - Use of safe work practices to
        - Avoid generation of aerosols or mists
        - Avoid inhalation of 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

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 could be implemented to manage the risk arising from exposure during manufacturing and formulation activities of the assessed chemical:
    - 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
- A copy of the SDS should be easily accessible to employees.

Environment

Information relating to safe introduction and use

- The packaging of heat transfer fluids 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.

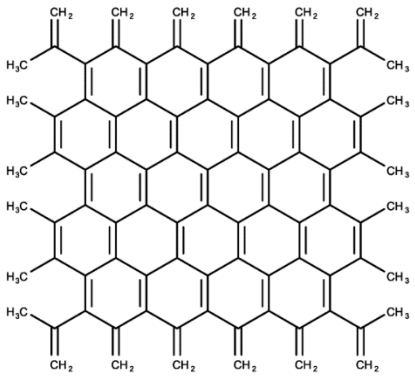
Considering the proposed means of managing risks, the Executive Director is satisfied that when the chemical is introduced and used in accordance with the terms of the assessment certificate the human health and environment risks can be managed within existing risk management frameworks. This is provided 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 greater than 95%.

Chemical name	Graphene
CAS No.	1034343-98-0
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 (lateral dimensions)	Range: 100 – 2000 nm d10 = 200 nm[ʃ] d50 = 370 nm[ʃ] d90 = 630 nm[ʃ]
Layer number (thickness)	Typical range: 2-4 layers d10 = 0.33 nm[ʃ] d50 = 1.00 nm[ʃ] d90 = 3.33 nm[ʃ]
Specific surface area	246.9 ± 15.4 m^2/g

Aspect ratio	Mean: 500: 1 Range: 30: 1 – 6000: 1
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	< 2%
Surface functionalisation	None

* Based on graphene analogues
∫ d10 is the 10th percentile of the particle size distribution; d50 is the median of the particle size distribution; d90 is the 90th percentile of the particle size distribution.

Human exposure

Workers

Transport and Storage

Transport and storage workers may come into contact with the assessed chemical in a liquid dispersion (at up to 15% concentration) only in the event of accidental rupture of containers.

Manufacture

Manufacturing of the assessed chemical using graphite at up to 100% concentration may cause worker exposure to dusts, aerosols or mists. Dermal, ocular and inhalation exposure (if aerosols or mists are formed) of workers to the assessed chemical at various concentrations may occur during transfer, exfoliation, quality control analysis, packaging, and cleaning and maintenance of equipment. Exposure to dusts is not expected from the liquid dispersion manufactured containing up to 15% concentration of the assessed chemical. 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 a fully enclosed/contained environment, followed by automated filling using sealed delivery systems into containers of various sizes. Dermal, ocular and inhalation (if aerosols or mists are formed) exposure of workers to the assessed chemical at up to 15% 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.

Research and Development (R&D)

Dermal, ocular and inhalation (if aerosols or mists are formed) exposure of workers to the assessed chemical (at up to 15% concentration) may occur during a variety of R&D activities using the liquid dispersion. The applicant states that exposure is expected to be minimised through the use of engineering controls (such as enclosed, automated processes where possible and sufficient ventilation) and PPE (such as protective clothing, impervious gloves, safety glasses and appropriate respiratory protection if inhalation exposure may occur).

Professional End Use

Dermal and ocular exposure to the assessed chemical (at up to 10% concentration) may occur during the use of heat transfer fluids containing the chemical. Inhalation exposure is not expected given the estimated very low vapour pressure of the assessed chemical and the liquid (dispersion) 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).

Public

Heat transfer fluids containing the assessed chemical at up to 10% concentration may be sold through the retail market to DIY users to replace or top-up heat transfer fluids in automobiles, photovoltaic batteries or computers. Dermal and ocular exposure to the assessed chemical at up to 10% concentration may occur to DIY users. Inhalation exposure to the assessed chemical is not expected, given the low vapour pressure of the assessed chemical and the liquid (dispersion) form of end use products. Protective gloves may not be used by DIY users during applications. However, the risk to DIY users from manual addition of liquid (dispersion) end use products containing the assessed chemical to automobiles, photovoltaic batteries or computers is considered low as only minimal dermal and ocular exposure could occur from infrequent DIY use. The applicant states that exposure to nano-sized particles of the assessed chemical is not expected during spills of the liquid during DIY use, as the nano particles will be bound with the other additives in end use products to form a waxy material when the heat transfer fluid is dried.

Health hazard information

For the purpose of this risk assessment ‘assessed chemical’ refers only to the physical form of graphene described 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

Inhalation

In an acute inhalation toxicity study (OECD TG 436), Sprague Dawley (SD) rats (3 rats/sex/dose) were exposed (nose-only) to an aerosol of graphene analogue 1 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 (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 a graphene analogue, 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 1 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 does not require classification 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 1 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 3 (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 (OECD TG not specified), rats were exposed (head-nose) to an aerosol of graphene analogue 4 (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 4 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, 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 5 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 1 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.

In a comet assay (OECD TG not specified) using cells from the lungs of rats repeatedly exposed to an aerosol of graphene analogue 3 (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 a component of heat transfer fluids to be used in automotive, computing, and photovoltaic (PV) industries.

The assessed chemical is manufactured within the liquid phase which may involve an open process. The suspension containing the assessed chemical will then be reformulated into the Fend use coolant liquids through blending processes. Environmental releases from the manufacture or reformulation of the substance are unlikely, as direct releases of the chemical are not expected to occur. Bunding will be employed, to contain any spills, that can then be collected and disposed in line with local government regulations.

The majority of the introduction volume is expected to be used in heat transfer liquid products for professional use. These applications will involve the filling and draining of sealed systems in the automotive, computing and PV industries by professional workers. Release to the environment is unlikely for these use cases, as any drained coolant is expected to be collected for re-use or correct disposal, and leakage from the sealed systems during use is expected to be minimal.

A portion of the introduction volume of the assessed chemical will be formulated into heat transfer liquid products available to DIY users for automotive, computing and PV applications. DIY computing and PV applications are expected to consume significantly smaller volumes of the assessed chemical compared to the DIY automotive market. While it is expected that DIY users will collect any drained heat transfer liquids for disposal, incorrect disposal of products containing the assessed chemical may occur. A 2013 report found that only 4% of households were disposing of motor oil (either correctly or incorrectly) in Australia (Aither 2013). This suggests that DIY users may make up a small portion of all consumers of vehicle maintenance products, and that the vast majority of vehicle maintenance is performed through professional mechanic services. On this basis, the worst-case exposure scenario for this introduction would be a situation where the entire introduction volume of the assessed chemical is solely for end use in automotive coolants, with all DIY users incorrectly disposing of coolant products. In this scenario, as 4% of households appear to be performing DIY automotive maintenance, the volume of the assessed chemical that may be released to sewer or surface waters is estimated to be 4% of the introduction volume.

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.

Su (2017) found that few-layered graphene (FLG) with lateral dimensions of 60–590 nm agglomerated readily compared to suspensions of FLG with lateral dimensions ranging 25–75 nm (Su et al. 2017). 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 assessed chemical is FLG (2-4 layers) with typical lateral dimensions of 370 nm and low oxygen content. Therefore, the assessed chemical is expected to agglomerate more readily than smaller graphene substances and more readily compared to graphene materials with higher levels of oxidisation.

Water chemistry has a significant impact on the surface charge of FLG. 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 FLG vary depending on the type of environmental water it is present in. In one experiment using environmentally relevant 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). As the assessed chemical is similar to the graphene used in this experiment, it can be inferred that the assessed 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 is likely to 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 sheets 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 FLG 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. FLG 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 much larger than 20–70 nm and is therefore not expected to be a bioaccumulation concern.

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. As the L-FLG used in this study is comparable to the assessed chemical in terms of size and surface groups, the assessed chemical is expected to have similar uptake behaviour in fish with none of the assessed chemical expected to cross intestinal membranes.

In another exposure study, uptake by neonatal (<1 day old) *Daphnia magna* was investigated through exposure to C^{14}-radiolabelled FLG (Guo et al. 2013). The FLG 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 FLG concentration of 250 µg/L. Uptake of the FLG was observed to occur solely within the digestive tract of the daphnia. Depuration of the FLG was

affected by the initial exposure concentration and the depuration medium. No depuration of FLG was observed in daphnia exposed to 50 µg/L after 24 hours in clean water, while after 24 hours, 46% and 64% of the FLG 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 FLG 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 FLG in higher level organisms can occur from consumption of FLG-contaminated biota (Dong et al. 2018). While higher body burdens were observed after uptake through diet, when compared to body burdens from exposure to FLG suspensions, levels of accumulation were not indicative of a biomagnification concern for *Daphnia magna* or zebrafish.

Predicted environmental concentration (PEC)

A predicted environmental concentration (PEC) for Australian waters was calculated assuming 4% of the introduction volume is released into sewage treatment plants (STP) and waterways. This calculated value is conservative as no removal of the assessed chemical in STP or removal through sedimentation has been considered. The calculation of the PEC is detailed in the table below:

Total Annual Introduction Volume	1000	kg/year
Proportion expected to be released to sewer	4 %	
Annual quantity of chemical released to sewer	40	kg/year
Days per year where release occurs	365	days/year
Daily chemical release	0.11	kg/day
Water use	200.0	L/person/day
Population of Australia	24.386	Million
Daily effluent production	4 877	ML/day
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River	0.022	µg/L
PEC - Ocean	0.002	µg/L

Environmental effects

As a nanomaterial, graphene will have unique physical and chemical properties, compared to non-nano size materials, 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, similar to 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).

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 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 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 and reproductive 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 similar 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.

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.

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 use in heat transfer liquids including in products for automotive, PV and computer cooling applications. These uses may result in release of the assessed chemical to environmental waters from DIY users.

Once released to the environment, agglomeration and sedimentation is expected to reduce the concentrations of the assessed chemical in suspension. 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 but is expected to be too large to cross the digestive membranes. 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.

Based on the PEC and PNEC values determined above, Risk Quotients (RQ = PEC ÷ PNEC) have been calculated for release of the assessed chemical to water, soil and sediment:

Compartment	PEC	PNEC	RQ
River	0.022 µg/L	0.5 µg/L	0.04
Ocean	0.002 µg/L	0.5 µg/L	< 0.01

For the riverine and marine environment, an RQ less than 1 indicates that the assessed chemical poses a low risk to the environment based on estimated emissions, as environmental concentrations are below levels that are likely to cause harmful developmental effects.

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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