File No: STD/1681

June 2019

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Graphene

(PureGRAPH™ 5, 10 & 20)

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act) and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the Department of Health, and conducts the risk assessment for public health and occupational health and safety. The assessment of environmental risk is conducted by the Department of the Environment and Energy.

This Public Report is available for viewing and downloading from the NICNAS website or available on request, free of charge, by contacting NICNAS. For requests and enquiries please contact the NICNAS Administration Coordinator at:

Street Address:	Level 7, 260 Elizabeth Street, SURRY HILLS NSW 2010, AUSTRALIA.
Postal Address:	GPO Box 58, SYDNEY NSW 2001, AUSTRALIA.
TEL:	+ 61 2 8577 8800
FAX:	+ 61 2 8577 8888
Website:	www.nicnas.gov.au

Director
NICNAS

TABLE OF CONTENTS

SUMMARY

The following details will be published in the NICNAS *Chemical Gazette:*

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1681	First Graphene Limited	Graphene (PureGRAPH™ 5, 10 & 20)	ND*	≤ 100 tonnes per annum	Component of industrial coatings and polymer composites

*ND = not determined

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Human Health Risk Assessment
Provided that the recommended controls are being adhered to, under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

When used in the proposed manner, the notified chemical is not considered to pose an unreasonable risk to public health.

Environmental Risk Assessment
Based on the currently available information for the aquatic hazards of graphene-based nanomaterials and the assessed use patterns, the notified chemical is not expected to pose an unreasonable risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical during manufacturing, reformulation and end use (if used as a powder):
  - Enclosed automated processes
  - Local exhaust ventilation fitted with high-efficiency particulate air (HEPA) filter

- A person conducting a business or undertaking at a workplace should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical in powder form:
  - Avoid inhalation of dust
  - Avoid generation of dust

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical in powder form:
  - Appropriate respiratory protection (such as a P2 respirator) if inhalation exposure may occur

  Guidance in selection of personal protective equipment can be obtained from Australian, Australian/New Zealand or other approved standards.

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

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)* as adopted for industrial chemicals in Australia, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation should be in operation.

Environment

- The following control measures should be implemented by manufacturers or users of the notified chemical to minimise environmental exposure during manufacture, formulation and use of the notified chemical:
  - The notified chemical in powder form should not be released to the aquatic environment

Disposal

- Where reuse or recycling are not appropriate, dispose of the notified chemical in an environmentally sound manner in accordance with relevant Commonwealth, state, territory and local government legislation.

Regulatory Obligations

Secondary Notification
This risk assessment is based on the information available at the time of notification. The Director may call for the reassessment of the chemical under secondary notification provisions based on changes in certain circumstances. Under Section 64 of the *Industrial Chemicals (Notification and Assessment) Act (1989)* the notifier, as well as any other importer or manufacturer of the notified chemical, have post-assessment regulatory obligations to notify NICNAS when any of these circumstances change. These obligations apply even when the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS).

Therefore, the Director of NICNAS must be notified in writing within 28 days by the notifier, other importer or manufacturer:

(1) Under Section 64(1) of the Act; if
  - the notified chemical is introduced with parameters significantly outside those stated in this notification, specifically particle size and size distribution, surface functionalisation, surface area, layer number, or impurities;
  - the notified chemical is intended for use in food packaging;
  - the notified chemical is intended for use in clothing other than footwear;
  - the use pattern of the notified chemical changes such that its exposure to workers, public or the environment is increased;
  - coating products containing the notified chemical are intended for retail sale to the public;

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from a component of industrial coatings and polymer composites, or is likely to change significantly;
  - the amount of chemical being introduced has increased, or is likely to increase, significantly;
  - the method of manufacture of the chemical in Australia has changed, or is likely to change, in a way that may result in an increased risk of an adverse effect of the chemical on occupational health and safety, public health, or the environment;
  - additional information has become available to the person as to an adverse effect of the chemical on occupational health and safety, public health, or the environment.

The Director will then decide whether a reassessment (i.e. a secondary notification and assessment) is required.

Safety Data Sheet
The SDS of the notified chemical provided by the notifier was reviewed by NICNAS. The accuracy of the information on the SDS remains the responsibility of the applicant.

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
First Graphene Limited (ABN: 50 007 870 760)
1 Sepia Close
HENDERSON WA 6166

NOTIFICATION CATEGORY
Standard: Chemical other than polymer (more than 1 tonne per year)

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
No details are exempt from publication.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Schedule data requirements are varied for melting point, boiling point, vapour pressure, water solubility, hydrolysis as a function of pH, partition coefficient, adsorption/desorption, dissociation constant, flash point, flammability, oxidising properties, acute dermal toxicity, skin and eye irritation, skin sensitisation, chromosome damage *in vitro*, genotoxic damage *in vivo* and ready biodegradation.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
European Union (2018)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Three different physical forms of the notified chemical will be introduced under the following marketing names:

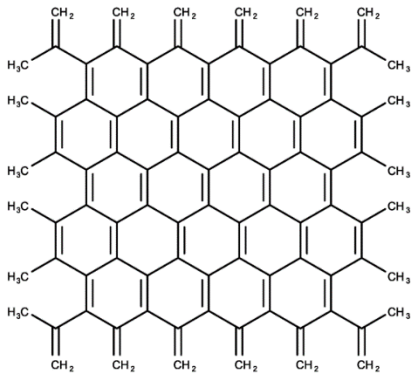
PureGRAPH™ 5
PureGRAPH™ 10
PureGRAPH™ 20

CHEMICAL NAME
Graphene

CAS NUMBER
1034343-98-0

MOLECULAR FORMULA
C

STRUCTURAL FORMULA



MOLECULAR WEIGHT
Not applicable

ANALYTICAL DATA

METHOD	Raman spectroscopy
Remarks	Raman spectroscopy conducted on all three forms (PureGRAPH™ 5, 10 & 20)
	Raman spectra were consistent with that expected for graphene with characteristic bands at 1350 cm^{-1} (D band), 1585 cm^{-1} (G band) and 2700 cm^{-1} (2D band). Ratio of l_D/l_G was < 0.2 indicating low defect graphene platelets.
TEST FACILITY	First Graphene (2018a)

COMPOSITION

DEGREE OF PURITY
> 98%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS
None

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (> 1% BY WEIGHT)
None

ADDITIVES/ADJUVANTS
None

3. COMPOSITION

DEGREE OF PURITY
> 98%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Black, solid powder

Property	***Value***	***Data Source/Justification***
Melting Point	~ 4,200 °C	Calculated
Boiling Point	Not determined	Study not required as melting point > 300 °C
Tapped Density	PureGRAPH™ 5: 61.8 kg/m^3 PureGRAPH™ 10: 124 kg/m^3 PureGRAPH™ 20: 251 kg/m^3	Measured
Vapour Pressure	Not determined	Study not required as melting point > 300 °C
Water Solubility	Not determined	Available data indicate that graphene is insoluble in water (Gottschalk *et al*., 2009)
Hydrolysis as a Function of pH	Not determined	The notified chemical is chemically inert under ambient conditions
Partition Coefficient (n-octanol/water)	Not determined	The notified chemical is composed of insoluble inorganic nanoparticles
Adsorption/Desorption	Not determined	The notified chemical is insoluble in water and is expected to adsorb to particulate matter - e.g. soil (Arvidsson *et al*., 2013; Batley & McLaughlin, 2008)
Dissociation Constant	Not determined	The notified chemical contains some surface carboxylic acid groups as indicated by XPS

Property	*Value*	*Data Source/Justification*
Particle Size (X/Y, Z)†‡ (Laser Diffraction)	PureGRAPH™ 5: X/Y: 5.63 µm Z: 1.5-3.3 nm PureGRAPH™ 10: X/Y: 9.92 µm Z: 3.3-5.2 nm PureGRAPH™ 20: X/Y: 19.2 µm Z: 3.3-5.2 nm	Measured
Particle Size (SEM)	Mean Primary Particle Size∫: PureGRAPH™ 5: 4.35 ± 2 µm	Measured
Zeta Potential (pH dependent)	PureGRAPH™ 5: 10 → -30 mV PureGRAPH™ 10: 0 → - 50 mV PureGRAPH™ 20: 0 → - 50 mV	Measured
Surface Area	PureGRAPH™ 5: 9.3821 m^2/g PureGRAPH™ 10: 9.2446 m^2/g PureGRAPH™ 20: 7.8127 m^2/g	Measured. Surface area indicates the particles are non-porous
Flash Point	Not determined	Study not required as inorganic solid
Flammability	Not determined	Not expected to be highly flammable
Autoignition Temperature	360 °C	Measured (graphene analogue)
Oxidising Properties	Not determined	Contains no functional groups that would imply oxidising properties
Dust Explosivity	Not explosive	Measured (graphene analogue)
Oxygen level (Unterzaucher method)	PureGRAPH™ 5: 1.41% PureGRAPH™ 10: 2.53% PureGRAPH™ 20: 3.31%	Measured
Oxygen level (XPS)	PureGRAPH™ 5: 14.67% PureGRAPH™ 10: 8.31% PureGRAPH™ 20: 5.22%	The notified chemical has low levels of oxidation

† X/Y dimensions given as a Dv(50) = 50% of particles less than the size specified. ǂ Z dimension is calculated from the formula Z = (*n* -1)*0.37 nm where *n* is the number of graphene layers specified by the notifier and 0.37 nm is the interspatial distance (Koh, Bae, Cahill, & Pop, 2011)
∫ later flake size
SEM: Scanning Electron Microscopy
XPS: X-ray Photoelectric Spectroscopy

As indicated by XPS, the notified chemical is present as sp^2 carbon platelets with low sp^3 defect levels and low oxidation levels (First Graphene, 2018b). Oxygen is present on the surface of the notified chemical as C-O (hydroxyl and epoxy) and O-C=O (carboxyl). No other impurities were detected on the surface of the chemical.

The notified chemical consists of graphene platelets ranging from 4 layers to 15 layers. No single layer (or mono-layer) graphene was observed in the size distribution of the notified chemical.

DISCUSSION OF PROPERTIES
For full details of tests on physical and chemical properties, refer to Appendix A.

Reactivity
The notified chemical is expected to be stable under normal conditions of use.

Physical Hazard Classification
Based on the submitted physico-chemical data depicted in the above table, the notified chemical is not recommended for hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)*, as adopted for industrial chemicals in Australia.

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will be manufactured in Australia from imported graphite. Three different forms of the notified chemical will be manufactured (PureGRAPH™ 5, 10 & 20).

MAXIMUM INTRODUCTION VOLUME OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	1 – 20	50	100	100	100

IDENTITY OF MANUFACTURER/RECIPIENTS
First Graphene Limited
1 Sepia Close
Henderson WA 6166

TRANSPORTATION AND PACKAGING
After manufacture, the notified chemical will be packaged into 500 g to 5 kg sealable plastic bags which will be packed into double lined, reinforced cardboard boxes or sealable plastic pails. Boxes and pails are stored at the notifier's warehouse and then distributed to end-use manufacturing sites by road, rail, sea and air. End-use products containing the notified chemical at ≤ 15% concentration will be in packaging appropriate for the end-use products.

USE
The notified chemical will be used as a component in fire retardant coatings at ≤ 15% concentration and in polymer composites (such as rubber, polyurethanes, epoxies) at ≤ 1% concentration. The notified chemical is not intended to be used in clothing items other than footwear, and is not intended for use in food packaging products.

OPERATION DESCRIPTION
Manufacture
The notified chemical is produced following the direct exfoliation of graphite using an electrochemical exfoliation process. Graphite is received on site in 25 kg triple lined bags stored in sealed wooden crates. The graphite is emptied into a hooded electrochemical cell in the presence of ducting to remove any potential dust. Captured dust is directed through a high efficiency polytetrafluoroethylene (PTFE) membrane filter followed by a wet cross flow scrubber before being released to the atmosphere through a stack that is > 3 m above the roof. The removal of dust is in accordance with Western Australia Department of Water and Environmental Regulation Works Approval Number W6050/2017/1.

Within the electrochemical cell, the graphite is exfoliated electrochemically in liquid sulphuric acid. Some fumes of low strength acid are expected to be released from the cell. These fumes will be extracted by a fume hood and directed to the crossflow fume scrubber for treatment prior to the waste emissions being released to the atmosphere through a stack that is > 3 m above the roof (in accordance with Western Australia Department of Water and Environmental Regulation Works Approval Number W6050/2017/1).

Following manufacture from graphite, the notified chemical will be transferred from the electrochemical cell and filtered via sonicated vibrating screen decks. The notified chemical will then be collected from the screens decks and finished through a sizing circuit to achieve the required particle sizes. The notified chemical will be washed and filtered to remove any acid solution before being dried in an oven. The notified chemical will then be blended and packaged into sealable plastic bags of 500 g to 5 kg in size. Blending and packaging will be performed under fume hoods in a dedicated, negative pressure clean room with any generated dust captured in a high efficiency PTFE membrane filter and wet cross flow scrubber before being released to the atmosphere through a stack that is > 3 m above the roof (in accordance with Western Australia Department of Water and Environmental Regulation Works Approval Number W6050/2017/1).

The sealed plastic bags will be packed into double lined, reinforced cardboard boxes or sealable plastic pails. The notified chemical will be stored at the notifier's warehouse prior to transport to other manufacturers.

Reformulation
The reformulation procedure will likely vary depending on the nature of the formulated products. However, these procedures are likely to be highly automated and use closed systems with adequate waste management and ventilation systems in place in accordance with individual state and territory regulations.

End-use
End-use products containing the notified chemical at ≤ 15% concentration will be available to industrial users for use in fire retardant coatings and polymer composites (such as those used in safety boots).

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Manufacturing	8	265
Transport	8	265

EXPOSURE DETAILS

Transport and Storage

Transport and storage workers may come into contact with the neat notified chemical in powder form only in the event of accidental rupture of containers. The notifier 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 notified chemical (at ≤ 100% concentration) may occur during transfer, electrochemical exfoliation, quality control analysis, packaging, and cleaning and maintenance of equipment. The notifier states that exposure is expected to be minimised through the use of enclosed and automated systems, high efficiency membrane filters and crossflow scrubbers, adequate ventilation and appropriate PPE for workers including protective clothing, chemical resistant gloves, safety glasses and appropriate respiratory protection such as a particle filter device (with filter type P2) if required.

Reformulation

During reformulation dermal, ocular and inhalation exposure of workers to the notified chemical (at ≤ 100% concentration) may occur during transfer, blending, quality control analysis, and cleaning and maintenance of equipment. Some reformulation will occur at the manufacturer's site, and exposure to workers is expected to be minimised using the same controls employed for the manufacturing process. The notifier states that external-reformulation sites are expected to have similar enclosed and automated systems, adequate ventilation and PPE for workers including protective clothing, chemical resistant gloves, safety glasses and appropriate respiratory protection such as a particle filter device (with filter type P2) if required.

End-use

Once in the manufactured product, the notified chemical at ≤ 15% concentration will be encapsulated within a matrix and will not be available for exposure.

6.1.2. Public Exposure

The notified chemical is for industrial use only. The public may come into contact with end-use products containing the notified chemical at ≤ 15% concentration, such as safety boots. However, as the notified chemical will be encapsulated within a matrix, it is not expected to be available for exposure. The notified chemical is not intended to be used in clothing items other than footwear, and is not intended for use in food packaging products.

6.2. Human Health Effects Assessment

For the purpose of this risk assessment 'notified chemical' refers only to the following three physical forms of graphene: PureGRAPH™ 5, 10 & 20. All other physical forms of graphene will be described as 'graphene analogues'.

No toxicological data for the notified chemical were provided. The results from toxicological investigations conducted on graphene analogues and other carbon-based nanomaterials [single walled carbons nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs)] have been used to estimate the toxicity of the notified chemical.

SWCNTs and MWCNTs are considered acceptable to estimate the acute toxicity of the notified chemical based on being composed of rolled graphene sheets and as such have the same composition as the notified chemical.

The results from toxicological investigations conducted on graphene analogues are summarised in the following table. Except for repeated dose inhalation toxicity, no details on the particle size of the test substance used in these studies were provided. For full details of the studies, refer to Appendix B.

Endpoint	*Result and Assessment Conclusion*
Acute oral toxicity – mouse (graphene analogue 1)	LD50 > 5,000 mg/kg bw; low toxicity
Acute oral toxicity – rat (graphene analogue 2)	LD50 > 300 mg/kg bw; low toxicity
Repeat dose inhalation toxicity – rat, 28 days (graphene analogue 3)	NOAEC > 1.88 mg/m^3
Genotoxicity – *in vitro* mammalian cell gene mutation test – Chinese Hamster V79 cells (HPRT locus) (graphene analogue 4)	non mutagenic

The results from toxicological investigations conducted on SWCNTs and MWCNTs are summarised in the following table. For details on the physical characteristics of the CNTs used in these studies, refer to Appendix D.

Endpoint (analogue)	*Method*	*Result and Assessment Conclusion*	*Comments*
*Acute oral toxicity – rat (MWCNT - A and B)	OECD TG 425	LD50 > 2,000 mg/kg bw; low toxicity	No unscheduled deaths. All animals made expected body weight gains. No signs of clinical or systemic toxicity.
*Acute dermal toxicity – rat (MWCNT - A and B)	OECD TG 402	LD50 > 2,000 mg/kg bw; low toxicity	No unscheduled deaths. All animals made expected body weight gains. No signs of clinical or systemic toxicity.
*Skin irritation – rabbit (MWCNT - A and B)	OECD TG 404	non-irritating	Animals exposed to 0.5 g MWCNT under semi-occlusive conditions. All animals made expected body weight gains. No signs of clinical toxicity. No dermal reactions observed.
†Skin irritation – rabbit (SWCNT - A and B; MWCNT - C and D)	OECD TG 404	non-irritating (SWCNT-A, SWCNT-B and MWCNT-D); minimally irritating (MWCNT-C)	Animals exposed to 0.5 g CNT paste containing 1% SWCNT-A, SWCNT-B or MWCNT-C, or 2% MWCNT-D in olive oil under occlusive conditions. All animals made expected body weight gains. No dermal reactions observed in animals exposed to SWCNT-A, SWCNT-B and MWCNT-B. All animals exposed to MWCNT-C exhibited very slight (barely perceptible) erythema 24 hour after exposure. Full recovery was exhibited in one animal at the 48 hour observation. At the 72 hour observation, all animals showed no signs of irritation.
*Eye irritation – rabbit (MWCNT – A and B)	OECD TG 405	slightly irritating	Animals exposed to 0.1 g of the test substance. Conjunctival redness, chemosis and discharge (score 1 or 2) observed from 1 hour onwards following exposure with full recovery on Day 5.
†Eye irritation – rabbit (SWCNT – A and B; MWCNT – C and D)	OECD TG 405	non-irritating (SWCNT-A, SWCNT-B and MWCNT-D): slightly irritating (MWCNT-C)	Animals exposed to 0.1 mL CNT paste containing 0.1% SWCNT-A, 0.5% SWCNT-B, 0.25% MWCNT-C or 1% MWCNT-D in olive oil. All animals made expected body weight gains. No signs of clinical toxicity. No ocular reactions observed in animals exposed to SWCNT-A, SWCNT-B and MWCNT-D. All animals exposed to MWCNT-C exhibited hyperanemia of some blood vessels of the conjunctiva at the 24 hour observation which was fully resolved at the 48 hour observation.

Endpoint (analogue)	*Method*	*Result and Assessment Conclusion*	*Comments*
*Skin sensitisation – guinea pig, Buehler test (MWCNT - A and B)	OECD TG 406	non sensitising	Induction: 0.4 g MWCNT Challenge: 0.2 g MWCNT Performed under occlusive conditions. No dermal reactions observed
†Skin sensitisation – guinea pig, Buehler test (SWCNT - A and B; MWCNT - C and D)	OECD TG 406	non sensitising	Induction and challenge concentrations: SWCNT-A –1% SWCNT-B – 1% MWCNT-C – 1% MWCNT-D – 2% Vehicle: olive oil for induction or white petrolatum for challenge Performed under occlusive conditions. No dermal reactions observed All animals made the expected body weight gains. No signs of clinical toxicity.

*Data from Balakrishna Murthy *et al.* (2011)
†Data from Ema *et al.* (2011)

Toxicokinetics
No information on the toxicokinetics of the notified chemical was provided.

In a review on dermal absorption of nanomaterials (predominantly three-dimensional nanomaterials) by the Danish EPA (Poland *et al*., 2013), it was concluded that whilst there are many conflicting results, on balance the literature seems to suggest that absorption of particles in the nano-range through the skin is possible, but it occurs to a very low degree. This is further supported by *in vitro* percutaneous absorption studies conducted with carbon black nanoparticles (particle size < 40 nm) that showed no dermal absorption (SCCS, 2013).

A review conducted by Fadeel *et al*. (2018) concluded that while there was evidence that various graphene-based materials are able to cross physiological barriers and reach secondary organs through the systemic circulation following intravenous administration, there was insufficient evidence to make conclusions about the potential bio-distribution profile of a graphene-based material based on its physicochemical features.

The notified chemical is a two-dimensional nanomaterial with only one dimension in the nanoscale. Therefore, dermal absorption of the notified chemical is not expected.

Acute Toxicity
No acute oral and dermal toxicity studies of the notified chemical have been provided.

Graphene analogues have been found to be of low acute oral toxicity based on two independent studies conducted in mice (graphene analogue 1) and rats (graphene analogue 2). Given the limited potential for dermal absorption, the notified chemical is expected to be of low acute dermal toxicity.

Furthermore, MWCNTs have been shown to be of low acute oral and dermal toxicity in studies conducted in rats (Balakrishna Murthy *et al*., 2011).

Irritation
No studies on skin and eye irritation of the notified chemical were provided.

SWCNTs and MWCNTs have been found to be non-irritating to the skin of rabbits, however slight eye irritation has been observed in some MWCNTs (Ema *et al*., 2011, Balakrishna Murthy *et al*., 2011).

Sensitisation
No study on skin sensitisation of the notified chemical was provided.

SWCNTs and MWCNTs have been shown not to be skin sensitisers in Guinea pigs using the Buehler method (Ema *et al*., 2011; Balakrishna Murthy *et al*., 2011).

Repeated Dose Toxicity
No studies on repeated dose toxicity of the notified chemical were provided.

In a 28-day repeated dose inhalation toxicity study with a 90-day recovery period, rats were exposed (nose-only) to an aerosol of graphene analogue 3 at measured concentrations of 0.12, 0.47 and 1.88 mg/m^3 for 5 days/week 6 hours/day. No significant toxicological changes 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 significantly elevated indicating that graphene had no effect on oxidative stress at the concentrations tested. The No Observed Adverse Effect Concentration (NOAEC) was established as > 1.88 mg/m^3 in this study, based on no toxicological effects in rats up to the highest dose tested.

In a 5-day repeated dose inhalation toxicity study with a 24 day recovery period, rats were exposed (head-nose) to an aerosol of graphene analogue 5 (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 (Ma-Hock *et al*., 2013). 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 toxicological relevant changes were observed regarding haematology and protein levels (α_2-macroglobulin and haptoglobin). There were no other reported effects on other organs. A NOAEC for graphene analogue 4 was not reported in this study.

Mutagenicity/Genotoxicity
Graphene analogue 4 tested negative in an *in vitro* mammalian cell gene mutation test with Chinese hamster V79 cells at the Hypoxanthine-Guanine Phosphoribosyl Transferase (HPRT) locus.

In a comet assay using cells from the lungs of rats repeatedly exposed to an aerosol of graphene analogue 3 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.

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

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
For nanomaterials, the inhalation route is generally considered of main concern in regard to potential toxicity. Considering the flake sizes of the notified chemical, and that they have only one dimension in the nano-size range, dermal absorption is not expected.

In a 28-day repeated dose inhalation toxicity study in rats with a 90-day recovery period, graphene analogue 3 showed no toxicological effects up to the highest dose tested (1.88 mg/m^3). However, in a 5-day repeated dose inhalation toxicity study in rats with a 24 day recovery period, graphene analogue 5 showed evidence of an inflammatory response and microgranulomas in the lungs at 3.05 and 10.1 mg/m^3.

Based on the available studies for graphene analogues, the notified chemical may have the potential for lung toxicity at high concentrations. 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, surface chemistry and impurities, there remains uncertainty as to the potential lung toxicity of the notified chemical. Therefore, exposure control measures should be in place to minimise inhalation exposure to workers to the notified chemical in powder form

including enclosed, automated processes with local exhaust ventilation fitted with high-efficiency particulate air (HEPA) filter, and workers using appropriate respiratory protection for nanoparticulates, such as a particle filter device (with filter type P2).

Provided that the recommended controls are being adhered to, under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

6.3.2. Public Health

The public may come into contact with end-use products containing the notified chemical at ≤ 15% concentration, such as safety boots. However, as the notified chemical will be encapsulated within a matrix, it is not expected to be available for exposure. Therefore, when used in the proposed manner, the notified chemical is not considered to pose an unreasonable risk to public health.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be manufactured in Australia (Henderson, WA) using a precursor imported from Sri Lanka. The entire manufacturing process takes place at a single site. The notifier has acquired a Works Approval Licence from the Department of Environment Regulation of Western Australia for the manufacture of the notified chemical (Works Approval Number W6050/2017/1).

Airborne release of the notified chemical is carefully controlled by the use of ventilated workspaces (including fume hoods) coupled to atmospheric scrubbers and high-efficiency membrane filters (PTFE) which capture particles larger than 2 µm with an efficiency of 99.9975%, before being released to the atmosphere through a stack that is more than 3 m above the roof. Overall, the release of airborne notified chemical on site is expected to be minimal (0.0025%) based on the stringent controls in place. The membrane filters are expected to be replaced periodically, in which case contaminated filters should be disposed-of as solid waste in accordance with State guidelines. Likewise, the notifier has advised that waste water from the washing cycle will be filtered with the treated waste water to be disposed of in accordance with local regulations.

Dry product produced at the manufacturing site will be sealed in plastic bags which are packed into double lined, reinforced cardboard boxes or sealable plastic pails. The notified chemical is then stored at the notifier's warehouse prior to transport to other manufacturers. Accidental spills of the products containing the notified chemical during transport or storage will only occur if the packaging is breached. Spillages of the notified chemical are expected to be physically collected for disposal, in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE

The notified chemical will be incorporated into fire retardant coatings, rubbers, elastomers, polyurethane, resins and polymers, epoxies including fibre glass and carbon fibre composites, and industrial footwear. The notifier may manufacture some end-use products (e.g. resins and polymers) incorporating the notified chemical at the same site of manufacture. Once the notified chemical is dispersed within the liquid phase and the mixture is cured to form a solid, the notified chemical becomes immobilised within the polymer chains of the solid material.

Since the notified chemical is to be used as an additive in tyres, abrasion during use has been considered as a release pathway. A standard Emission Scenario Document from the OECD indicates that physical abrasion is a potential pathway for the release of tyre material containing the notified chemical (OECD, 2004). Such wearing of the tyre is likely to release the notified chemical in free form or bound within a polymer matrix (as portions of the rubber abrade from the tyre), but there is currently no information on the proportion of free versus bound graphene. The notified chemical may ultimately be released from the encapsulating polymer at very long timescales (decades, centuries) because the notified chemical is expected to have a longer life-time in the environment than the polymeric host material (see Section 7.2). Existing information also indicates that tyre debris released from abrasion is localised within a distance of approximately 5 m from the edge of the road (Cadle & Williams, 1979).

According to the notifier, the notified chemical may also be used in a water-based formulation as part of a fire-retardant paint. This application has the potential to lead to environmental releases of the notified chemical if such

products become available to Do-It-Yourself (DIY) users. However, the notifier has indicated that there are no current plans for these products to be made available to DIY users. According to the notifier, the fire retardant paint products will be used by companies who are expected to have suitable waste disposal measures in place. These measures are expected to limit environmental exposure to levels compliant with State/Territory regulations. However, if the fire-retardant paint (or any other similar products containing the notified chemical) becomes commercially available to DIY users then emissions of the notified chemical to the environment may need to be reassessed.

Since the notified chemical is to be incorporated within fire-retardant materials, it may be released by the destruction of these materials during a fire. However, the combustion temperature of graphene is 350 ºC (Eftekhari & Jafarkhani, 2013), which is much lower than the temperatures reached during a building fire (Ariyanayagam & Mahendran, 2013). Where the notified chemical is released from the host material during a fire, it is expected to combust to produce oxides (e.g. carbon dioxide) and releases of the notified chemical from the coating in this scenario are, therefore, expected to be negligible.

RELEASE OF CHEMICAL FROM DISPOSAL

Based on the currently available information, no significant releases of the notified chemical to the aquatic or terrestrial environment are expected to occur as a result of the disposal of articles containing the notified chemical at the end of their useful life.

The majority of the notified chemical will be immobilised within an inert matrix of cured coatings or articles and is expected to be disposed of to landfill along with these materials at the end of their useful life. In Australia, approximately 5% of tyres are recycled or repurposed (Mountjoy, Hasthanayake, & Freeman, 2015). Common applications of these recycled materials include road base, construction, soft-surface products and adhesives. In each case the notified chemical is likely to be retained within the polymer matrix of the original rubber tyre and/or be further encapsulated by other material. In landfill, the notified chemical is also expected to remain associated with the composite matrix of which it is a component. Release of the notified chemical is possible under very severe (highly acidic or oxidising) conditions, but this is not expected to be a significant release pathway under typical environmental conditions.

7.1.2. Environmental Fate

No bioaccumulation, biodegradation or other fate studies were provided for the notified chemical. Based on information available in the scientific literature, the notified chemical is expected to be 'chemically stable' and 'not biodegradable' (Arvidsson, Molander, & Sandén, 2013).

Dispersion and dissipation in aquatic environments

The fate of graphene and other carbon-based nanomaterials in the aquatic environment is complex and still subject to on-going research. The four key properties of carbon-based nanomaterials which determine their behaviour in water are currently understood to be: *i*) particle size, *ii*) surface structure, *iii*) surface charge, and *iv*) functional groups on the surface of the nanoparticles (He *et al*., 2017).

The oxygen content on the surfaces of the notified chemical indicates that the surfaces are functionalised by small quantities of oxygen-containing groups (< 15% oxygen by XPS or < 4% by the Unterzaucher method). Characterisation of the notified chemical by Raman spectroscopy also indicates low levels of surface defects (< 20%). While graphene is insoluble in water, increasing surface functionalisation and increasing defect levels make it more dispersible in water (Gottschalk, Sonderer, Scholz, & Nowack, 2009; Sun *et al*., 2015). For example, graphene oxide has good dispersibility, but pristine graphene (which has no functional groups) is less dispersible (Gottschalk, Sonderer, Scholz, & Nowack, 2009; Sun *et al*., 2015). The low levels of both oxygen, and defects, in the notified chemical are expected to result in aqueous dispersibility that is in the range between that of pristine graphene and graphene oxide.

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 notified chemical (PureGRAPH^{TM} 5 and PureGRAPH^{TM} 10: 0 - 50 mV, PureGRAPH^{TM} 20: 10 - 30 mV) indicate that the notified chemical will have low to moderate stability in an aqueous dispersion in the pH range relevant for aquatic environments (pH 4 – 9).

Aggregation and agglomeration are processes which can reduce the mobility and bioavailability of nanoparticles in sediment and ground water (Batley & McLaughlin, 2008) because the larger particles are captured in the pores of soils and sediment. Graphene nanoparticles with large lateral dimensions (in the µm range) agglomerate rapidly in water to form larger colloids of loosely-bound particles (Su *et al*., 2017). This leads to deposition, and lower

bioavailability in aqueous environments. The notified chemical has lateral dimensions in the µm range (from SEM imaging) and is therefore expected to agglomerate rapidly when dispersed in water. Agglomeration of graphene is further promoted by dissolved salts in ground water (He *et al*., 2017). The relatively low measured surface areas of the notified chemical (~ 10 m^2/g) also indicate that aggregation and agglomeration are already significant in the solid phase; i.e. individual graphene nanoparticles spontaneously agglomerate during manufacture to form larger particles which have lower surface area-to-mass ratios.

Characterising the surface morphology of graphene is difficult experimentally, but the available data indicate that pristine graphene nanoparticles (with no surface functional groups) have well-ordered two-dimensional surfaces (Raman spectroscopy). These well-ordered surfaces facilitate intermolecular van der Waals interactions between graphene nanoparticles and other solids. Suspended particles with well-ordered surfaces adsorb to the surfaces of soil and sediment (He *et al*., 2017), which can contribute to dissipation of these particles from the water compartment. This behaviour in water is consistent with a recent study modelling the release of carbon-based nanomaterials in the UK which found that only very small quantities of carbon nanomaterials are present in the aquatic environment (PEC = 0.0005 µg/L), even though the manufacture and release scenarios involved much higher quantities of nanomaterials than are proposed in the current notification (Mueller & Nowack, 2008).

Overall, the notified chemical is likely to have some dispersibility in water, but over time dispersed particles in surface waters are expected to agglomerate and deposit with other suspended materials onto sediments. Any notified chemical which is released by tyre abrasion and degradation is expected to adsorb to soils and remain bound to the soil matrix. However, it is noted that no studies on the transport of the notified chemical or graphene analogues in soil environments were identified for this assessment.

Environmental transformation
Abiotic degradation of the notified chemical is expected to be slow. It does not undergo photolysis (Hou *et al*., 2015), contains no readily hydrolysable functional groups, and is mostly comprised of a network of strong C–C covalent bonds (bond dissociation energy ~ 111 kcal/mol). Strong oxidants coupled with acidic conditions are required to functionalise graphene and initiate biodegradation pathways (Hummers & Offeman, 1958; Marcano *et al*., 2010), but certain naturally occurring enzymes can reportedly biodegrade graphene (Liu *et al*., 2015). The notified 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).

Toxicokinetics in aquatic organisms
There are currently no generally applicable and validated methods to assess the bioaccumulation potential for nanoparticles. Rather, such considerations are made on a case-by-case basis taking into account the physical and chemical properties of the nanoparticles and the form in which they are likely to be bioavailable in the environment. Critical considerations will include how the nanoparticle interacts with the organisms (surface adsorption or ingestion, or both) and whether the nanoparticles can cross cell membranes (OECD, 2012a, OECD, 2012b).

Dong *et al*. (2018) have studied the interaction and uptake of graphene nanoparticles by a range of aquatic species. They demonstrated that these particles adsorb to the external membranes of bacteria. They also found that *Daphnia magna* (aquatic invertebrates) and *Danio rerio* (fish) ingested graphene when they consumed contaminated lower-level organisms. Ingested graphene was confined to the gut of these higher organisms, consistent with the observations of Lu *et al*. (2017). The amount of ingested graphene appeared to reach a steady-state in each of the studied organisms but the authors concluded that longer timescales were needed before any definitive conclusions from these studies can be drawn.

Recent research demonstrated that graphene (with similar dimensions to the notifier's product) can cross cell membranes *in vitro* through direct penetration (Li *et al*., 2013). The significance of this specific uptake pathway identified in cultured cells for the uptake of graphene nanoparticles in whole organisms has not yet been established. However, if future research demonstrates that graphene nanoparticles with comparable properties to the notified chemical cross the gut lining in higher animals then the environmental fate of the notified chemical may need to be reassessed.

The observation that graphene nanoparticles accumulate on the exterior surface of bacteria through membrane-adsorption may also be relevant to microorganisms living in soil. Consideration of the effects of graphene nanoparticles on terrestrial food-webs may be required if future research shows that associations between graphene and soil microorganisms has adverse effects on these organisms or the organisms that consume them in terrestrial food chains.

7.1.3. Predicted Environmental Concentration (PEC)

A predicted environmental concentration (PEC) for the notified chemical in the aquatic compartment has not been calculated. Direct release of the notified chemical (as the raw material) from the site of manufacture to the aquatic environment will be limited based on the collection of waste water from the washing cycle (removed suspended product) and disposed of in accordance with local regulations. No significant releases to the aquatic environment are expected from the use of the notified chemical in fire retardant coatings and polymer composites based on the currently available information.

A predicted environmental concentration (PEC) for the notified chemical in the terrestrial compartment has also not been calculated. Although the notified chemical may be released to the terrestrial environment due to abrasion of tyres containing the notified chemical during use, it is assumed that the majority of this release will be in the form of small fragments of tyre wherein the notified chemical remains strongly associated with the rubber matrix. However, it is noted that no information has been identified for this assessment which can be used to predict the long term fate of nano-sized particles of the notified chemical associated with tyre fragments in the terrestrial environment.

7.2. Environmental Effects Assessment

No ecotoxicological data for the notified chemical were provided. The assessment of the environmental effects of the notified chemical has been based on the effects of graphene analogues reported in the scientific literature. A summary of findings from these ecotoxicological studies are presented in the table below and details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Embryo Acute Toxicity* (graphene analogue 6)	96 h LC50 < 0.030 mg/L	Adversely affects fish embryos
Daphnia Acute Toxicity* (graphene analogue 7)	48 h EC50 > 16 mg/L	Adversely affects aquatic invertebrates
Daphnia Chronic Toxicity* (graphene analogue 7)	21 d NOAEC = 0.1 mg/L	Adversely affects the reproduction of aquatic invertebrates
Algal Acute Toxicity^{+} (graphene analogue 8)	96 h EC50 = 62.2 mg/L	Adversely affects algae

*Ecotoxicological tests were conducted using single-layered graphene (SLG)
$^{+}$ Ecotoxicological test was conducted using graphene nanoplatelets (GNP)

Only the algae test was performed on a directly comparable graphene analogue (GNP – graphene nanoplatelets; graphene analogue 8). Fish and daphnia studies were conducted using single layer graphene (SLG; graphene analogues 6 and 7), which is known to be significantly more toxic than the larger, multilayered graphene analogues (FLG – few layer graphene, or GNP) (Dasmahapatra, Dasari, & Tchounwou, 2019). No single layer graphene (SLG) is observed in the notified chemical. All reported end-points are based on nominal exposure concentrations.

In the fish embryo study it was found that exposure to levels of pristine graphene (graphene analogue 6) greater than 0.025 mg/L caused developmental effects (Manjunatha *et al.*, 2018). All zebrafish embryos exposed to higher concentrations (from 0.03 to 0.05 mg/L) of graphene analogue 6 died within 30 min to 2 hours. The study authors indicate that the underlying mechanisms for the toxic effects of pristine graphene are largely unclear. However, in a previous study it was found that nanomaterials such as SLG induce clogging of the chorion pores which affects embryonic development (Ong *et al*., 2014).

In the aquatic invertebrate study, a graphene analogue (graphene analogue 7) at higher concentrations (0.5 and 1.0 mg/L) inhibited the growth and reproduction of daphnids (Fan *et al.*, 2016). This was thought to occur due to disruption of the digestive system causing malnutrition. Single layer graphene exhibits an extremely sharp nanowall edge which is expected to cause mechanical damage upon direct contact with cells of organisms. It was noted that graphene analogue 7 heavily adsorbed on the surface of daphnids, may have severely limited normal activities such as swimming and filtering.

In the algae study, multilayered graphene (graphene analogue 8) showed no shading effect on algal growth due to their poor dispersibility, while nutrient depletion accounted for 27% of total toxicity. It was found that graphene sheets could penetrate algal cell walls and it was suggested that membrane damage was induced by both oxidative stress and physical penetration/extraction (Zhao *et al.*, 2017).

Muzi *et al.* (2016) showed that multilayer graphene had no effect on mortality (> 50 mg/L) of *Xenopus laevis* (African clawed frog) tadpoles. However, it did cause growth inhibition at concentrations of 10 – 50 mg/L.

The notified chemical and the analogues used in these studies are graphene based materials and, therefore, have unique physical and chemical properties compared to non-nano size carbon-based materials (*e.g.* graphite). The toxicity and toxicological mechanisms of graphene also differ from those of other carbon nanomaterials (Fan *et al.*, 2016). In general, the toxic effects of graphene depend on its physical size and surface area (Peralta-Videa *et al.*, 2011). In the aquatic environment, the toxicity of nanoparticles maybe strongly affected by dissolved organic matter, which has been shown in several studies to stabilise particles in suspension and to reduce the agglomeration/aggregation phenomena (Hyung *et al*., 2007; Loux and Savage, 2008).

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; UN 2009; UN 2014). Hence, the aquatic hazards of the notified chemical have not been classified for this assessment. Nevertheless, it is noted that ecotoxicity data evaluated for this assessment does show that graphene analogues of the notified chemical adversely affect aquatic life under certain exposure conditions.

7.2.1. Predicted No-Effect Concentration

A predicted no-effect concentration (PNEC) has not been calculated for the notified chemical as, based on its reported use pattern, significant quantities are not expected to be released to the aquatic or terrestrial environments.

7.3. Environmental Risk Assessment

The notified chemical will be manufactured in Australia. Stringent controls will be in place to minimise any release of the notified chemical to the environment. The notified chemical is to be incorporated into articles which are expected to be disposed of to landfill at the end of their useful lives and the majority of the notified chemical is therefore expected to end up in landfill. Some of the notified chemical incorporated into tyres is expected to be released into the environment through abrasion of tyres during use and a small proportion is expected to be recycled or repurposed. The notified chemical is expected to remain encapsulated by polymer in these recycled materials. Based on the current use pattern, aquatic organisms are not expected to be exposed to the notified chemical.

If the notified chemical is released, it is expected to adsorb to particulate matter including soil and sediment. In the aquatic environment, particles of the notified chemical are expected to adsorb to suspended particulate matter or agglomerate and deposit to the sediment.

Adverse effects have been observed in fish embryos, daphnia and algae exposed to graphene analogues of the notified chemical. The most toxic effects were observed with a single-layered graphene analogue which is considered to be the most toxic form of graphene based on the available ecotoxicology data. The notified chemical is a form of multilayered graphene which is expected to be less toxic than the single layered form. The observed ecotoxicological effects are thought to be due to both physical and chemical stressors and require the notified chemical to be dispersed in water (e.g. with chemical dispersants and sonication) for the effects to manifest. It is expected that if the notified chemical was released to the aquatic environment it would aggregate and/or agglomerate and eventually settle out to sediments. Based on the assessed use pattern, no significant release of the notified chemical to the aquatic environment is expected.

Based on the currently available information for the aquatic hazards and fate of graphene analogues and the assessed use patterns, the notified chemical is not expected to pose an unreasonable risk to the environment. However, if additional hazard information becomes available to indicate that the notified chemical has hazard characteristics of concern to the environment or if the use pattern changes such that aquatic or terrestrial organisms will be exposed to the notified chemical, then the risks to the environment posed by the notified chemical may need to be re-assessed.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point ~ 4,200 °C (4,510 K = 4236.85 °C)

Method Monte Carlo simulations based on the reactive bond order potential (LCBOPII: long-range carbon bond-order potential).

Remarks The calculated melting temperature sits within the range of values observed for chemical analogues graphite (~ 3,700 – 4,700 °C), fullerenes (~ 3,700 °C) and nanotubes (~ 4,500 °C).

Test Facility Los *et al*. (2015)

Tapped Density

PureGRAPH™ 5: 61.8 kg/m^3
PureGRAPH™ 10: 124 kg/m^3
PureGRAPH™ 20: 251 kg/m^3

Method The tapped density is an increased bulk density obtained by repeated mechanically tapping a graduated measuring cylinder or vessel containing the powder sample until there is little change in the volume or mass of the powder.

Remarks Instrument: DahoMeter DY-100D
Tap time: 12 minutes.
Temperature: room temperature

Test Facility First Graphene (2018c)

Particle Size Distribution

Dv(50) for lateral dimension:
PureGRAPH™ 5: 5.63 µm
PureGRAPH™ 10: 9.92 µm
PureGRAPH™ 20: 19.2 µm

Method Laser Diffraction using a Malvern Mastersizer 3000E. Samples were dispersed in water.

Product	*Dv(50) (µm)*	*Dv(90) (µm)*
PureGRAPH™ 5	5.63	12.5
PureGRAPH™ 10	9.92	21.1
PureGRAPH™ 20	19.2	51.1

Dv(50) = 50% of particles less than the size specified
Dv(90) = 90% of particles less than the size specified

Remarks Particle size distribution described by volume.
The three products show repeatable particle size distributions. The graphene products showed a narrow distribution of particle sizes; (Dv(90) values were typically < 2.5 times of Dv(50) values.

Test Facility First Graphene (2018d)

Particle Size PureGRAPH™ 5: Average flake size 4.35 ± 2 µm

Method Optical Microscopy and Scanning Electron Microscopy (SEM)

Remarks Lateral flake size distribution ranged from 3-15 µm. Flake size corresponds well with reported particle size (Dv(50) = 5.63 µm and Dv(90) = 12.5 µm) as determined by laser diffraction.

In a further study, SEM analysis of PureGRAPH™ 5, PureGRAPH™ 10 and PureGRAPH™ 20 also confirmed the particle size [Dv(50)] of the three products as determined by laser diffraction (First Graphene, 2018f). A uniform distribution of platelet sizes around the Dv(50) value was also indicated. Platelet thickness appears to be << 0.5 µm, although the SEM images were low-resolution. Re-stacking of platelets was not observed and the platelets presented as loosely bound agglomerates which are expected to disperse in solvents or polymer media.

Test Facility First Graphene (2018e)

Zeta Potential (pH dependent) PureGRAPH™ 5 ; 10 → -30 mV
PureGRAPH™ 10 ; 0 → -50 mV
PureGRAPH™ 20 ; 0 → -50 mV

Method Measured on a Nano-ZS Zetasizer (Malvern Instruments Ltd, UK) in electrophoretic light scattering mode using the Smoluchowski model.
Test Facility First Graphene (2019)

Surface Area PureGRAPH™ 5 – 9.3821 m^2/g
PureGRAPH™ 10 – 9.2446 m^2/g
PureGRAPH™ 20 – 7.8127 m^2/g

Method Brunauer-Emmett-Teller (BET) model based on measurements obtained using a Micrometrics TriStar II 3020 2.00. Adsorption gas was N_2. Sample density was 2.150 g/cm^3.

Product	*BET surface area (m^2/g)*	*Pore size (nm)*	*Pore volume (cm^3/g)*
PureGRAPH™ 5	9.3821	14.10	0.033256
PureGRAPH™ 10	9.2446	15.07	0.034830
PureGRAPH™ 20	7.8127	17.80	0.034916

Remarks All three products had isotherm plots which indicated that the chemical was non-porous and had a low surface area. The study authors indicated that this is similar to other graphene products.
Test Facility First Graphene (2018g)

Dust Explosivity Not explosive

Method Minimum Ignition Energy Test (MIE) (BS EN 13821:2002)
Minimum (Dust Cloud) Ignition Temperature (MIT) (BS EN 50281-2-1: 1999 Part 2-1: Method A)
Remarks A form of graphene (not the notified chemical) was tested.
Particle size: 10% < 127.760 µm; 50% < 384.467 µm, 90% < 853.755 µm, 3.36% < 63 µm. Powder was milled to create a suitable fine powder for testing. Particle size of powder tested: 83.75% < 63 µm.
MIE
Without inductance (< 25 µH) > 1,000 mJ
With inductance (1 mH) > 1,000 mJ
MIT > 1,000 °C
Test Facility DEKRA (2017)

Autoignition Temperature 360 °C

Method Layer Ignition Temperature Test (BS EN 50281-2-1: 1999 Part 2-1: Method A)

Remarks A form of graphene (not the notified chemical) was tested.
Particle size: 10% < 127.760 µm; 50% < 384.467 µm, 90% < 853.755 µm, 3.36% < 63 µm. Powder was milled to create a suitable fine powder for testing. Particle size of powder tested: 83.75% < 63 µm.
A 5 mm layer of dust is formed on a hot plate (at constant temperature) and observed for signs of self-heating or ignition. Temperature range tested: 20 °C – 1,000 °C
Test Facility DEKRA (2017)

Oxygen Content Low levels of oxygen present

Method Unterzaucher pyrolysis method. Samples were dried at 115 °C for 2 hours under vacuum at – 50 kPa/15 in Hg before being analysed.

Product	*Mean oxygen content (%)*
PureGRAPH™ 5	1.41
PureGRAPH™ 10	2.53
PureGRAPH™ 20	3.31

Remarks The graphene products have low levels of oxidation.
Test Facility First Graphene (2018h)

Oxygen Content Low levels of oxygen present

Method X-ray Photoelectron Spectroscopy (XPS)
Remarks Samples of PureGRAPH™ 5, PureGRAPH™ 10 and PureGRAPH™ 20 were dispersed in ethanol (1 mg/mL) and drop casted on a silicon substrate. Residual solvent was removed under vacuum at 60 °C prior to mounting the samples on a molybdenum holder for XPS measurements. Measurements were made with a no-monochromatic X-ray source (12 kV – 200 W) with magnesium anode under ultra-high vacuum conditions (base pressure of e^{-10} mbar).

	Percent (%)			
	Carbon	*Oxygen*	*sp^2*	*sp^3*
PureGRAPH™ 5	85.33	14.67	66.47	8.00
PureGRAPH™ 10	91.69	8.31	73.93	5.02
92.66	92.66	5.22	72.24	7.04

Remarks Oxygen is present on the surface of the products as C-O (hydroxyl and epoxy) and O-C=O (carboxyl) functionality. The relationship between sp^2 and oxidation levels may reflect a relationship between smaller platelet sizes and higher proportion of platelet edges.
Test Facility First Graphene (2018a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute Oral Toxicity – Mouse

TEST SUBSTANCE	Graphene analogue 1
METHOD	Chemical toxicity test instruction (2005), Ministry of Health of the People's Republic of China Similar to OECD TG 401 Acute Oral Toxicity – Limit Test
Species/Strain	Mice/ICR strain
Vehicle	Not provided
Remarks – Method	Characterisation of test substance not provided (particle size details are unknown). Animals were fasted overnight and dosed by oral gavage. Animals were observed at 1, 2, 3 and 4 hours after exposure and then at least once daily for two weeks. No control group recorded.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	10 M	5,000	0/10
2	10 F	5,000	0/10

LD50	> 5,000 mg/kg bw
Remarks – Results	No signs of systemic toxicity were observed during the course of the study. All animals survived to the end of the observation period.
CONCLUSION	The test substance is of low acute toxicity via the oral route.
TEST FACILITY	JPCDCP (2015)

B.2. Acute Oral Toxicity – Rat, Fixed Dose

TEST SUBSTANCE	Graphene analogue 2
METHOD	OECD TG 420 Acute Oral Toxicity – Fixed Dose Method Method B1 bis Acute Toxicity (Oral) of Commission Regulation (EC) No. 440/2008
Species/Strain	Rat/Wistar
Vehicle	Arachis oil BP
Remarks – Method	Characterisation of test substance not provided (particle size details are unknown). GLP compliant. All animals were dosed at the same time as the maximum achievable dose was 300 mg/kg bw and the notified chemical was expected to be non-toxic.

RESULTS

Main Study

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	5 F	300	0/5

Discriminating Dose	300 mg/kg bw
Signs of Toxicity	All five animals exhibited black faeces on days 1, 2 and 3 after exposure. This effect was not observed over the remainder of the study period (days 4 – 14). No signs of systemic toxicity were recorded.
Effects in Organs	No abnormalities observed.
Remarks – Results	No unscheduled deaths. All animals made the expected body weight gains

CONCLUSION	The test substance is of low acute toxicity via the oral route.
TEST FACILITY	Envigo (2018a)

B.3. Repeat Dose Inhalation Toxicity – Rat

TEST SUBSTANCE	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)
METHOD	OECD TG 412 Repeated Dose Inhalation Toxicity: 28-Day Study
Species/Strain	Rat/Sprague-Dawley
Route of Administration	Inhalation – nose only exposure
Exposure Information	Total exposure days: 28 days Dose regimen: 5 days per week Duration of exposure (inhalation): 6 hours/day Post-exposure observation period: 1, 28 and 90 days
Vehicle	Water
Physical Form	Aerosol
Mass median aerodynamic diameter (MMAD)	123 nm
Remarks – Method	The test concentration range was selected based on a previous study by the same group (Shin *et al.,* 2015) in which minimal toxic effects to the lungs were observed after a 5-day graphene inhalation study at a high concentration of 3.86 mg/m^3 and a low concentration of 0.68 mg/m^3. Aerosolized graphene nanoplatelets had various thicknesses ranging from 0.35 to 0.38 nm with an elemental composition of 96.28% Carbon and 3.72% Oxygen. No details were provided to indicate if the aerosolized graphene nanoplatelets were individual nanoplatelets or aggregates of the nanoplatelets. The daily deposited dose (mg/day) for the low-, mid- and high-dose groups was calculated as: Low dose group: 0.0006 mg/day Mid dose group: 0.0025 mg/day High dose group: 0.0099 mg/day The cumulative dose to the test substance over the 28-day exposure period was calculated as: Low dose group: 0.012 mg Mid dose group: 0.05 mg High dose group: 0.198 mg

Mass and number concentration of aerosolised graphene nanoparticles inside exposure chamber:

Group	*Dose/Mass Concentration (mg/m^3)*		*Number Concentration*
	Nominal	*Actual*	
Control	-	0.05 ± 0.02	1.92 ± 0.78
Low Dose	0.125	0.12 ± 0.00	$6.38 \times 10^3 \pm 8.57 \times 10^2$
Mid Dose	0.5	0.47 ± 0.03	$5.55 \times 10^4 \pm 1.04 \times 10^3$
High Dose	2	1.88 ± 0.18	$1.99 \times 10^5 \pm 3.87 \times 10^3$

Size distribution of aerosolised graphene nanoparticles inside the low- mid- and high-dose exposure chambers:

Group	*Particle size distribution (SNPS)*		*Particle size distribution (OPC)*	
	*Range**	*Peak*	*Range**	*Peak*
Low Dose		121.88 nm		265 nm
Mid Dose	7.37 – 289.03 nm	162.53 nm	265 nm – 34 μm	265 nm
High Dose		145.9 nm		325 nm

** The particle size distribution in the chambers was measured using SNPS and OPC. The study authors reported a range of values. The particle size distribution for each exposure chamber was not provided.*
SNPS – scanning nanoparticle spectrometer
OPC – Optical Particle Counter (dust monitor)

RESULTS

Group	*Number and Sex of Animals*	*Dose/Mass Concentration (mg/m^3)*		*Mortality*
		Nominal	*Actual*	
1-Day Recovery				
Control	5 M	-	0.05 ± 0.02	0/5
Low Dose	5 M	0.125	0.12 ± 0.00	0/5
Mid Dose	5 M	0.5	0.47 ± 0.03	0/5
High Dose	5 M	2	1.88 ± 0.18	0/5
28-Day Recovery				
Control	5 M	-	0.05 ± 0.02	0/5
Low Dose	5 M	0.125	0.12 ± 0.00	0/5
Mid Dose	5 M	0.5	0.47 ± 0.03	0/5
High Dose	5 M	2	1.88 ± 0.18	0/5
90-Day Recovery				
Control	5 M	-	0.05 ± 0.02	0/5
Low Dose	5 M	0.125	0.12 ± 0.00	0/5
Mid Dose	5 M	0.5	0.47 ± 0.03	0/5
High Dose	5 M	2	1.88 ± 0.18	0/5

Mortality and Time to Death
There were no unscheduled deaths.

Clinical Observations
Statistically significant body weight losses were observed in animals in the mid-dose groups at 2 weeks and in the high-dose group at 1, 5, 6, 11 and 13 weeks from the start of the exposure period when compared to the control group.

Statistically significant decreases in food consumption were observed in animals in the low-dose groups at 2, 6, 12, 16 and 17 weeks and in the high-dose groups at 1, 7, 9, 12, 13, 14, 16 and 17 weeks from the start of the exposure period when compared to the control group.

Laboratory Findings – Clinical Chemistry, Haematology and Coagulation
When compared to animals in the control group, animals exposed to the test substance with 1-day recovery exhibited a statistically significant decrease in lactate dehydrogenase levels in the low- and mid-dose groups, while animals in the high-dose group exhibited levels similar to those in control animals. Magnesium levels were low across all exposure groups (no dose-dependent relationship), but statistically significant only in the low- and mid-dose groups. Statistically significant higher levels of uric acid (mid-dose group), total bilirubin (mid-dose group), glucose (mid-dose group), potassium (mid-dose group) and haematocrit (low- and mid-dose groups) were observed compared to control animals. Red blood cell count and haemoglobin levels were higher across all exposure groups (compared to controls), but at statistically significant levels in the low-, and low- and mid-dose groups, respectively. Red blood cell count and haemoglobin levels were highest in the low-dose group, decreasing as the exposure dose increased.

When compared to animals in the control group, animals exposed to the test substance in the 28-day recovery group also exhibited a statistically significant decrease in lactate dehydrogenase levels in some groups (low- and high-dose). In the high-dose group, statistically significantly higher total bilirubin levels were observed as well as statistically significantly lower sodium and potassium levels. While chloride levels were higher than

those in the control group in the low- and mid-dose group and statistically significantly higher in the high-dose group, the increase observed was not in a dose-dependent manner.

When compared to animals in the control group, animals exposed to the test substance in the 90-day recovery groups exhibited a decrease (though not statistically significant) in lactate dehydrogenase levels in a dose-dependent manner (levels decreased as exposure dose increased). Levels of sodium and chloride were statistically significantly higher in the low-dose and high-dose groups, respectively. The albumin: globulin ratio was statistically significantly higher in animals in the mid-dose group.

No haematological data were available for animals within the 28-day recovery control and exposure groups. However, no statistically significant differences were observed in haematology parameters observed for those animals in the 1-day recovery group and the animals in the 90-day recovery group.

No significant differences in prothrombin time and activated partial thromboplastin time (blood coagulation time) were observed between control and exposed animals in the 1- or 90-day recovery groups (no measurements provided for animals in the 28-day recovery group).

Effects on Cytokines in Lung Tissue Lysates and BAL fluid

No significant differences in the cytokine levels in the lung lysate were observed between control and exposed animals in any of the treated groups with different recovery periods. Statistically significantly higher levels of interleukin-18 were observed in bronchoalveolar lavage fluid (BALF) in animals exposed to low- and mid-doses (with 28- and 1-day recovery periods) compared with the control groups. Lower levels of vascular endothelial growth factor were observed in all exposed animals in the 28- and 90-day recovery groups (statistically significantly lower in the mid-dose group with 28-day recovery period). Levels of vascular endothelial growth factor were also much lower (but not statistically significant) in the high-dose group with 1-day recovery.

When compared to the controls, the study authors advised that total cell counts and macrophage counts within the BALF were statistically significantly decreased in all exposed animals in low-, mid- and high-dose with in the 1- and 28-day recovery and lymphocyte counts were also statistically significantly decreased (when compared to control animals) in all exposed animals with 1-day recovery. Levels of microalbumin were statistically significantly decreased in the mid-dose with 28-day recovery, lactate dehydrogenase levels were statistically significantly increased in the low-dose (28-day recovery) when compared with control animals. No concentration related effects were observed in the BALF for inflammatory biomarkers (blood urea nitrogen, urea dilution factor, lactate dehydrogenase, microalbumin and micro-total protein) or oxidative stress biomarkers (hydrogen peroxide, glutathione and malondialdehyde). However, while malondialdehyde levels were significantly lower in the low- and mid-dose groups (with 90-day recovery) relative to control animals, there was no dose-dependent relationship observed.

A decrease in inflammatory cell counts was observed in low-, mid- and high-dose groups with 1-day recovery (total cell count, macrophages, and lymphocytes) and total cell count and macrophages decreased in low-, mid- and high-dose groups with 1- and 28-day recovery. Recovery from these effects were indicated after 90-day recovery.

Effects in Organs

When compared to animals in the control groups, statistically significant decreases in thymus weight (high-dose group, 1-day recovery), liver weight (mid-dose group, 28-day recovery) and lung weight (low- and mid-dose group, 28-day recovery) were observed, as well as statistically significant increases in brain weight (high-dose group with 90-day recovery). Statistically not significant brain weight increase was observed in the low- and mid-dose groups and thymus weight increase in low-dose group (90-day recovery).

No adverse effects were observed following gross examination of testes, kidneys, spleen, liver, lungs, thymus, eyes or brain in treated groups with 90-day recovery.

No inflammatory-related histological evidence, such as no increase and migration of polymorphonuclear cells or proliferation of pneumocytes, a change in thickness of the alveolar wall or the formation of granulomatous regions, were observed in the lungs of exposed animals in any of the treated groups.

Particles of graphene analogue 4 were observed in alveolar macrophages and in lung-associated lymph nodes in all treated groups with varying recovery periods. Deposition of graphene analogue 4 in the macrophages

was dose-dependent. Some deposition was observed in lung epithelial cells. Ingested graphene analogue 4 in the lung macrophages persisted beyond the 90-day post exposure period in all treated groups.

Comet Assay

No DNA damage was detected in the cells of the right lungs of animals treated with graphene analogue 4 (with 1- and 28-day recovery periods) following analysis using a Comet assay (data not available for the 90-day recovery group).

Remarks – Results

No significant toxicological changes were observed following exposure to the test substance.

Graphene analogue 4 was mostly deposited in lung macrophages with some deposition in lung epithelial cells. Translocation of graphene analogue 4 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 in treated groups with 90-day recovery) was reported in exposed animals. This finding was supported by an absence of any significant increases in inflammatory cells, inflammatory biomarkers or cytokines in the bronchoalveolar fluid or lung tissue lysate in all treated groups when compared to control animals. Furthermore, no oxidative stress markers (hydrogen peroxide, glutathione and malondialdehyde) were significantly elevated indicating that graphene analogue 4 had no effect on oxidative stress up to the highest dose tested.

CONCLUSION

The No Observed (Adverse) Effect Concentration (NOAEC) was established as > 1.88 mg/m^3 in this study, based on no adverse toxicological effects up to the highest dose tested.

REFERENCE Kim *et al*. (2016)

B.4. Genotoxicity – *In Vitro* Mammalian Cell Gene Mutation Test

TEST SUBSTANCE Graphene analogue 4

METHOD OECD TG 476 *In vitro* Mammalian Cell Gene Mutation Test

Species/Strain	Chinese Hamster
Cell Type/Cell Line	V79
Metabolic Activation System	S9 fraction from phenobarbital/β-naphtha flavone-induced rat liver
Vehicle	Minimal Essential Medium (MEM)
Remarks – Method	Characterisation of test substance not provided (particle size details are unknown). GLP compliant. No significant deviations from the protocol. Positive controls – Ethyl methane sulphonate (EMS) (absence of metabolic activation) and Dimethyl benzanthracene (DMBA) (presence of metabolic activation). A preliminary cytotoxicity test was performed on the test substance at concentrations of 0, 0.002, 0.004, 0.008, 0.015, 0.03, 0.06, 0.13, 0.25, 0.5 µg/mL. Precipitation was observed in the presence and absence of metabolic activation at 0.5 µg/mL following a 4 hour exposure period. No concentration related reductions in cloning efficiency were observed.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Period*	*Selection Period*
Absent				
Test 1	0*, 0.03*, 0.06*, 0.13*, 0.25*, 0.5*, 1.0	4 h	7 days	7 days
Present				
Test 1	0*, 0.03*, 0.06*, 0.13*, 0.25*, 0.5, 1.0	4 h	7 days	7 days

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 0.5	> 0.5	≥ 0.5	negative
Present				
Test 1	> 0.5	> 0.25	≥ 0.25	negative

Remarks – Results

Slight dose related decreases in cloning efficiency were observed in the presence of metabolic activation on day 0 of the expression period. No dose related decreases in cloning efficiency were observed at the end of the expression period (day 7) in the presence or absence of metabolic activation.

No toxicologically significant increases in mutation frequency were observed in the presence or absence of metabolic activation.

Positive and negative controls performed as expected.

CONCLUSION

The test substance was not mutagenic to V79 cells at the Hypoxanthine-Guanine Phosphoribosyl Transferase (HPRT) locus treated *in vitro* under the conditions of the test.

TEST FACILITY

Envigo (2018b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Ecotoxicological Investigations

C.1.1. Acute Toxicity to Fish

TEST SUBSTANCE	Graphene analogue 6
METHOD	Acute Toxicity Test on Fish Embryos – Semi Static
Species	*Danio rerio* (Zebrafish) embryos
Exposure Period	96 h
Auxiliary Solvent	Ethanol
Water Hardness	Not reported
Analytical Monitoring	None
Remarks – Method	Characterisation of test substance not provided (particle size details are unknown). Engineered pristine graphene (pG) monolayer flakes (liquid-phase exfoliation of graphite—dispersion in ethanol at 1 mg/L) were used for the study. Zebrafish (wild-type AB) embryos were collected after spawning at 4 hours post fertilization (hpf) and maintained at room temperature of 28.0 ± 0.5 °C, oxygen saturation of more than 85%, pH 7.0 ± 0.5 and 14 h:10 h light–dark cycle. Fertilized embryos were treated with pG at different concentrations (µg/L) by dilution of the stock solution (1 mg/L) for 96 hours.

RESULTS

Concentration (mg/L) Nominal	*Number of Embryos*	*Mortality 96 h*
Control	10	0*
0.001	10	0*
0.005	10	0*
0.010	10	0*
0.015	10	0*
0.020	10	0*
0.025	10	3*
0.030	10	10
0.035	10	10
0.040	10	10
0.050	10	10

*Read-off graph

LC50	< 0.030 mg/L at 96 hours
NOEC	0.020 mg/L at 96 hours
Remarks – Results	The physical and chemical conditions were maintained throughout the test. For the lower concentrations (< 0.02 mg/L), the mortality rate did not show significant difference from the control. However, all zebrafish embryos exposed to higher concentrations (from 0.03 to 0.05 mg/L) pG were deceased within 30 min to 2 h. Compared with the control, the hatching rate was delayed for the embryos exposed to 0.025 mg/L pG; it is less than 24% of the embryos hatched at 96 hpf. The results reveal that embryo exposure to pG causes irregular heartbeat and this may lead to cardiac arrhythmia. Additionally, exposure has an influence on numerous embryonic morphological defects compared to the control group. The mechanism of action however is not clearly apparent or discussed in the study.
CONCLUSION	The test substance adversely affects fish embryos.

TEST FACILITY Manjunatha *et al.* (2018)

C.1.2 Acute Toxicity to Aquatic Invertebrates

TEST SUBSTANCE Graphene analogue 7

METHOD Daphnia Acute Immobilisation Test – Static
Species *Daphnia magna*
Exposure Period 48 hours
Auxiliary Solvent Polyvinylpyrrolidone (PVP)
Water Hardness 250 mg/L $CaCO_3$
Analytical Monitoring None
Remarks – Method Particle size characterisation:
Lateral size of graphene: 0.6 – 1.7 μm
Thickness: 0.76 nm
BET Surface Area: 802.88 m^2/g
Purity of graphene: 99%
No information on diameter or length of particle was available.

The sensitivity of the daphnia was within the limits as specified in the GB/T 13266 guideline (Water quality–determination of the acute toxicity of substance to Daphnia issued by China's health ministry). The 24 h acute toxicity test of the daphnids was performed every month using the reference toxicant $K_2Cr_2O_7$. The stock solution was obtained by adding 10 mg of test substance to 100 mL of a 1 g/L PVP solution (PVP dissolved in SM7 medium) followed by sonication for 2 h at room temperature to obtain optimal particle dispersion. It was determined that PVP at high concentrations were not toxic. The PVP concentration in the test solutions were 160 mg/L. After preparation, the test substance was suspended and no precipitate was observed after sonication.

RESULTS

Concentration (mg/L) Nominal	*Number of D. magna*	*Number Immobilised 48 h*
Control	10	0
0.2	10	0
0.5	10	0
1.0	10	0
2.0	10	1*
4.0	10	1*
8.0	10	3*
16.0	10	4*

*Read-off graph

LC50 > 16 mg/L at 48 hours
NOEC 1.0 mg/L at 48 hours
Remarks – Results No daphnids were immobilised in the control. The test substance substantially induced daphnid mortality when its concentration exceeded 2 mg/L. The mortality induced by the test substance reached as high as 40 % at the maximum nominal test concentration (16 mg/L).

CONCLUSION The test substance adversely affects aquatic invertebrates.

TEST FACILITY Fan *et al.* (2016)

C.1.3 Chronic Toxicity to Aquatic Invertebrates

TEST SUBSTANCE Graphene analogue 7

METHOD	OECD TG 211 Daphnia magna Reproduction test
Species	*Daphnia magna*
Exposure Period	21 d
Auxiliary Solvent	Polyvinylpyrrolidone (PVP)
Water Hardness	250 mg/L $CaCO_3$
Analytical Monitoring	None
Remarks – Method	Particle size characterisation: Lateral size of graphene: 0.6 – 1.7 μm Thickness: 0.76 nm BET Surface Area: 802.88 m^2/g Purity of graphene: 99% No information on diameter or length of particle was available. The test was performed according to the OECD guidelines with slight modifications. The daphnia were cultured at 0.5 °C above the recommended maximum limit, however, the sensitivity of the daphnia was within the limits as specified in the GB/T 13266 guideline (Water quality–determination of the acute toxicity of substance to Daphnia issued by China's health ministry). The 24 h acute toxicity test of the daphnids was performed every month using the reference toxicant $K_2Cr_2O_7$. In a preliminary experiment, it was also shown that PVP at a concentration as high as 10 mg/L was not toxic and did not affect the growth and reproduction of 24 h-old daphnia compared with controls after 21 days of chronic exposure. The stock solution was obtained by adding 10 mg of the test substance to 100 mL of a 1 g/L PVP solution (PVP dissolved in SM7 medium) followed by sonication using a sonication bath for 2 h at room temperature to obtain optimal particle dispersion. The test substance was suspended and no precipitate was observed after sonication.

Nominal Test Concentration (mg/L)	Total No. offspring released by survived *Daphnia*
Control	~500*
0.1	~600*
0.5	~200*
1.0	~50*

*Read-off graph

Remarks – Results	The validity acceptance criteria for the test were not recorded. At 0.1 mg/L exposure, the offspring of the daphnids exhibited a significant increase compared with that of the control. At 0.5 and 1.0 mg/L, the test substance significantly decreased the number of daphnid offspring over time compared with the control. The effect of the test substance on the time of first brood of the daphnids led to earlier first parturition at 0.1 mg/L and an obvious time delay at 0.5 and 1 mg/L compared with the control. The test substance showed the most noticeable effect on time of first parturition (compared with the other carbon nanomaterials tested in the same study, but whose details are not reported here), in accordance with the effect of the test substance on the number of daphnid offspring.
CONCLUSION	The test substance from 0.5 mg/L adversely affects the reproduction of aquatic invertebrates.
TEST FACILITY	Fan *et al.* (2016)

C.1.4 Algal Growth Inhibition Test

TEST SUBSTANCE	Graphene analogue 8
METHOD	Growth Inhibition Test - Static
Species	*Chlorella pyrenoidosa*
Exposure Period	96 hours

Concentration Range	Nominal: 0 - 200 mg/L Actual: Not determined
Auxiliary Solvent	None
Water Hardness	Not reported
Analytical Monitoring	None
Remarks – Method	Particle size characteristics of the multi-layer graphene tested: Lateral size: 2.5 μm Thickness: 5.0 nm BET Surface area: 133 m^2/g Interlayer spacing: 0.34 nm Oxygen content: 5.34% Pore volume: 0.272 cm^3/g Zeta potential (in H_2O): -25.6 mV To investigate the effects of shading and agglomeration, exponentially growing algal cells in 250 mL conical flasks were placed into 1 L beakers which contained the test substance (50 mg/L) prepared in algal medium. The algal cell numbers in the conical flasks were counted after 96 h shading by the suspension of the test substance in the beaker.

RESULTS

Growth

EC50 (mg/L at 96 h)	*NOEC (mg/L)*
62.2	< 10.0*

*Read-off the graph

Remarks – Results	The validity criteria for the test were not recorded. The 96 h EC50 values of the test substance to freshwater algae (*Chlorella pyrenoidosa*) was 62 mg/L. The test substance showed no shading effect on algal growth due to their poor dispersibility while nutrient depletion led to 27% of the total toxicity. The test substance was poorly dispersible due to its lack of surface functional groups and it readily agglomerated with itself (homo-agglomeration) to form large particles which deposited/settled-out of the suspension. Around 43% of algal cells were co-settled with the test substance, suggesting strong hetero-agglomeration between algae and the test substance. The study authors suggested that membrane damage induced by both oxidative stress and physical penetration/extraction were important mechanisms for the observed effects of the test substance. Scanning electron micrographs showed evidence of penetration of the test substance through algal cell walls.
CONCLUSION	The test substance adversely affects algal growth.
TEST FACILITY	Zhao *et al.* (2017)

APPENDIX D: DETAILS OF CARBON NANOTUBES USED IN TOXICOLOGICAL STUDIES

Analogue	*Particle size*	*Comment*
From Balakrishna Murthy et al. (2011)		
MWCNT-A	5-8 µm in length, with inside diameter of 3-8 nm and outside diameter of 140 ± 30 nm (average size 166 nm; 901 nm in water).	Composed of 99.9% carbon with < 0.1% iron. Open or closed tube morphology
MWCNT-B	1-10 µm in length with inside diameter of 2-6 nm and outside diameter of 10-15 nm (average size 100 nm; 554 nm in water).	Composed of 99.9% carbon with < 0.1% iron. Open or closed tube morphology
From Ema et al. (2011)		
SWCNT-A	mean diameter 1.8 nm, BET surface are 878 m^2/g.	Composed of 43,700 ppm iron, 56 ppm rubidium, 22 ppm zinc, 12 ppm gallium, 10 ppm copper, 9 ppm nickel and 6 ppm lead.
SWCNT-B	mean diameter 3 nm, BET surface are 1064 m^2/g.	Composed of 145 ppm iron, 103 ppm nickel, 34 ppm chromium, 15 ppm manganese, and 12 ppm aluminium.
MWCNT-C	mean diameter 44 nm, BET surface are 69 m^2/g.	Composed of 176 ppm gallium, 80 ppm aluminium, 53 ppm iron, 16 ppm cadmium, and 0.5 ppm lithium.
MWCNT-D	mean diameter 60 nm, BET surface are 23 m^2/g.	Composed of 3,600 ppm iron, 14 ppm chromium, 6 ppm bismuth, and 4 ppm nickel.

BIBLIOGRAPHY

Ariyanayagam, A., & Mahendran, M. (2013). Fire Safety of Buildings Based on Realistic Fire Time-Temperature Curves

Arvidsson, R., Molander, S., & Sandén, B. A. (2013). Review of Potential Environmental and Health Risks of the Nanomaterial Graphene. Human and Ecological Risk Assessment: An International Journal, 19(4), 873-887

Batley, G. E., & McLaughlin, M. J. (2008). Fate of Manufactured Nanomaterials in the Australian Environment. Available from http://www.environment.gov.au/node/21212. Last accessed 20 May 2019

Balakrishna Murthy, P., Sairam Kishore, A., Surekha, P. (2011). Acute Toxicological Effects of Multi-Walled Carbon Nanotubes (MWCNT), Carbon Nanotubes - Growth and Applications, Dr. Mohammad Naraghi (Ed.), ISBN: 978-953-307-566-2, InTech. Available from: http://www.intechopen.com/books/carbon-nanotubes-growth-and-applications/acute-toxicological-effects-of-multi-walled-carbon-nanotubes-mwcnt-. Last accessed 15 May 2019

Cadle, S.H. & Williams, R.L. (1979). Gas and Particle Emissions from Automobile Tires in Laboratory and Field Studies. Rubber Chemistry and Technology, 52(1), 146 - 158

DEKRA (2017) AGM Graphene. Ignition Sensitivity & Thermal Stability Testing (Study No. S3016001104R1V1/2017, February, 2017). Hampshire, United Kingdom, DEKRA-Insight (Unpublished report submitted by the notifier)

Dong, S., Xia, T., Yang, Y., Lin, S., & Mao, L. (2018). Bioaccumulation of 14C-Labeled Graphene in an Aquatic Food Chain through Direct Uptake or Trophic Transfer. *Environmental Science & Technology*, *52*(2), 541-549. doi:10.1021/acs.est.7b04339

Eftekhari, A., & Jafarkhani, P. (2013). Curly Graphene with Specious Interlayers Displaying Superior Capacity for Hydrogen Storage. The Journal of Physical Chemistry C, 117(48), 25845-25851

Ema, M., Matsuda A., Kobayashi N., Naya M., & Nakanishi J. (2011). Evaluation of Dermal and Eye Irritation and Skin Sensitisation due to Carbon Nanotubes. Regulatory Toxicology and Pharmacology. 61: 276 - 281

Envigo (2018a) Graphene platelet (nano): Acute Oral Toxicity in the Rat – Fixed Dose Procedure (Study No. SL26MP, July, 2018). Derbyshire, United Kingdom, Envigo Research Limited (Unpublished report submitted by the notifier)

Envigo (2018b) Graphene platelet (nano): V79 HPRT Gene Mutation Assay (Study No. QG64VW, April, 2018). Derbyshire, United Kingdom, Envigo Research Limited (Unpublished report submitted by the notifier)

Fadeel, B., Bussy, C., Merino, S., Vázquez, E., Flahaut, E., Mouchet F., Evariste, L., Gauthier, L., Kovisto, A.J., Vogel, U., Martín, C., Delogu, L.G, Buerki-Thirnherr, T., Wick, P., Beloin-Saint-Pierre, D., Hischier, R., Pelin, M., Candotto Carniel, F., Tretiach, M., Cesca, F., Benfenati, F., Scaini, D., Ballerini, L., Kostarelos, K., Prato, & M., Bianco, A. (2018). Safety Assessment of Graphene-Based Materials: Focus on Human Health and the Environment. ACS Nano. 12: 10582 - 10620

Fan, W., Liu, Y., Xu, Z., Wang, X., Li, X., & Luo, S. (2016). The mechanism of chronic toxicity to *Daphnia magna* induced by graphene suspended in a water column. *Environ. Sci.: Nano*, 3, 1405

First Graphene (2018a) Raman Spectroscopy Analysis of PureGRAPH™ Graphene Products - PureGRAPH™ 5, PureGRAPH™ 10 and PureGRAPH™ 20 (Study No. 2018.0002, November, 2018). Henderson, Western Australia, First Graphene Ltd (Unpublished report submitted by the notifier)

First Graphene (2018b) X-ray Photoelectron Spectroscopy Analysis of PureGRAPH™ 5, PureGRAPH™ 10 and PureGRAPH™ 20 (Study No. 2018.0005, September, 2018). Henderson, Western Australia, First Graphene Ltd (Unpublished report submitted by the notifier)

First Graphene (2018c) Tapped Density Analysis of PureGRAPH™ 5, PureGRAPH™ 10 and PureGRAPH™ 20 (Study No. 2018.0004, November, 2018). Henderson, Western Australia, First Graphene Ltd (Unpublished report submitted by the notifier)

First Graphene (2018d) Particle Size Analysis of PureGRAPH™ Graphene Products - PureGRAPH™ 5, PureGRAPH™ 10 and PureGRAPH™ 20 (Study No. 2018.0001, November, 2018). Henderson, Western Australia, First Graphene Ltd (Unpublished report submitted by the notifier)

First Graphene (2018e) Optical Microscopy (OM) and Scanning Electron Microscopy (SEM) Analysis of PureGRAPH™ 5 (PG5) (Study No. 2018.0008, December, 2018). Henderson, Western Australia, First Graphene Ltd, First Graphene Ltd (Unpublished report submitted by the notifier)

First Graphene (2018f) Scanning Electron Microscopy Analysis of PureGRAPH™ 5, PureGRAPH™ 10 and PureGRAPH™ 20 (Study No. 2018.0003, November, 2018). Henderson, Western Australia, First Graphene Ltd (Unpublished report submitted by the notifier)

First Graphene (2018g) BET Surface Area Analysis of PureGRAPH™ 5, PureGRAPH™ 10 and PureGRAPH™ 20 (Study No. 2018.0007, December, 2018). Henderson, Western Australia, First Graphene Ltd (Unpublished report submitted by the notifier)

First Graphene (2018h) Oxygen by Elemental Microanalysis of PureGRAPH™ 5, PureGRAPH™ 10 and PureGRAPH™ 20 (Study No. 2018.0008, December, 2018). Henderson, Western Australia, First Graphene Ltd (Unpublished report submitted by the notifier)

First Graphene (2019) Zeta Potential Analysis of PureGRAPH™ 5, PureGRAPH™ 10 and PureGRAPH™ 20 in Water (Study No. 2019.0015, January, 2019). Henderson, Western Australia, First Graphene Ltd (Unpublished report submitted by the notifier)

Gottschalk, F., Sonderer, T., Scholz, R. W., & Nowack, B. (2009). Modeled Environmental Concentrations of Engineered Nanomaterials (TiO2, ZnO, Ag, CNT, Fullerenes) for Different Regions. Environ. Sci. Technol., 43(24), 9216-9222

He, K., Chen, G., Zeng, G., Peng, M., Huang, Z., Shi, J., & Huang, T. (2017). Stability, transport and ecosystem effects of graphene in water and soil environments. Nanoscale, 9(17), 5370-5388

Hou, W.-C., Chowdhury, I., Goodwin, D. G., Henderson, W. M., Fairbrother, D. H., Bouchard, D., & Zepp, R. G. (2015). Photochemical Transformation of Graphene Oxide in Sunlight. Environmental Science & Technology, 49(6), 3435-3443

Hummers, W. S., & Offeman, R. E. (1958). Preparation of Graphitic Oxide. Journal of the American Chemical Society, 80(6), 1339-1339

Hyung, H., Fortner, J.D., Hughes, J.B., & Kim, J-H. (2007). Natural organic matter stabilises carbon nanotubes in the aqueous phase, Environmental Science and Technology 41, pp.179–184

JPCDCP (2015) Acute Oral Toxicity Test Report. Test Material Graphene (Study No. 20150177, July, 2015). Nanjing, People's Republic of China, Jiangsu Provincial Centre for Diseases Control and Prevention (Unpublished report submitted by the notifier)

Kim J.K., Shin J.H., Lee J.S., Hwang J.H., Lee J.H., Baek J.E., Kim T.G., Kim B.W., Kim J.S., Lee G.H., Ahn K., Han S.G., Bello D., & Yu I.J. (2016) 28-Day Inhalation Toxicity of Graphene Nanoplatelets in Sprague-Dawley Rats. Nanotoxicology. 10(7): 891 – 901

Koh, Y. K., Bae, M.-H., Cahill, D. G., & Pop, E. (2011). Reliably Counting Atomic Planes of Few-Layer Graphene (n > 4). ACS Nano, 5(1), 269-274

Liu, L., Zhu, C., Fan, M., Chen, C., Huang, Y., Hao, Q., & Sun, D. (2015). Oxidation and degradation of graphitic materials by naphthalene-degrading bacteria. Nanoscale, 7(32), 13619-13628

Li, Y., Yuan, H., von dem Bussche, A., Creighton, M., Hurt, R. H., Kane, A. B., & Gao, H. (2013). Graphene microsheets enter cells through spontaneous membrane penetration at edge asperities and corner sites. *Proc. Natl. Acad. Sci. U.S.A., 110*(30), 12295-12300,S12295/12291-S12295/12211

Los, J.H., Zakharchenko K.V., Katsnelson M.I., Fasolino A. (2015). Melting Temperature of Graphene. Phys Rev B. 91, 045415

Losic D. & Goodwin A. (2018). The Structure and Physio-Chemical Properties of Graphene: A Comparison with Industrial Carbon Materials such as Graphite and Carbon Black. (Study No. 2018.0004, December, 2018). University of Adelaide/ First Graphene Ltd (Unpublished report submitted by the notifier)

Loux, N.T., & Savage, N. (2008). An assessment of the fate of metal oxide nanomaterials in porous media, Water, Air, and Soil Pollution 194, pp.227-241

Lu, K., Dong, S., Petersen, E. J., Niu, J., Chang, X., Wang, P., & Mao, L. (2017). Biological Uptake, Distribution, and Depuration of Radio-Labeled Graphene in Adult Zebrafish: Effects of Graphene Size and Natural Organic Matter. ACS Nano, 11(3), 2872-2885

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

Manjunatha, B., Ho Park, S., Kim, K., Kundapur R.R., & Joon Lee S. (2018). In vivo toxicity evaluation of pristine graphene in developing zebrafish (*Danio rerio*) embryos. Environ. Sci. Pollut. Res., 25, 12821-12829

Marcano, D. C., Kosynkin, D. V., Berlin, J. M., Sinitskii, A., Sun, Z., Slesarev, A., Tour, J. M. (2010). Improved Synthesis of Graphene Oxide. ACS Nano, 4(8), 4806-4814

Mountjoy, E., Hasthanayake, D., & Freeman, T. (2015). Stocks & Fate of End of Life Tyres - 2013-14 Study. Available from http://www.nepc.gov.au/resource/stocks-and-fate-end-life-tyres-2013-14-study. Last accessed 20 May 2019

Mueller, N. C., & Nowack, B. (2008). Exposure Modeling of Engineered Nanoparticles in the Environment. Environmental Science & Technology, 42(12), 4447-4453

Muzi L, Mouchet F, Cadarsi S, Janowska I, Russier J, Menard-Moyon C., Risuleo G, Soula B, Galibert AM, Flahaut E, Pinelli E, Gauthier L, & Bianco, A. (2016). Examining the impact of multi-layer graphene using cellular and amphibian models. 2D Mater 3:025009

OECD (2004). Emission Scenario Document on Additives in Rubber Industry. Available from https://read.oecd-ilibrary.org/environment/rubber-additives_9789264221192-en#page3. Last accessed 20 May 2019

OECD. (2012a). Important Issues on Risk Assessment of Manufactured Nanomaterials Retrieved from http://www.oecd.org/env/ehs/nanosafety/publications-series-safety-manufactured-nanomaterials.htm

OECD. (2012b). Guidance on Sample Preparation and Dosimetry for the Safety Testing of Manufactured Nanomaterials. Series on the Safety of Manufactured Nanomaterials No. 36

Ong, K.J., Zhao X, Thistle ME, Mac Cormack TJ, Clark RJ, Ma G, Martinez-Rubi Y, Simard B, Loo JSC, & Veinot JG (2014). Mechanistic insights in to the effect of nanoparticles on zebrafish hatch. *Nanotoxicology* 8(3):295–304

Peralta-Videa JR, Zhao L, Lopez-Moreno ML, de la Rosa G, Hong J, & Gardea-Torresdey JL (2011). Nanomaterials and the environment: a review for the biennium 2008–2010. *J Hazard Mater* 186(1):1–15

Poland, C.A., Read, S.A.K., Varet, J., Carse, G., Christensen, F.M., & Hankin, S.M., (2013). Dermal Absorption of Nanomaterials Part of the Better Control of Nano Initiative2012–2015. The Danish Environmental Protection Agency.

Shin J.H., Han S.G., Kim J.K., Kim B.W., Hwang J.H., Lee J.S., Lee J.H., Baek J.E., Kim T.G, Kim K.S., Lee H.S., Song N.W., Ahn K., & Yu I.J. (2015). 5-Day Repeated Inhalation and 28-Day Post-Exposure Study of Graphene. Nanotoxicology. 9(8): 1023 – 1031

Su, Y., Yang, G., Lu, K., Petersen, E. J., & Mao, L. (2017). Colloidal properties and stability of aqueous suspensions of few-layer graphene: Importance of graphene concentration. Environmental Pollution, 220, 469-477

Sun, T. Y., Conroy, G., Donner, E., Hungerbuhler, K., Lombi, E., & Nowack, B. (2015). Probabilistic modelling of engineered nanomaterial emissions to the environment: a spatio-temporal approach. *Environ. Sci.: Nano*, 2(4), 340-351

United Nations (2009) Globally Harmonised System of Classification and Labelling of Chemicals (GHS), 3rd revised edition. United Nations Economic Commission for Europe (UN/ECE), http://www.unece.org/trans/danger/publi/ghs/ghs_rev03/03files_e.html

United Nations (2014) Committee of Experts on the Transport of Dangerous Goods and on the Globally Harmonized System of Classification and Labelling of Chemicals (UN/SCEGHS/28/INF.28). https://www.unece.org/fileadmin/DAM/trans/doc/2014/dgac10c4/UN-SCEGHS-28-INF28e.pdf

Zhao J, Wang Z, White JC, & Xing B. (2014). Graphene in the aquatic environment: Adsorption, dispersion, toxicity and transformation. Environ Sci Technol 48:9995–10009.

Zhao J., Cao X., Wang Z., Dai Z., & Xing B. (2017). Mechanistic understanding toward the toxicity of graphene-family materials to freshwater algae. *Water Research* 111, 18-27