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February 2019

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Cuprate(2-), [[*N*,*N*′-1,2-ethanediylbis[*N*-[(carboxy-κ*O*)methyl]glycinato-κ*N*,κ*O*]](4-)]-, potassium (1:2), (*OC*-6-21)-

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:

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANTS	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1597	Yara Australia Pty Ltd and Akzo Nobel Pty Ltd	Cuprate(2-), [[*N*,*N*′-1,2-ethanediylbis[*N*-[(carboxy-κ*O*)methyl]glycinato-κ*N*,κ*O*]](4-)]-, potassium (1:2), (*OC*-6-21)-	Yes	≤ 1 tonne per annum	Component of micronutrient fertiliser

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is recommended for classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the following table.

Hazard classification	*Hazard statement*
Acute toxicity (Category 4)	H302 – Harmful if swallowed
Serious eye damage/Eye irritation (Category 2A)	H319 – Causes serious eye irritation

The environmental hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS) is presented below. Environmental classification under the GHS is not mandated in Australia and carries no legal status but is presented for information purposes.

Hazard classification	*Hazard statement*
Acute (Category 3)	H402 – Harmful to aquatic life

Human health risk assessment
Provided that the recommended controls are being adhered to, under the conditions of the occupational settings described, the notified chemical 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.

The public report of this assessment will be forwarded to Food Standards Australia New Zealand (FSANZ) for their information and consideration in future dietary surveys when estimating consumer exposure levels to nutrients such as copper.

Environmental risk assessment
On the basis of the PEC/PNEC ratio, comparison with Australian water and soil quality guideline limits for copper and the assessed use pattern, the notified chemical and its transformation products are not considered to pose an unreasonable risk to the environment provided, good agricultural practices ensure that the wastage and potential contamination of water bodies from overspray, drift or run-off are minimised. For spray-drift it is regarded as good agricultural practice to not apply chemicals when wind speed is less than 3, or more than 20 kilometres per hour, at the application site.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - H302 – Harmful if swallowed
  - H319 – Causes serious eye irritation

The above should be used for products containing the notified chemical, if applicable, based on the concentration of the notified chemical present.

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 in powder form:
  - Adequate ventilation

- 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
  - Minimise dust generation where possible

  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

- Good agricultural practice of not applying chemicals by spray application when wind speed is less than 3 or more than 20 kilometres per hour at the application site.

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.

Storage

- The handling and storage of the notified chemical should be in accordance with the Safe Work Australia Code of Practice for *Managing Risks of Hazardous Chemicals in the Workplace* (SWA, 2012) or relevant State or Territory Code of Practice.

Emergency procedures

- Spills or accidental release of the notified chemical should be handled by containment, physical collection and subsequent safe disposal.

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(2) of the Act; if
- the function or use of the chemical has changed from component of micronutrient fertiliser, or is likely to change significantly;
- the amount of chemical being introduced has increased, or is likely to increase, significantly;
- the chemical has begun to be manufactured in Australia;
- 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.

No additional secondary notification conditions are stipulated.

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANTS
Yara Australia Pty Ltd (ABN: 77 076 301 221)
Level 1, 6 Holt Street
McMAHONS POINT NSW 2060

Akzo Nobel Pty Ltd (ABN: 59 000 119 424)
8 Kellaway Place
WETHERILL PARK NSW 2164

NOTIFICATION CATEGORY
Standard: Chemical other than polymer.

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: analytical data, degree of purity, use details, and import volume.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed for all physico-chemical properties.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANTS
None

NOTIFICATION IN OTHER COUNTRIES
Canada, China, EU, Japan, New Zealand and USA

2. IDENTITY OF CHEMICAL

MARKETING NAMES
Dissolvine CXK (product containing the notified chemical at < 5% concentration)
Rexolin CXK (product containing the notified chemical at < 5% concentration)

CAS NUMBER
15170-14-6

CHEMICAL NAME
Cuprate(2-), [[*N,N'*-1,2-ethanediylbis[*N*-[(carboxy-κ*O*)methyl]glycinato-κ*N*,κ*O*]](4-)]-, potassium (1:2), (*OC*-6-21)-

OTHER NAMES
Cuprate(2-), [(ethylenedinitrilo)tetraacetato]-, dipotassium (8CI)

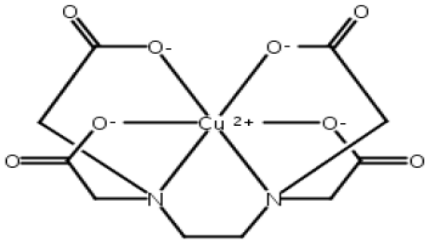
Cuprate(2-), [[*N,N'*-1,2-ethanediylbis[*N*-(carboxymethyl)glycinato]](4-)-*N,N',O,O',O*^{N},*O*$^{N'}$]-, dipotassium, (*OC*-6-21)-

Cuprate(2-), [[*N,N'*-1,2-ethanediylbis[*N*-[(carboxy-κ*O*)methyl]glycinato-κ*N*,κ*O*]](4-)]-, dipotassium, (*OC*-6-21)-

Ethylenediaminetetraacetic acid, copper dipotassium complex

MOLECULAR FORMULA
$C_{10}H_{12}CuN_2O_8.2K$

STRUCTURAL FORMULA



$2K^+$

MOLECULAR WEIGHT
430.0 g/mol

ANALYTICAL DATA
Reference MS and UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 95%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Blue microgranules

Property	Value	Data Source/Justification
Melting Point/Freezing Point	Decomposes without melting at > 200 °C	Information provided by notifier. No study details
Boiling Point	Decomposes without boiling at > 200 °C	Information provided by notifier. No study details
Density	1,725 kg/m^3 at 20 °C	Measured*
Vapour Pressure	Not determined	Expected to be low based on chemical structure
Water Solubility	736 g/L at 20 °C	Measured§
Hydrolysis as a Function of pH	Not determined	Expected to be hydrolytically stable based on the structure
Partition Coefficient (n-octanol/water)	Not determined	Expected to partition to the water phase based on high water solubility of organic moiety
Adsorption/Desorption	Not determined	Expected to be mobile in soil systems due to the high water solubility
Dissociation Constant	Not determined	pKa1 =11.24; pKa2= 6.04; pKa3 =3.74; pKa4 =1.78 estimated by using ACD/Labs
Particle Size	Inhalable fraction (< 100 µm): 35 - 50% Respirable fraction (< 10 µm): 0%	Measured*
Flash Point	Not determined	Inorganic solid
Flammability	Not determined	Not expected to be highly flammable
Autoignition Temperature	> 400 °C	Information provided by notifier. No study details
Explosive Properties	Not determined	Contains no functional groups that would imply explosive properties
Oxidising Properties	Not determined	Contains no functional groups that would imply oxidising properties

* For the product Dissolvine CXK containing the notified chemical at < 5% concentration
§ Full study report was not provided

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 cannot be classified 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 not be manufactured in Australia. The notified chemical will be imported as a component of micronutrient fertiliser mixture in powder form at < 5% concentration. There will be no reformulation or repackaging in Australia prior to sale to farmers.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	≤ 1	≤ 1	≤ 1	≤ 1	≤ 1

PORT OF ENTRY
Melbourne, Sydney, Brisbane, Adelaide, Perth, Hobart and Darwin

IDENTITY OF RECIPIENTS
Yara Australia Pty Ltd
AkzoNobel Pty Ltd

TRANSPORTATION AND PACKAGING
Products containing the notified chemical at < 5% concentration will be imported as a component of finished soil fertiliser in powder form in 5 kg or 40 kg lined paper bags and transported by road within Australia to regional centres for sale to farmers.

USE
The notified chemical will be used as a component of a micronutrient fertiliser that will be applied only in Cu deficient conditions as determined by foliar and soil testing. The product containing the notified chemical will be applied to vegetables, cut-flowers, potted flowers and pot plants grown in glasshouses and arable crops, including soy-bean, cereals, cotton, maize, oilseed and lucerne, and horticultural crops, including citrus, apple, grapes, peach and plums grown in open fields either as a foliar application or a soil application.

Foliar application
For crops grown in glasshouses, the product containing the notified chemical will be applied at a rate of ≤ 1 g of product/L of water (equivalent to ≤ 0.05 g/L notified chemical). For crops grown in open fields the product containing the notified chemical will be applied at a rate of ≤ 2 kg (equivalent to ≤ 100 g notified chemical) of the product/hectare.

Soil application
Products containing the notified chemical will be applied at a rate of ≤ 15 kg (equivalent to ≤ 750 g notified chemical) of the product/hectare.

In general, the number of applications depends on the type of crop grown and up to four applications in a year may be required with a two-week interval in between.

OPERATION DESCRIPTION
Farmers or farmworkers will move the bags containing the fertiliser mixture (containing the notified chemical at < 5% concentration) to the loading area, weigh-out the required amount of the product and manually add to the make-up tank. The tank will be connected to the spray equipment (boom spray only) or to the on-site drip fertigation system. When boom spray is used for method of application, the farmer will drive the tractor while

the fertigation water containing the notified chemical at < 0.005% concentration is sprayed onto the soil or plant foliage to be treated.

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)*
Transport and storage	2 - 6	12 - 24
Farmers and farm workers	1 - 3	1 - 4

EXPOSURE DETAILS

Transport and storage

Transport and storage workers may come into contact with the notified chemical at < 5% concentration only in the event of an unlikely accidental rupture of bags containing the notified chemical.

End-use

Farmers or farmworkers will move the bags containing the fertiliser mixture (containing the notified chemical at < 5% concentration) to the loading area, weigh-out the required amount of the product and manually add to the make-up tank. The tank will be connected to the spray equipment (boom spray only) or to the on-site drip fertigation system. When boom spray is used for method of application, the farmer will drive the tractor while the fertigation water containing the notified chemical at < 0.005% concentration is sprayed onto the soil or plant foliage to be treated.

The principal route of exposure to the notified chemical will be dermal. However, as the fertiliser mixture is in powder form, during weighing and loading into make-up tank, exposure to the notified chemical via dust through inhalation route and to a lesser extent through ocular route is also possible.

The notifier has confirmed that, if the product is applied via spray, only boom sprayers will be used. No other methods, such as air-blast or handheld or backpack applicators or aerial application, will be used. As low energy/low pressure equipment will be used in the boom spray, a very low amount of fine spray particles will be generated during spraying. The notifier stated that the end-users are expected to adhere to Australian Pesticides and Veterinary Medicines Authority's (APVMA) operating principals to prevent spray drifts (APVMA, 2008).

The notifier stated that such workers will use appropriate PPE, including impervious gloves, coveralls, safety glasses and dust masks to minimise repeated exposure. Moreover, good hygiene practices are expected to be in place.

6.1.2. Public Exposure

The product containing the notified chemical will not be made available to the public. Application of product containing the notified chemical by ground-boom application may lead to unintended bystander exposure via chemical spray drift. This may be in the form of single random exposure or repeat exposure of residents who reside adjacent to areas being treated with the product.

As low energy/low pressure equipment will be used in the boom spray, a very low amount of fine spray particles will be generated during spraying. The notifier stated that the end-users are expected to adhere to Australian Pesticides and Veterinary Medicines Authority's (APVMA) operating principals to prevent spray drifts (APVMA, 2008).

The products containing the notified chemical will be applied via foliar spray and soil application to various food producing crops grown in fields (including soybeans, apples, plums, peaches and grapes) and glasshouses (including various vegetables), up to four times in a growing season. It will be applied during various stages, including fruit and vegetable formation and maturation phases, of crop growth. As the notified chemical will be applied via foliar spray during fruit and vegetable formation and maturation phases, public exposure to the notified chemical and its residues may occur via ingestion of sprayed fresh produce if consumed unwashed.

6.2. Human Health Effects Assessment

The results from toxicological investigations conducted on the notified chemical and an analogue chemical are summarised in the following table. For full details of the studies, refer to Appendix B.

Endpoint	*Test substance*	*Result and Assessment Conclusion*
Rat, acute oral toxicity	Notified chemical	LD50 = 500 mg/kg bw; harmful
Rat, acute inhalation toxicity	Analogue chemical 1	LC50 > 5.30 mg/L/4 hour; low toxicity
Skin irritation (*in vitro*)	Notified chemical	inconclusive
Rabbit, skin irritation	Analogue chemical 1*	non-irritating
Eye irritation (*in vitro*)	Notified chemical	inconclusive
Rabbit, eye irritation	Analogue chemical 1*	irritating
Mouse, skin sensitisation – Local lymph node assay	Analogue chemical 1	no evidence of sensitisation
Rat, repeated dose oral toxicity with reproductive/developmental toxicity screening – 90 days	Analogue chemical 1	NOAEL (parental) < 150 mg/kg bw/day NOEL (reprod/develop) ≥ 500 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	Analogue chemical 1	non mutagenic
Genotoxicity – *in vitro* mammalian micronucleus test	Analogue chemical 1	genotoxic

*Test substance 50% aqueous solution

Analogue chemical 1 (EDTA-$CuNa_2$; CAS No. 14025-15-1) is a metal complex of ethylenediaminetetraacetic acid (EDTA) similar to the notified chemical. Both contain copper and only differ by the nature of the alkali metal.

Toxicokinetics, metabolism and distribution

No toxicokinetic data on the notified chemical were submitted.

EDTA salts are poorly absorbed by the oral and dermal route (ECCC/HC, 2017). Following administration of 50 mg radiolabeled EDTA-$CaNa_2$ to rats via oral gavage, absorption after 24 hours was 10% and 6% in males and females, respectively, based on urinary excretion.

In humans (males) exposed to 1.5 or 2 mg radiolabeled EDTA-$CaNa_2$ by the oral or dermal route, recovery in urine was only 5% through the oral route and 0.001% through the dermal route after 24 hours. In addition, it has been reported that EDTA and 23 of its salts did not absorb through the skin (ECCC/HC, 2017).

The notified chemical is a highly water soluble (736 g/L) copper compound with the potential to release copper (II) ions following systemic absorption. However, upon dissolution, a negatively charged Cu-EDTA complex and 2 positively charged potassium (I) ions will exist. The EDTA moiety surrounds and protects the Cu (II) ion and modifies its reactivity. The Cu-EDTA complex can be absorbed following oral exposure, but it does not readily penetrate the skin following dermal exposure. However, based on the low oral absorption of EDTA from the gut (<5%), the oral absorption of the Cu-EDTA complex from the gut is expected to be limited (<5%). The notifier stated that the amount of Cu (II) ions absorbed from the gut will depend on the physiological need of the individual. Following inhalation exposure, in view of the relative large particle size, mucociliary clearance from the respiratory tract will generate oral exposure following inhalation, as particles can be returned from the lungs to the back of the throat and swallowed.

Human true absorption rates of 30-65% associated with 'copper-adequate' diets were concluded from several toxicokinetics studies (ECHA, 2007). Highest concentrations of copper occur in liver, brain, heart and kidney. The liver is the main organ involved in copper distribution and plays important role in homeostasis by regulating the release of copper. Faecal excretion is main route of elimination for copper.

Two *in vitro* studies are available which have reported on the dermal absorption of copper in human skin (ECHA, 2007). Based on these two studies, a dermal absorption factor of 0.3% is proposed for copper substances in solution or suspension.

Data on systemic absorption of copper from the respiratory tract for humans are sparse and of little relevance to human risk assessment (ECHA, 2007). With regard to absorption of copper in animals following inhalation exposure, only one study is available. This study showed the potential for systemic absorption of copper in rats

following inhalation of copper-containing dust, but provided no reliable quantitative data which are relevant for human health risk assessment.

Acute toxicity
The notified chemical was found to be harmful in a rat acute oral toxicity study.

No studies were submitted for acute dermal toxicity. The notified chemical is not expected to be toxic by the dermal route as significant dermal absorption is not expected.
No studies were submitted for acute inhalation toxicity for the notified chemical. Analogue chemical 1 was found to be of low acute inhalation toxicity in rats. Respiratory irritation effects, such as breathing difficulties, were reported in exposed rats. The notified chemical is expected to be of low acute toxicity via the inhalation route.

Irritation and sensitisation
In an *in vitro* skin irritation study (conducted in three independent experiments using a human skin model), highly variable relative tissue viability values (13%, 58% and 75%) were obtained for the notified chemical. Therefore the results were considered to be inconclusive. In a study conducted in rabbits, analogue chemical 1 at 50% concentration was found to be non-irritating in rabbits. Therefore, by inference, the notified chemical is not expected to be irritating to the skin at up to 50% concentration.

In an *in vitro* eye irritation study using the bovine corneal opacity and permeability test, the notified chemical in two independent tests induced mean *in vitro* irritation scores (IVIS) of 3.1 and 4.0. As these values are between > 3 and ≤ 55 no eye irritation classification can be made according to the test guidelines. In an eye irritation study conducted in rabbits, analogue chemical 1 at 50% concentration was found to be irritating to eyes. Moderate conjunctival irritation and corneal opacity (grade 1) were observed in all animals which persisted in one animal to the end of the study (Day 8). Based on the effects observed, analogue chemical 1, and by inference the notified chemical, is classified as a Category 2A eye irritant under the GHS.

No studies were submitted for skin sensitisation for the notified chemical. Analogue chemical 1 was determined not to be a skin sensitiser in a mouse local lymph node assay (LLNA). Therefore, by inference, the notified chemical is not expected to be a skin sensitiser.

Repeated dose toxicity and toxicity for reproduction
No data were submitted for the notified chemical.

In a 90-day combined repeated dose oral toxicity study with the reproduction/developmental screening test in rats, analogue chemical 1 was administered daily by gavage (in water) during a premating period of 10 weeks and during mating, gestation and lactation until postnatal day 4 for females and 90 days for males. The dose levels were 150, 500, and 1,500 mg/kg bw/day. Due to mortalities in the high-dose group, the high-dose was reduced to 1,050 mg/kg bw/day from day 9.

Three males in the mid-dose group were euthanised *in extremis* during or at the end of the mating period and in the high-dose group, two females died on days 4 and 6, and a female was euthanised *in extremis* on day 7 of treatment. All animals in the high-dose group were either euthanised *in extremis* or found dead before the start of the mating period (from day 11 to day 65).

At 500 mg/kg bw/day, treatment related effects observed included tubular necrosis and degeneration, tubular epithelial cell karyomegaly, and accumulation of brown pigment in the kidneys and hepatocellular karyomegaly, brown pigment accumulation, bile duct hyperplasia and multifocal infiltration of mononuclear inflammatory cells observed in the liver. In the 150 mg/kg bw/day dose group, 6/10 males showed tubular epithelial brown pigment in the kidneys and 6/10 males and 3/10 females showed mononuclear cell infiltrate in the liver.

Based on treatment related histopathological effects observed in kidneys and liver in the low dose group, a No Observed Adverse Effect Level (NOAEL) for parental toxicity was not established by the study authors. However, given only limited effects were observed in the low-dose group, a No-Observed Effect Level (NOEL) for parental toxicity close to 150 mg/kg bw/day was speculated by the study authors.

The study authors established a NOEL of ≥ 500 mg/kg bw/day for reproductive and developmental toxicity, based on the absence of treatment related effects on fertility parameters, reproductive performance and development.

Mutagenicity/Genotoxicity
There were no data available on mutagenicity or genotoxicity for the notified chemical. Only limited data were provided for the analogue chemical.

Analogue chemical 1 tested negative in a bacterial reverse mutation assay but a positive response was obtained from 62.5 µg/mL in an *in vitro* micronucleus test with human lymphocytes. However, it was reported that the percentage of binucleated cells containing micronuclei were only slightly higher than the historical control range of the test facility, although the analogue chemical was reported to be aneugenic to human lymphocytes from 62.5 µg/mL.

Health hazard classification
Based on the available information, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the following table.

Hazard classification	*Hazard statement*
Acute toxicity (Category 4)	H302 – Harmful if swallowed
Serious eye damage/Eye irritation (Category 2A)	H319 – Causes serious eye irritation

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the available information, the critical health effects of the notified chemical for risk characterisation include local effects (eye irritation), acute oral toxicity and systemic long-term toxic effects following repeated exposure to high doses. Toxicity via the dermal route is not expected given the limited potential for dermal absorption.

Farmers and farmworkers may be exposed to the notified chemical at < 5% concentration. At the low proposed use concentration, eye irritation effects are not expected.

During weighing and loading into the make-up tank, exposure to dust of the product containing the notified chemical at < 5% concentration is possible. The product contains a significant fraction of inhalable particles (36.7% < 100 µm) but only a very small fraction of respirable particles (1.7% < 10 µm). If inhaled, the notified chemical will therefore likely to be deposited in the mucus lining of the upper respiratory tract where it will dissolve and be removed upwards by the mucociliary escalator and subsequently swallowed. Given the low proposed use concentration, the risk of systemic effects via inhalation is not expected. Furthermore, the notifier has stated that workers will use dust masks when handling the product containing the notified chemical in powder form which should further minimise the risk.

It is expected that the spray operations will be low energy/low pressure, therefore inhalation exposure to vapours, mists or aerosols during spray application is not likely to occur. Furthermore, the concentration of the notified chemical in spray solution is very low (< 0.005%), therefore inhalation exposure to the notified chemical will not be significant.

Provided control measures are in place to limit inhalation exposure to dusts of the notified chemical, the risk to workers is not considered to be unreasonable.

6.3.2. Public Health
The product containing the notified chemical will not be made available to the public. Bystander risk is possible, but is expected to be limited based on the proposed use pattern. Potential routes of exposure for bystanders are dermal, inhalation and ocular during or immediately after a spraying event, while dermal exposure is the most likely route of exposure during re-entry situations. Workers adherence to good agricultural practice will minimise potential risks for the public.

Products containing the notified chemical will be applied via foliar spray and soil application to various food producing crops grown in fields, including soybeans, apples, plums, peaches and grapes, and grown in glasshouses, including various vegetables, up to four times in a growing season. It will be applied during various stages, including fruit and vegetable formation and maturation phases, of crop growth.

As the notified chemical will be applied via foliar spray during fruit and vegetable formation and maturation phases, copper residues of the notified chemical could be present on fresh fruits and vegetables. Copper is an essential trace element; however, in excess it can lead to adverse effects (ECHA, 2007). The main target organ for toxicity is the liver. This is consistent with the effects observed in the repeated dose toxicity study for the analogue chemical. The acceptable daily intake (ADI) for copper is 0.2 mg/kg bw/day (Department of Health, 2016).

In case of soybean, cereals and oilseeds, the presence of pod or husk will act as a protective barrier against the notified chemical reaching consumable parts, such as seeds. In case of fruits, foliar application will target leaves, and therefore only a portion of the applied product is expected to be present on the fruits and vegetables.

The notifier has provided a worst case consumer exposure scenario through ingestion of copper residues of the notified chemical from unwashed butter lettuce (lettuce) treated by four foliar applications of the notified chemical:

- Product containing the notified chemical could be applied as a foliar spray on lettuce for 4 times per season at a rate of 2 kg/ha per spray application (8 kg/ha in total per crop season).
- A hectare of lettuce plantation will yield ~ 10,000 to 12,000 kg of lettuce heads. One kg of lettuce will contain 0.8 g of the product (i.e. 8 kg of the product ÷ 10,000 kg of lettuce).
- As the product contains approximately 0.74% copper, 1 kg of lettuce will contain 0.006 g of copper (i.e. 0.0074× 0.8 g).
- An edible part of lettuce head weighs ~ 360 g and a person would consume ~100 g of unwashed lettuce/day.
- Therefore, 0.6 mg of copper will be available in 100 g of unwashed lettuce.
- This equates to 0.01-0.009 mg of copper/kg bw/day for a 60-70 kg person.
- This value is below the acceptable daily intake (ADI) level of 0.2 mg/kg bw/day for copper.

Members of the public may consume various fruits and vegetables containing copper residues of the notified chemical. However, exposure to copper residues at significant levels (as calculated above with four spray applications and unwashed) is not expected due to:

- low concentration (< 0.005%) of the notified chemical in one spray application;
- watering between spray applications will wash off residues on crops; and
- watering prior to harvest, or rain following application will wash off residues on crops.

The public report of this assessment will be forwarded to Food Standards Australia New Zealand (FSANZ) for their information and consideration in future dietary surveys when estimating consumer exposure levels to nutrients such as copper when applied on fresh fruits and vegetables as a foliar spray.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will not be manufactured in Australia. It will be imported as a component of micronutrient fertiliser mixture in powder form. There will be no reformulation or repackaging in Australia prior to sale to farmers. Any accidental spills during transport are expected to be collected and recycled or disposed of to landfill.

RELEASE OF CHEMICAL FROM USE

The notified chemical will be used as a component of a micronutrient fertiliser that will be used in micronutrient deficient soils. The product containing the notified chemical will be applied either as a foliar or a soil application. For foliar application, it will be applied at a rate of ≤ 1 g of product/L of water (equivalent to ≤ 0.05 g/L notified chemical) or at a rate of ≤ 2 kg (equivalent to 100 g notified chemical) of product/hectare. For soil application, it will be applied at a rate of ≤ 15 kg (equivalent to ≤ 750 g notified chemical) of product/hectare. Up to four applications may be required with a two-week interval in between. The fertiliser mixture containing the notified chemical will be mixed with water and connected to the spray equipment (boom spray only) or to on-site drip fertigation system. When boom spray is used for method of application, the farmer will drive the

tractor while the fertigation water containing the notified chemical is sprayed onto the soil or plant foliage to be treated. Notified chemical residues remaining in application equipment are expected to be delivered to soil during subsequent use of the equipment.

RELEASE OF CHEMICAL FROM DISPOSAL
During use, all the notified chemical is expected to be applied to the soil or plant foliage as the fertiliser. However, unwanted, unused fertiliser is likely to be disposed of by an authorised waste disposal company.

7.1.2. Environmental Fate

All the notified chemical is expected to be applied to plant foliage and topsoil as fertiliser. The submitted study conducted on an analogue (analogue chemical 2; EDTA-$CaNa_2$) indicates that the organic moiety of the notified chemical is not readily biodegradable within 28 days but shows high biodegradation rate in natural waters at pH=8. Generally, EDTA-metal complexes are considered to be inherently biodegradable. For details of the environmental fate study refer to Appendix C. Supplementary environmental fate characteristics of the notified chemical were sourced from published documents including the report provided by the notifier which is based on the European Union Risk Assessment Report (2004). In addition, metal ion exchange reactions may occur in soils. The notified chemical (EDTA-CuK_2) may be converted to Fe or Ca-EDTA complexes depending on the pH in the environment (European Union Risk Assessment, 2004). The Ca-EDTA complex is susceptible to biodegradation at pH > 8, whereas the Fe-EDTA complex is susceptible to photodegradation (European Union Risk Assessment, 2004). The half-life of Cu-EDTA complex was reported to be 2 days at pH of 7.85 and 5-25 days at pH of 5.7-7.3 in soil systems (Norvell and Lindsay, 1969).

Generally, copper sorbs strongly onto soil particulates and may be rapidly converted to the organically-bonded complexes or inorganic precipitates. A significant proportion of copper is expected to be organically complexed with abundant organic ligands and retained in the surface soil. The distribution of copper between solution and solid phases is related to the soil pH, redox conditions, salinity, nature and concentration of complexing agents, and cation exchange capacity. Copper bioavailability and toxicity is affected by biotic and abiotic factors including organism age and size, water hardness, pH, and dissolved organic carbon.

The microelements contained in liquid fertilisers such as copper are essential elements necessary for plant metabolism. Therefore, the notified chemical in the form of bioavailable copper species is expected to be taken up by plants and crops in nutrient deficient soils. Factors affecting the availability of copper in soils to plants are pH, organic matter composition, clay content, redox conditions, microbial activity in the rhizosphere, soil moisture status, concentrations of other trace elements and macronutrients, and climate.

The notified chemical is expected to be highly soluble and may reach aquatic environment from overspray, spray-drift or run-off. The notified chemical will transform under environmental conditions and may form different copper species which may have variable solubility and bioavailability in complex soil, aquatic and sediment systems. However, a significant proportion of copper will partition to the solid phase and efficient and economic use of fertilisers, in addition to good farming practices, are expected to minimise loss of the notified chemical to the aquatic environment.

Copper is an essential element and aquatic organisms are known to bioconcentrate copper from water. While most aquatic organisms internally regulate copper concentrations, elevated levels of copper in water can overwhelm homeostasis mechanisms leading to toxicity. The concentration at which copper is homoeostatically regulated is species-specific and the external copper concentration at which regulation breaks down depends on both intrinsic (e.g., species) and extrinsic (e.g., temperature, pH, presence of other metals) factors. Accumulation of copper to meet physiological requirements can be mistaken for trophic transfer, however, copper is not biomagnified.

7.1.3. Predicted Environmental Concentration (PEC)

The notified chemical is intended as part of a nutrient supplement program for agricultural land and actual application rates will depend on specific crop nutrient requirements. The product containing the notified chemical will be applied either as a foliar or a soil application. For foliar application, it will be applied at a rate of ≤ 2 kg/ha of product (equivalent to ≤ 100 g notified chemical)/hectare. For soil application, it will be applied at a rate of ≤ 15 kg of product (equivalent to ≤ 750 g notified chemical)/hectare. Up to four applications may be required with a two-week interval in between.

Since the notified chemical may be ion exchanged with Fe or Ca depending on the pH, the predicted environmental concentrations were calculated based on the application rates of the notified chemical and total copper.

Soil compartment
The notified chemical will be released into soils as a result of its application to agricultural soils by ground boom sprayer and drip irrigation. The recommended annual maximum application rate of the notified chemical [0.75kg notified chemical/ha×4times]$_{soil}$+[4 times×0.1kg notified chemical/ha]$_{foliar}$ = 3.4 kg notified chemical/hectare or 3.4 kg/ha×[63.55/430.0] = 0.50 kg of copper/hectare results in a worst case PEC_{soil} [notified chemical] = 2.3 µg/kg and PEC_{soil} [Cu_{Total}] = 0.33 µg/kg in the 10 cm of the soil system assuming soil density of 1500 g/cm^3. PEC_{soil} values decrease further with soil depth. The increase in copper in soil is considered well within natural variability of this element in Australian rural surface soil (1 and 190 mg/kg; Naidu et al., 1996).

Aquatic compartment
The notified chemical may reach aquatic environments from overspray, spray drift during application by ground boom sprayer, or in run-off. Direct overspray is unlikely based on the reported use pattern. For run-off a worst-case edge-of-field scenario may be considered assuming a 100 mm rainfall event with 20 mm of run-off and 5% of the applied chemical contained in the run-off water (https://apvma.gov.au/node/805). This does not consider the uptake by crops, or degradation and mobility of the notified chemical. Given the notified chemical may be persistent for several weeks, the resulting concentration from a run-off event after a final annual application is 850 µg/L {[(3.4 kg/ha) × 0.05] ÷ 200 m^3}. However, a more realistic scenario would be from a single application given significant proportion of the notified chemical will be taken by the plants. Therefore, the resulting concentration from a run-off event after a single application is 212.5 µg/L.

Copper speciation will change in the soil system and is not expected to significantly contribute to the background copper concentrations in water and sediment from the run-off route, given copper is ubiquitous in the environment.

Exposure to the aquatic compartment from spray drift as a result of application by ground boom sprayer can be modelled using the AgDRIFT® model (AgDRIFT Spray Drift Task Force Spray Software, Version 2.0.09). The PEC arising from spray drift is calculated for both the notified chemical and total copper assuming a water body 15 cm deep and 3 m wide (≡ 1500 m^3 per hectare). The variables in the model that affect spray drift are the droplet size and height of the boom. Since the notified chemical will be used as a fertiliser, a coarse droplet size is likely to be used, and a high boom height is assumed based on the worst-case scenario. Generally, off-target exposure is increased with fine droplets size and increased boom height. The percent drift at 0 m, 1 m and 5 m is 18%, 6.79% and 2.1% of the nominal application rate, respectively. With the worst-case application rate of 3.4 kg of notified chemical/hectare $PEC_{spray\ drift}$ for a water body at 0 m, 1 m and 5 m are 410 µg/L, 150 µg/L, and 47 µg/L, respectively. With an application rate of 0.5 kg Cu/ha the $PEC_{spray\ drift}$ for a water body at 0 m, 1 m and 5 m are 61 µg Cu/L, 23 µg Cu/L, and 7 µg Cu/L.

A significant proportion of the notified chemical and its transformed products will be lost due to uptake in plants or may be associated with the solid phase, therefore, the calculated PEC for both the notified chemical and total copper is an overestimate of the aquatic exposure.

A more realistic scenario, therefore, would be from a single application, as the long-term concentration of copper ions is expected to be determined by the environmental conditions. The more realistic concentration of copper after a single application for a water body at 0 m, 1 m and 5 m are 15 µg copper/L, 5.7 µg copper/L, and 1.7 µg copper/L ([0.75 +0.1 kg notified chemical/hectare] × 63.55/430. × 18%, 6.79% or 2.1% ÷ 1500 m^3). Similarly the concentration of the notified chemical at 0 m, 1 m and 5 m would be 100 µg/L, 38 µg/L, and 12 µg/L, respectively.

7.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on analogue chemical 1 (EDTA-$CuNa_2$) are summarised in the table below. Details of these studies can be found in Appendix C. In some cases, additional ecotoxicological endpoints have been sourced from the published literature to supplement the submitted information. A small proportion of released copper may be bioavailable to aquatic organisms and the toxicity of the notified chemical may be attributed to the soluble form of copper (Cu (II)). Both released copper and un-complexed EDTA show higher toxicity to aquatic life than Cu-EDTA complex (Sorvari and Sillanpää, 1996). However, direct effects caused by the intrinsic toxicity of EDTA are not expected in surface waters, where a stoichiometric surplus of metal ions is present (European Union Risk Assessment, 2004). Probable

transformation complexes such as Fe and Ca-EDTA are not expected to be harmful to aquatic life based on the information submitted by the notifier.

Endpoint	*Result*	*Assessment Conclusion*
Analogue chemical 1		
Acute toxicity		
Fish Toxicity	96 h LC50 = 555* mg/L	Not harmful to fish
Daphnia Toxicity	24 h EC50 = 38[§] mg/L	Harmful to aquatic invertebrates
Algal Toxicity	72 EC50 > 1000* mg/L	Not harmful to algae
Cu (II)		
Acute toxicity		
Fish Toxicity	96 h LC50 = 9.3-720[¥] µg/L	Very toxic to fish
Daphnia Toxicity	24 h EC50 = 22[§] µg/L	Very toxic to aquatic invertebrates
Water Flea Toxicity	48-72 h EC50 = 1.1-41 [¥] µg/L	Very toxic to aquatic invertebrates
Algae Toxicity	48-72 h EC50 = 0.8-42 [¥ €] µg/L	Very toxic to algae

[*] The results should be interpreted with care (See Attachment C.2.1. and C.2.2 for more details)
[§] Sorvari and Sillanpaa (1996)
[¥] Markich et al (2002)
[€] Based on population growth

Based on the above ecotoxicological endpoints for analogue chemical 1, the notified chemical is expected to be harmful to daphnia. Therefore, the notified chemical is classified as 'Acute Category 3: Harmful to aquatic life' according to the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009). The notified chemical is expected to transform to other products under the environmental conditions and has low potential for bioaccumulation. Therefore, the notified chemical is not formally classified under the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)* (United Nations, 2009) for chronic toxicities.

Copper species in ionic form may be very toxic to aquatic life. However, copper species are not expected to be present in ionic form in significant amounts in the natural environment therefore, the ANZECC/ARMCANZ (2000) guideline limits for copper in surface waters for ecosystem protection will be considered as more representative for risk characterisation purposes.

No ecotoxicity data was provided to describe toxicity of the notified chemical and copper species to soil- and sediment-dwelling organisms.

7.2.1. Predicted No-Effect Concentration

The predicted no-effect concentration (PNEC) for the notified chemical has been calculated from the most sensitive endpoint for daphnia. An assessment factor of 250 was used given acute endpoints for three trophic levels are available, but one study on daphnia has not been reviewed and is for a shorter duration than the standard study.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
EC50 (Daphnia)	38	mg/L
Assessment Factor	250	
Mitigation Factor	1.00	
PNEC:	152	µg/L

7.3. Environmental Risk Assessment

The application of the notified chemical to fields by ground boom sprayer or drip irrigation has the potential to result in exposure to aquatic organisms in the nearby water bodies. The risk quotient (RQ) for the aquatic compartment for the notified chemical may be estimated for direct exposure scenarios, spray-drift and run-off based on a single application rate.

Estimated RQ for spray-drift and run-off, based on a PNEC of 152 µg/L

	Spray drift	Run-off
PEC (µg/L)	100	212.5
RQ	0.66	1.40

The risk posed to the aquatic environment from spray-drift is not expected to be unreasonable (RQ < 1). Although the risk from run-off indicates a potential risk (RQ > 1), the actual concentration of the notified chemical is expected to be a significant overestimate, as in reality copper will be rapidly converted to copper species that are not bioavailable, or be taken up by plants as a nutrient. Therefore, the RQ from run-off is expected to be below 1 under realistic conditions.

Different environmental factors and characteristics of soils, such as pH, can result in changes to the mobility and bioavailability of copper in soils over time. The contribution of the notified chemical as an anthropogenic source of copper, at the use pattern, is compared to Australian water and soil quality guideline limits. In Australia, the ANZECC/ARMCANZ (2000) WQG guideline limit for copper in surface waters for ecosystem protection is 1.4 µg/L in freshwater at a hardness of 30 mg $CaCO_3$/L. Background copper concentrations in Australian surface waters have been reported as 0.11 µg/L in fresh water (ANZECC/ARMCANZ, 2000). At the proposed application rate the notified chemical may lead to the maximum predicted environmental concentrations in surface waters of 15 µg copper/L per hectare from spray drift, with a 5 m downwind no-spray zone this concentration is reduced to 1.7 copper µg/L. In addition, total copper concentration is not a good predictor of its bioavailability, and copper as the EDTA-CuK_2 complex is less toxic than ionic copper to the most sensitive aquatic species tested.

In Australia, the soil ecological investigation level for copper is 100 mg/kg dry solids (NEPC, 1999). Background copper concentrations in Australian soils range from 2-100 mg/kg (Berkman, 1989). At the proposed application rate and use pattern, assuming no copper uptake by crops the notified chemical may lead to a 3.4 µg/kg increase in copper concentrations in agricultural soils over a 10 year period due to the application of fertilisers. This accounts for 0.003% of the soil ecological investigation level for copper. Therefore, the contribution of the notified chemical as an anthropogenic source of copper is not expected to result in a significant increase to the concentration of copper in soils with respect to Australian environmental trigger values. Similarly the concentration of copper in sediment will be dependent on its fate and behaviour in the whole aquatic system including the overlying water and no significant increase in environmental levels of copper in sediment is expected.

Realistic consideration of concentrations of the notified chemical in the environment is expected to be below the PNECs, for spray drift and run-off. Copper was similarly in concentrations that did not significantly alter background concentrations or were below the ANZECC/ARMCANZ (2000) soil and water quality guidelines (WQG), except in the case of spray-drift. However, this was only marginally above the WQG with a 5 m downwind no-spray zone, with no consideration of reduced toxicity of the EDTA-CuK_2 complex.

Therefore on the basis of the PEC/PNEC ratio, comparison with Australian water and soil quality guideline limits for copper and the assessed use pattern, the notified chemical and its transformation products are not considered to pose an unreasonable risk to the environment provided, good agricultural practices ensure that the wastage and potential contamination of water bodies from overspray, drift or run-off are minimised. For spray-drift it is regarded as good agricultural practice to not apply chemicals when wind speed is less than 3, or more than 20 kilometres per hour, at the application site.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Density 1,725 kg/m^3 at 20 °C

Method OECD TG 109 Density of Liquids and Solids.
Remarks The density was determined using a pycnometer.
Test Facility AkzoNobel (2013a)

Particle Size Inhalable fraction (< 100 μm): 36.7%
Respirable fraction (< 10 μm): 1.7%

Method OECD TG 110 Particle Size Distribution/Fibre Length and Diameter Distributions.

Range (μm)	*Mass (%)*
< 100	36.7
< 10	1.7
< 5	0.9

Remarks Determined using laser light scattering method.
Test Facility AkzoNobel (2013b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical (92.7% purity)
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Wistar Crl:WI (Han)
Vehicle	Water
Remarks - Method	Group 1 animals were exposed to the notified chemical at a concentration of 1,965 mg/kg bw instead of 2,000 mg/kg bw as the correction for purity was not performed accurately.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 F	1,965	3*/3
2	3 F	300	0/3
3	3 F	300	0/3

*- one animal was euthanised on day 3.

LD50	500 mg/kg bw
Signs of Toxicity	At 1965 mg/kg bw two animals were found dead on day 1 and 2 and one animal was euthanised *in extremis* on day 3. Lethargy, abnormal posture, hunched posture, uncoordinated movements, piloerections, diarrhoea, pale appearance, ptosis, hypothermia, and/or green faeces were observed in animals treated at 1965 mg/kg bw between days 1 and 3. At 300 mg/kg bw, hunched posture and/or piloerection was noted between days 1 and 5. Body weight gain observed in rats at 300 mg/kg bw was similar to that expected for rats of same age and strain.
Effects in Organs	Bluish discolouration of the gastro intestinal tract was observed in rats treated at 1965 mg/kg bw and this effect was considered to be due to the colour of the test substance.
Remarks - Results	The LD50 was established to be within the range of 300-1965 mg/kg bw. According to the OECD 423 test guideline, the LD50 is considered to be 500 mg/kg bw.
CONCLUSION	The notified chemical is harmful via the oral route.
TEST FACILITY	WIL Research (2014a)

B.2. Acute toxicity – inhalation

TEST SUBSTANCE	Analogue chemical 1 (92.7% purity)
METHOD	OECD TG 436 Acute Inhalation Toxicity – Acute Toxic Class Method
Species/Strain	Rat/Wistar RccHan
Vehicle	Water
Method of Exposure	Nose-only exposure
Exposure Period	4 hours
Physical Form	Liquid aerosol
Particle Size	Mass median aerodynamic diameter (MMAD): 4.85 to 5.38 µm
Remarks - Method	The MMAD of the test substance (4.85-5.38 µm) exceeded the upper limit of the range recommended in the OECD guideline 436 (MMAD 1-4 µm).

The study authors indicated that during extensive preliminary testing a MMAD meeting the test guidelines could not be achieved at a test concentration of 5 mg/m^3.

RESULTS

Group	*Number and Sex of Animals*	*Concentration (g/m^3)*		*Mortality*
		Nominal	*Actual*	
1	3 per sex	5.0	5.30	1/6

LC50	> 5.30 mg/L/4 hours
Signs of Toxicity	One male died two days after exposure to the test substance. All exposed animals showed shallow breathing, decreased breathing rate and restlessness during exposure. On the day of exposure, all animals showed hunched posture, piloerection and abnormal contraction of the eyelid muscles. Increased respiratory rate was noted between days 2 and 5 in all surviving animals. One male had soft faeces on days 3-5. Reduction in body weight gain was observed in all animals up to day 3 and in one male and one female this was extended to day 7. This was followed by catch-up growth in the second week.
Effects in Organs	Red and poorly collapsed lung and red thymus was observed in the male that died on day 2 of exposure. The other two males showed treatment related bilaterally enlarged kidneys and one of these males also showed pale lung with white patches and a few red spots. All three females showed treatment related pale lung with white patches and a few petechiae (spots caused by bleeding) and one of these females also showed large red patch on left and cranial lobe.
CONCLUSION	The analogue chemical 1 is of low acute toxicity via inhalation.
TEST FACILITY	TNO (2012)

B.3. Irritation – skin (*in vitro*)

TEST SUBSTANCE	Notified chemical (92.7% purity)
METHOD	OECD TG 439 In vitro Skin Irritation: Reconstructed Human *Epidermis* Test Method
Vehicle	Nil. The solid test substance was applied directly on the top of the tissue.
Remarks - Method	Phosphate buffered solution and 5% sodium dodecyl sulphate were used as negative control and positive control, respectively. A pre-test was conducted by adding 19.4 mg of the test substance to 2 mL of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution. Due to hygroscopic nature of the test substance, in the pre-test, the tissue was not completely covered with the test substance therefore this test was repeated (results of this study are in first table). The study authors indicated that as the test was performed in 6-well plates instead of 12-well plates, negative control values were not within the historical control data range (results of this study are in second table), and this study was repeated. Standard deviation of the relative mean viability was not provided.

RESULTS

Test material	*Mean OD_{570} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	1.103	100	Not provided
Test substance	0.831	75	Not provided
Positive control	0.385	35	Not provided

Test material	*Mean OD_{570} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	0.416	100	Not provided
Test substance	0.056	13	Not provided
Positive control	0.086	21	Not provided

Test material	*Mean OD_{570} of triplicate tissues*	*Relative mean Viability (%)*	*SD of relative mean viability*
Negative control	1.058	100	Not provided
Test substance	0.608	58	Not provided
Positive control	0.425	40	Not provided

OD = optical density; SD = standard deviation

Remarks - Results: As the relative tissue viability of the notified chemical was 13% to 75% in the three independent experiments, the results are inconclusive.

CONCLUSION: Based on the results obtained, the skin irritancy potential of the notified chemical cannot be made.

TEST FACILITY: WIL Research (2014b)

B.4. Irritation – skin

TEST SUBSTANCE: Analogue chemical 1 (50% aqueous solution)

METHOD: OECD TG 404 Acute Dermal Irritation/Corrosion.
- Species/Strain: Rabbit/White Vienna
- Number of Animals: 3 (1 M and 2 F)
- Vehicle: Distilled water
- Observation Period: 72 h
- Type of Dressing: Not stated.
- Remarks - Method: No protocol deviations.

Remarks - Results: No erythema or oedema was observed in all treated animals at any observation point.

CONCLUSION: The analogue chemical 1 is non-irritating to the skin at 50% concentration.

TEST FACILITY: BASF (1985a)

B.5. Irritation – eye (*in vitro*)

TEST SUBSTANCE: Notified chemical (92.7% purity)

METHOD: OECD TG 437 Bovine Corneal Opacity and Permeability Test Method for Identifying i) Chemicals Inducing Serious Eye Damage and ii) Chemicals Not Requiring Classification for Eye Irritation or Serious Eye Damage
- Vehicle: Water
- Remarks - Method: Physiological saline solution and 20% imidazole were used as negative control and positive control respectively.
  No protocol deviations.

RESULTS

Test material	*Mean opacities of triplicate tissues*	*Mean permeabilities of triplicate tissues*	*IVIS*
Vehicle control	0	0.000	0.0
*Test substance**	4	-0.001	4.0
*Positive control**	92	2.986	137

SD = Standard deviation; IVIS = in vitro irritancy score
*Corrected for background values

Test material	*Mean opacities of triplicate tissues*	*Mean permeabilities of triplicate tissues*	*IVIS*
Vehicle control	0	0.000	0.0
*Test substance**	3	0.007	3.1
*Positive control**	114	2.384	150

SD = Standard deviation; IVIS = in vitro irritancy score
*Corrected for background values

Remarks - Results

Since one out of three corneas showed an IVIS below 3, the test was repeated.

The test substance showed IVIS scores of 6, 0 and 4.9 in the first experiment, resulting in a mean IVIS score of 4.0.

In the second experiment, the test substance showed IVIS scores of 4.1, -0.1 and 5.3, resulting in a mean IVIS score of 3.1.

The negative and positive controls gave satisfactory results confirming the validity of the test system.

In two independent experiments, the test substance induced an IVIS of 4.0 and 3.1 and these values are between > 3 and ≤ 55 therefore no prediction on the classification can be made.

CONCLUSION

Based on the results obtained, the eye irritancy potential of the notified chemical cannot be made.

TEST FACILITY

WIL Research (2014c)

B.6. Irritation – eye

TEST SUBSTANCE

Analogue chemical 1 (50% aqueous solution)

METHOD

OECD TG 405 Acute Eye Irritation/Corrosion.

Species/Strain: Rabbit/White Vienna
Number of Animals: 3 (2 M and 1 F)
Observation Period: 8 days
Remarks - Method: No significant protocol deviations.

RESULTS

Lesion	*Mean Score** *Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva: redness	1.7	1.3	2.0	2.0	8 days	0
Conjunctiva: chemosis	0.3	1.0	1.3	2.0	< 8 days	1.0
Conjunctiva: discharge	-	-	-	-	-	-
Corneal opacity	1.0	0.7	1.0	1.0	8 days	1.0
Iridial inflammation	0.0	0.0	0.7	1	< 8 days	0

* Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.

Remarks - Results	The analogue chemical produced a mean corneal opacity score of 1 in two animals therefore the analogue chemical is considered to be an eye irritant.
CONCLUSION	The analogue chemical 1 is irritating to the eye at 50% concentration.
TEST FACILITY	BASF (1985b)

B.7. Skin sensitisation – mouse local lymph node assay (LLNA)

Test Substance	Analogue chemical 1 (92.5% purity)
Method	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain	Mouse/CBA/J
Vehicle	Water with 1% pluronic L92
Preliminary study	Yes
Positive control	α-Hexylcinnamaldehyde (not conducted in parallel)
Remarks - Method	A preliminary study was conducted using 25% and 50% of the test substance. Very slight erythema was observed on one animal at a concentration of 50%. Variation in ear thickness during the observation period was less than 25% from day 1. Based on these results, the highest concentration selected for the main study was 50%.

Results

Concentration (% w/w)	*Number and sex of animals*	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance			
0 (vehicle control)	5 F	513	1.0
10	5 F	466	0.9
25	5 F	571	1.1
50	5 F	612	1.2
Positive Control			
0 (vehicle control)*	5 F	300	1.0
5	5 F	518	1.7
10	5 F	502	1.7
25	5 F	1423	4.7

*Acetone/Olive oil (4:1)

Remarks - Results	White/grey test substance residues were present in the dorsal surface of the ears on both animals on days 1-5 in the preliminary study and in all animals on days 1-3 and one animal on day 4 in the main study. The study authors asserted that this effect did not affect scoring of the skin reactions.
Conclusion	There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the test substance.
Test Facility	WIL Research (2013)

B.8. Repeat dose toxicity–with reproductive/developmental toxicity screening

Test Substance	Analogue chemical 1 (92.7% purity)
Method	OECD TG 422 Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test.
Species/Strain	Rats/Wistar
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: Males: 90 days (10 weeks premating, during mating and up to the day of scheduled sacrifice) Females: 10 weeks premating, during mating and gestation, and up to day 4

	of lactation
Vehicle	Tap water
Remarks - Method	No significant protocol deviations

Results

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
control	12/sex	0	0/24
low dose	12/sex	150	0/24
mid dose	12/sex	500	3M/24
high dose	12/sex	1,500 (until day 8 of treatment) and 1050 (from day 9 of treatment)*	24/24

* due to mortalities on day 9 of treatment, the dose level was reduced to 1050 mg/kg bw/day

Mortality and Time to Death
Three males from mid-dose group were euthanised *in extremis* during or at the end of mating period (one animal on day 75 and two animals on day 77).

In the high-dose group, two females died on day 4 and 6 and a female was euthanised *in extremis* on day 7 of treatment and these animals were replaced with reserve animals. All animals were either euthanised *in extremis* or found dead before the start of the mating period (from day 11 to day 65).

Clinical Observations
During pre-mating period, high-dose group animals and to a lesser extent mid-dose group animals showed thin appearance, hunched posture, piloerection, abnormal contraction of the eyelid muscles, swollen abdomen, soft faeces and green watery discharge. Further, during gestation, mid-dose group animals showed swollen abdomen and soft-faeces.

Statistically significant reduction in mean body weight was observed in high-dose group males on day 14 onwards and in high-dose females on days 21 and 42. On day 1 and day 4 of lactation, statistically significant increase in mean body weight was observed in low and mid-dose group animals.

During first week of the premating period, statistically significant reduction in food consumption was observed in both sexes in high-dose group and males in the mid-dose group. Food consumption remained relatively low in high-dose group males during pre-mating period.

Laboratory Findings – Clinical Chemistry, Haematology
Nine out of 12 females and all males in the high-dose group had either died or were euthanised *in extremis* on the blood collection day (day 65 for females and day 85 for males). Remaining three females in high-dose group were euthanised *in extremis* on day 65. The study authors therefore indicated that the results obtained in females in the high-dose group may be of limited value.

Clinical chemistry
Statistically significant increase in alkaline phosphate activity, aspartate aminotransferase activity, alanine aminotransferase activity and gamma glutamyl transferase activity were observed in mid-dose group males. High-dose group females also showed increased aspartate aminotransferase activity. Alkaline phosphate activity and alanine aminotransferase activity, however, was decreased in mid- and high-dose groups and mid-dosed group females, respectively. Statistically significant increase in bilirubin, creatinine, urea, inorganic phosphate and calcium was observed in the mid-dose group males and in the high-dose group females.

Mid-dose group females and males showed statistically significant increase in potassium levels and sodium levels, respectively. Statistically significant reduction in chloride was observed in the high-dose group females. Statistically significant reduction in albumin was observed in high-dose group females.

Alanine aminotransferase activity was decreased in low-dosed group females. At low dose, statistically significant reductions of potassium level in males and sodium level in females were reported.

Haematology
High-dose group females showed statistically significant increased reticulocytes and thrombocytes. The mid-dose group animals showed statistically significant reduction in prothrombin and males showed statistically

significant increase in red blood cell count and reduction in mean corpuscular volume and mean corpuscular haemoglobin. Mid-dose group males and high-dose group females showed increased total white blood cell counts and absolute neutrophils and monocytes count.

Effects in Organs
Males were euthanised on day 90 and females on day 4 of lactation. During this time both sexes in high-dose and 3 out of 12 males in mid-dose groups had either died or was euthanised *in extremis.*
The following statistically significant changes were observed:

- Both sexes exposed to mid-dose of the test substance showed decrease in absolute and relative heart weight
- Relative weight of kidneys was increased in mid-dose group males, however, females in this group showed increased absolute kidney weights.
- Mid-dose group females showed absolute and relative weight increases in spleen and absolute and relative weights decreases in ovaries.
- Mid-dose group males showed decreased absolute thymus weight and females in this group showed increased adrenal weights.

Macroscopic examination in mid and high-dose group animals showed treatment related enlarged intestines with green/watery contents, pale and/or green appearance of the liver and kidneys, small epididymides and seminal vesicles, enlarged dark spleen, small thymus and a variety of changes in the stomach. A small thymus was considered secondary to poor health conditions.

Microscopic examination showed the following treatment related findings in the mid-dose group:

- Tubular necrosis and degeneration, tubular epithelial cell karyomegaly, and accumulation of brown pigment in kidneys.
- Hepatocellular karyomegaly, brown pigment accumulation, bile duct hyperplasia, periportal macrophages (especially in males) and multifocal infiltration of mononuclear inflammatory cells in liver.
- Accumulation of brown pigment and macrophages in the white pulp of the spleen.

In the low dose group, 6/10 males showed very slight tubular epithelial brown pigment in the kidneys, and 6/10 males and 3/10 females showed mononuclear cell infiltrate in the liver. Statistically significant reductions in the absolute and relative weights of the epididymides were also observed in males of the low dose group but this finding was not confirmed as a significant change in the mid-dose group.

Reproductive and developmental effects
The male fertility parameters (epididymal sperm motility, sperm count and sperm morphology, and testicular sperm count and daily sperm production) were not affected by the test substance at all doses tested. Two mid-dose group males failed to become fertile and were euthanised *in extremis* and the study authors indicated that their conditional decline may have prevented successful copulation.

Mid-dose group pups showed statistically significant increase in skeletal anomalies. The study authors stated that this finding was due to three pups of the same litter showing two or more wavy ribs and asserted that this effect was not treatment related.

The incidence of incompletely ossified frontal skull in the low dose group was slight, though statistically significant. Given this finding was not confirmed at the mid dose level, the effect was not considered treatment related by the study authors.

Remarks – Results
Given only limited histopathological effects were observed in the liver and kidneys in the low-dose group, a No-Observed Effect Level (NOEL) for parental toxicity close to 150 mg/kg bw/day was speculated by the study authors.

Conclusion
The No Observed Effect Level (NOEL) for parental toxicity was established by the study authors as ~150 mg/kg bw/day, based on toxicity findings observed in the kidneys and liver at 500 mg/kg bw/day.

The NOEL for reproductive and developmental toxicity was established as ≥ 500 mg/kg bw/day in this study, based on the absence of treatment related effects on fertility parameters, reproductive performance and development.

Test Facility TNO (2013a)

B.9. Genotoxicity – bacteria

TEST SUBSTANCE Analogue chemical 1 (47.2% aqueous solution)

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
Plate incorporation (Test 1) and pre-incubation procedure (Test 2)
Species/Strain *Salmonella typhimurium*: TA1535, TA1537, TA100 and TA98
Metabolic Activation System S9 mix from Aroclor1254-induced rat liver
Concentration Range in Main Test a) With metabolic activation: 100 – 10,000 µg/plate
b) Without metabolic activation: 100 – 10,000 µg/plate
Vehicle Distilled water
Remarks - Method Negative control: distilled water
Positive control:
with S9-mix: 2-aminoanthracene (TA100, TA98, TA1537 and TA1535)
without S9-mix: *N*-methyl-N'-nitro-N-nitrosoguanidine (TA100 and TA1535); 4-nitro-phenylendiamine (TA98); and 9-aminoacridine chloride monohydrate (TA1537).

Preliminary toxicity test was not conducted.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:* *Cytotoxicity in Preliminary Test*	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	-	> 10,000	> 10,000	Negative
Test 2	-	> 10,000	> 10,000	Negative
Present				
Test 1	-	> 10,000	> 10,000	Negative
Test 2	-	> 10,000	> 10,000	Negative

Remarks - Results In Test 1, due to high spontaneous rate of revertants (> 50%) no evaluation was performed for TA98 strain.

No biologically relevant increases in revertant colony numbers of any of the tester strains were observed during the test in either the presence or absence of metabolic activation.

The positive controls induced a distinct increase of revertant colonies during the study indicating the validity of the test system.

CONCLUSION The test substance was not mutagenic to bacteria under the conditions of the test.

TEST FACILITY BASF (1992)

B.10. Genotoxicity - In vitro mammalian cell micronucleus test

TEST SUBSTANCE Analogue chemical 1 (92.7% purity)

METHOD	OECD TG 487 *In Vitro* Mammalian Cell Micronucleus Test.
Species/Strain	Human
Cell Type/Cell Line	Lymphocytes
Metabolic Activation System	S9 mix from Aroclor 1254-induced rat liver
Vehicle	Culture medium
Remarks - Method	Negative control: culture medium Positive control (clastogen): Without S9: mitomycin C With S9: cyclophosphamide Aneugenic positive control: vinblastine sulphate Preliminary cytotoxicity test was not conducted.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	7.8, 15.6, 31.3, 62.5, 125, 250, 500, 1000*, 2000*, 3977*	4 h	24 h
Test 2	62.5*, 125*, 250*, 500, 750, 1000, 1500, 2000, 2500, 3000, 3977	20 h	48 h
Present			
Test 1	7.8, 15.6, 31.3, 62.5, 125, 250, 500*, 1000, 2000*, 3977*	4 h	24 h

*Cultures selected for micronuclei 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	-	≥ 3977	> 3977	Negative
Test 2	-	≥ 125	> 3977	Positive
Present				
Test 1	-	≥ 2000	> 3977	Negative

Remarks - Results

In Test 1 (without S9), 41% cytotoxicity was observed in cells treated at 3,977 µg/mL.

In Test 1 (with S9), 45%, 35% and 18% cytotoxicity was observed at 3,977, 2,000 and 1,000 µg/mL, respectively. At lower concentrations cytotoxicity fluctuated between 6% and 13%.

In Test 1 (with or without S9) the test substance did not show a statistically significant increase in the number of binucleated cells containing micronuclei, at any of the concentrations analysed.

In Test 2 (without S9), only dead cells were observed at the four high concentrations (1,500, 2,500, 3,000 and 3,977 µg/mL). Some binucleated cells were detected at 750 and 1,000 µg/mL. At 500, 250 and 125 µg/mL cytotoxicity was 77%, 53% and 35%, respectively. At 62.5 µg/ml concentration, the test substance was not cytotoxic to the cells when compared to the concurrent negative control.

In Test 2 (without S9) a dose dependent statistically significant increase in the number of binucleated cells containing micronuclei was observed at 250, 125 and 62.5 µg/mL. However the percentage of binucleated cells containing micronuclei at these concentrations were reported to be only slightly higher than the historical control range of the test facility.

The proportion of the large and small micronuclei induced by the test substance was reported as not statistically different from the response of the aneugen vinblastine sulphate. The observed similar proportions of large

	and small micronuclei are considered to be an indication for aneugenic effects of the test substance at ≥ 62.5 µg/mL.
CONCLUSION	Analogue chemical 1 was aneugenic to human lymphocytes treated *in vitro* at ≥ 62.5 µg/mL, under the conditions of the test.
TEST FACILITY	TNO (2013b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE: Analogue chemical 2

METHOD
- Inoculum: Lake, Ditch and River Water
- Exposure Period: 49 days
- Auxiliary Solvent: None
- Analytical Monitoring: Theoretical Oxygen Demand ($ThOD_{NH3}$)
- Remarks - Method: The Closed Bottle tests are performed according to modified OECD Test Guidelines. To assess the potential of natural ecosystem biodegradation the test substance was added at a concentration of 8 mg/L into biological oxygen demand (BOD) bottles filled with water obtained from 3 different aquatic systems in the Netherlands, the shallow freshwater lake Ketelmeer, the river IJssel near Arnhem and a ditch near Zevenaar. Biodegradation was measured by following the course of the oxygen decrease. Tests were run over 49 days at both pH 6.5 and 8.0, adjusted with 1 N HCl.
Inhibition of the endogenous respiration of the inoculum by the test substance was not detected, therefore inhibition of biodegradation due to initial high concentration of the test substance is not expected.
River, lake and ditch water biodegradation tests, without test substance added, were run in parallel to the test substance aqueous inoculum biodegradation tests. The actual concentration of the test solution was not determined. Deviations from the closed bottle test procedure were: the complete filling of the bottles with river, lake and ditch water, respectively, instead of dilution of the inoculum into a mineral salts medium with activated sludge; the adjustment of pH to 6.5 and 8.0 instead of 7.0: the extended test time to 49 days.

RESULTS

EDTA- $CaNa_2$ in River Water			*EDTA- $CaNa_2$ in Lake Water*			*EDTA- $CaNa_2$ in Ditch Water*		
Day	*% Degradation*		*Day*	*% Degradation*		*Day*	*% Degradation*	
	pH 6.5	pH 8.0		pH 6.5	pH 8.0		pH 6.5	pH 8.0
0	0	0	0	0	0	0	0	0
7	0	0	7	2	0	7	4	0
14	0	9	14	0	0	14	4	4
21	2	47	21	2	8	21	4	8
28	12	72	28	2	53	28	6	62
35	47	75	35	17	79	35	11	89
42	83	-	42	45	-	42	70	-
49	-	-	49	60	-	49	81	-

- Remarks - Results: The validity of the test was demonstrated by high endogenous respiration and oxygen concentrations > 0.2 mg/L in all bottles during the test period. Biodegradation of > 60% was found within 28 days in alkaline (pH 8.0) river and ditch water inoculum tests and at 35 days in the lake water inoculum test. There was a longer lag phase to the onset of biodegradation in the pH 6.5 tests which had a > 60% degradation after 42 days in the river and ditch water inoculum tests and at day 49 for the lake water. On the basis of these the prolonged closed bottle tests, the test substance is considered to be somewhat biodegradable at pH 8.0 and pH 6.5 in the natural waters tested, but does not meet the 10-day window test criteria for ready biodegradability under the OCDE 301 D guideline.

CONCLUSION: The analogue chemical 2 is not readily biodegradable in natural waters.

TEST FACILITY	AkzoNobel (1999)

C.2. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Versene AG 7.5% copper (analogue chemical 1- active ingredient 45.5%)
METHOD	US EPA-660/3-75-009 Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians (1975) - static
Species	*Lepomis macrochirus* (bluegill)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	103 mg $CaCO_3$/L
Analytical Monitoring	Not determined
Remarks – Method	The test method adhered to the guidelines of the US EPA-660/3-75-009 'Methods for Acute Toxicity Tests with Fish, Macroinvertebrates and Amphibians' of the (Committee on Methods for Toxicity with Aquatic Organisms 1975). Test fish were acclimated for 10 days in the Lake Huron water used in the tests. The tests were run at 22ºC in a 16 hour light / 8 hour dark cycle. Between 5 and 10 different concentrations of the test substance are reported as tested but the actual concentrations are not given. No analysis of test substance concentrations was done. Mean test substance pH was 7.8. Ten fish were exposed to each test solution concentration for 96 hours. Fish were not fed for the 3 days prior and during the exposures.
RESULTS	
LC50	555 (95% CI: 487-640) mg/L at 96 hours.
NOEC	320 mg/L at 96 hours.
Remarks – Results	As this testing was undertaken prior to the advent of GLP standards these are not relevant. Some shortcomings in the test reporting are the lack of information on test substance concentrations used, no analysis of test substance concentrations and no reporting of controls. The results of the testing should, therefore, be interpreted with care. The reported LC_{50} and NOEL for the test substance Versene AG 7.5% copper were both >100 mg/L.
CONCLUSION	The analogue chemical 1 is not expected to be harmful to fish.
TEST FACILITY	Environmental Sciences Research Laboratory (Batchelder et al., 1980)

C.2.2. Algal growth inhibition test

TEST SUBSTANCE	Analogue chemical 1
METHOD	OECD TG 201 Alga, Growth Inhibition Test
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 0, 1, 10, 100, 1000 mg/L Actual: Not determined
Auxiliary Solvent	None
Water Hardness	Not determined
Analytical Monitoring	None
Remarks - Method	This was an initial screening test not a full study. GLP is not claimed for this test as a quality control inspection for the test was not done. Tests were conducted to an approved study plan in a GLP accredited laboratory

following the OECD guidelines. No chemical analysis was undertaken.

RESULTS

Biomass		*Growth*	
E_bC50 (mg/L at 72 h)	*NOEC (mg/L)*	*ErC50 (mg/L at 72 h)*	*NOEC (mg/L)*
ND	ND	>1000	10

Remarks - Results

The study met the validity criteria of average increase in absorbance in the control, which was a factor of 168 over 72 h and the EC_{50} values of the reference compound, potassium dichromate, were in the range of 0.25 - 2.0 mg/L. As this was a screening study the quality criteria of control growth rates could not be measured and therefore, this criterion was not applicable. Light variation in the incubator, caused by a high number of testing vessels, resulted in higher growth variation in controls than recommended in the OECD 201 guideline. The calculations are based on screening test results and should be interpreted with care. Definitive tests are required to determine these endpoints accurately. Calculated on the basis of the nominal test substance concentrations the ErC_{10} was 70.4 mg/L, the ErC_{50} was >1000 mg/L and the NOEC was 10.

CONCLUSION

The analogue chemical 1 is not expected to be harmful to algae.

TEST FACILITY

AkzoNobel (2009)

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