File No: STD/1598

February 2019

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Zincate(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	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1598	Yara Australia Pty Ltd and Akzo Nobel Pty Ltd	Zincate(2-), [[*N*,*N*′-1,2-ethanediylbis[*N*-[(carboxy-κ*O*)methyl]glycinato-κ*N*,κ*O*]](4-)]-, potassium (1:2), (*OC*-6-21)-	ND*	≤ 10 tonnes per annum	Component of micronutrient fertiliser

*ND = not determined

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available information, the notified chemical is not recommended for classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals* (GHS), as adopted for industrial chemicals in Australia.

Human health risk assessment
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.

Environmental risk assessment
On the basis of the PEC/PNEC ratio, comparison with Australian water and soil quality guideline limits for zinc 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

CONTROL MEASURES

Occupational Health and Safety

- No specific engineering controls, work practices or personal protective equipment are required for the safe use of the notified chemical itself. However, these should be selected on the basis of all ingredients in the formulation.

  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.

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 (more than 1 tonne per year).

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 endpoints.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

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

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Dissolvine CXK (product containing the notified chemical at < 30% concentration)
Rexolin CXK (product containing the notified chemical at < 30% concentration)

CAS NUMBER
14689-29-3

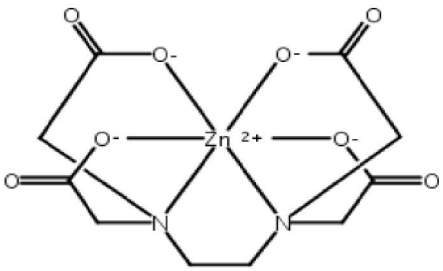
CHEMICAL NAME
Zincate(2-), [[*N,N*′-1,2-ethanediylbis[*N*-[(carboxy-κ*O*)methyl]glycinato-κ*N*,κ*O*]](4-)]-, potassium (1:2), (*OC*-6-21)-

OTHER NAMES
Potassium [(ethylenedinitrilo)tetraacetato]zincate (7CI); Zincate(2-), [[*N,N*′-1,2-ethanediylbis[*N*-(carboxymethyl)glycinato]](4-)-*N,N′,O,O′*,O^{N},$O^{N'}$]-, dipotassium, (*OC*-6-21)-

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

MOLECULAR FORMULA
$C_{10}H_{12}N_2O_8Zn.2K$

STRUCTURAL FORMULA



$2K^+$

MOLECULAR WEIGHT
431.8 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: White microgranules

Property	Value	Data Source/Justification
Melting Point/Freezing Point	Decomposes without melting at 245 °C	Information provided by notifier. No study details
Boiling Point	Decomposes without boiling at 245 °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 structure
Water Solubility	746 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	pKa_1 =11.24; pKa_2= 6.04; pKa_3 =3.74; pKa_4 =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 based on analogue chemical
Autoignition Temperature	277 °C	Information provided by notifier. No study details
Explosive Properties	Not determined	Contain no functional groups that would imply explosive properties
Oxidising Properties	Not determined	Contain no functional groups that would imply oxidising properties

* For the product Dissolvine CXK containing the notified chemical at < 30% 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 fertiliser mixture in powder form at < 30% 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	2-10	2-10	2-10	2-10	2-10

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

IDENTITY OF MANUFACTURER/RECIPIENTS
Yara Australia Pty Ltd
AkzoNobel Pty Ltd

TRANSPORTATION AND PACKAGING
Products containing the notified chemical at < 30% 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 Zn 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 ≤ 0.3 g/L of the 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 ≤ 600 g of the notified chemical) of the product/hectare.

Soil application
Products containing the notified chemical will be applied at a rate of ≤ 15 kg (equivalent to ≤ 4.5 kg of the 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 < 30% 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 < 0.03% 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 farmworkers	1 - 3	1 - 4

EXPOSURE DETAILS

Transport and storage

Transport and storage workers may come into contact with the notified chemical at < 30% 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 < 30% 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 < 0.03% 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 exposures 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 grown in glasshouses, including various vegetables, up to four times, including during fruit formation and maturation, per crop season. 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 analogue chemicals 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 > 1,970 mg/kg bw; low toxicity
Rat, acute inhalation toxicity	Analogue chemical 1	LC50 > 5.16 mg/L; low toxicity
Skin irritation (*in vitro*)	Notified chemical	non-irritating
Eye irritation (*in vitro*)	Notified chemical	non-irritating
Mouse, skin sensitisation – Local lymph node assay	Analogue chemical 2	no evidence of sensitisation
Mutagenicity – bacterial reverse mutation	Analogue 2	non mutagenic
Genotoxicity – *in vitro* mammalian cell gene mutation test	Analogue 1	non genotoxic
Genotoxicity – *in vitro* mammalian micronucleus test	Analogue 1	non genotoxic
Genotoxicity – *in vitro* mammalian micronucleus test	Analogue 2	genotoxic
Mutagenicity – bacterial reverse mutation	Analogue 2	non mutagenic

The analogue chemicals 1 (EDTA-$MnNa_2$; CAS No. 15375-84-5) and 2 (EDTA-$CuNa_2$; CAS No. 14025-15-1) are metal complexes of ethylenediaminetetraacetic acid (EDTA) similar to the notified chemical.

Toxicokinetics, metabolism and distribution

No toxicokinetic data on the notified chemical were submitted.

The notified chemical is a highly water soluble (746 g/L) zinc compound. Upon dissolution, a negatively charged Zn-EDTA complex and 2 positively charged potassium (I) ions will exist. The EDTA moiety surrounds and protects the Zn (II) ion and modifies its reactivity. The Zn-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 Zn-EDTA complex from the gut is expected to be limited (<5%). The notifier stated that the amount of Zn (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.

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).

Acute toxicity

The notified chemical was found to be of low acute oral toxicity in rats.

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 2 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

The notified chemical was found to be non-irritating to the skin and eyes based on the results from *in vitro* studies.

No studies were submitted for skin sensitisation for the notified chemical. Analogue chemical 2 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
No data were submitted for the notified chemical.

The toxicological profile of EDTA salts is related to their ability to associate and dissociate metal ions, in particular the removal of essential trace metals from the body. The metal ions present in the notified chemical are important for the functioning of human biological systems and there are no known maximum tolerable daily intakes for these metal ions. Similar EDTA salts to the notified chemical are used in cosmetics and as food additives (ECCC/HC, 2017). An acceptable daily intake (ADI) of 2.5 mg/kg for EDTA-$CaNa_2$ was established by the FAO/WHO (1974). Therefore based on the available information for similar EDTA salts and low potential for absorption, the notified chemical is expected to be of low repeated dose toxicity.

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

Analogue chemical 1 tested negative in an *in vitro* micronucleus test in human lymphocytes and in an *in vitro* cell gene mutation test in mouse lymphoma cells. Analogue chemical 2 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.

Based on the weight of evidence, the notified chemical is not expected to be genotoxic.

Health hazard classification
Based on the available information, the notified chemical is not recommended for classification 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
Based on the available information, the notified chemical is expected to be of low hazard.

Farmers and farmworkers may be exposed to the notified chemical at < 30% concentration as a powder. The principal routes of exposure are inhalation and dermal. The notifier has stated that workers will use appropriate PPE, including impervious gloves, coveralls, safety glasses and dust masks, to minimise exposure. Moreover, good hygiene practices are expected to be in place.

It is expected that the spray applications will be low energy/low pressure therefore inhalation exposure to vapours, mists or aerosols during spraying is not likely to occur. Furthermore, the concentration of the notified chemical in spray solution is low (< 0.03%).

Overall under the occupational settings described and expected low hazard 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. Exposure to bystanders 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 to 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, public exposure to the notified chemical and its residues may occur via ingestion of sprayed fresh produce if consumed unwashed.

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.

Members of the public may consume various fruits and vegetables containing residues of the notified chemical. However, exposure to the notified chemical at significant levels is not expected due to:

- low concentration (< 0.03%) 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.

Therefore based on the expected low hazard of the notified chemical and low exposure potential, the risk to public health is not considered to be unreasonable.

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 application or a soil application. For foliar application, it will be applied at a rate of ≤ 1 g of product/L of water (equivalent to ≤ 0.3 g/L of the notified chemical) or ≤ 2 kg (equivalent to ≤ 600 g notified chemical) of product/hectare. For soil application, it will be applied at a rate of ≤ 15 kg (equivalent to ≤ 4.5 kg notified chemical) 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 the 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 3; 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-ZnK_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 the notified chemical complex was reported to be 2 days at pH of 7.85 and 5 to > 30 days at pH of 5.7 to 7.3 in soil systems (Norvell and Lindsay, 1969). Zinc may readily partition to

the solid phase through sorption onto hydrous iron and manganese oxides, clay minerals and organic matter. Generally, zinc sorbs strongly onto soil particulates and may be rapidly converted to the organically-bonded or inorganic precipitates. The distribution of zinc between solution and solid phases is related to the soil pH, redox conditions, salinity, nature and concentration of complexing agents, and cation exchange capacity. Soil conditions not suitable for zinc sorption may lead to leaching, for example, low pH, low organic carbon and high ionic strength of the leaching solution may favour desorption. Studies on zinc speciation in soils affected by industrial and agricultural activities indicated that a significant proportion of zinc was associated with the recalcitrant fraction (ATSDR, 2005). However, some zinc was found in exchangeable and carbonate fractions indicating that it may be bioavailable. Generally, zinc bioavailability is affected by biotic and abiotic factors including organism age and size, water hardness, pH, dissolved organic carbon and temperature (WHO, 2001).

The microelements contained in liquid fertilisers such as zinc are essential elements necessary for plant metabolism. Therefore, the notified chemical in the form of bioavailable zinc species is expected to be taken up by plants and crops in nutrient deficient soils. Factors affecting the availability of zinc 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 form different zinc species which may have variable solubility and bioavailability in complex soil, aquatic and sediment systems. However, efficient and economic use of fertilisers, in addition to good farming practices, is expected to minimise loss of the notified chemical to the aquatic environment.

Zinc is an essential element and aquatic organisms are known to bioconcentrate zinc from water. While most aquatic organisms internally regulate zinc concentrations, elevated levels of zinc in water can overwhelm homeostasis mechanisms leading to toxicity. The concentration at which zinc is homoeostatically regulated is species-specific and the external zinc 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 zinc to meet physiological requirements can be mistaken for trophic transfer, however, zinc is not biomagnified (WHO, 2001).

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 ≤ 2 kg of product (equivalent to ≤ 600 g notified chemical)/hectare. For soil application, it will be applied at a rate of ≤ 15 kg of product (equivalent to ≤ 4.5 kg 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 iron or calcium depending on the pH, the predicted environmental concentrations were calculated based on the application rates of the notified chemical and total zinc.

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 [4.5 kg notified chemical/ha×4times]$_{soil}$+[4 times×0.6 kg notified chemical/ha]$_{foliar}$=20.4 kg of the notified chemical/hectare or 20.4 kg/ha×[65.38/431.80] = 3.1 kg of zinc/hectare results in a worst case PEC_{soil} [notified chemical] = 13.6 µg/kg and PEC_{soil} [$zinc_{Total}$] = 2.1 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 zinc in soil is considered well within natural variability of this element in Australian rural surface soil (< 2 and 200 mg/kg; Naidu et al., 1996). Background zinc concentrations in Australian soils range 1-263 mg/kg, with a calculated median zinc concentration in soil of 39 mg/kg.

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 5,100 µg/L {[(20.4 kg/hectare) × 0.05] ÷ 200 m^3}. However, a more realistic scenario would be from a single application (4.5 + 0.6 kg/ha) 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 1275 µg/L.

Zinc speciation will change in the soil system and is not expected to significantly contribute to the background zinc concentrations in water and sediment from the run-off route, given zinc 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 zinc 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 20.4 kg of the notified chemical/hectare $PEC_{spray\ drift}$ for a water body at 0 m, 1 m and 5 m are 2,480 µg/L, 920 µg/L, and 280 µg/L, respectively. With the worst-case application rate of 20.4 kg of the notified chemical/hectare $PEC_{spray\ drift}$ for a water body at 0 m, 1 m and 5 m are 2,500 µg/L, 920 µg/L, and 280 µg/L, respectively. With an application rate of 3.1 kg zinc /hectare the $PEC_{spray\ drift}$ for a water body at 0 m, 1 m and 5 m are 380 µg zinc /L, 140 µg zinc /L, and 43 µg zinc /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 zinc is an overestimate of the aquatic exposure.

A more realistic scenario, therefore, would be from a single application, as the long-term concentration of zinc ions is expected to be determined by the environmental conditions. The more realistic concentration of zinc after a single application (4.5+0.6kg notified chemical/ha×[65.38/431.80] ÷ 1500 m^3) for a water body at 0 m, 1 m and 5 m are 94 µg zinc /L, 35 µg zinc /L, and 11 µg zinc /L. Similarly the concentration of the notified chemical at 0 m, 1 m and 5 m would be 620 µg/L, 230 µg/L, and 70 µg/L, respectively.

7.2. Environmental Effects Assessment

The results from eco-toxicological investigations conducted on an analogue (analogue chemical 3; EDTA-$ZnNa_2$) are summarised in the table below. Details of these studies can be found in Appendix C. In some cases, additional eco-toxicological endpoints have been sourced from the published literature to supplement the submitted information. A small proportion of released zinc may be bioavailable to aquatic organisms and the toxicity of the notified chemical may be attributed to the soluble form of zinc (Zn (II)). Both released zinc and un-complexed EDTA show higher toxicity to aquatic life than Zn-EDTA complex (Sorvari and Sillanpää, 1996). However, direct effects caused by the intrinsic toxicity of EDTA is 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 complexes are not expected to be harmful to aquatic life based on the information submitted by the notifier.

Endpoint	*Result*	*Assessment Conclusion*
Analogue chemical 3		
Acute toxicity		
Fish Toxicity	Very Soft Water: 96 h LC50 = 940* mg/L Medium Hard Water: 96 h LC50 = 685* mg/L Very Hard Water: 96 h LC50 = 513* mg/L	Not harmful to fish
Daphnia Toxicity	24 h EC50 = 910[§] mg/L	Not harmful to aquatic invertebrates
Algal Toxicity	72 EC50 > 1000* mg/L	Not harmful to algae
Zn (II)		
Acute toxicity		
Fish Toxicity	96 h LC50 =0.14-38[¥] mg/L	Very Toxic or toxic or harmful to fish
Daphnia Toxicity	24 h EC50 = 5.5[§] mg/L	Toxic to aquatic invertebrates
Water Flea Toxicity	48 h EC50 =0.01-0.47[¥] mg/L	Very toxic to aquatic invertebrates
Algae Toxicity	48-72 h EC50 =0.027-3.2[¥] mg/L	Very toxic or 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 the above ecotoxicological endpoints for analogue chemical 3, the notified chemical is not expected to be harmful to aquatic life. Therefore, the notified chemical is not formally classified under the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)* (United Nations, 2009) for acute toxicity. 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.
Zinc species in ionic form may be very toxic to aquatic life. However, zinc 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 zinc 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 zinc species to soil- and sediment-dwelling organisms.

7.2.1. Predicted No-Effect Concentration (PNEC)

The predicted no-effect concentration (PNEC) for the notified chemical has been calculated from the most sensitive endpoint for fish. 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		
LC50 (Fish)	513	mg/L
Assessment Factor	250	
Mitigation Factor	1.00	
PNEC:	2,052	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.

Estimated RQ for spray-drift and run-off, based on a PNEC of 2,052 µg/L

	Spray drift	Run-off
PEC (µg/L)	620	1275
RQ	0.30	0.62

The risk posed to the aquatic environment from spray-drift and run-off is not expected to be unreasonable (RQ < 1). In addition, the actual concentration of the notified chemical is expected to be a significant overestimate, as in reality zinc will be rapidly converted to zinc species that are not bioavailable, or be taken up by plants as a nutrient.

Different environmental factors and characteristics of soils, such as pH, can result in changes to the mobility and bioavailability of zinc in soils over time. The contribution of the notified chemical as an anthropogenic source of zinc, at the proposed import volume and use pattern, is compared to Australian water and soil quality guideline limits. In Australia, the ANZECC/ARMCANZ (2000) WQG guideline limit for zinc in surface waters for ecosystem protection is 8 µg/L in freshwater at a hardness of 30 mg $CaCO_3$/L. Background zinc concentrations in Australian surface waters have been reported as 0.9 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 94 µg zinc /L per hectare from spray drift, with a 5 m downwind no-spray zone this concentration is reduced to 11 µg zinc /L. However, total zinc concentration is not a good predictor of its bioavailability, and zinc as the EDTA-ZnK_2 is not expected to be harmful to the aquatic species.

In Australia, the soil ecological investigation level for zinc is 200 mg/kg dry solids (NEPC, 1999). Background zinc concentrations in Australian soils range 1-263 mg/kg, with a calculated median background zinc

concentration in soil of 39 mg/kg (Berkman, 1989). At the proposed application rate and use pattern, assuming no zinc uptake by crops the notified chemical may lead to a 21 µg/g or 0.02 mg/kg increase in zinc concentrations in agricultural soils over a 10 year period due to the application of fertilisers. This represents a 0.05% increase with respect to the median background concentration over 10 years and accounts for 0.01% of the soil ecological investigation level for zinc. Therefore, the contribution of the notified chemical as an anthropogenic source of zinc is not expected to result in a significant increase to the concentration of zinc in soils with respect to Australian environmental trigger values. Similarly the concentration of zinc 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 zinc, 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. Zinc was similarly in concentrations that did not significantly alter background concentrations or were below the ANZECC/ARMCANZ (2000) 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 notified chemical.

Therefore on the basis of the PEC/PNEC ratio, with Australian water quality guideline limits for zinc 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 (purity 91.4%)

METHOD: OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
- Species/Strain: Rat/Wistar Crl:WI (Han)
- Vehicle: Water (exposed via gavage)
- Remarks - Method: The study authors stated that animals were dosed at 1,970 mg/kg bw instead of 2,000 mg/kg bw because the correction for purity was not done accurately.

  There were no significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	3 F	1,970	0/3
2	3 F	1,970	0/3

- LD50: > 1,970 mg/kg bw
- Signs of Toxicity: All animals showed hunched posture on days 1 and 2 and piloerection on days 1 and/or 2. No unscheduled mortalities were observed during the study.
- Effects in Organs: No abnormalities were noted at macroscopic examination.
- Remarks - Results: The body weights were within the range commonly recorded for this strain and age of rats.

CONCLUSION: The notified chemical is of low acute toxicity via the oral route.

TEST FACILITY: WIL Research (2014a)

B.2. Acute toxicity – inhalation

TEST SUBSTANCE: Analogue chemical 1 (92.3% purity)

METHOD: OECD TG 436 Acute Inhalation Toxicity – Acute Toxic Class Method
- Species/Strain: Rat/Wistar WU (Crl;[W1]WU)
- Vehicle: Water
- Method of Exposure: Nose-only
- Exposure Period: 4 hours
- Physical Form: Liquid aerosol
- Particle Size: Mass median aerodynamic diameter (MMAD): 3.3-3.4 µm
- Remarks - Method: No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Concentration (g/m^3)*		*Mortality*
		Nominal	*Actual*	
1	3 per sex	29.9	5.16	0/6

- LC50: > 5.16 mg/L/4 hours
- Signs of Toxicity: Slight sniffing was heard shortly after exposure in one male and two females and all three females showed slightly soiled head fur. No treatment related abnormalities were observed after day 2.

  Reduction in body weight gain was observed in all animals on days 1 and 3.
- Effects in Organs: Petechiae (spots caused by bleeding) were observed on both lobes of the

	thymus in one male and on one side of the lungs of one female.
Remarks - Results	Effects of exposure were limited to decreased body weight gain up to day 3 and breathing abnormalities shortly after exposure. No mortality occurred.
CONCLUSION	The analogue chemical 1 is of low acute toxicity via inhalation.
TEST FACILITY	TNO (2010a)

B.3. Irritation – skin (in vitro)

TEST SUBSTANCE	Notified chemical (91.4% purity)
METHOD	OECD TG 439 In vitro Skin Irritation: Reconstructed Human *Epidermis* Test Method
Vehicle	Nil
Remarks - Method	Positive and negative controls were run in parallel with the test substance: Phosphate buffered saline (PBS) and 5% sodium dodecyl sulphate (SDS) were used as negative and positive controls respectively. A pre-test was conducted by adding 18.6 mg of the test substance to 4 mL of the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution. Due to hygroscopic nature of the test substance, the tissue was not completely covered with the test substance and this test was not repeated. The study authors asserted that a clear negative response was obtained in this study therefore this deviation had no impact on the study integrity. Standard deviation of the relative mean viability was not provided.

RESULTS

Test material	*Mean OD_{570} of triplicate tissues*	*Relative mean cell viability (%)*	*SD of relative mean viability*
Negative control	1.103	100	Not stated
Test substance	1.099	100	Not stated
Positive control	0.385	35	Not stated

OD = optical density; SD = standard deviation

Remarks - Results	The test substance was shown not to directly reduce MTT. As the relative mean tissue viability for the test substance was above 50%, it is considered a non-irritant. The positive and negative controls gave satisfactory results, confirming the validity of the test. The SD of relative mean viability scores was not provided.
CONCLUSION	The notified chemical was non-irritating to the skin under the conditions of the test.
TEST FACILITY	WIL Research (2014b)

B.4. Irritation – eye (in vitro)

TEST SUBSTANCE	Notified chemical (91.4% purity)

METHOD	OECD TG 437 (adopted July 2013) 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 was used as a negative control and 20% (w/v) imidazole was used as a positive control. SD values were not provided.

RESULTS

Test material	*Mean opacities of triplicate tissues*	*Mean permeabilities of triplicate tissues*	*IVIS*
Vehicle control	0	0.000	0.0
*Test substance**	2	0.007	2.1
*Positive control**	89	1.576	113

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

Remarks - Results	A mean *in vitro* irritancy score of 2.1 was obtained for the test substance. As this value is below ≤ 3, the test substance is a not an eye irritant. The positive and negative controls gave satisfactory results confirming the validity of the test.
CONCLUSION	The notified chemical was non irritating to the eye under the conditions of the test.
TEST FACILITY	WIL Research (2014c)

B.5. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Analogue chemical 2 (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.6. Genotoxicity – bacteria

Test Substance	Analogue chemical 2 (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.7. Genotoxicity – in vitro mammalian cell gene mutation test

Test Substance	Analogue chemical 1 (91% purity)
Method	OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.
Species/Strain	Mouse
Cell Type/Cell Line	Lymphoma/L5178Y tk +/- 3.7.2C line
Metabolic Activation System	S9 mix from Aroclor 1254-induced rat liver
Vehicle	Culture medium (RPMI 1640)
Remarks - Method	No significant protocol deviations. Negative control: culture medium (RPMI 1640). Positive control: Without S9: methyl methanesulphonate With S9: 3-methyl-cholantrene Preliminary cytotoxicity test was not conducted.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest time*
Absent			
Test 1	14, 28, 56, 112, 224*, 320*, 458*, 654*, 934*, 1335*, 1907*, 2724*, 3891*	24 h	48 h
Test 2	14*, 28, 56*, 112*, 224*, 320*, 458*, 654*, 934*, 1335*, 1907, 2724, 3891	24 h	48 h
Test 3	13, 26, 51*, 102*, 204*, 292, 417*, 595, 851*, 1215, 1736*, 2480*, 3543*	4 h	48 h
Present			
Test 1	14, 28, 56, 112, 224, 320*, 458*, 654*, 934*, 1335*, 1907*, 2724*, 3891*	4 h	48 h

*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	-	≥ 14	> 3891	Negative
Test 2	-	≥ 14	> 3891	Negative
Test 3	-	> 3543	> 3543	Negative
Present				
Test 1	-	≥ 934	> 3891	Negative

Remarks - Results

In Test 1, without metabolic activation, an increase in the mean mutant frequency (MF) by more than 88 but less than 126 mutants/1,000,000 clonable cells was observed for cells treated with the test substance at 934, 1335, 1907, 2724 and 3891 µg/mL. However a clear dose response relationship was not observed.

In Test 2, without metabolic activation, an increase in mean MF (more than 88 but less than 126 mutants/1,000,000 clonable cells) was observed for cells treated with the test substance at 934 and 1335 µg/mL.

An increase in mean MF by more than 88 but less than 126 mutants/1,000,000 clonable cells is considered an equivocal result under the test guideline.
The increases in the MF occurred at concentrations causing high cytotoxicity (relative total growth ≤ 12% in Test 1 and < 10% in Test 2). The study authors therefore considered that these increases were not biologically relevant.

In Test 3 without metabolic activation no increase in MF was observed, further supporting the conclusion from Test 1 and 2. No increase in MF was also observed in Test 1 with metabolic activation.

The study authors considered the high cytotoxicity to be caused by chelation of essential metals.

Conclusion: The analogue chemical 1 was not clastogenic to mouse lymphoma L5178Y cells treated in vitro under the conditions of the test.

Test Facility: TNO (2015)

B.8. Genotoxicity – In vitro mammalian cell micronucleus test

Test Substance: Analogue chemical 1 (92.3% 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: A preliminary cytotoxicity test was not conducted.

Negative control: culture medium
Positive control (clastogen):
Without S9: mitomycin C
With S9: cyclophosphamide
Aneugenic positive control: vinblastine sulphate

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	125, 250, 500, 1000*, 2000*, 3891*	4 h	24 h
Test 2	1000, 1500, 2000, 2500*, 3000*, 3891*	20 h	48 h
Present			
Test 1	125, 250, 500, 1000*, 2000*, 3891*	4 h	24 h

*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	-	≥ 3891	> 3891	Negative
Test 2	-	≥ 3000	> 3891	Negative
Present				
Test 1	-	≥ 3891	> 3891	Negative

Remarks - Results: In Test 1 (in the presence of S9-mix) and Test 2 (in the absence of S9-mix), the highest concentration (3,891 µL/mL) tested showed cytotoxicity (20% and 31% respectively). Slight to no cytotoxicity was observed at all other concentrations.

In Test 2 (in the absence of S9-mix), 26% and 35% cytotoxicity was observed at 3,000 and 3,891 µL/mL, respectively. Slight cytotoxicity was noted at all other concentrations.

The test substance did not induce a dose dependent statistically significant increase in the number of binucleated cells containing micronuclei at any concentrations analysed.

	The positive controls behaved as expected, confirming the validity of the test system.
Conclusion	The analogue chemical 1 was not clastogenic to human lymphocytes treated in vitro under the conditions of the test.
Test Facility	TNO (2010b)

B.9. Genotoxicity – In vitro mammalian cell micronucleus test

Test Substance	Analogue chemical 2 (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	The analogue chemical 2 was aneugenic to human lymphocytes treated in vitro under the conditions of the test.
Test Facility	TNO (2013)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE: Analogue chemical 3

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 3 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 1 lb zinc (analogue chemical 3 active ingredient 53.0%)
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 22^{o}C 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. The tests were run in very soft (pH 8.1), medium hard ($CaCO_3$ 103 mg/L, pH 7.9) and very hard (pH 8.4) test solutions, the $CaCO_3$ of the soft and the very hard test solutions are not reported. 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	Water hardness Very Soft: 940 (95% CI: 869-1050) mg/L at 96 hours. Medium Hard: 685 (95% CI: 584-831) mg/L at 96 hours. Very Hard: 513 (95% CI: 454 -578) mg/L at 96 hours.
NOEC	Very Soft: 560 mg/L at 96 hours. Medium Hard: 320 mg/L at 96 hours. Very Hard: 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 and water hardness characterisation 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 1 lb zinc at all water harnesses were >100 mg/L. The test substance was less toxic to fish at greater water hardness.
CONCLUSION	Analogue chemical 3 is not expected to be harmful to fish.
TEST FACILITY	Batchelder, T. L., et al (1980)

C.2.2. Algal growth inhibition test

TEST SUBSTANCE	Analogue chemical 3
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 131.1 mg/L, the ErC_{50} was >1000 mg/L and the NOEC was 10.
CONCLUSION	Analogue chemical 3 is not expected to be harmful to algae.
TEST FACILITY	AkzoNobel (2009)

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