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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Chemical in Liquitint Aztec Yellow

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
LTD/2028	Milliken (Australia) Pty Ltd	Chemical in Liquitint Aztec Yellow	ND*	< 1 tonne per annum	Colouring agent for fertilisers

*ND = not determined

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Human health risk assessment
Under the conditions of the occupational settings described, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

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

Environmental risk assessment
On the basis of the PEC/PNEC ratio, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- A person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical during reformulation:
  - Enclosed and/or automated processes, where possible
  - Exhaust 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 during reformulation:
  - Avoid skin and eye contact
  - Avoid breathing aerosols and mists

- A person conducting a business or undertaking at a workplace should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical during reformulation:
  - Protective clothing
  - Eye protection
  - Impervious gloves
  - Respiratory protection

  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.

Regulatory Obligations

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

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

(1) Under Section 64(1) of the Act; if
  - the importation volume exceeds one tonne per annum notified chemical;

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from a colouring agent for fertilisers, 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.

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT
Milliken (Australia) Pty Ltd (ABN: 35 605 606 148)
171 Briens Road,
NORTHMEAD NSW 2152

NOTIFICATION CATEGORY
Limited-small volume: Chemical other than polymer (1 tonne or less per year)

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details claimed exempt from publication: chemical name, other names, CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, impurities, additives/adjuvants, and use details.

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

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT
None

NOTIFICATION IN OTHER COUNTRIES
Canada (2017), US

2. IDENTITY OF CHEMICAL

MARKETING NAME
Liquitint Aztec Yellow (product containing the notified chemical at < 40% concentration)

MOLECULAR WEIGHT
Number Average Molecular Weight (Mn) is > 500 g/mol

ANALYTICAL DATA
Reference GPC and UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 95%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Dark orange liquid*

Property	Value	Data Source/Justification
Melting Point	Not determined	Introduced in aqueous solution
Boiling Point	Not determined	Introduced in aqueous solution
Relative Density*	1,116 kg/m^3	SDS
Vapour Pressure	Not determined	Expected to be low based on chemical structure and molecular weight
Water Solubility	75.3 g/L at 20 °C	Calculated using WSKOW v1.42, EPI Suite v4.1 (US EPA, 2018)
Hydrolysis as a Function of pH	Not determined	Contains no functional groups susceptible to hydrolysis
Partition Coefficient (n-octanol/water)	log Pow = - 3.02 at 20 °C	Calculated using KOWWIN v1.68, EPI Suite v4.1 (US EPA, 2010)
Adsorption/Desorption	log K_{oc} = -1.47 – 0.32	Calculated using KOCWIN v2.00, EPI Suite v4.1 (US EPA, 2010)

Dissociation Constant	Not determined	Will remain in the ionised state in the environmentally relevant range (pH 4-9)
Flash Point	Not determined	Introduced in aqueous solution
Flammability	Not determined	Introduced in aqueous solution
Autoignition Temperature	Not determined	Introduced in aqueous solution
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 product containing the notified chemical at < 40% concentration in aqueous solution

DISCUSSION OF PROPERTIES

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

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. The notified chemical will be imported into Australia as a component of a fertiliser additive at < 10% concentration.

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
Sydney and Melbourne

IDENTITY OF RECIPIENT
Milliken (Australia) Pty Ltd

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a component of a fertiliser additive in 1,000 L International Bulk Containers (IBC). Within Australia the IBCs will be transported by road or rail to the warehouse for storage and later distributed to fertiliser manufacturers by road. Finished end use fertilisers containing the notified chemical at < 0.1% concentration will be packaged in 25 kg or 1,000 kg packages, or ~2,200 kg bulk bags, and transported by road or rail within Australia to regional centres for sale to farmers.

USE
The notified chemical will be used as a colouring agent in fertilisers at < 0.1% concentration.

OPERATION DESCRIPTION
Reformulation of the fertiliser additive product containing the notified chemical at < 10% concentration into finished end use fertilisers may involve both automated and manual transfer steps. Typically, reformulation processes may incorporate blending operations that are highly automated and occur in a fully enclosed/contained environment, followed by automated packaging of the finished end-use fertilisers.

The fertilisers containing the notified chemical at < 0.1% concentration will be applied in solid form to soil via spreaders by broad-acre farmers and farmworkers. The fertilisers will be used for various field crops such as cereal grains, wheat, barley oats, canola, chickpeas, sorghum, sugarcane, cotton and rice. Application rate depends on crop and required application rate. However, the ranges of total treated fertiliser ranges from 98 kg/ha for cereal grains to 652 kg/ha for sugarcane and cotton. The number of applications ranges from once a year for cereal grains to up to five times per year for grasses.

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	1	10
Blenders	1	20
Equipment maintenance	1	10
Farmers and farm workers	0.5	4

EXPOSURE DETAILS

Transport and storage

Transport and storage workers may come into contact with the notified chemical as a component of the imported fertiliser additive (< 10% concentration) or finished end-use fertiliser (< 0.1% concentration), only in the unlikely event of accidental rupture of the packaging.

Reformulation

During reformulation, dermal, ocular and inhalation exposure of workers to the notified chemical at < 10% concentration may occur during weighing and transfer stages, blending, quality control analysis and cleaning and maintenance of equipment. The notifier stated that exposure is expected to be minimised through the use of mechanical ventilation and/or enclosed systems, and through the use of personal protective equipment (PPE) such as protective clothing, eye protection, impervious gloves and respiratory protection.

End-use

Farmers or farmworkers will move the bags containing the fertiliser (containing the notified chemical at < 0.1% concentration) to the loading area, weigh-out the required amount of the product and manually add to the hopper. The farmer will drive the spreader and incorporate the fertiliser mixture into the soil.

The principal route of exposure to the notified chemical will be dermal. However, as the fertiliser is in solid form, during weighing and loading into the hopper, inhalation exposure to the notified chemical via dust and to a lesser extent ocular exposure are also possible. The notifier states that appropriate personal protective equipment (PPE) are expected to be used to minimise exposure.

6.1.2. Public Exposure

End use fertilisers containing the notified chemical at < 0.1% concentration will not be made available to the public. Public exposure is therefore not expected.

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity*	LD50 > 2,000 mg/kg bw; low toxicity
Rabbit, skin irritation*	non-irritating
Mouse, skin sensitisation – Local lymph node assay*	no evidence of sensitisation
Mutagenicity – bacterial reverse mutation*	non mutagenic
Mutagenicity – bacterial reverse mutation (incorporating Prival and Mitchell modification for azo dyes)	non mutagenic
Genotoxicity – *in vitro* mammalian cell micronucleus test (peripheral blood lymphocytes)	non genotoxic
Genotoxicity – *in vitro* cell gene mutation test (mouse lymphoma L5178Y)	non mutagenic**

*for the notified chemical at 33% concentration in aqueous solution
**study author's conclusion

Toxicokinetics
Given the relatively high molecular weight (> 500 g/mol), water solubility (75.3 g/L at 20 °C) and low partition coefficient (log Pow = - 3.02 at 20 °C) of the notified chemical, dermal absorption is expected to be limited. However, the notified chemical is an azo compound. Bacterial skin microflora has been reported to be able to break down azo compounds into smaller species, which may be more readily absorbed through azo reduction (SCCNFP, 2002).

Absorption through the gastrointestinal (GI) tract is also expected to be limited, based on the above physico-chemical properties. However, azo compound reduction in the small intestine with possible absorption of the reduction products through the GI tract cannot be ruled out.

Acute toxicity
The notified chemical at a concentration of 33% is of low acute oral toxicity based on a study conducted in rats.

No studies were submitted for acute dermal toxicity. No signs of systemic toxicity were observed in a dermal irritation study or in a mouse local lymph node assay (LLNA).

No studies were submitted for acute inhalation toxicity on the notified chemical.

Irritation and sensitisation
The notified chemical at a concentration of 33% is non-irritating to the skin of rabbits.

No eye irritation studies were submitted on the notified chemical.

The notified chemical was determined not to be a skin sensitiser in a mouse LLNA at up to 33% concentration.

Repeated dose toxicity
No studies were submitted for repeated dose toxicity on the notified chemical. Based on its physico-chemical properties, the notified chemical is likely to have limited potential for absorption; however metabolism to smaller species could occur on the skin or small intestine. Hence the potential for systemic toxicity cannot be ruled out.

Mutagenicity/Genotoxicity
The notified chemical is an azo dye. Azo dyes are a concern for their potential induction of mutagenicity and carcinogenicity. Liver azo reductase enzymes reductively cleave the molecule into component amines. This reduction may contribute to the carcinogenicity of many azo dyes. The notified chemical is not expected to be reductively cleaved to release any of the aromatic amines classified as carcinogens in the EU and identified in the REACH list of 22 aromatic amines in Annex XVII Appendix 8 (European Commission, 2006).

The notified chemical tested negative both in a standard bacterial reverse mutation study and in a modified bacterial reverse mutation assay for azo dyes (Prival MJ and Mitchell VD, 1982). This modified test is thought to yield a greater detection of mutagenic azo dyes as it utilises a reductive pre-incubation step (during which the azo dye is reduced to amine species) before the test is carried out.

The notified chemical also tested negative in an *in vitro* mammalian cell micronucleus test (peripheral blood lymphocytes and in an *in vitro* mouse lymphoma cell gene mutation test (L5178Y). In the mouse lymphoma test, a statistically significant linear increase in the mutation frequency (MF) was observed without metabolic activation. However, these observations were not considered biologically relevant by the study authors as the MF did not exceed the sum of the MF and the global evaluation factor (GEF, 126 mutants per 10^6 viable cells) in both experiments. The significance of increases in mutant frequencies was assessed according to the recommendations of the Mouse Lymphoma Workshop, Aberdeen (2003) and does not form part of the OECD test guideline.

Overall, the available evidence indicates that the notified chemical is unlikely to be mutagenic or genotoxic.

Health hazard classification
Based on the available information, the notified chemical cannot be classified according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia.

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety

Based on the available information, the notified chemical is expected to be of low acute toxicity and is not expected to be genotoxic; however, the potential for eye irritation and acute inhalation toxicity is unknown. Based on its estimated physico-chemical properties, the notified chemical is likely to have limited potential for absorption; however metabolism to smaller species could occur on the skin or small intestine. Hence the potential for systemic toxicity cannot be ruled out.

During reformulation workers may be exposed to the notified chemical at < 10% concentration. At this proposed use concentration significant toxic effects are not expected. In addition, exposure is expected to be minimised through the expected use of PPE and engineering controls (i.e. mechanical ventilation and/or enclosed systems).

Although farmers may be exposed to the notified chemical when handling fertilisers containing the notified chemical, exposure levels will be low given the low concentration of the notified chemical (< 0.1%) in fertilisers.

Overall, given the expected use of PPE and engineering controls by reformulation workers and the low use concentration in fertilisers, the notified chemical is not considered to pose an unreasonable risk to the health of workers.

6.3.2. Public Health

End use fertilisers containing the notified chemical at < 0.1% concentration will not be made available to the public. Hence given the low use concentration and limited potential for exposure, the risk of the notified chemical to the public 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 a fertiliser additive at < 10% concentration for formulation of finished end use fertilisers in Australia prior to sale to farmers. There is unlikely to be any significant release to the environment from transport and storage, except in the case of accidental spills and leaks. In the event of spills, the product containing the notified chemical is expected to be collected and disposed of to landfill in accordance with local government regulations.

The reformulation process will involve blending operations and is expected to occur within a fully enclosed environment. Therefore, significant release of the notified chemical from this process to the environment is not expected. Release of the notified chemical during reformulation in Australia is expected to be limited to accidental spills or leaks and residue in import containers. These releases are expected to be collected and disposed of in accordance with local government regulations or re-worked into other fertiliser products.

RELEASE OF CHEMICAL FROM USE

End-use fertilisers containing the notified chemical at < 0.1% concentration are intended to be spread on agricultural fields to increase crop yields. The environmental release from this practice is described below. Any spills during transport are likely to be collected for re-use to the extent practicable, or disposed of in accordance with any applicable regulations.

RELEASE OF CHEMICAL FROM DISPOSAL

Any waste fertiliser additive is expected to be collected for disposal by an approved waste management company. Any waste fertiliser containing the notified chemical is expected to be disposed of in accordance with any applicable regulations and/or good farming practices.

7.1.2. Environmental Fate

The notified chemical is not readily biodegradable. For the details of the environmental fate study refer to Appendix B. However, there is some evidence of biodegradation and similar chemicals are known to biodegrade slowly in aerobic conditions (NICNAS, 2016). The fertiliser pellets in which the notified chemical is contained are expected to rapidly dissipate and the notified chemical is expected to become distributed on the soil. Here,

the notified chemical is expected to slowly degrade in biotic and abiotic processes to form oxides of carbon, sulfur, nitrogen and water. The notified chemical is likely to be mobile in soil based on its Koc and may leach deeper into soil or run-off during rainfall events.

7.1.3. Predicted Environmental Concentration (PEC)

After application, rainfall events can lead to run-off of the notified chemical from soil. The method for estimating the concentration of the notified chemical in run-off is calculated based on the method used by the APVMA for pesticides (APVMA, 2016). The method uses an OECD based model (Probst et al., 2005), which considers the application rate, topography, in particular the slope of the field to which the chemical is applied, the magnitude of the rainfall and run-off events, and the persistence and mobility of the chemical. In addition placement of the pesticide, an allowance for the heterogeneity of fields and pesticide bound to suspended sediment are also considered. The percentage of the applied chemical that is estimated to run-off is presented as follows:

L%run-off = (R/P) × Crsoil_surface × f1slope × f2bufferzone × f3foliar_application × heterogeneity_factor × 100 + suspended_substance.

Where:
L% run-off is the percentage of application dose available in runoff water as dissolved substance
R is the quantity of run-off water (mm/day)
P is the daily precipitation (mm/day)
f1slope = 0.02153 × slope + 0.001423 × slope^2 for slope < 20%
Crsoil_surface = exp(-3 ln2 ÷ DT50) × (1/(1 + Kd)
Kd = Koc × percent organic carbon (OC%) ÷ 100%
DT50 is the half-life of the chemical on soil in days
Kd is the solid/water partition coefficient
f3foliar_application is equal to (1-F_{ret}), where $F_{ret} = F_{int} \times 0.5$. For weeds and bare soil $F_{ret} = 0$.

Table 1: Parameters Used in Run-off model

Parameter	Value		Comment
P	100 mm		Likely worst case, based on brief survey of Australian weather data
R	20 mm		Based on available evidence especially ANRA (2001) and other sources
Heterogeneity Factor	0.5		Based on Dunne & Black (1970)
F_{ret}	0		Bare soil
Transport suspended substance	0		For pesticides with water solubility ≥ 1 mg/L based on Grover R. ed., (1989)
Slope	12.5%	0.5	Worst case scenario
Buffer	1		Default Value (No effect)
DT50	3000 days		Based on EPHC (2009a)*
Koc	0.03		Supplied data
OC%	1		Default Based on ANRA (2001)

* A value of 3000 days was used to reflect that the notified chemical is not regarded as inherently biodegradable but showed some degradability. Therefore an additional factor of 10 was applied to the soil degradation value recommended by EPHC (2009a).

The percentage run-off calculated using this methodology is 5.0%.

The application rate of the notified chemical may be calculated from the application rate of the fertiliser pellets (up to 652 kg/ha) and the percentage of notified chemical in the fertiliser (< 0.1%). This results in application rate of 652 g/ha. Although applications may be made up to five times for grasses, the PEC was modelled on a maximum application rate of 652 g/ha. This is because the highest rate exceeds the maximum rate of nitrogen recommended for sugarcane (343 g urea/ha/annum; Bell, 2014), and the notified chemical is not expected to accumulate between applications. The basis for the latter is the high mobility of the notified chemical which will result in field dissipation through leaching and run-off.

The resulting PEC from a single application of the notified chemical at a rate of 652 g/ha at the edge of field is 163 µg/L [0.05 × 652 g/ha ÷ 200 m^3/ha).

The effect of the "edge of field" run-off water entering an existing environmental water body is considered using an adaptation of the US EPA (2004) model. Consideration is given to a 1500 m^3 water body of environmental significance. This could be represented by a 1 ha pond, 15 cm deep or a low flow (~ 0.03 – 0.06 m/sec; ~ 0.1-0.2 km/hr) primary stream with 1500 m^3 per day flow having approximate dimensions of ~ 2 m wide and ~ 25 cm deep (based on Vietz *et al*., 2003). In a worst case scenario this water body is considered to be fed entirely by the largest likely field to be 100% treated at the maximum rate. The considered field size is 10 ha (US EPA, 2004).

In most realistic circumstances either the amount of run-off from 10 ha will result in a water body larger than 1500 m^3, or to support such a water body a much larger watershed will need to be considered (*ibid*). The concentration in the water body may be calculated assuming that 200 m^3 of water contaminated with the notified chemical from each hectare for a total of 10 ha flows into the 1500 m^3 water body resulting in a total water body of 3500 m^3. The resulting PEC in the water body is < 93 µg/L.

The PEC in soil may be calculated using a single application of 652 g/ha, a default density of soil of 1500 kg/m^3 and distribution in the top 15 cm for mobile chemicals (EPHC 2009b). This results in a PEC of 0.29 mg/kg soil [652 g/ha ÷ (0.15 m × 100 m × 100 m × 1500 kg/m^3)].

7.2. Environmental Effects Assessment

The result from an ecotoxicological investigation conducted on the notified chemical is summarised in the table below. Details of this study can be found in Appendix B.

Endpoint	*Result*	*Assessment Conclusion*
Daphnia Toxicity	EC50 > 33 mg/L NOEC ≥ 33 mg/L	Not harmful to aquatic invertebrates

Based on the above ecotoxicological data, the notified chemical is not 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 and chronic toxicity.

7.2.1. Predicted No-Effect Concentration

The Predicted No-Effect Concentration (PNEC) has been calculated from the acute toxicity data (daphnia) for the notified chemical. As an EC50 could not be established the NOEC of ≥ 33 mg/L was used. Normally an assessment factor of 1000 is applied to the EC50 for when only one trophic level is available. However, according to Mayo-Bean (2011), the no-effect concentration of chemicals is usually in the range of 4-10 times less than the EC50 for aquatic species. Therefore to take account of using a NOEC an assessment factor of 250 was used.

Predicted No-Effect Concentration (PNEC) for the Aquatic Compartment		
	≥ 33	mg/L
Assessment Factor	250	
Mitigation Factor	1.00	
PNEC:	> 132	µg/L

7.3. Environmental Risk Assessment

The risk quotient (Q = PEC/PNEC) was calculated using the PEC of the notified chemical in an environmental waterbody after a worst case run-off event (< 93 µg/L) and the PNEC of > 132 µg/L. This results in a Q value of

< 0.70. This is indicative of no unreasonable risk. Furthermore this value is considered a worst case as the toxicity endpoint is based on a limit test and no effects were observed at that level.

Although no earthworm toxicity studies were submitted, the PEC of 0.29 mg/kg indicates that the notified chemical would have to be toxic to earthworms with an LC50 < 29 mg/kg using an assessment factor of 100. On the basis of the low toxicity to aquatic invertebrates this would appear to be unlikely.

Therefore based on the PEC/PNEC ratio, the notified chemical when used as dye in fertiliser, is not considered to pose an unreasonable risk to the environment.

APPENDIX A: TOXICOLOGICAL INVESTIGATIONS

A.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical (33% aqueous solution)

METHOD: OECD TG 420 Acute Oral Toxicity – Fixed Dose Procedure
- Species/Strain: Rat/Sprague-Dawley CD
- Vehicle: Nil
- Remarks - Method: No protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	4 F	2,000	0/4

- LD50: > 2,000 mg/kg bw
- Signs of Toxicity: No unscheduled mortalities or adverse clinical signs were recorded 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 test substance is of low acute toxicity via the oral route.

TEST FACILITY: SPL (2007a)

A.2. Irritation – skin

TEST SUBSTANCE: Notified chemical (33% aqueous solution)

METHOD: OECD TG 404 Acute Dermal Irritation/Corrosion
- Species/Strain: Rabbit/New Zealand White
- Number of Animals: 3 M
- Vehicle: Nil
- Observation Period: 72 hours
- Type of Dressing: Semi-occlusive
- Remarks - Method: No 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			
Erythema/Eschar	0	0	0	0	Nil	0
Oedema	0	0	0	0	Nil	0

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

Remarks - Results: Yellow staining was observed at the treated skin sites during the study and the study authors state that this did not affect the evaluation of skin reactions.

No signs of irritation or toxicity were observed in any of the animals during the study.

CONCLUSION: The test substance is non-irritating to the skin.

TEST FACILITY: SPL (2007b)

A.3. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE	Notified chemical (33% aqueous solution)
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay
Species/Strain	Mouse/CBA CA
Vehicle	Distilled water with 1% pluronic L92
Preliminary study	Yes
Positive control	Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using 2,4-dinitrobenzenesulfonic acid.
Remarks - Method	A preliminary study was conducted using 100% of the test substance. No signs of systemic toxicity were observed. Based on the results, the highest concentration selected for the main study was 100%.

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	428	1.0
25	5 F	433	1.01
50	5 F	556	1.30
100	5 F	571	1.34
Positive Control			
0 (vehicle control)*	5 (sex not specified)	Not provided	1.0
1*	5 (sex not specified)	Not provided	1.80
5*	5 (sex not specified)	Not provided	4.32
10*	5 (sex not specified)	Not provided	11.98

*1% pluronic L92 in distilled water

Remarks - Results	No unscheduled mortalities or signs of systemic toxicity were observed during the study period. The stimulation indices were 1.01, 1.30 and 1.34 at 25%, 50% and 100% concentrations, respectively, indicating the test substance as negative for skin sensitisation. The positive control behaved as expected, confirming the validity of the test system.
CONCLUSION	There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the test substance.
TEST FACILITY	SPL (2008a)

A.4. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical (33% aqueous solution)
METHOD	OECD TG 471 Bacterial Reverse Mutation Test
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA98 and TA100 *Escherichia coli*: WP2uvrA
Metabolic Activation System	S9 mix from phenobarbitone/β-naphthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 50, 150, 500, 1,500 and 5,000 µg/plate b) Without metabolic activation: 50, 150, 500, 1,500 and 5,000 µg/plate
Vehicle	Distilled water
Remarks - Method	Negative control: distilled water Positive control: with S9-mix: 2-aminoanthracene (TA100, TA1535, TA1537 and WP2uvrA) and benzo(a)pyrene (TA98)

without S9-mix: *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (TA100, TA1535 and WP2uvrA); 9-aminoacridine (TA1537) and 4-nitroquinoline-1-oxide (TA98)

Test 1 (without metabolic activation) was repeated due to contamination and technical error in the original test and Test 2 (both with and without metabolic activation) were repeated due to defective top-agar batch.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5,000	> 5,000	> 5,000	Negative
Test 2		> 5,000	> 5,000	Negative
Present				
Test 1	> 5,000	> 5,000	> 5,000	Negative
Test 2		> 5,000	> 5,000	Negative

Remarks - Results

Yellow staining was observed in the 150 µg/plate colony and the study authors state that this staining did not prevent the scoring of the revertant colonies.

No substantial increase in revertant colony numbers of any of the five tester strains was observed following treatment with the test substance at any dose level, in the presence or absence of metabolic activation. There were also no dose dependent increases in mutation rates.

The positive controls gave satisfactory responses, confirming the validity of the test system.

CONCLUSION

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

TEST FACILITY

SPL (2008b)

A.5. Genotoxicity – bacteria (Prival and Mitchell modification)

TEST SUBSTANCE

Notified chemical (19% aqueous solution)

METHOD

OECD TG 471 Bacterial Reverse Mutation Test

Species/Strain

Salmonella typhimurium: TA98, TA100, TA102, TA1535 and TA1537

Metabolic Activation System

S9 mix from Aroclor1254-induced rat liver (for tests 1 and 2) un-induced hamster liver post-mitochondrial fraction (for test 3)

Concentration Range in Main Test

Test 1
With metabolic activation: 1.6, 8, 40, 200, 1,000 and 5,000 µg/plate
Test 2
Without metabolic activation: 1.6, 8, 40, 200, 1,000 and 5,000 µg/plate
Test 3
With metabolic activation: 156.3, 312.5, 625, 1,250, 2,500 and 5,000 µg/plate

Vehicle

Distilled water

Remarks - Method

The concentrations used in the study were adjusted for the purity of the notified chemical.

Negative control: distilled water
Positive control:
with S9-mix: 2-aminoanthracene (TA100, TA1535, TA1537 and TA102); benzo(a)pyrene (TA98) and Congo red (TA98

and TA100)
without S9-mix: 2-nitrofluorene (TA98); sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537) and mitomycin C (TA102)

Test 3 was performed in the absence of metabolic activation using a concentration range of 156.3 to 5,000 µg/plate.

Preliminary test (with or without metabolic activation) was conducted with TA100 only.

No significant protocol deviations.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5,000	> 5,000	> 5,000	Negative
Test 2		> 5,000	> 5,000	Negative
Present				
Test 1	> 5,000	> 5,000	> 5,000	Negative
Test 2		> 5,000	> 5,000	Negative
Test 3		> 5,000	> 5,000	Negative

Remarks - Results

In Test 3 (with S9-mix), statistically significant increase (1.48 fold) in revertant colonies were observed in TA98 strain at 2,500 µL/plate. Slight to no increase in revertant colonies was observed in all other strains and at all other concentrations.

The test substance did not induce a dose dependent increase in the number of revertant colonies at any concentration tested.

The positive controls behaved as expected, confirming the validity of the test system.

CONCLUSION

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

TEST FACILITY

CLL (2008a)

A.6. Genotoxicity – *in vitro* mammalian cell gene mutation test

TEST SUBSTANCE

Notified chemical (19% aqueous solution)

METHOD

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

Species/Strain: *Mouse*

Cell Type/Cell Line: Lymphoma L5178Y/*tk* locus

Metabolic Activation System: S9 mix from Aroclor1254-induced rat liver

Vehicle: Purified water

Remarks - Method: The concentrations used in the study were adjusted for the purity of the notified chemical.

A preliminary test at concentrations range of 156.3 – 5,000 µg/mL (with or without metabolic activation, 3 hour exposure) and 19.53 – 5,000 (without metabolic activation, 24 hour exposure) was conducted. Although cytotoxicity was observed at ≥ 156.3 µg/mL, no significant osmolality or pH changes were observed in the preliminary tests compared to concurrent vehicle control tests.

Vehicle and positive control studies were conducted in parallel with the main study.

Negative control: purified water
Positive control: With metabolic activation: benzo(a)pyrene
Without metabolic activation: 4-nitroquinoline1-oxide

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	100, 200, 400, 500, 600, 650, 700, 800, 1,000, 1,200	3 h	48 h
Test 2	50, 100, 150, 200, 250, 275, 300, 325, 350, 450	24 h	48 h
Present			
Test 1	250, 500, 1,000, 2,000, 3,000, 4,000, 5,000	3 h	48 h
Test 2	300, 600, 1,000, 2,000, 3,000, 4,000, 5,000	3 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	≥ 312.5	≥ 400	Not stated	Negative
Test 2	≥ 156.3	≥ 200	Not stated	Negative
Present				
Test 1	≥ 5,000	≥ 5,000	Not stated	Negative
Test 2		≥ 4,000	Not stated	Negative

Remarks - Results

In Tests 1 and 2, without metabolic activation, a statistically significant linear increase in the mutation frequency (MF) was observed. However, these observations were not considered biologically relevant by the study authors as the MF did not exceed the sum of the MF and the global evaluation factor (GEF, 126 mutants per 10^6 viable cells) in both experiments. The significance of increases in mutant frequencies was assessed according to the recommendations of the Mouse Lymphoma Workshop, Aberdeen (2003).

No significant increase in MF was observed in Test 1 and Test 2 with metabolic activation.

CONCLUSION

The test substance was negative to mouse lymphoma L5178Y treated *in vitro* under the conditions of the test and when assessed according to the recommendations of the Mouse Lymphoma Workshop, Aberdeen (2003).

TEST FACILITY

CLL (2008b)

A.7. Genotoxicity - *In vitro* mammalian cell micronucleus test

TEST SUBSTANCE

Notified chemical (19% aqueous solution)

METHOD

OECD TG 487 *In Vitro* Mammalian Cell Micronucleus Test.
Species/Strain: Human Lymphocytes
Metabolic Activation System: S9 mix from Aroclor 1254-induced rat liver
Vehicle: Culture medium
Remarks - Method: The concentrations used in the study were adjusted for the purity of the notified chemical.

A preliminary test using a concentration range of 18.14 to 5,000

μg/mL was conducted.

Negative control: purified water
Positive control: 4-nitroquinoline1-oxide, cyclophosphamide and vinblastine

Metabolic Activation	*Test Substance Concentration (μg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	25, 50, 100*, 200, 400*, 600, 800, 1,000, 1,200, 1,400*, 1,600, 1,800, 2,000, 2,500, 3,000	20 h	28 h
Test 2	250*, 500, 700*, 900, 1,100*, 1,400, 1,700, 2,000, 2,250, 2,500, 3,000, 4,000	20 h	28 h
Present			
Test 1	100, 200, 400, 600, 1,000, 2,000, 3,000*, 4,000*, 5,000*	3 h	45 h
Test 2	250, 500, 1,000, 2,000, 3,000*, 4,000*, 5,000*	3 h	45 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	> 5,000	100 and 400	> 3,000	Negative
Test 2	> 5,000	≥ 700	> 4,000	Negative
Present				
Test 1	> 5,000	≥ 4,000	> 5,000	Negative
Test 2	> 5,000	≥ 3000	> 5,000	Negative

Remarks - Results

In Test 1 (in the absence of S9-mix), the lowest two concentrations (100 and 400 μL/mL) tested showed statistically significant increase in micronucleated binucleated cells (MNBN) (1.10% and 1.05% increase respectively). The frequency, however, is within the historical control range. Slight cytotoxicity was observed at other concentrations (1,400 μL/mL in the absence of S9-mix and 3,000, 4,000 and 5,000 μL/mL in the presence of S9-mix) tested.

In Test 2 (in the absence of S9-mix), 1.0% and 0.95% increase (statistically significant) in MNBN were observed at two concentrations (700 and 1,100 μL/mL). The MNBN frequency exceeded historical control range at a concentration of 1,100 μL/mL in one of the replicate cultures. In the presence of S9-mix, 1.05% and 1.15% increase (statistically significant) in MNBN cells was observed at two highest concentrations (4,000 and 5,000 μL/mL) tested. The MNBN frequency exceeded historical control range for one of two replicate cultures for 3,000, 4,000, and 5,000 μL/mL. Slight cytotoxicity was noted at all other concentrations tested. The study authors state that MNBN frequencies in the replicate cultures at these concentrations were within the normal range and therefore these findings have no biological relevance.

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

The positive controls behaved as expected, confirming the validity of the test system.

CONCLUSION	The test substance was not clastogenic to human lymphocytes treated in vitro under the conditions of the test.
TEST FACILITY	CLL (2008c)

APPENDIX B: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

B.1. Environmental Fate

B.1.1. Ready biodegradability

TEST SUBSTANCE: Notified chemical (33% aqueous solution)

METHOD: OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test

Inoculum: Activated sewage sludge
Exposure Period: 29 days

Remarks - Method: A preliminary test was conducted using stock solutions of the test item, a negative control, a toxicity control and a reference substance (sodium benzoate). No replicates were used. The test was conducted for seven days. The test item was not toxic to the inoculum at a nominal concentration of 20 mg carbon/L.

On the basis of the preliminary test, a definitive test was conducted using the same test design as used in the preliminary test, excepting duplicates for the negative and test treatments and the inclusion of an abiotic control (test substance, no inoculum).

RESULTS

Test substance		*Reference Substance*	
Day	*% Degradation*	*Day*	*% Degradation*
2	0.0	2	26.5
11	0.4	11	79.2
29	2	29	88.0

Remarks - Results: All validity criteria were met. The CO_2 production in the inoculum control was 10.89 mg CO_2/L within 29 days. The degradation in the toxicity control was 45.2%, indicating that the notified chemical had no inhibitory effect on the activated sludge.

CONCLUSION: The test substance was not biodegradable.

TEST FACILITY: CRL (2016)

B.2. Ecotoxicological Investigations

B.2.1. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE: Notified chemical (33% aqueous solution)

METHOD: OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test - Static
Method C.2 of European Commission Directive 92/69/EEC

Species: *Daphnia magna*
Exposure Period: 48 hours *[acute study]*

Water Hardness: 250 mg $CaCO_3$/L

Remarks - Method: A range finding test was conducted by exposing *Daphnia* to the nominal concentrations ranging from 0.1 to 100 mg/L of the test item, and a control. No immobilisation was recorded at any of the concentrations.

Based on the results of this preliminary range test, a definitive (limit) test was conducted with a test concentration of 100 mg/L and a negative control, using four replicates of five *Daphnia*, for each concentration.

A positive control test with a reference substance, potassium dichromate, was performed under static conditions for 48 h less than 6 months prior to this study.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h [acute]*	*48 h [acute]*
100	99 - 100	20	0	0

EC50 > 100 mg/L at 48 hours (nominal value)

NOEC (or LOEC) ≥ 100 mg/L at 48 hours (nominal value)

Remarks - Results All validity criteria were met. The 48 h EC50 for the reference substance was 0.75 mg/L (within the accepted range). The concentration of oxygen in the 100 mg/L test item in the definitive test was 8.8 mg/L at 0 h and 8.7 mg/L at 48 h.

CONCLUSION The test substance is not toxic to aquatic invertebrates.

TEST FACILITY SPL (2007)

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