File No: LTD/1844

November 2015

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Benzenamine, *N,N*-diethyl-3-methyl-4-[2-(5-nitro-2-thiazolyl)diazenyl]-

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.

For the purposes of subsection 78(1) of the Act, this Public Report may be inspected at our NICNAS office by appointment only at Level 7, 260 Elizabeth Street, Surry Hills NSW 2010.

This Public Report is also 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/1844	Epson Australia Pty Ltd	Benzenamine, *N,N*-diethyl-3-methyl-4-[2-(5-nitro-2-thiazolyl)diazenyl]-	Yes	≤ 1 tonne per annum	Component of ink

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard classification	*Hazard statement*
Flammable solid (Category 1)	H228 – Flammable solid
Skin sensitisation (Category 1)	H317 – May cause an allergic skin reaction
Specific target organ toxicity (Category 2)	H373 – May cause damage to organs through prolonged or repeated use

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004) with the following risk phrase:

R10: Flammable
R43: May cause sensitisation by skin contact
R48/22: Harmful: danger of serious damage to health by prolonged exposure if swallowed

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

When used in clothing textiles as proposed, the notified chemical is considered to pose an unreasonable risk to public health.

When used in non-clothing articles such as soft signage and promotional items, the notified chemical is not considered to pose an unreasonable risk to public health.

Environmental risk assessment
On the basis of its limited aquatic exposure and the reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

REGULATORY CONTROLS

Hazard Classification and Labelling

- The notified chemical should be classified as follows:
  - Flammable solid (Category 1): H228 – Flammable solid
  - Skin sensitisation (Category 1): H317 – May cause an allergic skin reaction

- Specific target organ toxicity (Category 2): H373 – May cause damage to organs through prolonged or repeated use

The above should be used for products/mixtures containing the notified chemical, if applicable, based on the concentration of the notified chemical present and the intended use/exposure scenario.

- Due to the flammable properties of the notified chemical, the notifier should consider their obligations under the Australian Dangerous Goods Code.

Health Surveillance

- As the notified chemical is a skin sensitiser, employers should carry out health surveillance for any worker who has been identified in the workplace risk assessment as having a significant risk of sensitisation.

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:
  - Local exhaust ventilation
  - Printers fitted with filters to capture any aerosols
  - Use of enclosed, automated processes, where possible

- 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:
  - Avoid contact with skin and eye

- 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:
  - Impervious gloves, goggles and coveralls

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

- A copy of the (M)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.

Public Health

- The notified chemical should not be used in products where there is the potential for significant public exposure, such as clothing textiles.

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 physical containment, 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(1) of the Act; if
- the importation volume exceeds one tonne per annum notified chemical;
- the notified chemical is applied to clothing textiles;
- the notified chemical is used on products other than soft signage and promotional items; ;
- information becomes available on the sensitisation, mutagenicity and/or carcinogenicity of the notified chemical.

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from Component of ink, 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.

AICS Entry

- When the notified chemical is listed on the Australian Inventory of Chemical Substances (AICS), it should be annotated with the following condition of use:
  - The notified chemical must not be applied to clothing textiles.

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

ASSESSMENT DETAILS

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT
Epson Australia Pty Ltd (ABN: 91 002 625 783)
3 Talavera Road
NORTH RYDE NSW 2113

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

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

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows: dissociation constant, flash point and reactivity.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
Low Volume Chemical Permit (2015)

NOTIFICATION IN OTHER COUNTRIES
None

2. IDENTITY OF CHEMICAL

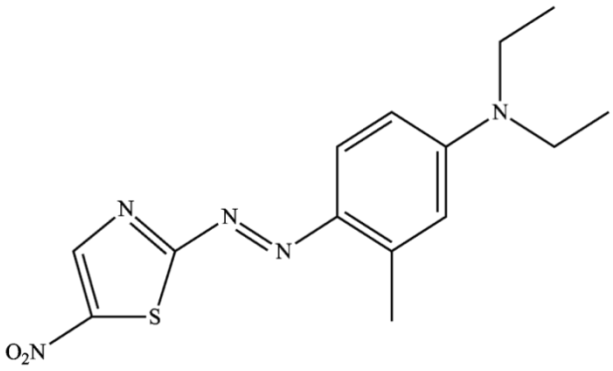
MARKETING NAME(S)
Disperse Blue 360

CAS NUMBER
70693-64-0

CHEMICAL NAME
Benzenamine, *N,N*-diethyl-3-methyl-4-[2-(5-nitro-2-thiazolyl)diazenyl]-

MOLECULAR FORMULA
$C_{14}H_{17}N_5O_2S$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
319.38 Da

ANALYTICAL DATA
Reference NMR spectra was provided.

3. COMPOSITION

DEGREE OF PURITY
98.2%

HAZARDOUS IMPURITIES
None

NON HAZARDOUS IMPURITIES

Chemical Name	Water		
CAS No.	7732-18-5	*Weight %*	1.8

ADDITIVES/ADJUVANTS
None

4. ANALOGUE DATA

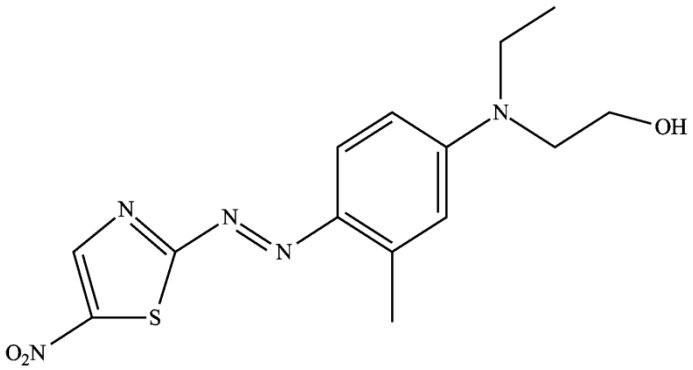
Analogue 1
CAS NUMBER
68516-81-4

CHEMICAL NAME
Ethanol, 2-[ethyl[3-methyl-4-[2-(5-nitro-2-thiazolyl)diazenyl]phenyl]amino]-

OTHER NAME
Disperse Blue 106

MOLECULAR FORMULA
$C_{14}H_{17}N_5O_3S$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
335.38 Da

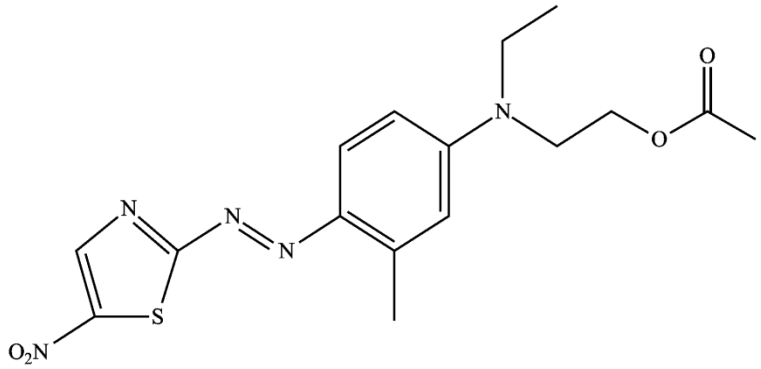
Analogue 2
CAS NUMBER
15141-18-1

CHEMICAL NAME
Ethanol, 2-[ethyl[3-methyl-4-[2-(5-nitro-2-thiazolyl)diazenyl]phenyl]amino]-, 1-acetate

OTHER NAME
Disperse Blue 124

MOLECULAR FORMULA
$C_{16}H_{19}N_5O_4S$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
377.42 Da

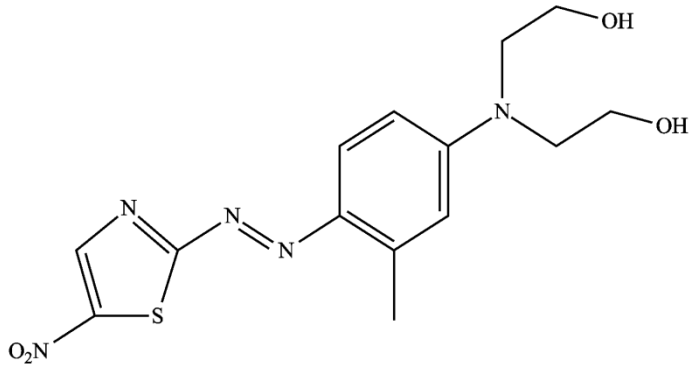
Analogue 3
CAS NUMBER
72987-42-9

CHEMICAL NAME
Ethanol, 2,2'-[[3-methyl-4-[2-(5-nitro-2-thiazolyl)diazenyl]phenyl]imino]bis-

OTHER NAME
Disperse Blue 96

MOLECULAR FORMULA
$C_{14}H_{17}N_5O_4S$

STRUCTURAL FORMULA



MOLECULAR WEIGHT
351.38 Da

5. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: blue/green powder

Property	Value	Data Source/Justification
Melting Point/Freezing Point	> 224 °C	Measured; the notified chemical decomposes during melting
Boiling Point	> 225 °C	Measured; the notified chemical decomposes before boiling
Relative Density	1.38 at 20 °C	Measured
Vapour Pressure	< 4.7×10^{-8} kPa at 25 °C	Measured
Water Solubility	< 1×10^{-4} g/L at 20 °C	Measured
Hydrolysis as a Function of pH	$t_{½}$ = 153 days at pH 7	Measured
Partition Coefficient	log Pow = 3.49 at 20 °C	Measured

(n-octanol/water)		
Adsorption/Desorption	log K_{oc} = 4.04 at 20 °C	Measured
Dissociation Constant	pKa = 2.8 ± 0.4 (strongest base)	Calculated using I-Lab v2.0
Particle Size	Inhalable fraction (< 100 μm): 5.6 % Respirable fraction (< 10 μm): 6.81 %	Measured
Flash Point	Not determined	The notified chemical decomposes before boiling
Flammability	Highly flammable	Measured
Autoignition Temperature	> 224 °C	Measured
Explosive Properties	Not explosive	Measured
Oxidising Properties	Not oxidising	Measured

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

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

Physical hazard classification
Based on the submitted physico-chemical data depicted in the above table, the notified chemical is recommended for hazard classification according to the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS),* as adopted for industrial chemicals in Australia. The recommended hazard classification is presented in the following table.

Hazard classification	***Hazard statement***
Flammable solid (Category 1)	H228 – Flammable solid

6. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured and / or reformulated in Australia. The notified chemical will be imported into Australia as a component (up to 2% concentration) of ink formulations to be used in commercial inkjet printing systems.

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
Brisbane, Melbourne, Perth and Sydney

TRANSPORTATION AND PACKAGING
The notified chemical will be imported as a component (up to 2% concentration) of inkjet printing ink in 1L purpose-designed sealed foil bags packed in boxes.

USE
The notified chemical will be used as a component of a commercial inkjet printing system. The materials that are expected to be printed on include garments, sports ware, soft signage and promotional items with the print area ranging from small logos to whole garments.

OPERATION DESCRIPTION
The notified chemical will be imported in ink formulations at a concentration of up to 2% in 1 L purpose built foil bags packed in boxes. The boxes will be stored in the warehouse and will be distributed to the print houses as required. At the site of use, the ink formulations will be manually emptied into the on-board ink reservoirs in the printer. The ink will then be used for printing an image onto a substrate material which can be paper or cloth that will be allowed to dry before handling. The image will then be transferred via sublimation with a heat press on to material that contains a polyester base or has received polyester pre-treatment.

7. HUMAN HEALTH IMPLICATIONS

7.1. Exposure Assessment

7.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Storage and Transport	4	50
Printer operator	1	50
Service technician	6	200

EXPOSURE DETAILS
Storage and transport workers are not expected to be exposed to the notified chemical as a component of ink formulations at ≤ 2% concentration except in the unlikely event of an accident.

Printer operators may be exposed to the notified chemical during addition of the ink into the reservoir of the printing machine and when using a heat press to transfer the printed images to fabric. Service technicians may be exposed to the notified chemical during cleaning and maintenance of the printing system. The principal route of exposure would be dermal and the use of personal protective equipment (PPE) such as impervious gloves, coveralls and safety glasses as predicted by the notifier should minimise exposure. Inhalation exposure is expected to be limited as the printers will be fitted with filters to capture any aerosols with local exhaust ventilation also in place.

7.1.2. Public Exposure

The printing ink containing the notified chemical is intended for industrial use only. Therefore, the main source of exposure for the public is expected to be through the use of printed garments. This exposure will be dermal and repeated.

No data or quantitative estimate was provided by the notifier on the migration of the notified chemical from the fabrics to which it will be applied. There was also no data provided on the amount of dye used per area of fabric. The notifier has stated that "The inks have been designed for colourfastness and migration of the ink components to the skin is not expected to occur even under heavy sweating conditions" based on observation and garment/print durability testing. However, it has been reported that disperse dyes do not chemically bond to the fibres of the material they are applied to, and their small, lipophilic molecules can therefore easily migrate onto the skin of the person who is wearing the garment (Malinauskiene *et al*., 2013).

The notified chemical was shown to have a high wash fastness (grade 5 on reduction or alkali cleared material and grade 4-5 on dyed samples that had just been rinsed without any clearing treatment) on polyester materials (Choi *et al*., 1999). The dye fastness of the notified chemical was lower on diacetate, nylon and silk; there was no quantitative measurement of dye migration undertaken in this study (Choi *et al*., 1999).

Studies measuring migration of textile dyes corresponding to good fastness properties (≥ 4) have shown migration rates of 0.01 to 0.03 μg/cm^2 (ETAD, 2004; Kimber *et al*., 2005). For dyes of poor fastness the migration rate can be up to 0.3 μg/cm^2, with a proposed model suggesting a peak migration rate of 0.5 μg/cm^2 based on the assumption that a standard textile garment of 100 g/m^2 is dyed with 1% active dye ingredient, i.e. 1 g/m^2 (ETAD, 2004; Kimber *et al*., 2005). Other studies have shown that dynamic processes (friction) can have a significant effect on the amount of dye migration, with the amount of dye released during the simulation of wear conditions not necessarily correlating with colour fastness (BfR, 2012). The migration rate of dyes rapidly decreases with < 10% migration after 28 hours of simulated wash/wear cycles (BfR, 2012).

Therefore, a conservative estimate of the peak migration rate per surface area of the fabric, which is assumed to be the same as the skin which it will be in contact with, is 0.5 μg/cm^2. Although the migration rates of textile dyes corresponding to good fastness properties have been shown to be much lower than this value, it is considered appropriate to take into account the potential for dynamic processes to increase the migration and also the unknown amount of dye that will be applied to the fabric. If the fabrics to which the notified chemical is

applied are washed prior to use the potential peak migration rate is expected to be significantly lower than 0.5 µg/cm^2.

For chronic dermal exposure the systemic dose can be calculated using the model proposed by the German Federal Institute for Risk Assessment based on the following formula (Krätke and Platzek, 2004):

$$EFmi = \frac{G}{100} \times TG \times EH \times MA \times KF \times \frac{PF}{KG}$$

Where EFmi is the mean systemic dose per wear event; G is the concentration of the chemical in the dye (2%); TG is the amount of dye applied to the fabric (10^8 µg/m^2)*; EH is the area of fabric to which the dye is applied (1 m^2)*; MA is the migration correction factor (0.0025); KF is the correction factor for the decrease in migration rate following repeated washings (0.1); PF is the fraction of material that is expected to be absorbed through the skin (0.01)*; and KG is the default body weight (60 kg).
* These values are not known for the notified chemical so the default values from the model were used.

Calculating EFmi using the above formula results in a mean systemic dose of 83 ng/kg bw/wear event. Although a penetration factor of 0.01 (1%) used in the model may be less than the actual skin absorption, the final mean systemic dose is still considered to be a conservative value as the following factors were not considered in the model. The notified chemical is predominantly intended for printing logos rather than whole garments. The printed area may contain many colours not all of which will incorporate the notified chemical. Fabrics containing the notified chemical may be worn over other garments limiting skin contact. Additionally when calculating the margin of exposure (MOE) by comparing the EFmi to the NOAEL, it is comparing a daily exposure to a wear event with wear events very unlikely to occur on a daily basis over a long period of time.

When inks containing the notified chemical are applied to non-clothing articles such as soft signage and promotional items the potential for public exposure is expected to be considerably lower than for garments due to the smaller potential contact area and shorter contact time.

7.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rat, acute dermal toxicity	LD50 > 2,000 mg/kg bw; low toxicity
Rabbit, skin irritation[1]	slightly irritating
Eye irritation (in vitro)	non-irritating
Rabbit, eye irritation[1]	slightly irritating
Guinea pig, skin sensitisation – Bühler test[1]	evidence of sensitisation
Guinea pig, skin sensitisation – Magnuson and Kligman test[1]	evidence of sensitisation
Guinea pig, skin sensitisation – Magnuson and Kligman test[2]*	no evidence of sensitisation
Guinea pig, skin sensitisation – Bühler test[3]*	no evidence of sensitisation
Guinea pig, skin sensitisation – Magnuson and Kligman test[3]*	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days	NOAEL = 15 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	mutagenic
Genotoxicity – in vitro mouse lymphoma test	genotoxic
Genotoxicity – in vivo mouse micronucleus test	non genotoxic
Genotoxicity – in vivo unscheduled DNA synthesis test	non genotoxic

1 – Study conducted on analogue 1
2 – Study conducted on analogue 2
3 – Study conducted on analogue 3
* – Full study reports not sighted

Toxicokinetics, metabolism and distribution.
No information on the toxicokinetics of the notified chemical was provided. For dermal absorption, molecular weights below 100 Da. are favourable for absorption and molecular weights above 500 Da. do not favour

absorption (ECHA, 2014). Dermal uptake is likely to be low if the water solubility is below 1 mg/L, however Log P values between 1 and 4 favour dermal absorption (ECHA, 2014). In addition evidence of skin sensitisation or irritation increase the probability of dermal absorption occurring (ECHA, 2014). Based on the water solubility (< 1×10^{-4} g/L at 20 °C), partition coefficient (log Pow = 3.49 at 20 °C) and low molecular weight (< 500 Da) of the notified chemical, dermal absorption cannot be ruled out. Absorption across the gastrointestinal tract of the notified chemical can be confirmed by the systemic effects seen in the 28 day repeated dose toxicity study (see below).

Acute toxicity.
The notified chemical was found to be of low toxicity via the oral and dermal routes in rats with LD50 values of > 2,000 mg/kg body weight.

Irritation.
An *in vitro* eye irritation study conducted on the notified chemical suggests it is unlikely to have the potential to cause severe ocular irritancy *in vivo*. Studies conducted on analogue 1 show it to be slightly irritating to the skin and eyes of rabbits. Based on the results for analogue 1 and the *in vitro* study, the notified chemical has the potential to be a slight irritant but severe irritation is not expected.

The notifier has classified the notified chemical as: Serious eye damage/eye irritation (Category 2A): H319 – Causes serious eye irritation. However, this classification does not appear to be supported by the data that was provided.

Sensitisation.
No sensitisation data was provided on the notified chemical. Two skin sensitisation studies carried out on analogue 1 found it to be a skin sensitiser when tested at concentrations of 50% and 75%. Other skin sensitisation studies carried out on analogue 2 (challenge concentrations 10% and 25%) and analogue 3 (challenge concentrations 25% and 50%) showed no evidence of sensitisation, however these studies are considered to be of low reliability as the full test reports were not sighted.

Analogue 1 and analogue 2 have been shown to be highly potent skin sensitisers in a number of animal studies reported in the literature. Analogue 1 was found to be a very strong sensitiser in guinea pigs at a challenge concentration of 0.001%, which was the lowest concentration tested (Hausen and Brandão, 1986). Analogue 2 was shown to be sensitising in a guinea pig maximisation test at 1%, which was the lowest dose tested (Hausen and Sawall, 1989). In LLNA assays in mice with analogue 1 where the EC3 values were 0.012% and 0.017% and the study authors determined that the concentration of analogue 1 on the skin when the EC3 value was reached would be approximately 16.4 µg/cm^2 based on a comparison with 2,4-dinitrochlorobenzene (Betts *et al*., 2005). In a biphasic murine LLNA protocol with analysis of lymphocyte subpopulations by flow cytometry both analogue 1 and analogue 2 caused a statistically significant increase in cell numbers in auricular lymph nodes of mice at a concentration of 0.003% (lowest dose tested), which corresponds to an area dose of 0.75 µg/cm^2 (Ahuja *et al*., 2010). In a loose-fit coculture-based sensitisation assay (LCSA) the substance concentration that led to half-maximal increase of CD86 expression (EC50) was 0.25 µmol/L (9.4×10^{-5} g/L; 8.5×10^{-6} % w/w) and 2.5 µmol/L (8.4×10^{-4} g/L; 7.6×10^{-5} % w/w) for analogue 2 and analogue 1 respectively (Sonnenburg *et al*., 2012).

There is a substantial body of evidence showing that disperse dyes are one of the most prevalent causes of textile related allergic contact dermatitis with analogue 1 and analogue 2 being shown to have particularly high rates of positive responses in allergy screening studies at 1.9% and 1.7% respectively (Malinauskiene *et al*., 2013). Although there is evidence that analogue 1 and analogue 2 are rarely used in textiles today (Malinauskiene *et al*., 2012) there have been numerous examples of them causing sensitisation in people who have worn clothing dyed with them, both for the public (Brandão *et al*., 1985; Hausen, 1993; Pecquet *et al*., 1999; Wong *et al*., 2011) and in occupational settings (Mota *et al*., 2000). There are also reports of both analogue 1 and analogue 2 causing sensitisation within the Australian population (Dawes-Higgs and Freeman, 2004; Slodownik *et al*., 2011). There is evidence of some level of sensitisation cross reactivity between analogue 1 and analogue 2 (Brandão and Hausen, 1987; Uter *et al*., 2001), although some of the cross reactivity may be over-estimated in clinical settings due to previous co-exposure (Uter *et al*., 2007) or impurities in the patch test preparations (Ryberg *et al*., 2009). As the concentration (wt/wt) of analogue 1 or analogue 2 decreased from 1% to 0.1% in patch tests there was a decrease in the number of positive responses in allergy screening studies from 1.9% - 0.2% and 1.7% – 0.2% for analogue 1 and analogue 2 respectively (Malinauskiene *et al*., 2013). Nonetheless, analogue 1 and analogue 2 have produced positive responses in patch tests at concentrations down to 0.01 µg/mL (Ryberg *et al*., 2009).

In conclusion there is a considerable amount of evidence showing that analogue 1 and analogue 2 are very strong sensitisers both from animal studies and human data. Therefore, the notified chemical is also expected to have strong skin sensitisation potential. In addition there is evidence that there is sensitisation cross reactivity between analogue 1 and analogue 2, which is of concern considering the high degree of structural similarity to the notified chemical and the expected prevalence of sensitisation to the analogues within the Australian population.

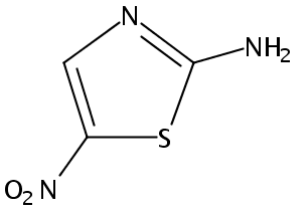
Repeated dose toxicity.
A 28 day repeated dose toxicity study via oral gavage was conducted to assess the toxicity potential of the notified chemical. Three concentrations 15 mg, 150 mg and 1,000 mg/kg body weight were assessed. Adverse effects were observed in animals exposed to 150 and 1,000 mg/kg bw/day. The effects included but were not limited to enlargement of liver and kidneys, histopathological changes in liver, kidney, spleen, thyroids, stomach, thymus, bone marrow and reproductive organs. Weight gains were also adversely effected suggesting the notified chemical to be toxic to the animals when administered by gavage. Based on the adverse effects seen at mid and high concentrations and no adverse effects seen at 15 mg/kg bw, a no observed adverse effect level (NOAEL) of 15 mg/kg bw/day was established.

Mutagenicity/Genotoxicity/Carcinogenicity.
In vitro and *in vivo* mutagenicity/genotoxicity studies were carried out on the notified chemical. The notified chemical was found to be mutagenic in a bacterial reverse mutation test (OECD TG 471), with a dose dependent increase in all of the *Salmonella typhimurium* strains both in the presence and absence of metabolic activation. The notified chemical was also found to be genotoxic in an *in vitro* mouse lymphoma assay (OECD TG 476), although only in the presence of metabolic activation. The notified chemical was found to be non-genotoxic in *in vivo* mammalian erythrocyte micronucleus (OECD TG 474) and mammalian liver unscheduled DNA synthesis (OECD TG 486) tests. The studies OECD TG 471 and 476 look at gene mutations in bacterial and mammalian cells respectively, and can detect both base pair substitutions and frame-shift mutations (EFSA, 2012). The *in vivo* mammalian erythrocyte micronucleus (OECD TG 474) study can identify substances that cause structural and numerical chromosomal damage in somatic cells (EFSA, 2012), but is not intended to detect gene mutations such as those seen in the *in vitro* studies. While the *in vivo* mammalian liver unscheduled DNA synthesis (OECD TG 486) test allows the investigation of genotoxic effects of substances in the liver and is indicative of DNA adduct removal by nucleotide excision repair in liver cells (EFSA, 2012). The *in vivo* mammalian liver unscheduled DNA synthesis (OECD TG 486) test is an indicator test for DNA damage and not a surrogate test for gene mutations per se, and its sensitivity has been questioned (EFSA, 2012). Both of the *in vitro* tests on the notified chemical were positive and looked at gene mutations, while the *in vivo* tests looked at DNA damage/genotoxic effects and not gene mutations. Therefore, the negative results in the *in vivo* studies do not necessarily negate the positive results in the *in vitro* studies and unless further evidence is provided that the notified chemical does not introduce gene mutation(s), the possibility of the chemical being a mutagen cannot be ruled out based on the above studies only. Additional evidence could be in the form of a study on the notified chemical or a suitable analogue, which specifically measures chemical mediated introduction of gene mutations. One such assay is the *in vivo* transgenic rodent somatic and germ cell gene mutation assay (OECD TG 488).

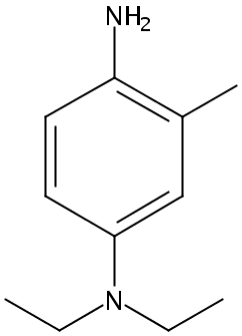
Additionally the notified chemical is an azo compound and may break down to its component amines. Azo bond reduction and cleavage occurs by an enzyme-mediated metabolism in the liver, skin and intestines. In the liver, metabolism is facilitated by cytosolic and microsomal enzymes (Platzek *et al*., 1999), including NADH cytochrome P450 reductase, NAD(P)H quinone oxidoreductase, and cytochrome P450s (OEHHA, 2012). Bacterial strains in human faeces have been shown to cleave azo dyes, suggesting the important role of the intestinal microflora in azo reduction (Platzek *et al*., 1999).

Although azo reduction occurs favourably in anaerobic conditions, several in vitro and in vivo studies indicated that this process could also occur aerobically when azo dyes are applied to the skin (SCCP, 2005). In vitro, the skin microflora of mouse, guinea pig and human caused reductive cleavage of the azo dyes, followed by percutaneous absorption (SCCNFP, 2002). In addition, non-biological processes, such thermal and photochemical degradation, have also been reported to break azo linkages (Engel *et al*., 2009).

The notified chemical may be metabolised or broken down by azo reduction to release the arylamines, 2-thiazolamine, 5-nitro- (CAS No. 121-66-4) and 1,4-benzenediamine, N^4,N^4-diethyl-2-methyl- (CAS No. 148-71-0).



2-Thiazolamine, 5-nitro- (CAS No. 121-66-4)



1,4-Benzenediamine, N^4,N^4-diethyl-2-methyl- (CAS No. 148-71-0)

Neither 2-thiazolamine, 5-nitro- (CAS No. 121-66-4) or 1,4-benzenediamine, N^4,N^4-diethyl-2-methyl- (CAS No. 148-71-0) are on the European Union (EU) Regulation on Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) list of 22 carcinogenic aromatic amines in Annex XVII Appendix 8 (European Commission, 2006).

Carcinogenicity studies have been conducted on 2-thiazolamine, 5-nitro- (CAS No. 121-66-4) in both rats (two studies) and mice (one study) (IARC, 1983). There was no evidence of carcinogenicity found in a 104 week feeding study with B6C3F1 mice (IARC, 1983). In one rat study there was increased incidence of benign mammary tumours, while in the second rat study there were increased incidences of malignant lymphomas, lymphocytic and undifferentiated leukaemias, and granulocytic leukaemias in male rats (IARC, 1983). Additionally 2-thiazolamine, 5-nitro- (CAS No. 121-66-4) was found to be mutagenic in *Salmonella typhimurium* strain TA100 both in the presence and absence of mutagenic activation (IARC, 1983). IARC concluded that "There is limited evidence for the carcinogenicity of 2-amino-5-nitrothiazole in experimental animals. In the absence of epidemiological data, no evaluation of the carcinogenicity of 2-amino-5-nitrothiazole to humans could be made" (IARC, 1983); IARC has given 2-thiazolamine, 5-nitro- (CAS No. 121-66-4) a Group 3 classification (IARC, 1987). In the study reports for the carcinogenicity studies the study authors "concluded that under the conditions of this bioassay, the occurrence of tumours of the hematopoietic system, i.e., lymphoma and granulocytic leukaemia, in dosed male Fischer 344 rats was associated with administration of 2-amino-5-nitrothiazole" (NIH, 1978). Recently, it has been recommended that further research be conducted on 2-thiazolamine, 5-nitro- (CAS No. 121-66-4) to determine the risks of using it in clothing textiles (Brüschweiler *et al*., 2014).

Overall, based on the mutagenic effects of the notified chemical *in vitro* that were not necessarily negated by negative *in vivo* studies the potential that the notified chemical is mutagenic and/or carcinogenic to humans cannot be ruled out.

Health hazard classification

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

Hazard classification	*Hazard statement*
Skin sensitisation (Category 1)	H317 – May cause an allergic skin reaction
Specific target organ toxicity (Category 2)	H373 – May cause damage to organs through prolonged or repeated use

Based on the available information, the notified chemical is recommended for hazard classification according to the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004), with the following risk phrase(s):

R43: May cause skin sensitisation by skin contact
R48/22: Harmful: danger of serious damage to health by prolonged exposure if swallowed

7.3. Human Health Risk Characterisation

7.3.1. Occupational Health and Safety

The notified chemical causes significant adverse effects following repeated exposure and has the potential to be mutagenic and/or carcinogenic to humans. In addition, based on analogue data it is expected to be a slight skin and eye irritant and a strong skin sensitiser. Therefore, control measures are required to mitigate possible adverse health effects to the workers who may come into contact with the notified chemical.

Workers most at risk of exposure to products containing the notified chemical at ≤ 2% concentration include printer operators and service technicians when adding the ink into the reservoir of the printing machine, when using a heat press to transfer the printed images to fabric, and during cleaning and maintenance of printing machine. The notifier anticipates that the use of PPE such as impervious gloves, coveralls and goggles, in addition to printers fitted with filters to capture any aerosols and local exhaust ventilation will minimise exposure.

Overall, provided that control measures are in place to minimise worker exposure to the notified chemical, including the use of PPE and well ventilated environments, the risk to the health of workers from use of the notified chemical for printing operations is not considered to be unreasonable.

7.3.2. Public Health

The ink formulations containing the notified chemical will not be sold to the public. However, the public will be repeatedly exposed to the notified chemical through the use of garments, sports ware, soft signage and promotional items.

Local effects/sensitisation

The notified chemical is expected to be a strong sensitiser based on analogue data from animal studies and human patch testing. The analogues were found to be sensitising in LLNA studies, with EC3 values down to 0.012% (Betts *et al*., 2005) and evidence of sensitisation at 0.003% (Ahuja *et al*., 2010), additionally analogue 1 was shown to be a sensitiser in guinea pigs at a challenge concentration of 0.001% (Hausen and Brandão, 1986). In the studies by Ahuja *et al*. (2010) and Hausen and Brandão (1986) effects were seen at the lowest concentrations tested and therefore these values should be considered to be the Lowest Observed Effect Level (LOEL) with the No Observed Effect Level (NOEL) unknown. In the LLNA study by Betts *et al*. (2005) on analogue 1 the concentration of the analogue on the skin when the EC3 value was reached was determined by the study authors to be approximately 16.4 μg/cm^2. The skin concentration value in the LLNA study by Betts *et al*. (2005) is based on a comparison with 2,4-dinitrochlorobenzene, however this could lead to an underestimation of the potency as the NOEL for 2,4-dinitrochlorobenzene was determined to be 1.4 μg/cm^2 (Kimber *et al*., 2005), while at a skin concentration of 0.75 μg/cm^2 sensitising effects were seen in the study by Ahuja *et al*. (2010). Calculating the skin concentration of analogue 1 at the EC3 concentration of 0.012% in the study by Betts *et al*. (2005) gives a result of 4.14 μg/cm^2.

Methods for the quantitative risk assessment of dermal sensitisation have been proposed and been the subject of significant discussion (see for example, Api *et al*., 2008 and RIVM, 2010). As a worst case scenario, the Consumer Exposure Level (CEL) is estimated to be the same as the peak migration rate of 0.5 μg/cm^2. Although the skin concentration at which sensitising effects were seen is lower in the LLNA study by Ahuja *et al*. (2010) no EC3 values were calculated in this study and hence the derivation of an Acceptable Exposure Level (AEL) was based on the LLNA study by Betts *et al*. (2005) (EC3 of 0.012% and skin concentration of 4.14 ug/cm2), which results in an AEL of 0.0146 μg/cm^2. In this instance, the safety factors employed included an interspecies factor (3), intraspecies factor (10), a matrix factor (1), a use and time factor (3.16) and a database factor of 3 (given data on an analogue, with significant uncertainty), giving an overall safety factor of > 284. A matrix factor of 1 was selected because the notified chemical will not be part of a cosmetic product or other mixture that could enhance skin penetration.

As the CEL > AEL, the risk to the public of the induction of sensitisation that is associated with the use of the notified chemical in clothing textiles would generally be considered to be unreasonable. Comparing this CEL to

the skin concentration of 0.75 μg/cm^2 where statistically significant sensitising effects were seen in the study by Ahuja *et al*. (2010) also suggests that there would essentially be no margin of exposure (MOE) with a peak migration rate of 0.5 μg/cm^2. However, in this instance, it is noted that the CEL is based on a maximum peak migration rate that is likely to be a considerable overestimate of the migration rate of dyes which have a good fastness properties, which is expected to be no more than 0.03 μg/cm^2. Additionally if the printed clothing textile is washed prior to use the potential peak migration rate is expected to be significantly reduced. However, even with a CEL of 0.03 μg/cm^2 the CEL > AEL and hence the risk to the public of the induction of sensitisation that is associated with the use of the notified chemical in clothing textiles would still be considered to be unreasonable.

In addition to the animal toxicity studies, patch testing in humans has shown that a significant proportion of the Australian public may already be sensitised to analogue 1 and analogue 2. Combined with the potential for cross sensitisation and the low concentrations at which allergic reactions have been seen in people there is a possibility that the use of the notified chemical in clothing textiles may generate allergic reactions in those members of the population that are already sensitised to analogue 1 and analogue 2.

It should be noted that due to the risk of sensitisation the German Federal Institute for Risk Assessment (BfR) made the following statement about analogues 1 and 2 "The use of these two highly potent sensitising disperse dyes in garment textiles is therefore inacceptable in the opinion of the BfR" (BfR, 2012). Kimber *et al*. (2005) stated that "Therefore, it is expected that under good fastness conditions induction of sensitization can essentially be precluded. Nevertheless, it is not recommended to use Disperse Blue 106 [analogue 1] on textiles with potential consumer exposure, especially not on substrates with limited fastness properties like polyamide or polyacetate.", this statement was based on a comparison with the NOEL for 2,4-dinitrochlorobenzene and hence may underestimate the risk. Additionally after conducting their LLNA studies Ahuja *et al*. (2010) came to the following conclusion "With regard to the disperse dyes tested, it is concluded that at least for Disperse Blue 106 [analogue 1] and Disperse Blue 124 [analogue 2] their use for clothing textiles is irresponsible".

In conclusion, the risk of sensitisation from the use of the notified chemical in clothing textiles is considered to be unreasonable based on the both CEL being > the AEL and the evidence that analogues have induced significant levels of sensitisation in the public under similar exposure scenarios.

If additional information were to become available on the notified chemical that showed it to be a significantly weaker sensitiser than either analogue 1 or analogue 2 such as an EC3 value for the notified chemical determined through an LLNA study and/or data on the expected migration rate of the notified chemical from textiles to skin then it may be possible to re-evaluate the risk to the public from the use of the notified chemical in clothing textiles.

The risk to the public of the induction of sensitisation that is associated with the use of the notified chemical in non-clothing articles such as soft signage and promotional items is not considered to be unreasonable due to the smaller potential contact area and shorter contact time, when compared to clothing textiles.

Systemic effects

The potential systemic exposure to the public from the use of the notified chemical in clothing textiles was estimated to be 83 ng/kg bw/wear event. Using a NOAEL of 15 mg/kg bw/day, the margin of exposure (MOE) was estimated to be 180,723. A MOE value greater than or equal to 100 is considered acceptable to account for intra- and inter-species differences, therefore, the MOE is considered to be acceptable. Additionally even if the expected absorption was increased to 100% this would result in a calculated systemic exposure of 8.3 μg/kg bw/wear event, which would still give an acceptable MOE of 1,807.

Mutagenicity/Genotoxicity/Carcinogenicity

The notified chemical has the potential to be mutagenic and/or carcinogenic to humans and therefore any use of the notified chemical where the potential for public exposure leading to systemic exposure should be avoided. The maximum systemic exposure to the notified chemical is expected to be 83 ng/kg bw/wear event, and although this value is low, in the absence of sufficient data to show otherwise such levels of exposure may still produce mutagenic and /or carcinogenic effects in humans. Therefore, the risk to the public from the use of the notified chemical in clothing textiles is considered to be unreasonable. Additional information on the notified chemical showing that it is not likely to cause gene mutations, as described in section 7.2 above, may be sufficient to re-evaluate the risk posed to the public from the use of the notified chemical in clothing textiles.

The risk to the public of mutagenic and/or carcinogenic effects that are associated with the use of the notified chemical in non-clothing articles such as soft signage and promotional items is not considered to be unreasonable due to the smaller potential contact area and shorter contact time, when compared to clothing textiles.

Public Health Assessment Conclusion.
The notified chemical is considered to pose an unreasonable risk to the public when used in clothing textiles, due to the expected strong sensitisation and the potential for mutagenic and/or carcinogenic effects.

The risk to the public from the use of the notified chemical in non-clothing articles such as soft signage and promotional items is not considered to be unreasonable.

8. ENVIRONMENTAL IMPLICATIONS

8.1. Environmental Exposure & Fate Assessment

8.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical will be imported as a component of finished inkjet printing ink formulations, and will not be reformulated or repackaged in Australia. Therefore, no environmental release is expected from manufacturing or reformulation in Australia.

Release of the notified chemical during transport and storage is expected to be limited to accidental spills or leaks, and residue in import packaging. Spills or accidental release of the products containing the notified chemical are expected to be contained and collected using absorbents, and disposed of to landfill in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE
The majority of the notified chemical is expected to be stable within an inert ink matrix on printed paper substrates once it is cured. Cured ink containing the notified chemical on paper substrates will then be heat transferred onto textiles, with no residue remaining on the paper. Once transferred, the ink containing the notified chemical is expected to be stable and predominantly remain fixed to the textile substrate.

Release of the notified chemical to the environment during use is expected to be limited to accidental spills or leaks, and from disposal of empty packaging containing residual printing ink. Accidental spills or leaks during use will be contained and collected using absorbents, and disposed of to landfill in accordance with local government regulations.

RELEASE OF CHEMICAL FROM DISPOSAL
The notified chemical will be used in inkjet printing inks to be printed onto paper, and subsequently transferred onto textiles. The notified chemical is therefore expected to share the fate of the printed textile articles, which are expected to be disposed of to landfill at the end of their useful life. It is estimated that a maximum of 2% (or $\leq$ 20 kg) of the notified chemical may remain in empty packaging. Empty packaging containing residues of the notified chemical are expected to be disposed of to landfill in accordance with local government regulations.

8.1.2. Environmental Fate
No environmental fate studies were submitted for the notified chemical. An estimate of the biodegradability of the notified chemical has been calculated using BIOWIN v4.10 (US EPA, 2011). Based on its molecular structure, the notified chemical is not expected to be readily biodegradable. However, the notified chemical is not expected to be bioaccumulative, based on its low water solubility and partition coefficient (log P_{OW} = 3.49). This is supported by the low bioconcentration factor (BCF = 52.34), calculated using BCFBAF v3.01 (US EPA, 2011).

The majority of the notified chemical in printing ink will be bound to the inert ink matrix which, once transferred onto textile substrates, is expected to have low mobility. At the end of their useful life, textile articles to which the notified chemical is bound are expected to be disposed of to landfill. In landfill, the notified chemical is expected to adsorb to soil and sediment, based on its low water solubility and high adsorption coefficient (log K_{OC} = 4.04). The majority of the notified chemical disposed of to landfill is expected to eventually degrade by biotic and abiotic processes to form water and oxides of carbon and nitrogen.

8.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has not been calculated for the notified chemical, since no significant release of the notified chemical to the aquatic environment is expected from the reported use pattern.

8.2. Environmental Effects Assessment

The results from ecotoxicological investigations conducted on the notified chemical are summarised in the table below. Details of these studies can be found in Appendix C.

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	96 h LL50 > 0.017 mg/L (WAF*)	Not harmful to fish up to limit of water solubility
Daphnia Toxicity	48 h EL50 > 0.0086 mg/L (WAF*)	Not harmful to *Daphnia* up to limit of water solubility
Algal Toxicity	72 h E_rL50 > 0.0029 mg/L (WAF*)	Not harmful to algae up to limit of water solubility
Inhibition of Bacterial Respiration	3 h IC50 > 1000 mg/L	Not inhibitory to bacterial respiration

* Water Accommodated Fraction

Based on the above ecotoxicological endpoints for the notified chemical, it is not considered to be harmful to fish, daphnids, and algae up to the limit of its solubility in water. Therefore, under the Globally Harmonised System of Classification and Labelling of Chemicals (GHS) (United Nations, 2009), the notified chemical is not formally classified for acute and chronic toxicities.

8.2.1. Predicted No-Effect Concentration

A predicted no-effect concentration (PNEC) for the aquatic compartment has not been calculated since the notified chemical is not considered to be harmful to aquatic organisms up to the limit of its solubility in water, and no significant release of the notified chemical to the aquatic environment is expected.

8.3. Environmental Risk Assessment

The Risk Quotient (Q = PEC/PNEC) of the notified chemical has not been calculated, since the neither the PEC nor PNEC are available, and due to its low potential for release to the aquatic compartment. The majority of the notified chemical will be printed onto paper then bound to textiles following heat transfer. After their useful life, the majority of the textiles containing the notified chemical are expected to be disposed of to landfill. In landfill, the notified chemical is expected to adsorb to soil and sediment, based on its low water solubility and high log K_{OC}. Release of the notified chemical to the aquatic compartment is unlikely based on the reported use pattern. On the basis of its limited aquatic exposure and assessed use pattern, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point/Freezing Point > 224 °C

Method EC Council Regulation No 440/2008 A.1 Melting/Freezing Temperature.
Remarks Differential scanning calorimetry method. Decomposition was observed with melting
Test Facility SPL (2000a)

Boiling Point > 225 °C

Method EC Council Regulation No 440/2008 A.2 Boiling Temperature.
Remarks Differential scanning calorimetry method. Peak decomposition was observed at 225 °C at 101.83 kPa, with the onset of decomposition at 188 °C.
Test Facility SPL (2000a)

Relative Density 1.38 at 20 °C

Method EC Council Regulation No 440/2008 A.3 Relative Density.
Remarks Gas comparison pycnometer method.
Test Facility SPL (2000a)

Vapour Pressure $< 4.7\times10^{-8}$ kPa at 25 °C

Method EC Council Regulation No 440/2008 A.4 Vapour Pressure.
Remarks Determined using a vapour pressure balance system.
Test Facility SPL (2000b)

Water Solubility $< 1 \times 10^{-4}$ g/L at 20 °C

Method EC Directive 92/69/EEC Method A6.
Remarks Column Elution Method
Test Facility SPL (2000a)

Hydrolysis as a Function of pH $t_{½}$ = 153 days at pH 7 at 25 °C

Method EC Directive 92/69/EEC Method C7.

pH	*T (°C)*	$t_{½}$
4	25	Not determined
7	25	153 days
9	25	Not determined

Remarks An initial test under accelerated conditions of 50 °C was first conducted at pH 4, 7, and 9. A secondary test was conducted under accelerated conditions of 40 °C at pH 4 and 7, and 25 °C at pH 9. A rate constant and half-life at pH 4 and 9 could not be determined after 190 hours. The half-life of the notified chemical at pH 7 was determined to be $t_{½}$ = 153 days.
Test Facility SPL (2000a)

Partition Coefficient (n-octanol/ water) log Pow = 3.49 at 20 °C

Method EC Directive 92/69/EEC Method A8.
Remarks HPLC Method
Test Facility SPL (2000a)

Adsorption/Desorption log K_{oc} = 4.04 at 20 °C

Method OECD TG 106 Estimation of the Adsorption Coefficient (K_{OC}) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC).
Remarks HPLC Method

Test Facility SPL (2000a)

Particle Size Inhalable fraction (< 100 μm): 5.6 %
Respirable fraction (< 10 μm): 6.81 %

Method Particle Size Distribution, Fibre Length and Diameter Distributions.

Range (μm)	*Mass (%)*
< 10	6.81
≥ 10 and < 100	5.60

Remarks The screening test was conducted using sieve method and the definitive test was conducted using cascade impactor method. The different methods account for the inhalable fraction being smaller than the respirable fraction.
Test Facility SPL (2000a)

Flammability Highly flammable

Method EC Council Regulation No 440/2008 A.10 Flammability (Solids).
Remarks The average time taken for propagation of combustion over 100 mm after an initial burning distance of 80 mm was 23 s. The mean moisture content was determined to be 0.180%.
Test Facility SPL (2000c)

Autoignition Temperature > 224 °C

Method EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids.
Remarks The test substance was determined not to have a relative autoignition temperature below its melting temperature.
Test Facility SPL(2000d)

Explosive Properties Not explosive

Method EC Council Regulation No 440/2008 A.14 Explosive Properties.
Remarks The test substance was tested using BAM fall hammer, BAM friction and Koenen steel tube test methods.
Test Facility SPL (2000d)

Oxidizing Properties Not oxidising

Method EC Council Regulation No 440/2008 A.17 Oxidizing Properties (Solids).
Remarks Positive result was obtained in standard test using cellulose. A confirmatory test conducted using celite showed the results of standard test to be false-positive due to combustion of the test material itself.
Test Facility SPL (2000d)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
Species/Strain	Rat/Crl:CD(SD) IGS BR
Vehicle	Arachis oil BP
Remarks - Method	No significant deviations from the OECD guideline.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	3 Female	2,000	0/3
2	3 Male	2,000	0/3

LD50	> 2,000 mg/kg bw
Signs of Toxicity	No signs were noted
Effects in Organs	No effects were noted
Remarks - Results	Dark purple coloured staining of the fur was noted in all female rats one day after dosing. No other clinical signs were noted during the study period. body weights and weight gains were no affected.
CONCLUSION	The notified chemical is of low toxicity via the oral route.
TEST FACILITY	SPL (2000e)

B.2. Acute toxicity – dermal

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity – Limit Test.
Species/Strain	Rat/Crl:CD(SD) IGS BR
Vehicle	Arachis oil
Type of dressing	Semi-occlusive.
Remarks - Method	No significant deviations from the OECD guideline.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 Female & 5 Male	2,000	0/10

LD50	> 2,000 mg/kg bw
Signs of Toxicity - Local	No erythema or oedema noted.
Signs of Toxicity - Systemic	No signs of toxicity were noted.
Effects in Organs	No effects were observed.
Remarks - Results	Staining was noted at the treatment sites of all animals one to six days after dosing. The body weights and weight gains were not affected during the study. No abnormalities were noted at necropsy.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	SPL (2000f)

B.3. Irritation – skin

TEST SUBSTANCE	Analogue 1

METHOD	Consumer Product Safety Commission of the U.S.A., Code of Federal Regulations, Title 16, Section 1500.41
Species/Strain	Rabbit/ strain not specified
Number of Animals	Six
Vehicle	Distilled water
Observation Period	72 hours
Type of Dressing	Semi-occlusive.
Remarks - Method	The test facility was not stated, the concentration of the material is unclear and the grading scale is not explained in the test report. Observations were only reported/taken at 24 hours and 72 hours. The test material was applied to both intact and abraded sites. Very little detail about the test methodology is provided in the report.

RESULTS

Lesion	*Mean Score**						*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3	4	5	6			
Erythema/Eschar	0	0	0.5	0.5	0	0	1	< 72 hours	0
Oedema	0	0	0	0	0.5	0	1	< 72 hours	0

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

Remarks - Results	Grade 1 erythema was seen in 2/6 animals at the 24 hour observation. Grade 1 oedema was seen in 1/6 animals at the 24 hour observation. No skin irritation in any animal was present at the 72 hour observation.
CONCLUSION	The test substance is slightly irritating to the skin.
TEST FACILITY	Unknown (1979a)

B.4. Irritation – eye (in vitro)

TEST SUBSTANCE	Notified chemical
METHOD	Rabbit Enucleated Eye Test (REET; study conducted in place of the OECD TG 405 Acute Eye Irritation/Corrosion test).
Observation Period	4 hours
Remarks - Method	Five enucleated rabbit eyes were excised and allowed to equilibrate for 30 mins in a Perspex clamp placed within a superfusion chamber. Saline solution was used to irrigate the surface of the cornea via a saline drip in the rear of the chamber. The eyes were re-examined after approximately 30 mins of equilibration to ensure that they had not been damaged during the excision. Any eyes with corneal swelling greater than 10% of the pre-enucleation measurement or stained with fluorescein were discarded. Following inspection, 3 eyes held by Perspex clamps were removed from the superfusion chamber and placed horizontally into a petri dish and 0.1 mL of the test substance was applied evenly to the surface of each of the cornea. After 10 seconds the test substance was rinsed off using a minimum 20 mL of saline solution. The remaining 2 eyes remained untreated (i.e. saline solution only) and served as negative controls. The thickness of the cornea was measured using an ultrasonic pachymeter under at pre-enucleation, post-equilibration and after 1, 2, 3 and 4 hours following treatment. For each enucleated eye a measurement was made at the optical centre, and at four other locations at the apex of the cornea and a mean value was calculated based on these measurements. The corneal thickness for each eye following treatment was used to calculate the

percentage change compared with the corneal thickness pre-treatment.

The condition of the corneal epithelium was assessed using a slit-lamp biomicroscope at 1, 2, 3 and 4 hours following treatment. Uptake of fluorescein by the corneal epithelium was assessed pre-enucleation, post-equilibration and approximately 4 hours following treatment using a cobalt blue filter of the split-lamp biomicroscope after application of fluorescein sodium drops.

RESULTS

Remarks - Results

No corneal effects were noted in the test eyes during the study period.

Mean corneal swelling of the test eyes increased by 0.7, 3.2 and 9.4% at 1, 2 and 4 hours respectively. Whereas the mean corneal swelling in the control eye was recorded as 0.6, 0.1 and 4.0% at 1, 2, and 4 hours respectively. According to the study author, the increase in the corneal swelling seen with the test eyes failed to reach statistical significance and was similar to historical control values.

The condition of the corneal epithelium of the test eyes and control eyes appeared normal during the study period.

No fluorescein uptake was noted in the test eyes or control eyes 4 hours following test substance application.

Collectively due to the comparable effects in test and control eyes, the study author considered the chemical unlikely to have the potential to cause severe ocular irritancy *in vivo*.

CONCLUSION

The notified chemical is non-irritating to the eye.

TEST FACILITY

SPL (2000g)

B.5. Irritation – eye

TEST SUBSTANCE

Analogue 1

METHOD

Consumer Product Safety Commission of the U.S.A., Code of Federal Regulations, Title 16, Section 1500.42.

Species/Strain: Rabbit/New Zealand White

Number of Animals: Six

Observation Period: 7 days

Remarks - Method: The test facility was not stated, and the concentration of the material is unclear. There was not a day 14 observation despite there being signs of irritation present at day 7. Very little detail about the test methodology is provided in the report.

RESULTS

Lesion	*Mean Score**						*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3	4	5	6			
Conjunctiva: redness	1	1.3	0.3	1	0.3	1.7	2	> 7 days	1
Conjunctiva: chemosis	1.7	1.7	0.6	1	1	2	2	< 7 days	0
Conjunctiva: discharge	0.7	1	0.3	0.7	0.7	2	3	< 7 days	0
Corneal opacity	0	0	0	0	0	0	0	-	0
Iridial inflammation	0	0	0	0	0	0	0	-	0

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

Remarks - Results	Although the corneal opacity was not assigned a value under the Draize grading scale used, dulling of the cornea was noted by the study authors. Conjunctival redness, chemosis and discharge was noted in all animals at the 24 hour observation. By the 7 day observation signs of irritation were present in only one animal, where minimal conjunctival redness was present.
CONCLUSION	The test material is slightly irritating to the eye.
TEST FACILITY	Unknown (1979b)

B.6. Skin sensitisation

TEST SUBSTANCE	Analogue 1
METHOD	OECD TG 406 Skin Sensitisation – Bühler Test.
Species/Strain	Guinea pig/Ibm:GOHI
PRELIMINARY STUDY	Maximum Non-irritating Concentration: topical: 50%
MAIN STUDY	
Number of Animals	Test Group: 20 Control Group: 10
Vehicle	PEG 400
Positive control	Not conducted in parallel with the test substance, but had been conducted previously in the test laboratory using α-Hexylcinnamaldehyde.
INDUCTION PHASE	Induction Concentration: topical: 50%
Signs of Irritation	None
CHALLENGE PHASE	
Challenge	topical: 50%
Remarks - Method	No significant deviations from the OECD guideline.

RESULTS

Animal	*Number of Animals Showing Skin Reactions with erythema score ≥ 1 after challenge*	
	24 h	*48 h*
Test Group	16/20	15/20
Control Group	0/10	0/10

Remarks - Results	Observation for erythema could not be conducted during the induction phase due to the dark-blue discoloration produced by the test substance. No oedema was observed during the induction and challenge phase in any of the test and control animals. At the 24 hour or 18 hour observations the remaining 4 or 5 animals respectively that had erythema readings less than grade 1 still had some form of slight patchy erythema (grade ±).
CONCLUSION	There was evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	RCC (1998)

B.7. Skin sensitisation

TEST SUBSTANCE	Analogue 1
METHOD	OECD TG 406 Skin Sensitisation – Magnusson and Kligman Maximisation Test.
Species/Strain	Guinea pig/ Albino Dunkin-Hartley
PRELIMINARY STUDY	Maximum Non-irritating Concentration: intradermal: 1% (w/v)

	topical: 75% (w/w)
MAIN STUDY	
Number of Animals	Test Group: 20 Control Group: 10
Vehicle	Distilled water
Positive control	Not conducted in parallel with the test substance.
INDUCTION PHASE	Induction Concentration: intradermal: 1% (w/v) topical: 75% (w/w)
Signs of Irritation	None reported
CHALLENGE PHASE	
Challenge	topical: 75% and 50% (w/w)
Remarks - Method	No significant deviations from the OECD guideline.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions with erythema score ≥ 1 after challenge*	
		24 h	*48 h*
Test Group	50%	14/20	12/20
	75%	0/20	0/20
Control Group	0	0/10	0/10

Remarks - Results	Dark blue coloured staining was observed in all test animals 1, 24 and 48 hour after topical application of the test substance during induction phase and this interfered with evaluation of erythema in 3 animals. Staining was also observed in challenge phase but did not interfere with scoring. Test sites with 75% test substance application did not show any signs of erythema whereas test sites with 50% test substance showed erythema suggesting reaction to test substance. The reason for not seeing any response at the higher concentration of 75% was attributed to the suitability of the test substance formulation for topical application. According to the study authors, the 75% formulation did not maintain very good skin contact and the results do not accurately reflect the sensitisation potential of the test material. Body weights and weight gains in test animals were comparable to control animals over the entire study period.
CONCLUSION	There was evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	SPL (1993)

B.8. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents.
Species/Strain	Rat/Crl:CD BR
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: none
Vehicle	Arachis oil BP
Remarks - Method	No significant deviations from the OECD guidelines.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
control	5 Female & 5 Male	0	0/10
low dose	5 Female & 5 Male	15	0/10
mid dose	5 Female & 5 Male	150	0/10
high dose	5 Female & 5 Male	1,000	0/10

Mortality and Time to Death

No deaths occurred during the course of the study.

Clinical Observations

Test animals exposed to high dose showed signs of toxicity including hunched posture, dehydration, emaciation, pilo-erection and tiptoe gait. Splayed gait, increased activity and ataxia were also observed to a lesser extent in male animals only. Convulsion of hind limbs was observed once in a test animal from the high dose group.

No clinical signs of toxicity were observed in the mid dose group.

One test animal from low dose group exhibited laboured respiration on one occasion. No other signs of toxicity were observed.

Animals from all treatment groups showed pink staining on the cage tray-liners and / or dark faeces due to the excretion of the test substance. This was considered normal by the study authors due to the colouring nature of the test substance.

Body weights on day 28 in male animals were decreased by 8.9%, 11.0% and 42.4% in the low, mid and high dose groups respectively. In female animals body weights on day 28 were decreased only in the high dose group (↓31.4%). Bodyweight gains in male animals showed statistically significant reductions in the mid dose group on weeks 3 and 4 and the high dose group across all the weeks. In female animals statistically significant reductions in bodyweight gain were only seen in the high dose group on weeks 1, 2 and 4. Food consumption was reduced for both sexes across all four weeks in the high dose groups (↓20-55% males; ↓33-42% females) and for males on week four (↓17%) in the mid dose group. The food efficiency ratio which is the ratio of body weight gain to dietary intake was also adversely affected with reductions evident in over the first three weeks of the study. Water consumption showed no differences between the control and treated animals.

Functional Observations

Test animals from the high dose group showed reduced functional performance as measured by fore- and hind-limb grip strength. The animals also exhibited an increase in startle reflex parameters suggesting impairment of sensory reactivity.

No signs of toxicity were observed in low and mid dose group animals.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Test animals from the high dose group showed reductions in haemoglobin, erythrocyte count, haematocrit, mean corpuscular volume and mean corpuscular haemoglobin indicative of anaemia. One male from mid dose was also found to be anaemic. The test animals also showed statistically significant reduction in plasma glucose levels. Female rats had reduced total plasma protein and albumin with the albumin/globulin ratio achieving a statistically significant reduction when compared to control. Increased plasma cholesterol in both sexes and increase in aspartate aminotransferase in male rats were also noted.

Male rats from the mid dose group showed a statistically significant reduction in plasma glucose levels.

Animals from all test groups showed statistically significant reductions in plasma bilirubin levels. No other changes were reported in the test animals from the low dose group.

Effects in Organs

Treatment related increases in relative weights of the liver and kidneys were noted with the effects attributed to the test substance by the study authors. In addition the relative thymus weight in female rats from high dose group was significantly reduced. All the test animals from high dose group and male rats from mid dose group also had increased relative brain weights. The increase in brain weight was attributed to the reduction in body

weight gain rather than a direct effect of the test substance by the study authors.

No adverse effects were observed in the low dose group of animals.

Histopathological changes

Histopathological changes attributed to test substance were observed in various organs in animals from the high dose and mid dose group. No test substance related histopathological changes were observed in animals from low dose group.

Spleen – a slight increase in the severity of haemosiderin pigment was observed in animals from high dose group.

Liver – treatment related centrilobular / generalized hepatocyte enlargement was observed in animals from mid and high dose groups.

Kidneys – tubular basophilia, cellular extrafoliation / pigmentation and accumulation of granular pigment in the tubular epithelial cells were seen in animals from high dose group. Tubular basophilia and vacuolation were also seen in female rats from mid dose group.

Thyroids – follicular cell hypertrophy and associated depletion of colloid were seen in test animals from high dose group.

Stomach – a mild degree of mucosal atrophy was observed in two male and one female rat from high dose group.

Thymus – atrophy was observed in animals from high dose group.

Bone marrow – treatment related myeloid atrophy was seen in animals from high dose group and one male from mid dose group.

Reproductive organs – atrophy of seminiferous tubules and an associated reduction in the spermatozoal content of the epididymides was observed in two male rats from high dose group. In addition, reduced secretory content of the prostate gland was observed in three males from high dose group. Four male rats from high dose group also had significantly reduced secretory content of seminal vesicles.

Remarks – Results

Significant bodyweight reductions and reductions in bodyweight gain in both sexes in the high dose group and males in the mid dose group along with the increases in the relative weights of the liver and kidneys, anaemic effects and a range of histopathological changes in the high and mid dose groups were observed. Collectively the data shows that the test substance had adverse effects at mid and high doses with systemic toxicity.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was established as 15 mg/kg bw/day in this study, based on adverse effects observed in the mid and high dose group test animals.

TEST FACILITY	SPL (2000h)

B.9. Genotoxicity – bacteria

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 471 Bacterial Reverse Mutation Test. Plate incorporation procedure
Species/Strain	*S. typhimurium*: TA1535, TA1537, TA98, TA100 *E. coli*: WP2uvrA$^-$
Metabolic Activation System	S9 fraction from phenobarbital/β-naphthoflavone induced rat liver
Concentration Range in Main Test	a) With metabolic activation: 5–5,000 µg/plate b) Without metabolic activation: 5–5,000 µg/plate
Vehicle	Dimethyl formamide
Remarks - Method	No significant deviations from the OECD guideline.

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 µg/plate	> 5,000 µg/plate	≥ 500 µg/plate	Positive
Test 2		> 5,000 µg/plate	≥ 500 µg/plate	Positive
Present				
Test 1	> 5,000 µg/plate	> 5,000 µg/plate	≥ 500 µg/plate	Positive
Test 2		> 5,000 µg/plate	≥ 500 µg/plate	Positive

Remarks - Results

A pink/brown colour was observed at and above 50 µg/plate but did not interfere with colony counting.

A clear dose dependent increase in the number of revertant colonies was observed for all tested *Salmonella typhimurium* strains both in the absence and presence of metabolic activation and the numbers reached statistical significance indicating the test substance to be mutagenic to tested bacterial strains. There was no statistically significant increase in the number of revertant colonies in the *Escherichia coli* strain.

The positive controls produced satisfactory responses, thus confirming the activity of S9-mix and the sensitivity of the bacterial strains. The spontaneous mutation rates for the negative controls were within historical values and considered to be acceptable by the study authors.

CONCLUSION

The notified chemical was mutagenic to bacteria under the conditions of the test.

TEST FACILITY

SPL (2000i)

B.10. Genotoxicity – in vitro

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 476 In vitro Mammalian Cell Gene Mutation Test.

Species/Strain: Mouse

Cell Type/Cell Line: Lymphoma L5178Y TK +/-

Metabolic Activation System: S9 fraction from phenobarbital/β-naphthoflavone induced rat liver

Vehicle: Acetone

Remarks - Method: No significant deviations from the OECD guideline.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	0, 50, 100, 200, 400, 600, 800	3 h	2 days	10-14 days
Test 2	0, 2, 4, 8, 16, 32, 64	24 h	2 days	10-14 days
Present				
Test 1	0, 50, 100, 200, 400, 600, 800	3 h	2 days	10-14 days
Test 2	0, 50, 100, 200, 400, 600, 800	3 h	2 days	10-14 days

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*		
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Genotoxic Effect*
Absent			
Test 1	> 800 µg	≥ 100 µg	Negative
Test 2	≥ 100 µg	≥ 32 µg	Negative

Present			
Test 1	≥ 400 µg	≥ 50 µg	Positive
Test 2		≥ 100 µg	Positive

Remarks - Results

The test substance induced a dose dependent increase in the mutant frequency with metabolic activation in both experiments. The increase was statistically significant and close to 2 fold at highest test concentration of 800 µg/plate in experiment 1 and greater than 2 fold starting from 100 µg/plate in experiment 2.

The increase in mutant frequency was partly due to small colony formation, suggesting clastogenic activity resulting in structural chromosomal damage.

CONCLUSION

The notified chemical was clastogenic to Mouse Lymphoma L5178Y TK +/- cells treated in vitro under the conditions of the test.

TEST FACILITY

SPL (2000j)

B.11. Genotoxicity – in vivo

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 474 Mammalian Erythrocyte Micronucleus Test.

Species/Strain

Mouse/Crl:CD-1^{TM}(ICR)BR

Vehicle

Arachis oil

Remarks - Method

No significant deviations from the OECD guideline. The sex, test doses and route of administration were chosen on the basis of a range-finding study. Male mice were selected for the study as there was no sex related difference in toxicity/response.

Group	*Route of Administration*	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control 1)	Oral	7 Male	0	24
II (low dose)	Intraperitoneal	7 Male	50	24
III (mid dose)	Intraperitoneal	7 Male	100	24
IV (high dose 1)	Intraperitoneal	7 Male	200	24
V (positive control, CP)	Oral	5 Male	50	24
VI (vehicle control 2)	Oral	7 Male	0	48
VII (high dose 2)	Intraperitoneal	7 Male	200	48

CP=cyclophosphamide

RESULTS

Doses Producing Toxicity

In the range finding study the test substance administered intraperitoneally at 500, 1,000 and 2,000 mg/kg bw resulted in the death of all animals within 48 hours of administration. When notified chemical was administered intraperitoneally at 360 mg/kg bw 2/2 male animals and 1/2 female animals died. Clinical signs of toxicity such as hunched posture, lethargy, laboured respiration, ataxia and ptosis were also observed in these animals. No deaths were reported for test animals exposed to 2,000 mg/kg bw orally however they showed signs of toxicity such as hunched posture and lethargy.

Clinical signs of toxicity including hunched posture, lethargy, ptosis and tiptoe gait were observed in animals dosed with the test substance at and above 100 mg/kg bw in both the 24 and 48 hour groups. A premature death was observed in group VII.

Genotoxic Effects

No statistically significant change in polychromatic erythrocyte to normochromatic erythrocyte (PCE/NCE) ratio was observed when

compared to vehicle only controls. However, the presence of premature death and clinical signs of toxicity were taken to indicate that systematic absorption had occurred.

Remarks - Results

No evidence of the test substance reaching the bone marrow, the site of action, was evident from the study.

The positive control gave a satisfactory response and the negative controls were comparable with historical data, confirming the validity of the test system.

CONCLUSION

The notified chemical was not clastogenic under the conditions of this in vivo bone marrow micronuclei test.

TEST FACILITY

SPL (2001a)

B.12. Genotoxicity – in vivo

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 486 Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo.

Species/Strain: Rat/Crl:CR(SD)IGS BR

Route of Administration: Intraperitoneal

Vehicle: Arachis oil

Remarks - Method: No significant deviations from the OECD guidelines. The sex and test doses were chosen on the basis of a range-finding study. Male mice were selected for the main study as there was no sex related difference in toxicity/response.

Experiment 1

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Perfusion Time (hours after dosing)*
I (vehicle control)	6 Male	0	16
II (low dose)	4 Male	53.3	16
III (high dose)	4 Male	160	16
IV (positive control)	4 Male	50	16

Positive control – 2-Acetylaminofluorene

Experiment 2

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Perfusion Time (hours after dosing)*
I (vehicle control)	6 Male	0	2
II (low dose)	4 Male	53.3	2
III (high dose)	4 Male	160	2
IV (positive control)	4 Male	40	2

Positive control – N,N'-Dimethylhydrazine dihydrochloride

RESULTS

Doses Producing Toxicity

In a preliminary range finding study, animals dosed with the test substance via the intraperitoneal route showed clinical signs of toxicity consistent with the maximum tolerated dose having effectively been achieved at a concentration of 160 and 200 mg/kg bw. Therefore the maximum tolerated dose of the test substance for use in the main test was set as 160 mg/kg bw.

In the main test clinical signs observed in animals dosed with 160 mg/kg bw included hunched posture, pilo-erection, staining around the snout and blue coloration of the extremities.

Genotoxic Effects

The test substance did not induce any marked or toxicologically

	significant increases in the incidence of cells undergoing unscheduled DNA synthesis in isolated rat hepatocytes following *in vivo* exposure for 2 hours or 16 hours. Both of the different positive controls induced a significant increase in the percentage of cells in repair confirming the sensitivity of the test system.
CONCLUSION	The notified chemical was not clastogenic under the conditions of this in vivo Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells.
TEST FACILITY	SPL (2004)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Ecotoxicological Investigations

C.2.1. Acute toxicity to fish

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test – Semi-static. EC Directive 92/69/EEC Method C1 Fish, Acute Toxicity Test.
Species	*Oncorhynchus mykiss* (rainbow trout)
Exposure Period	96 hours
Auxiliary Solvent	Acetone
Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	No significant deviation in protocol.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*					
Nominal	*Actual*		*3 h*	*6 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	Control	10	0	0	0	0	0	0
0.1	0.017	10	0	0	0	0	0	0

LL50	> 0.017 mg/L (WAF) at 96 hours.
NOEL	0.017 mg/L (WAF) at 96 hours.
Remarks – Results	All validity criteria for the test were satisfied. The test solutions were renewed every 24 hours during the 96 h test period. The 96 h LL50 and NOEL for fish were determined to be > 0.017 mg/L and 0.017 mg/L, respectively, based on measured concentrations.
CONCLUSION	Under the study conditions, the notified chemical is not considered to be toxic to fish up to the limit of its water solubility.
TEST FACILITY	SPL (2000k)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test – Static.
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	Acetone
Water Hardness	250 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks - Method	No significant deviation in protocol.

RESULTS

Concentration mg/L		*Number of D. magna*	*Cumulative Immobilised (%)*	
Nominal	*Actual*		*24 h*	*48 h*
Control	Control	40	0	0
0.1	0.0086	40	0	0

EL50	> 0.0086 mg/L at (WAF) 48 hours
NOEL	0.0086 mg/L at (WAF) 48 hours
Remarks - Results	All validity criteria for the test were satisfied. The test solutions were not renewed during the 48 h test period. The 48 h EL50 and NOEL for daphnids were determined to be > 0.0086 mg/L and 0.0086 mg/L,

respectively, based on measured concentrations.

CONCLUSION Under the study conditions, the notified chemical is not considered to be harmful to daphnids up to the limit of its water solubility.

TEST FACILITY SPL (2000l)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Freshwater Alga and Cyanobacteria, Growth Inhibition Test.

Species	*Scenedesmus subspicatus* (green alga)
Exposure Period	72 hours
Concentration Range	Nominal: 0.01-0.1 mg/L Actual: 0.0029-0.0695 mg/L
Auxiliary Solvent	Acetone
Water Hardness	Not reported
Analytical Monitoring	HPLC
Remarks - Method	No significant deviation in protocol.

RESULTS

Biomass		*Growth*	
E_bL50	*NOE_bL*	*E_rL50*	*NOE_rL*
mg/L at 72 h	*mg/L*	*mg/L at 72 h*	*mg/L*
> 0.0029	Not determined	> 0.0029	0.0029

Remarks - Results All validity criteria for the test were satisfied. The 72 h E_bL50 and E_rL50 were both determined to be > 0.0029 mg/L, based on measured concentrations. The 72 h NOE_rL was determined to be 0.0029 mg/L.

CONCLUSION Under the study conditions, the notified chemical is not considered to be harmful to algae up to the limit of its water solubility.

TEST FACILITY SPL (2000m)

C.2.4. Inhibition of microbial activity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 209 Activated Sludge, Respiration Inhibition Test.

Inoculum	Aerated activated sludge from a domestic wastewater treatment plant (Derbyshire, UK).
Exposure Period	3 hours
Concentration Range	Nominal: 100-1000 mg/L Actual: Not determined
Auxiliary Solvent	Dimethylformamide
Water Hardness	100 mg $CaCO_3$/L
Remarks – Method	No significant deviation in protocol. Chemical 3,5-dichlorophenol was used as the reference control. The respiration rate was determined by measurement of Biochemical Oxygen Demand during the test after 3 hours of exposure.

RESULTS

IC50	> 1000 mg/L at 3 hours
Remarks – Results	All validity criteria for the test were satisfied. No significant inhibition of respiration rates were observed at 1000 mg/L. The 3 h EC50 was determined to be > 1000 mg/L, based on nominal concentrations. The

	notified chemical is not considered to be inhibitory to sludge microbial activity.
CONCLUSION	The notified chemical is not inhibitory to microbial activity.
TEST FACILITY	SPL (2000n)

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