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February 2019

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, C_{13-15}-branched and linear alkyl esters

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

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

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Director
NICNAS

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SUMMARY

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

ASSESSMENT REFERENCE	APPLICANT(S)	CHEMICAL OR TRADE NAME	HAZARDOUS CHEMICAL	INTRODUCTION VOLUME	USE
STD/1675	DIC Australia Pty Ltd	Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, C_{13-15}-branched and linear alkyl esters	No	≤ 20 tonnes per annum	Component of industrial printing ink

CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard Classification
Based on the available information, the notified chemical is not 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 as a component of industrial printing ink at a maximum concentration of 5%, the notified chemical is not considered to pose an unreasonable risk to public health.

Environmental Risk Assessment
Based on the low hazard and reported use pattern, the notified chemical is not considered to pose an unreasonable risk to the environment.

Recommendations

CONTROL MEASURES

Occupational Health and Safety

- 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.

Emergency procedures

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

Disposal

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

Regulatory Obligations

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

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

(1) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a component of industrial printing 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.

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
DIC Australia Pty Ltd (ABN: 12 000 079 0550)
42 Sunmore Close
HEATHERTON VIC 3202

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

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

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Schedule data requirements are not varied.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Europe (2008)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
ANOX® 1315

CAS NUMBER
171090-93-0

CHEMICAL NAME
Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, C_{13-15}-branched and linear alkyl esters

OTHER NAMES
A mixture of: esters of C_{14}-C_{15} branched alcohols with 3,5-di-t-butyl-4-hydroxyphenyl propionic acid, C_{15} branched and linear alkyl 3,5-bis(1,1-dimethylethyl)-4-hydroxybenzenepropanoate, C_{13} branched and linear alkyl 3,5-bis(1,1-dimethylethyl)-4-hydroxybenzenepropanoate

Benzene propanoic acid, 3,5-bis (1,l-dimethyl ethyl) 4-hydroxy, isomeric mixture of tetradecyl and pentadecyl esters

3,5-Bis(1,1-dimethylethyl)-4-hydroxybenzenepropanoic acid, branched and linear alkyl($C_{13\ 15}$) esters

C_{13} branched and linear alkyl 3,5-bis(1,1-dimethylethyl)-4-hydroxybenzenepropanoate

C_{15} branched and linear alkyl 3,5-bis(1,1-dimethylethyl)-4-hydroxybenzenepropanoate

Reaction mass of: esters of C_{14}-C_{15} branched alcohols with 3,5-di-t-butyl-4-hydroxyphenyl propionic acid

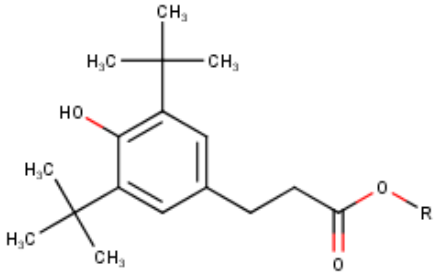
Reaction mass of: esters of C_{14}-C_{15} branched alcohols with 3,5-di-t-butyl-4-hydroxyphenyl propionic acid|C_{15} branched and linear alkyl 3,5-bis(1,1-dimethylethyl)-4-hydroxybenzenepropanoate|C_{13} branched and linear alkyl 3,5-bis(1,1-dimethylethyl)-4-hydroxybenzenepropanoate

MOLECULAR FORMULA
Unspecified

The notified chemical is a substance of Unknown, of Variable Composition, or of Biological Origin (UVCB)

The notifier provided the following:
$C_{30}H_{52}O_3$ – $C_{32}H_{56}O_3$

STRUCTURAL FORMULA
Representative structural formulae were provided by the notifier.



Where R = $C_{13–15}$ branched and linear alkyl chains

The tetradecyl (C_{14}) and pentadecyl (C_{15}) ester derivatives are the main components of the notified chemical. The typical alkyl chain length distribution is listed below.

R-group chain length	*Weight %*
Dodecyl (C_{12})	≤ 1
Tridecyl (C_{13})	1 – 5
Tetradecyl (C_{14})	50 – 60
Pentadecyl (C_{15})	35 – 45
Hexadecyl (C_{16})	≤ 0.2

MOLECULAR WEIGHT
460.74 – 488.79 g/mol

ANALYTICAL DATA
Reference NMR, IR, GCMS, UV spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
97.3%

HAZARDOUS IMPURITIES

Chemical Name Benzenepropanoic acid, 3,5-bis(1,1-dimethylethyl)-4-hydroxy-, methyl ester
CAS No. 6386-38-5 *Weight %* 2.66
Hazardous Properties Not listed on HCIS. Notifier supplied the following:
H411 (Toxic to aquatic life with long-lasting effects)

Chemical Name Alcohols, C_{14-16}
CAS No. 68333-80-2 *Weight %* < 1
Hazardous Properties Not listed on HCIS. ECHA website lists the following:
H400 (Very toxic to aquatic life)

NON HAZARDOUS IMPURITIES (> 1% BY WEIGHT)
None

ADDITIVES/ADJUVANTS
None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Yellow viscous liquid

Property	*Value*	*Data Source/Justification*
Glass Transition Temperature	-56.3 °C	Measured
Boiling Point	220 – 245 °C at 6.7×10^{-2} kPa	Measured
Density	939 kg/m^3 at 20 °C	Measured
Vapour Pressure	0.166 kPa at 20 °C	Measured
Water Solubility	0.33 mg/L at 25 °C	Measured
Fat solubility	79.35%	Measured
Hydrolysis as a Function of pH	Not determined	Contains hydrolysable ester functionality but is not expected to hydrolyse due to low water solubility
Partition Coefficient (n-octanol/water)	log P_{ow} = 3.56 at 25 °C	Measured; unlikely to bioaccumulate
Surface tension	62.05 mN/m at 20 °C (at concentration of 0.29 mg/L)	Measured
Adsorption/Desorption	log K_{oc} = > 5.0 at 20 °C	Measured
Dissociation Constant	Not determined	Contains phenolic functionalities, which can dissociate in the environmentally relevant pH range (4 – 9)
Flash Point	229 ± 1 °C	Measured
Flammability	Not determined	Not expected to be a flammable liquid based on the flash point
Autoignition Temperature	338 ± 2 °C	Measured
Thermal sensitivity	No explosion observed	Measured
Shock sensitivity	No explosion observed	Measured
Oxidising Properties	Not determined	Contains no functional groups that would imply oxidative properties

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

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

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

5. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. It will be imported as a component of finished industrial ink products (at concentrations of ≤ 5%) and local repackaging is not expected. Neat form of the notified chemical will not be imported and no local reformulation will occur in Australia.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	≤ 20	≤ 20	≤ 20	≤ 20	≤ 20

PORT OF ENTRY
Melbourne or Sydney

IDENTITY OF RECIPIENTS
DIC Australia

TRANSPORTATION AND PACKAGING
Typical packaging of finished ink products containing the notified chemical will include 200 kg drums and 1,000 kg bulk bags excluding intermediate bulk container (IBC) tankers. The ink products will be distributed by road for commercial sale.

USE
The notified chemical is a phenolic antioxidant used as a carrier at concentrations of ≤ 5% in printing inks for direct use in large scale industrial print presses.

OPERATION DESCRIPTION
Finished printing ink products containing the notified chemical at concentrations of ≤ 5% will be handled by workers. The ink product in 200 kg drums or 1,000 kg bulk bags will be transferred either via special drum pumps directly to industrial printing presses or by gravity into larger capacity (> 1,000 kg) bulk tanks for further processes.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	2 – 4	150
Repackaging	4 – 8	200
Service technicians	8	200
Office	8	200

EXPOSURE DETAILS

Transport and Storage
Transport and storage workers will handle the notified chemical at concentrations of ≤ 5% in sealed bulk containers. Dermal or ocular exposure to the notified chemical may occur in the unlikely event of an accident when the containers are breached.

End Use
Workers may come into contact with printing ink products containing the notified chemical at concentrations of ≤ 5%. Dermal or ocular exposure of workers to the notified chemical may occur during the transfer of printing inks from original containers into industrial printer presses or into larger ink tanks (> 1,000 kg). Dermal or ocular exposure is also possible during cleaning or maintaining of the printers, or in the unlikely event of printer ink leaks. According to the notifier, exposure is likely to be reduced by the use of automated processes and appropriate PPE including safety glasses, impervious gloves and coveralls.

In addition, dermal exposure to the notified chemical may occur when workers handle printed pages before the ink dries or if ink-stained parts of printers are touched. Exposure to the notified chemical will be reduced once the ink dries, as the notified chemical will be bound to the matrix of the substrates and is not expected to be available for further exposure.

Inhalation exposure to the notified chemical is not expected, unless ink aerosols are formed during printer operations.

6.1.2. Public Exposure
The printing ink products containing the notified chemical will not be sold to the general public for home and office use. Therefore, direct public exposure to the notified chemical is unlikely to occur.

Members of the public may come into contact with printed materials. However, once the ink dries, the notified chemical will be bound to the matrix of the substrates and is not expected to be available for exposure.

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 B.

Endpoint	*Result and Assessment Conclusion*
Acute oral toxicity – rat	LD50 > 5,000 mg/kg bw; low toxicity
Acute dermal toxicity – rat	LD50 > 2,000 mg/kg bw; low toxicity
Acute inhalation toxicity – rat	LC50 > 7.53 mg/L/4 hour; low toxicity
Skin irritation – rabbit	slightly-irritating
Eye irritation – rabbit	slightly-irritating
Skin sensitisation – guinea pig, Buhler test	no evidence of sensitisation
Repeat dose oral toxicity – rat, 28 days	NOEL [a] = 10 mg/kg bw/day (NOAEL [b] = 100 mg/kg bw/day)
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – *in vitro* chromosome aberration Test	non clastogenic
Genotoxicity – *in vitro* mammalian cell gene mutation test	non mutagenic
Genotoxicity – *in vivo* mouse micronucleus test	non genotoxic
Reproductive and developmental toxicity – rat	NOEL [a] (parental, F0) = 10 mg/kg bw/day (NOAEL [b] = 50 mg/kg bw/day) NOEL [a] (first filial generation, F1) = 50 mg/kg bw/day (NOAEL [b] > 50 mg/kg bw/day but < 1,000 mg/kg bw/day)

[a] No observed effect level (NOEL)
[b] No observed adverse effect level (NOAEL)

Toxicokinetics, Metabolism and Distribution

No toxicokinetic data on the notified chemical were submitted. Given the low molecular weight (< 500 g/mol) of the notified chemical and a log P_{ow} of 3.56, absorption across biological membranes is likely to occur.

Acute Toxicity

The notified chemical is of low acute oral, dermal and inhalation toxicity based on studies conducted in rats.

In the acute inhalation toxicity study, 4 hour exposure to a gravimetric aerosol concentration of 7.53 mg/L of the notified chemical resulted in unkempt appearance in the test animals. One animal appeared subdued and showed hunched posture for 24 hours.

Irritation and Sensitisation

Based on results from eye and skin irritation studies conducted in rabbits, the notified chemical was considered to be slightly-irritating. Minor irritation effects observed one hour after exposure in the animals included slight erythema (grade 1) of the skin and slight conjunctival redness (grade 1) of the eyes. The notified chemical was not classified as an irritant under GHS.

No evidence of sensitisation for the notified chemical was observed in a Buehler test conducted in guinea pigs.

Repeated Dose Toxicity

In a 28 day repeated dose oral toxicity study, the notified chemical was administered to rats at dosages of 10, 100 and 1,000 mg/kg bw/day. Treatment related increased liver weight and decreased leucocyte count were observed in the mid and high dose group rats. Statistically significant increase in liver weights in male rats in the high dose group (116% of absolute organ weight compared to control group) were reported while the increases in female rats were slight. The increase in liver weights was associated with dose-related hepatic centrilobular hypertrophy. However, no statistically significant changes in the liver enzyme (alkaline phosphatase) were noted. The effects on the liver were fully reversed in the recovery group treated at high dose after 28 days without treatment indicating it as an adaptive response to the treatment.

A decrease in leucocyte number was observed in all treated males and in females in the high dose group. On discontinuation of treatment, the leucocyte number in recovery animals treated at high dose was still reduced compared to the controls. This change reached statistical significance in females.

The study authors concluded that the above liver and leucocyte effects were likely to be adaptive.

Based on the reported treatment related effects in rats in the mid and high dose groups, the study authors considered the no observed effect level (NOEL) to be 10 mg/kg bw/day. The no observed adverse effect level (NOAEL) could be considered as 100 mg/kg bw/day (or higher) based on liver weights and decreased leucocytes in recovery group females at 1,000 mg/kg bw/day.

Mutagenicity/Genotoxicity
The notified chemical was determined to be not mutagenic to the limit of the water solubility in an *in vitro* bacterial reverse mutation assay. The notified chemical was not considered to be clastogenic in an *in vitro* chromosome aberration test using Chinese hamster ovary cells. In an *in vitro* mammalian cell gene mutation test using mouse lymphoma cells, although limited equivocal results were seen in one of the test cultures without metabolic activation, the notified chemical was not considered to be mutagenic as the results were negative in a repeated test. The study authors of an *in vivo* mammalian micronucleus test conducted in rats concluded that the notified chemical was not genotoxic. However, there was no evidence recorded to support that the notified chemical reached the bone marrow of the treated rats.

Reproductive Toxicity
In a one-generation reproductive toxicity study, the notified chemical was administered by oral gavage doses of 10, 50 and 1,000 mg/kg bw/day to male rats during the pre-mating and mating periods, and to female rats during the pre-mating, mating, gestation and lactation periods. One female in the high dose group died due to difficult parturition. No other treatment related mortality or clinical signs were noted during the study.

At the high dose, the study authors noted maternal toxicity, including an increase (not statistically significant) of early resorptions associated with a decrease of live foetuses and an increase of still births. There was no clear indication as to whether these effects were caused by secondary non-specific consequences of systemic toxicity. However, no effects on postnatal survival and development of the first filial generation (F1) live pups were noted at any dose. No pathological changes were observed at the autopsy examinations on the parents (F0) and the F1 pups.

Decreases in mean daily food consumption and mean body weight gain were noted in F0 females in the mid and high dose groups. There were no treatment related effects reported in treated F0 males.

The study authors reported a NOEL of 10 mg/kg bw/day for F0 generation based on reduced weight gain in females at 50 mg/kg bw/day. The NOAEL could be 50 mg/kg bw/day as there were no adverse signs reported at this dose, other than reduced body weight gain due to reduced food consumption. The NOEL for developmental toxicity in F1 pups was reported to be 50 mg/kg bw/day due to developmental effects observed at 1,000 mg/kg bw/day. The NOAEL for developmental toxicity could be > 50 and < 1,000 mg/kg bw/day.

Health Hazard Classification
Based on the available information, the notified chemical is not 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
The notified chemical is not considered to be hazardous based on the information provided, except at very high doses (for instance, at 1,000 mg/kg bw/day). It will only be imported in printing inks at concentrations of ≤ 5%. Therefore repeated or prolonged exposure to high concentrations is unlikely based on the assessed use pattern. Safe work practices, engineering controls and use of personal protective equipment (PPE) are expected to minimise exposure to the notified chemical.

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

6.3.2. Public Health
Direct public exposure to the notified chemical is unlikely to occur as the printing ink products containing the notified chemical will not be sold to the general public for home and office use. Members of the public may come into contact with materials printed with ink containing the notified chemical; however, once the ink dries,

the notified chemical will be bound to the matrix of the substrates and is not expected to be available for exposure.

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

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE
The notified chemical is not manufactured or reformulated in Australia; therefore no environmental release is expected from this category. Accidental spills of the notified chemical during import, transport or storage are expected to be adsorbed onto a suitable material and collected for disposal, in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE
The notified chemical will be used within ink products which will be bound to substrates once dried. The release of the notified chemical may occur from leakage of ink during use, installation or replacement of ink containers. Any releases are expected to be adsorbed onto a suitable material and collected for disposal, in accordance with local government regulations.

RELEASE OF CHEMICAL FROM DISPOSAL
Bulk containers are expected to contain residue which accounts for approximately 2% of the import volume which will be disposed of in accordance with government regulations during the cleaning and recycling of bulk packaging.

Most of the notified chemical is expected to share the fate of the printed substrates to which it has been applied, either subjected to substrate recycling processes, or being disposed of to landfill at the end of their useful lives. As estimated by the notifier, printing on paper accounts for most of the import volume of the notified chemical. A recent Australian waste report states an average paper recycling rate of 60% (Blue Environment Ltd., 2016). In the worst case scenario, up to 60% of the import volume of the notified chemical could be released to the aquatic environment from paper recycling processes.

7.1.2. Environmental Fate

As a result of its use pattern, most of the notified chemical is expected to share the fate of the substrates to which it has been applied, either subjected to substrate recycling processes, or being disposed of to landfill at the end of their useful lives. Waste plastic items may be recycled, but eventually plastic items containing the notified chemical will be disposed of to landfill. In landfill, the notified chemical will be present as cured solids and will be neither bioavailable nor mobile.

During paper recycling processes, waste paper is repulped using a variety of chemical treatments which, amongst other things, enhance ink detachment from the fibres. Waste water from paper recycling processes containing the notified chemical is expected to be treated at an onsite wastewater treatment plant before potential release to sewers or surface waters.

Based on the log P_{ow} and its low water solubility, the notified chemical is expected to associate with the sludge in the wastewater treatment plant. The waste sludge containing the notified chemical will be sent to landfill for disposal or to agricultural land for remediation. The notified chemical is expected to bind to soil or sludge based on its predicted high log K_{oc} and low solubility in water. In landfill, soil, sludge and water, the notified chemical is inherently biodegradable according to manometric respirometry studies (> 30% degradation after 28 days) and is expected to eventually degrade via biotic and abiotic processes to form water and oxides of carbon.

The notified chemical is not expected to bioaccumulate based on a bioaccumulation factor (BCF) estimate of 7.7 – 23.3 L/kg wet-weight calculated using the log P_{ow} value (log P_{ow} = 3.56) in QSAR modelling (US EPA On-Line EPI Suite™ v4.11 model BCFBAF v3.01).

Further study details are located in Appendix C.

7.1.3. Predicted Environmental Concentration (PEC)

The worst-case predicted environmental concentration (PEC) has been calculated to assume 100% of the import volume of the notified chemical will be used on paper and cardboard substrates and 60% of this would be potentially released to sewers through paper recycling processes. As paper recycling occurs at facilities located throughout Australia, it is anticipated that such releases will occur over 260 working days per annum into the Australian effluent volume. It is also assumed under the worst-case scenario that there is no removal of the notified chemical during wastewater treatment processes.

Predicted Environmental Concentration (PEC) for the Aquatic Compartment		
Total Annual Import/Manufactured Volume	20,000	kg/year
Proportion expected to be released to sewer	60%	
Annual quantity of chemical released to sewer	12,000	kg/year
Days per year where release occurs	260	days/year
Daily chemical release:	46.15	kg/day
Water use	200	L/person/day
Population of Australia (Millions)	24.386	million
Removal within STP	0%	
Daily effluent production:	4,877	ML
Dilution Factor - River	1.0	
Dilution Factor - Ocean	10.0	
PEC - River:	9.46	µg/L
PEC - Ocean:	0.95	µg/L

The predicted concentration of the notified chemical in soils was calculated using worst-case SimpleTreat STP modelling (Struijs et al. 1991) which assumes a 92% removal rate during sewage treatment, based on the physical and chemical properties of the notified chemical. Partitioning to biosolids in STPs Australia-wide may result in an average biosolids concentration of 15.141 mg/kg (dry weight). Biosolids are applied to agricultural soils, with an assumed average rate of 10 t/ha/year. Assuming a soil bulk density of 1,500 kg/m^3 and a soil-mixing zone of 10 cm, the concentration of the notified chemical may approximate 0.101 mg/kg in applied soil. This assumes that degradation of the notified chemical occurs in the soil within 1 year from application. Assuming accumulation of the notified chemical in soil for 5 and 10 years under repeated biosolids application, the concentration of notified chemical in the applied soil in 5 and 10 years may approximate 0.505 mg/kg and 1.01 mg/kg, respectively.

7.2. 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 (acute)	Not determined at limit of water solubility (0.19 mg/L)	Not acutely toxic to fish to the limit of water solubility
Fish toxicity (chronic)	Not determined at limit of water solubility	Not chronically toxic to fish
Daphnia Toxicity	Not determined at limit of water solubility	Not acutely toxic to invertebrates
Algal Toxicity	Not determined at limit of water solubility (0.17 mg/L)	Not acutely toxic to algae
Inhibition of Bacterial Respiration	EC50 > 100 mg/L	Not likely to be inhibitory to microbial activity
Acute earthworm toxicity	> 1,000 mg/kg (dry soil)	Not toxic to earthworms

Based on the above ecotoxicological endpoints for the notified chemical, it is not expected to be harmful to aquatic organisms. Therefore, the notified chemical is not formally classified under the *Globally Harmonised System of Classification and Labelling of Chemicals (GHS)* for acute and chronic toxicities (United Nations, 2009).

7.2.1. Predicted No-Effect Concentration

The Predicted No-Effect Concentration was not calculated as the notified chemical is not toxic at the limit of water solubility.

7.3. Environmental Risk Assessment

On the basis of no toxicity at the limit of water solubility, the notified chemical is not considered to pose an unreasonable risk to the aquatic environment. In addition, on the basis of low toxicity to earthworms, the notified chemical is not considered to pose unreasonable risk to the soil environments.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Glass Transition Temperature -56.3 °C

Method	Differential thermal analysis
Remarks	An accurately measured quantity of the notified chemical was placed in a container and the quantity of heat absorbed during glass transition was measured. The test was conducted in a nitrogen atmosphere.
Test Facility	EniChem (1992a)

Boiling Point 220 – 245 °C at 67 Pa

Method	Low pressure distillation with quantitative determination of the distillate
Remarks	The notified chemical was heated at a vacuum of 133 Pa. The temperature in the heater was increased up to 200 °C and the vacuum was further lowered to 67 Pa. The temperature was then increased by 2 °C/min until the boiling temperature for the sample was reached. Fractions were collected and quantified.
Test Facility	EniChem (1992b)

Density $D^{20}_4 = 939$ kg/m^3

Method	Similar to EC Council Regulation No 440/2008 A.3 Relative Density
Remarks	Measurement of relative density using a Anton Paar K.G. DMA 46 digital micro-densimeter
Test Facility	EniChem (1992c)

Vapour Pressure 1.663×10^{-1} kPa at 20 °C

Method	EEC Guideline N. L. 251 part A: Method A4
Remarks	The static method was used.
Test Facility	Istituto Guido Donegani (1992a)

Water Solubility 0.33 mg/L at 25 °C

Method	Similar to OECD TG 105 Water Solubility
Remarks	Column Elution Method
Test Facility	EniChem (1992d)

Fat (or n-Octanol) Solubility 79.35%

Method	EEC Guideline N. L. 251 part A: Method A7
Remarks	Analytical Method: the test substance and the standard fat (Natec HB) were mixed in various ratios (16 – 79% concentration of test substance in fat) and the solubility estimated by checking the presence of either one or two phases.
Test Facility	Istituto Guido Donegani (1992a)

Partition Coefficient (n-octanol/water) $\log P_{ow} = 3.56$ at 25 °C

Method	Analytical Method: Three water/n-octanol ratios were saturated with the test substance at 25 °C. The water/n-octanol ratios used were 20/1, 10/1 and 10/2.
Remarks	HPLC Method
Test Facility	EniChem (1992e)

Surface Tension 62.05 mN/m at 20 ± 0.5 °C

Method	EEC Guideline N. L. 251 part A: Method A5
Remarks	Concentration: 0.29 mg/L
Test Facility	Istituto Guido Donegani (1992b)

Adsorption/Desorption – screening test

log K_{oc} = 5.0 at 20 °C

Method	OECD TG 121 Estimation of the Adsorption Coefficient (Koc) on Soil and on Sewage Sludge using High Performance Liquid Chromatography (HPLC)
Remarks	Dead time t_0 was determined using thiourea and methanol/ 0.1M citrate-buffer mobile phase was used.
Test Facility	Istituto di Ricerche Biomediche (1997a)

Flash Point 229 ± 1 °C

Method	EEC Guideline N. L. 251 part A: Method A9
Remarks	SetaFlash Closed Cup tester was used.
Test Facility	Istituto Guido Donegani (1992a)

Autoignition Temperature 338 ± 2 °C

Method	EEC Guideline N. L. 251 part A: Method A15
Remarks	The notified chemical was injected into a uniformly heated 500 mL glass flask containing air at a predetermined temperature to observe the lowest temperature at which autoignition occurs.
Test Facility	Istituto Guido Donegani (1992a)

Thermal sensitivity No explosion observed

Method	EEC Guideline N. L. 251 part A: Method A14
Remarks	The test substance was heated in a steel tube with nozzle-plates of different diameters of orifice that provide various degree of confinement to determine whether the test substance is liable to explode under thermal stress.
Test Facility	Istituto Guido Donegani (1992a)

Shock sensitivity No explosion observed

Method	EEC Guideline N. L. 251 part A: Method A14
Remarks	The test substance after drying in a $CaCl_2$ desiccator was placed in a standard holder (die device) and subjected to the shock of a falling hammer on a steel anvil. A drop-hammer of 10 kg was dropped on samples from a height of 0.4 m.
Test Facility	Istituto Guido Donegani (1992a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute Oral Toxicity – Rat

TEST SUBSTANCE	Notified chemical
METHOD	European Economic Community Guidelines - VI Amendment, Annex V, Directive 84/449/EEC
Species/Strain	Rat/Sprague Dawley Crl:CD (SD) BR
Vehicle	0.5% methylcellulose (400 cP) in water
Remarks – Method	Test substance was administered once by oral gavage. The post-treatment observation period was 14 days. Stability and concentration analysis of the test substance in the vehicle was not conducted.

RESULTS

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

LD50	> 5,000 mg/kg bw
Signs of Toxicity	Piloerection was observed 2 hours after administration of the test substance in 2 male and 3 female animals. Recovery of all the treated animals occurred 4 hours after treatment.
Effects in Organs	The mean body weight of all animals increased within the normal range throughout the study period. There were no macroscopic pathological findings in the animals sacrificed at the end of the observation period.
Remarks – Results	No mortalities occurred
CONCLUSION	The notified chemical is of low acute toxicity via the oral route.
TEST FACILITY	Istituto di Ricerche Biomediche (1991a)

B.2. Acute Dermal Toxicity – Rat

TEST SUBSTANCE	Notified chemical
METHOD	European Economic Community Guidelines - VI Amendment, Annex V, Directive 84/449/EEC
Species/Strain	Rat/Sprague Dawley Crl:CD (SD) BR
Vehicle	None
Type of dressing	Semi-occlusive
Remarks – Method	Test substance used as supplied and a single dose was applied uniformly to the skin. The post-treatment observation period was 14 days.

RESULTS

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

LD50	> 2,000 mg/kg bw
Signs of Toxicity – Local	No signs of local toxicity were observed
Signs of Toxicity – Systemic	No signs of systemic toxicity were observed
Effects in Organs	The mean body weight of all animals increased within the normal range throughout the study period.

	There were no macroscopic pathological findings in the animals sacrificed at the end of the observation period.
Remarks – Results	No mortality or clinical signs of toxicity in animals treated with the test substance were observed.
CONCLUSION	The notified chemical is of low acute toxicity via the dermal route.
TEST FACILITY	Istituto di Ricerche Biomediche (1991b)

B.3. Acute Inhalation Toxicity – Rat

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 403 Acute Inhalation Toxicity – Limit Test
Species/Strain	Sprague-Dawley rats
Vehicle	None
Method of Exposure	Oro-nasal exposure
Exposure Period	4 hours
Physical Form	Liquid aerosol
Particle Size	< 3.5 μm (94% of test aerosol particles)
Remarks – Method	Nominal calculation assumed that relative test substance density equals 1.

RESULTS

Group	*Number and Sex of Animals*	*Concentration (mg/L)*		*Mortality*
		Nominal	*Actual*	
1	10 (5 F/5 M)	87.85	4.07	0/10
2	10 (5 F/5 M)	70.14	7.53	0/10

LC50 (4 hours)	> 7.53 mg/L
Signs of Toxicity	An unkempt appearance was noted for all Group 1 animals immediately after exposure to the notified chemical. One animal in Group 2 appeared subdued and showed hunched posture for 24 hours after exposure.
Effects in Organs	Slightly mottled lungs in all but 2 animals in Group 2 were observed. Pale and discoloured lungs were noted in 1 female and 1 male in Group 2. The study authors deemed these changes to be in accordance with normal background findings in acute rat studies at the test facility and not attributable to the test substance. There were no other macroscopic pathological findings in the animals sacrificed at the end of the observation period.
Remarks – Results	No mortalities occurred
CONCLUSION	The notified chemical is of low acute toxicity via inhalation.
TEST FACILITY	IRI (1991)

B.4. Skin Irritation – Rabbit

TEST SUBSTANCE	Notified chemical
METHOD	European Economic Community Guidelines - VI Amendment, Annex V, Directive 84/449/EEC
Species/Strain	Rabbit/New Zealand White
Number of Animals	3
Vehicle	None
Observation Period	72 hours
Type of Dressing	Occlusive
Remarks – Method	Test substance was used undiluted

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	1	< 24 h	0
Oedema	0	0	0	0	–	0

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

Remarks – Results: Slight erythema (grade 1) was observed in all treated animals 1 hour after patch removal.

No other dermal reactions were noted at the 24 – 72 hour observations in any animal.

CONCLUSION: The notified chemical is slightly-irritating to the skin.

TEST FACILITY: Istituto di Ricerche Biomediche (1991c)

B.5. Eye Irritation – Rabbit

TEST SUBSTANCE: Notified chemical

METHOD: European Economic Community Guidelines-VI Amendment, Annex V, Directive 84/449/EEC

Species/Strain: Rabbit/New Zealand White
Number of Animals: 3
Observation Period: 72 hours
Remarks – Method: Test substance was used undiluted and as supplied

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva – Redness	0	0	0	1	< 24 h	0
Conjunctiva – Chemosis	0	0	0	0	–	0
Conjunctiva – Discharge	0	0	0	0	–	0
Corneal Opacity	0	0	0	0	–	0
Iridial Inflammation	0	0	0	0	–	0

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

Remarks – Results: No clinical signs of toxicity were noted in the treated animals.

Locally induced slight conjunctival redness (grade 1) was observed in all animals 1 hour after administration of the test substance.

No other ocular reactions were noted at the 24 – 72 hour observations in any animal.

No evidence of epithelial defects were noted in any of the treated animals.

CONCLUSION: The notified chemical is slightly-irritating to the eye.

TEST FACILITY: Istituto di Ricerche Biomediche (1991d)

B.6. Skin Sensitisation – Guinea Pig, Buhler test

TEST SUBSTANCE: Notified chemical

METHOD	OECD TG 406 Skin Sensitisation – Buhler test (1981)
Species/Strain	Guinea pig (male)/Dunkin Hartley albino
PRELIMINARY STUDY	Maximum non-irritating concentration: Topical: 100%
MAIN STUDY	
Number of Animals	Test Group: 10 Control Group: 10
Vehicle	None
Positive Control	Not conducted in parallel with the test substance
INDUCTION PHASE	Induction concentration: Topical: 100%
Signs of Irritation	No signs of irritation observed in any treated animals
CHALLENGE PHASE	
1st Challenge	Topical: 100%
Remarks – Method	Test substance was used undiluted and applied topically by occlusive patch.

RESULTS

Animal	*Challenge Concentration*	*Number of Animals Showing Skin Reactions after Challenge*	
		24 h	*48 h*
Test Group	100%	0/10	0/10
Control Group	0%	0/10	0/10

Remarks – Results	No animal treated with the test substance showed positive reactions during the challenge phase.
CONCLUSION	There was no evidence of reactions indicative of skin sensitisation to the notified chemical under the conditions of the test.
TEST FACILITY	Istituto di Ricerche Biomediche (1992a)

B.7. Repeat Dose Oral Toxicity – Rat

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents (1981) EEC Guidelines (EEC Directive 84/449–Annex 5 to EEC Directive 79/831)
Species/Strain	Sprague Dawley Crl:CD (SD) BR
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 28 days (recovery)
Vehicle	Corn oil
Remarks – Method	Analyses of the stability and concentration of the formulated test substance were performed.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	10 (5 F/5 M)	0	0/10
Low Dose	10 (5 F/5 M)	10	1/10
Mid Dose	10 (5 F/5 M)	100	0/10
High Dose	10 (5 F/5 M)	1,000	1/10
Control Recovery*	10 (5 F/5 M)	0	0/10
High Dose Recovery*	10 (5 F/5 M)	1,000	0/10

* Control Recovery Group and High Dose Recovery Group were combined with Control Group and High Dose Group respectively in the treatment period.

Mortality and Time to Death

One female in the low dose group and one male in the high dose group died before the end of the treatment

period due to incorrect administration of the test substance into the lungs.

Clinical Observations

No clinical changes were noted in mid and low dose groups.

In the high dose group, episodes of salivation were observed after the administration in some rats of both sexes starting from the third week of the study till the end of the administration period.

One female in the high dose group had fur loss between the second and third week of treatment. This was considered by the study authors as incidental and unrelated to the treatment.

There were no reported treatment related changes on body weights or food consumption in the test animals.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

There were no treatment related adverse effects on clinical chemistry reported.

The study authors noted from an evaluation of the haematological data that there was a decrease in the leucocyte number in males in all treatment groups and in females in the high dose group. This change did not reach statistical significance during the treatment period. After 28 days recovery, the leucocyte number in treated animals of both sexes was still reduced in comparison to the control recovery group and reached statistical significance in females in the high dose recovery group.

A slight increase in the specific gravity of urine were reported in all treated males that reached statistical significance in the mid and high dose groups. Urinalysis revealed a slight increase in the frequency of urinary leucocytes in some males and females in the high dose group. This increase in urinary leucocytes, coincident with decrease in blood leucocytes, was still evident in the high dose recovery group after 28 days without treatment.

Effects in Organs

Statistically significant increase in mean absolute and relative liver weights in males in the high dose group (116% of absolute organ weight compared to control group) were reported. In females of the same group a slight increase in the liver weights was also noted. The increase in liver weights was associated with dose related hepatic centrilobular hypertrophy, indicative of hepatic functional changes. However, the study authors reported that there were no statistically significant changes in the liver enzyme alkaline phosphatase in either sex. Animals in the recovery groups were fully recovered at the end of the recovery period. The study authors considered the changes in the liver to be adaptive in origin.

Remarks – Results

Oral administration of the test substance to rats for a period of 28 consecutive days at dosages of 10, 100 and 1,000 mg/kg bw/day resulted in some treatment related effects as noted above. The study authors considered these changes likely to be adaptive. A no observed effect level (NOEL) was regarded to be 10 mg/kg bw/day for both sexes.

CONCLUSION

The study authors concluded that the NOEL was 10 mg/kg bw/day in the repeated dose oral toxicity study based on the liver and leucocyte effects observed at 50 mg/kg bw/day and above.

TEST FACILITY	Istituto di Ricerche Biomediche (1992b)

B.8. Genotoxicity – Bacteria

TEST SUBSTANCE	Notified chemical	
METHOD	OECD TG 471 Bacterial Reverse Mutation Test (1981) Plate incorporation procedure	
Species/Strain	*Salmonella typhimurium*: TA1535, TA1537, TA1538, TA98, TA100	
Metabolic Activation System	Aroclor 1254 induced rat liver S9 mix	
Concentration Range in Main Test	a) With metabolic activation:	9.3 – 93,000 µg/plate
	b) Without metabolic activation:	9.3 – 93,000 µg/plate
Vehicle	Water (for serial dilutions)	

Remarks – Method	No preliminary test was conducted. The test substance was assayed undiluted and at 4 serial 1 in 10 dilutions (1:10, 1:100, 1:1,000, 1:10,000) using water. The density of the test substance is 0.93 g/cm^3 and resulted in a test concentration range of 9.3 – 93,000 µg/plate for the serially diluted solutions. The main test was conducted in duplicate and the test substance was added to both base-pair substitution type (TA100 and TA1535) and frameshift type (TA98, TA1537 and TA1538) tester strains. Tests with negative control and positive controls were run concurrently. Positive controls were: - With metabolic activation: 2-Aminofluorene (TA1538, TA98 and TA100) - Without metabolic activation: hydrazine sulphate (TA1535); 9-aminoacridine HCl monohydrate (TA1537), doxorubicine HCl (TA1538, TA98, TA100). The negative control was acetone. No major deviations from the test guideline were reported.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*		
	Cytotoxicity in Test	*Precipitation**	*Genotoxic Effect*
Absent			
Test 1	Not reported	Not reported	Negative
Test 2	Not reported	Not reported	Negative
Present			
Test 1	Not reported	Not reported	Negative
Test 2	Not reported	Not reported	Negative

* Based on the physical and chemical properties (Appendix A), the solubility of the notified chemical in the vehicle (water) is 0.33 mg/L at 25 °C.

Remarks – Results	The test substance at any tested concentrations did not result in an increase of more than twice the number of revertant colonies in comparison to the negative control. No dose-related response was observed in any test strains with or without metabolic activation. The positive and negative controls provided a satisfactory response confirming the validity of the test system.
CONCLUSION	The notified chemical was not mutagenic to bacteria under the conditions of the test.
TEST FACILITY	Istituto di Ricerche Biomediche (1991e)

B.9. Genotoxicity – *In Vitro* Mammalian Chromosome Aberration Test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test (1981) EC Directive 92/69/EEC and 67/548/EEC B.10 Mutagenicity – *In vitro* Mammalian Chromosome Aberration Test (1992)
Species/Strain	Hamster
Cell Type/Cell Line	Chinese hamster ovary cells (CHO)
Metabolic Activation System	Aroclor 1254 induced rat liver S9 mix
Vehicle	Acetone

Remarks – Method

The preliminary cytotoxicity test with and without metabolic activation was performed with a concentration range of 5 – 5,000 µg/mL.

At the dosage levels of 500, 1,500 and 5,000 µg/mL, the test substance was cytotoxic both with and without metabolic activation, resulting in very few metaphases on the slides at harvesting.

Based on the preliminary cytotoxicity test, the concentrations 15, 50 and 150 µg/mL were selected for metaphase analysis in the main tests, with and without metabolic activation.

Ethylmethane sulphonate (EMS) and cyclophosphamide (CP) were used as positive controls. The vehicle was used as the negative control.

No major deviations from the test guideline were reported.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Preliminary Test	5, 15, 50, 150, 500, 1500, 5000	3 h	20 h
Main Test 1	15*, 50*, 150*	3 h	20 h
Main Test 2	15*, 50*, 150*	18 h	20 h
Main Test 3	150*	24 h	44 h
Present			
Preliminary Test	5, 15, 50, 150, 500, 1500, 5000	3 h	20 h
Main Test 1	15*, 50*, 150*	3 h	20 h
Main Test 2	15*, 50*, 150*	3 h	20 h
Main Test 3	150*	24 h	44 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				
Preliminary Test	≥ 500	–	≥ 500	Not tested
Main Test 1	–	> 150	> 150	Negative
Main Test 2	–	> 150	> 150	Negative
Main Test 3	–	> 150	> 150	Negative
Present				
Preliminary Test	≥ 500	–	≥ 500	Not tested
Main Test 1	–	> 150	> 150	Negative
Main Test 2	–	> 150	> 150	Negative
Main Test 3	–	> 150	> 150	Negative

Remarks – Results

In the preliminary toxicity test when the test article was added to the incubation mixture, visible droplets formed at concentrations ≥ 500 µg/mL of the test substance. At concentrations ≤ 150 µg/mL small droplets of the test substance distributed throughout the incubation mixture were visible under microscope.

The test substance did not induce any statistically significant increases in the frequency of cells with chromosome aberrations either in the absence or presence of metabolic activation.

The positive and negative (vehicle) controls provided a satisfactory response confirming the validity of the test system.

CONCLUSION

The notified chemical was not clastogenic to Chinese hamster ovary cells treated *in vitro* under the conditions of the test.

TEST FACILITY Istituto di Ricerche Biomediche (1997b)

B.10. Genotoxicity – *In Vitro* Mammalian Cell Gene Mutation Test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 476 *In vitro* Mammalian Cell Gene Mutation Test (1998)
Species/Strain Mouse
Cell Type/Cell Line L5178Y lymphoma cells
Metabolic Activation System Aroclor 1254 induced rat liver S9 mix
Vehicle Ethanol
Remarks – Method Two preliminary cytotoxicity tests were performed to determine concentration range to be used in the main test. In the first test cells were exposed to the test substance for 4 hours at a concentration range of 0.5 – 5,000 µg/mL in the absence and presence of metabolic activation. In the second test, cells were exposed for 24 hours at a concentration range of 0.25 – 2,500 µg/mL of the test substance in the absence of metabolic activation.

Based on the results of the preliminary toxicity assay, the concentration range chosen for Main Test was 5 to 150 µg/mL, in both the presence and absence of metabolic activation.

Methyl methanesulfonate (MMS) was used as the positive control for the tests in the absence of metabolic activation. In the presence of metabolic activation 7,12-Dimethyl-benz(a)anthracene (7,l2 ZDMBA) was used as the positive control. The vehicle (ethanol) was use as the negative control.

In the study, the negative control mutant frequency was 110 mutants per $l0^6$ clonable cells for 4 hour exposure in the absence of metabolic activation. The study authors reported that this deviation had no adverse effect on the integrity or conclusions of this study.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Preliminary Test 1	0.5, 1.5, 5, 15, 50, 150, 500, 1500, 5000	4 h	2 d	10 – 14 d
Preliminary Test 2	0.25, 0.75, 2.5, 7.5, 25, 75, 250, 750, 2500	24 h	2 d	10 – 14 d
Main Test 1	50*, 75*, 100*, 125*, 150*	4 h	2 d	10 – 14 d
Main Test 2	3.75*, 7.5*, 18.75*, 37.5*, 75*, 187.5	24 h	2 d	10 – 14 d
Present				
Preliminary Test 1	0.5, 1.5, 5, 15, 50, 150, 500, 1500, 5000	4 h	2 d	10 – 14 d
Main Test 1	50*, 75*, 100*, 125*, 150*	4 h	2 d	10 – 14 d

* Cultures selected for colony analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Preliminary Test	> 5,000	–	≥ 150	Not tested
Preliminary Test	> 2,500	–	≥ 250	Not tested
Main Test 1	–	> 150	≥ 150	Equivocal*
Main Test 2	–	> 75	≥ 75	Negative
Present				
Preliminary Test	> 5,000	–	≥ 150	Not tested
Main Test 1	–	> 150	≥ 150	Negative

* Equivocal results were only observed at 125 µg/mL dose level.

Remarks – Results	One culture tested at 125 µg/mL dose level without metabolic activation exhibited a mutant frequency significantly higher than that of the vehicle control. No dose-response trend was observed. As the results were equivocal, an independent repeat assay was performed for a 24 hour exposure period only in the absence of metabolic activation. The repeat assay (Main Test 2) showed negative results. The positive and negative (vehicle) controls provided a satisfactory response confirming the validity of the test system.
CONCLUSION	The notified chemical was not mutagenic to L5178Y/TK$^{+/-}$ mouse lymphoma cells treated *in vitro* under the conditions of the test.
TEST FACILITY	BioReliance (2003)

B.11. Genotoxicity – Rat, *In Vivo* Micronucleus Induction in Bone Marrow Cells

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 474 Mammalian Erythrocyte Micronucleus Test (1981)
Species/Strain	Sprague Dawley Crl:CD (SD) BR
Route of Administration	Oral – gavage
Vehicle	0.5% concentration methylcellulose water solution (0.5% MC)
Remarks – Method	Positive control was mitomycin C. The vehicle control and the test substance were administered by oral gavage, while the positive control was administered by the intraperitoneal route.

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Sacrifice Time (hours)*
Vehicle control	30 (15 F/15 M)	20 [a]	18 h, 42 h, 66 h [b]
Test substance	30 (15 F/15 M)	5,000	18 h, 42 h, 66 h [b]
Positive control, M	10 (5 F/5 M)	8	42 h

M = mitomycin C.
[a] Vehicle control administered was 20 mL/kg bw
[b] Animals were sacrificed at 3 time intervals (18 h, 42 h and 66 h). At each time interval 10 animals treated with the test substance or negative control were sacrificed.

RESULTS

Doses Producing Toxicity	No cytotoxic effects on bone marrow cells were observed at a dose of 5,000 mg/kg bw. No clinical signs of toxicity were reported.
Genotoxic Effects	Negative
Remarks – Results	The positive and vehicle controls provided a satisfactory response confirming the validity of the test system. It was noted that the study authors did not determine if the test substance reached the bone marrow of the treated rats. The test substance did not induce any statistically significant increase in the frequency of micronucleated cells in the bone marrow under the test conditions.
CONCLUSION	The study authors reported that the notified chemical was not clastogenic under the conditions of this *in vivo* micronucleus test.
TEST FACILITY	Istituto di Ricerche Biomediche (1991f)

B.12. Toxicity to Reproduction – Rat, One Generation Study

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 415 One-Generation Reproduction Toxicity Study (1981)
Species/Strain	Rat/Sprague Dawley Crl:CD (SD) BR
Route of Administration	Oral – gavage
Exposure Information	Exposure period – female: From 14 days pre-mating to the end of lactation Exposure period – male: From 70 days pre-mating to the end of mating)
Vehicle	Com oil
Remarks – Method	The dosages were selected based on a previous 28 day repeated oral dose toxicity study in rats. The highest dose corresponds to the maximum tolerated dose and the lowest dose is the no observed effect level (NOEL) established in the study. The test substance was administered to 30 male rats (parental, F0) per group for approximately 70 days covering pre-mating and mating, and to 30 female rats (parental, F0) per group from 14 days prior to mating, during pregnancy and during lactation (for approximately 58 days). Analyses of the stability and concentration of the formulated test substance were performed.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	60 (30 F/30 M)	0	0/30
Low Dose	60 (30 F/30 M)	10	1/30
Mid Dose	60 (30 F/30 M)	50	0/30
High Dose	60 (30 F/30 M)	1,000	1/30

Mortality and Time to Death

One female rat in the high dose group died owing to difficult parturition (dystocia) and another female in the low dose group died before the end of the treatment period due to incorrect administration of the test substance.

Effects on Parental (P) animals:

No clinical signs or behavioural changes were noted in any experimental group during the pre-mating, mating, gestation and lactation periods.

No differences were noted in the mating and fertility indices nor in the mean mating time among the different experimental groups.

No body weight gain changes observed in treated males compared to control males during pre-mating. Female body weight gains were slightly reduced during the pre-mating period in the high dose group and statistically significantly reduced during the gestation and lactation in the mid and high dose groups. The body weight reductions in the females were associated with reduced food consumptions.

No interferences were found on parental reproductive performance and no effects were observed on the weight of the gonads. The absolute weights of testes were significantly increased in males of the high dose group while the relative weights were similar in all experimental groups. No treatment-related changes were seen histologically either in testes or in the epididymitis of males in the high dose group. The weights of ovaries in females of the high dose group were slightly lower than that of the control females, without reaching statistical significance.

No dams with late resorptions, 100% resorptions or dead foetuses were observed in any experimental group. In females of the high dose group, the frequency of early resorptions was statistically significantly higher compared to control females. An increase in number of still births with a related decrease in number of live births was also observed. For live foetuses, both the total number per group and the mean number per litter were significantly lower in the high dose group compared to controls. The length of parturition was slightly increased. Difficult parturition was also observed in one female in this dose group. The study authors also noted an apparent increase

in frequency of female foetuses in necropsy which they did not consider to be treatment related. No effects were reported on the postnatal survival and development of the first filial generation (F1) live pups.

Effects on 1st Filial Generation (F1)

One pluri-malformed foetus was observed at the external examination in the high dose group, having ablefaria, acrania, exencephaly, exophthalmia and macrophthalmia. One foetus with hydroureter and related hydronephrosis was noted in the control group and 3 foetuses with hydroureter were found in the high dose group. A significant increase was observed in the frequencies per group of visceral variants in the high dose group, all related to the urinary tract. No skeletal malformations were found.

No significant changes were found in the mean body weight of pups from treated and control groups during the lactation and post-lactation periods.

Remarks – Results

No pathological changes were observed at the autopsy examination done on F0 parents and F1 pups.

The study authors considered that oral administration of the test substance to male rats during the pre-mating and mating periods and to female rats during the pre-mating, mating, gestation and lactation periods did not induce any apparent toxic effects in male animals. However, they noted that females in the mid and high dose groups had reduced mean body weight gain associated with reduced mean daily food consumption.

Maternal toxicity was present in females in the high dose group as increases in early resorptions and still births in this treatment group were observed.

No effects on postnatal survival and development of the F1 live pups were noted in the study.

CONCLUSION

The study authors concluded that a NOEL of 10 mg/kg bw/day may be regarded for F0 parents based on reduced body weight gain noted at 50 mg/kg bw/day and above. A NOEL of 50 mg/kg bw/day may be considered for F1 pups based on developmental effects observed at 1,000 mg/kg bw/day dose level.

TEST FACILITY Istituto di Ricerche Biomediche (1998a)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

Contradictory results were reported between the modified MITI study and the manometric respirometry studies. HPLC analysis conducted in the modified MITI test showed an unknown peak which may be evidence of a degradant and confounded the results of this test. The two manometric respirometry studies returned similar results for biodegradability. Therefore the overall consideration for biodegradability of the notified chemical was based on the results from the manometric respirometry studies.

C.1.1. Ready Biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 C Ready Biodegradability: Modified MITI Test (I)
Inoculum	Mixed liquor suspended solid
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	BOD and HPLC
Remarks – Method	As per OECD test guidelines. No deviations to the test guideline were noted.

RESULTS

Test Substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	0	7	62
14	0	14	71
28	0	28	71

Remarks – Results	All validity criteria were met. Reference substance reached 62% after 7 days and 71% after 14 days, and the difference in extremes of the test substances was less than 20% at Day 10. Oxygen consumption in the control test was 6.7 mg/L at Day 28, it is noted that this is outside of the expected 20 – 30 mg/L range. An additional peak was detected during the HPLC analysis of the test concentrations.
CONCLUSION	The notified chemical is not biodegradable under the conditions of the modified MITI test. However, the test substance may have been modified under the study conditions.
TEST FACILITY	Mitsubishi Chemical Safety Institute Ltd. (2001)

C.1.2. Ready Biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 F Manometric Respirometry
Inoculum	Activated sludge
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Biochemical Oxygen Demand (BOD)
Remarks – Method	As per OECD guidelines. No deviations to the test guideline were noted.

RESULTS

Test Substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
7	8.75	7	71.6
14	18.3	14	81.2
21	30.5	21	86.2
28	36.2	28	87.9

Remarks – Results

Most validity criteria were met. The reference substance reached 81.2% degradation after 14 days. The difference in replicate extremes of the test substance was less than the 20% at Day 10.

It is noted that oxygen consumption in the inoculum blank was slightly above 60 mg/L limit (mean 60.8 mg/L); however this does not invalidate the test.

CONCLUSION

The notified chemical is inherently biodegradable (36.2%) under the conditions of the manometric respirometry test.

TEST FACILITY

Huntingdon Life Sciences Ltd. (1998)

C.1.3. Ready Biodegradability

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 301 F Manometric Respirometry

Inoculum: Unspecified
Exposure Period: 28 days
Auxiliary Solvent: None
Analytical Monitoring: Chemical Oxygen Demand (COD)
Remarks – Method: As per OECD guidelines. No deviations to the test guideline were noted.

RESULTS

Test Substance		*Sodium Benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
5	0	5	75.4
9	0.4	9	89.7
14	6.9	14	90.0
23	32.0	23	96.5
28	34.5	28	96.8

Remarks – Results

Control sample data were not provided, and therefore not all of the validity criteria could be verified.

The following validity criteria were verified:

- The difference in extremes of the test substance was less than 20% at Day 10.
- Reference substance reached 75.4% degradation after 5 days and 90% after 14 days.

CONCLUSION

The notified chemical is inherently biodegradable (34.5%) under the conditions of the manometric respirometry test.

TEST FACILITY

Istituto Guido Donegani (1992c)

C.2. Ecotoxicological Investigations

C.2.1. Acute Toxicity to Fish

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 203 Fish, Acute Toxicity Test – semi-static
EC Council Regulation No 440/2008 C.1 Acute Toxicity for Fish – semi-static

Species	*Cyprinus carpio*
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	180 mg $CaCO_3$/L
Analytical Monitoring	Ultra HPLC (UPLC)
Remarks – Method	As per OECD test guidelines. No deviations to the test guideline were noted. Water was renewed daily. Oversaturation was observed when attempting to create a water accommodated fraction (WAF) of 10 mg/L, and therefore a WAF of 1.0 mg/L was used for this study. A reference test was conducted using pentachlorophenol.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
Control	0	7	0	0	0	0	0
1.0	0.19	7	0	0	0	0	0

LC50	> 0.19 mg/L (measured) at 96 hours
NOEC (or LOEC)	0.19 mg/L (measured) at 96 hours
Remarks – Results	All validity criteria were met. Temperature was maintained at 22 °C, pH was maintained within 1 unit and the dissolved oxygen concentration was maintained at > 60% of air saturation. Concentrations of the test substance were maintained at > 80% of the nominal concentration. Reference test concluded a 96 h LC50 of 0.24 mg/L.

CONCLUSION: The notified chemical is not toxic to fish at the limit of water solubility.

TEST FACILITY: WIL (2015a)

C.2.2. Acute Toxicity to Aquatic Invertebrates

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test – static
EC Council Regulation No 440/2008 C.2 Acute Toxicity for Daphnia – static

Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	180 mg $CaCO_3$/L
Analytical Monitoring	UPLC
Remarks – Method	As per OECD guidelines. No deviations to the test guideline were noted. Oversaturation was observed when attempting to create WAFs of 10 mg/L

and 100 mg/L, and therefore a WAF of 1.0 mg/L was used for this study.

A reference test was conducted using potassium dichromate.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h [acute]*	*48 h [acute]*
Control	0	20	0	1
1.0	0.17	20	0	0

LC50 > 0.17 mg/L at 24 hours
> 0.17 mg/L at 48 hours

NOEC (or LOEC) 0.17 mg/L at 48 hours

Remarks – Results All validity criteria were met. The dissolved oxygen was maintained at > 3 mg/L, pH was maintained at 8.1 and the temperature was maintained at 21 – 22 °C

Reference test indicated an EC50 (24 h) of 0.70 mg/L and an EC50 (48 h) of 0.41 mg/L (within the accepted range).

CONCLUSION The notified chemical is not toxic at the limit of water solubility.

TEST FACILITY WIL (2015b)

C.2.3. Chronic Toxicity to Aquatic Invertebrates

TEST SUBSTANCE Notified chemical

METHOD OECD TG 204 Fish, Prolonged Toxicity Test: 14-Day Study – semi-static

Species *Brachydanio rerio*

Exposure Period 21 days

Auxiliary Solvent Acetone

Water Hardness Total hardness was 236 mg $CaCO_3$/L

Analytical Monitoring Unspecified

Remarks – Method As per OECD guidelines when conducted (OECD TG 204 has been deleted as of 2nd April 2014), test timeframe was extended from 14 days to 21 days.

Water was renewed daily.

Concentration (mg/L)		*Number of Fish*	*Mortality*			
Nominal	*Actual*		*1 d*	*7d*	*14 d*	*21d*
Control	0	10	0	0	0	0
Control (solvent)	0	10	0	0	0	0
0.033	BDL	10	0	0	0	0
0.104	BDL	10	0	0	0	0
0.330	0.240	10	0	0	0	0

BDL = Below Detection limit

LC50 > 0.330 mg/L at 21 days based on nominal values.

NOEC (or LOEC) 0.240 mg/L at 21 days

Remarks – Results The test met the validity criteria (at the time of test completion). Oxygen content was maintained above 50% of air saturation value and substance concentration was maintained throughout the test.

The concentrations in the nominal samples 0.033 and 0.104 could not be confirmed as the concentration was below the detectable level.

CONCLUSION The notified chemical is not chronically toxic to fish.

TEST FACILITY Istituto di Ricerche Biomediche (1998b)

C.2.4. Algal Growth Inhibition Test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 201 Alga, Growth Inhibition Test
EC Council Regulation No 440/2008 C.3 Algal Inhibition Test

Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 1.0 mg/L Actual: 0.17 mg/L
Auxiliary Solvent	None
Water Hardness	240 mg $CaCO_3$/L
Analytical Monitoring	UPLC

Remarks – Method Initial concentration of test substance was measured as 0.437 mg/L from a nominal initial WAF of 1.0 mg/L and this is assumed to be the limit of water solubility of the notified chemical.

A positive control was run using potassium dichromate.

RESULTS

Growth rate		*Yield*	
ErC50 (mg/L at 72 h)	*NOEC (mg/L)*	*EyC50 (mg/L at 72 h)*	*NOEC (mg/L)*
> 0.17	0.17	> 0.17	0.17

Remarks – Results The measured concentration deteriorated to 11% of the initial concentration at the end of the study. Therefore a time weighted average was used to determine the exposure concentration of 0.17 mg/L.

All validity criteria were met.

Control cell density increased by a factor of at least 16 per day. The coefficient of variation for both the section-by-section growth and average specific growth rate was 18%.

CONCLUSION The notified chemical does not inhibit algal growth at the limit of water solubility.

TEST FACILITY WIL (2015c)

C.2.5. Inhibition of Microbial Activity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 209 Activated Sludge, Respiration Inhibition Test
EC Directive 88/302/EEC C.11 Biodegradation: Activated Sludge Respiration Inhibition Test

Inoculum	Activated sludge
Auxiliary solvent	Tween 80
Exposure Period	30 minutes
Concentration Range	Nominal: 3.2 – 100 mg/L
Remarks – Method	A reference test was conducted using 3,5-dichlorophenol.

Deviations from the OECD test guidelines include the use of "Tween 80" as an emulsifier for the test substance. The samples were also aerated for 30 minutes rather than the specified 3 hours.

	An upper range sample of 1,000 mg/L was not included in this test.
RESULTS	
IC50	> 100 mg/L
NOEC	100 mg/L
Remarks – Results	Test was repeated twice as validity criteria were not met. Only the third test was reported which met all validity criteria. The coefficient of variation between control samples was 13.4% and the EC50 of the reference test was 12.2 mg/L. Inhibition in the 10 mg/L sample could not be calculated as there was rapid oxygen consumption in this sample.
CONCLUSION	The notified chemical is not likely to be inhibitory to microbial activity
TEST FACILITY	RCC UMWELTCHEMIE AG. (1993)

C.2.6. Acute Toxicity to Earthworms

TEST SUBSTANCE	Notified chemical
METHOD	Equivalent to OECD TG 207 Acute Earthworm Toxicity Test
Species	*Eisenia foetida*
Exposure Period	14 days
Remarks – Method	As per OECD guidelines. No deviations to the test guideline were noted.
RESULTS	
EC50	> 1,000 mg/kg
NOEC	1,000 mg/kg
Remarks – Results	A limit test at a concentration of 1,000 mg/kg was conducted which showed no mortality or abnormalities in either the treated group or the control after the testing period.
CONCLUSION	Notified chemical is not toxic to earthworms.
TEST FACILITY	Istituto di Ricerche Biomediche (1998c)

BIBLIOGRAPHY

BioReliance (2003) In Vitro Mammalian Cell Gene Mutation Test (L5178Y/TK+/- Mouse Lymphoma Assay), (Study No. AA80MY.704.BTL, December, 2003), BioReliance, Rockville, MD, (Unpublished report submitted by the notifier)

Blue Environment Pty Ltd (2016) Australian National Waste Report 2016. Canberra, Australia.

Enichem (1992a) Determination of the Vitreous (Glass) Transition of a Sample of ANOX BF (Study No. ABF 8/1, February 1992) Enichem Synthesis, Milano, Italy (Unpublished report submitted by the notifier)

Enichem (1992b) Determination of Interval for the Boiling Point of a Sample of ANOX BF (Study No. ABF 9/1, March 1992) Enichem Synthesis, Milano, Italy (Unpublished report submitted by the notifier)

Enichem (1992c) Determination of the Relative Density of a Sample of ANOX BF (Study No. ABF 6/1, February 1992) Enichem Synthesis, Milano, Italy (Unpublished report submitted by the notifier)

Enichem (1992d) Determination of Water Solubility of a Sample of ANOX BF (Study No. ABF 4/1, March 1992) Enichem Synthesis, Milano, Italy (Unpublished report submitted by the notifier)

Enichem (1992e) Determination of Coefficient of Distribution of a Sample of ANOX BF (Study No. ABF 5/1, March 1992) Enichem Synthesis, Milano, Italy (Unpublished report submitted by the notifier)

Huntingdon Life Sciences Limited (1998) ANOX BF Assessment of Inherent Biodegradability by Manometric Respirometry (Study No. 960694, August 1998) Huntingdon Life Sciences Limited, Suffolk, England (Unpublished report submitted by the notifier)

IRI (1991) ANOX BF Acute Inhalation Toxicity Study in Rats (Limit Test) (Study No. 650927, June 1991) Inveresk Research International, Tranent, Scotland (Unpublished report submitted by the notifier)

Istituto di Ricerche Biomediche (1991a) Acute Oral Toxicity Study in Rats Treated with the Test Article ANOX BF (Study No. 910014, April 1991) Istituto di Ricerche Biomediche “Antoine Marxer” RBM S.p.A, Colleretto Giacosa, Italy (Unpublished report submitted by the notifier)

Istituto di Ricerche Biomediche (1991b) Acute Dermal Toxicity Study in Rats Treated with the Test Article ANOX BF (Study No. 910015, April 1991) Istituto di Ricerche Biomediche “Antoine Marxer” RBM S.p.A, Colleretto Giacosa, Italy (Unpublished report submitted by the notifier)

Istituto di Ricerche Biomediche (1991c) ANOX BF Acute Dermal Irritation Study in Rabbits (Occlusive Patch) (Study No. 910016, April 1991) Istituto di Ricerche Biomediche “Antoine Marxer” RBM S.p.A, Colleretto Giacosa, Italy (Unpublished report submitted by the notifier)

Istituto di Ricerche Biomediche (1991d) Acute Eye Irritation Study in New Zealand White Rabbits Treated with the Test Article ANOX BF (Study No. 910017, April 1991) Istituto di Ricerche Biomediche “Antoine Marxer” RBM S.p.A, Colleretto Giacosa, Italy (Unpublished report submitted by the notifier)

Istituto di Ricerche Biomediche (1991e) Study of the Capacity of the Test Article ANOX BF to Induce Gene Mutations in Strains of Salmonella Typhimurium (Study No. 910018, March 1991) Istituto di Ricerche Biomediche “Antoine Marxer” RBM S.p.A, Colleretto Giacosa, Italy (Unpublished report submitted by the notifier)

Istituto di Ricerche Biomediche (1991f) Micronucleus Induction in Bone Marrow Cells of Rats Treated by Oral Route with the Test Article ANOX BF (Study No. 910416, December 1991) Istituto di Ricerche Biomediche “Antoine Marxer” RBM S.p.A, Colleretto Giacosa, Italy (Unpublished report submitted by the notifier)

Istituto di Ricerche Biomediche (1992a) Skin Sensitization Test in Guinea Pigs of the Test Article ANOX BF (Buhler Test), (Study No. 910417, April 1992) Istituto di Ricerche Biomediche “Antoine Marxer” RBM S.p.A, Colleretto Giacosa, Italy (Unpublished report submitted by the notifier)

Istituto di Ricerche Biomediche (1992b) ANOX BF 4-Week Oral Toxicity Study in Rats Followed by 4-Weeks of Recovery, (Study No. 910415, June 1992) Istituto di Ricerche Biomediche “Antoine Marxer” RBM S.p.A, Colleretto Giacosa, Italy (Unpublished report submitted by the notifier)

Istituto di Ricerche Biomediche (1997a) ANOX BF Adsorption-Coefficient on Soil Screening Test (Study No. 960695, September 1997) Istituto di Ricerche Biomediche “Antoine Marxer” RBM S.p.A, Colleretto Giacosa, Italy (Unpublished report submitted by the notifier)

Istituto di Ricerche Biomediche (1997b) ANOX BF In Vitro Cytogenetic Assay in CHO Cells (Study No. 960696, September 1997) Istituto di Ricerche Biomediche “Antoine Marxer” RBM S.p.A, Colleretto Giacosa, Italy (Unpublished report submitted by the notifier)

Istituto di Ricerche Biomediche (1998a) ANOX BF Fertility and Reproduction Study by Oral Route in Male and Female Rats (F1), (Study No. 960689, July 1998) Istituto di Ricerche Biomediche “Antoine Marxer” RBM S.p.A, Colleretto Giacosa, Italy (Unpublished report submitted by the notifier)

Istituto di Ricerche Biomediche (1998b) ANOX BF Prolonged Toxicity Study in Fish (14 + 7 Days) (Semi-Static Test) (Study No. 960693, July 1998) Istituto di Ricerche Biomediche “Antoine Marxer” RBM S.p.A, Colleretto Giacosa, Italy (Unpublished report submitted by the notifier)

Istituto di Ricerche Biomediche (1998c) ANOX BF "Artificial Soil" Acute Toxicity Study in Earthworms (Study No. 960692, July 1998) Istituto di Ricerche Biomediche “Antoine Marxer” RBM S.p.A, Colleretto Giacosa, Italy (Unpublished report submitted by the notifier)

Istituto Guido Donegani (1992a) ANOX BF Physical-Chemical Characteristics (Study No. 034/91, April, 1992) Istituto Guido Donegani, Novara Italy (Unpublished report submitted by the notifier)

Istituto Guido Donegani (1992b) ANOX BF Surface Tension (Study No. 034/91-Addendum, October, 1992) Istituto Guido Donegani, Novara Italy (Unpublished report submitted by the notifier)

Istituto Guido Donegani (1992c) Ready Biodegradability and Chemical Oxygen Demand of ANOX BF (Study No. 008/92, June, 1992) Istituto Guido Donegani, Novara Italy (Unpublished report submitted by the notifier)

Mitsubishi Chemical Safety Institute Ltd. (2001) Ready Biodegradability of ANOX BF (Study No. A010251, December, 2001) Mitsubishi Chemical Safety Institute Ltd., Yokohama, Japan (Unpublished report submitted by the notifier)

RCC UMWELTCHEMIE AG. (1993) Assessment of the Acute Toxicity of ANOX BF on Aerobic Waste-Water Bacteria (Study No. 351764, October, 1993) RCC UMWELTCHEMIE AG, Switzerland (Unpublished report submitted by the notifier)

Struijs J, Stoltenkamp J, Van de Meent D. 1991. A Spreadsheet-Based Box Model to Predict the Fate of Xenobiotics in a Municipal Wastewater Treatment Plant. Water Res 25:891–900.

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

WIL (2015a) 96-Hour Acute Toxicity Study in Carp with ANOX® 1315 (Semi-Static) (Project No. 504488, January, 2015). DD 's-Hertogenbosch, The Netherlands, WIL Research Europe B.V. (Unpublished report submitted by the notifier).

WIL (2015b) Acute Toxicity Study in Daphnia Magna with ANOX® 1315 (Static) (Project No. 504490, February, 2015). DD 's-Hertogenbosch, The Netherlands, WIL Research Europe B.V. (Unpublished report submitted by the notifier).

WIL (2015c) Fresh Water Algal Growth Inhibition Test with ANOX® 1315 (Project No. 504491, February, 2015). DD 's-Hertogenbosch, The Netherlands, WIL Research Europe B.V. (Unpublished report submitted by the notifier).