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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

PUBLIC REPORT

Paliotol Yellow K 1750

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/1700	IMCD Australia Ltd	Paliotol Yellow K 1750	Yes	≤ 20 tonnes per annum	Colourant for plastics

CONCLUSIONS AND REGULATORY OBLIGATIONS

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

Hazard Classification	*Hazard Statement*
Skin irritation (Category 2)	H315 – Causes skin irritation

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

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

As the notified chemical will be used in packaging with direct food contact, the public report of this assessment will be forwarded to Food Standards Australia New Zealand (FSANZ) for their consideration.

Environmental Risk Assessment
On the basis of the low hazard and the 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 person conducting a business or undertaking at a workplace should implement the following engineering controls to minimise occupational exposure to the notified chemical as introduced:
  - Local exhaust ventilation and/or appropriate dust extraction systems

- 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 as introduced:
  - Avoid contact with skin and eyes
  - Avoid inhalation of dust

- 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 as introduced:
  - Impervious gloves
  - Coveralls
  - Respiratory protection

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

- In the interest of occupational health and safety, the following precaution should be observed for use of the notified chemical as introduced in powder form:
  - The level of atmospheric nuisance dust should be maintained as low as possible. The Safe Work Australia exposure standard for atmospheric dust is 10 mg/m^3.

- 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(1) of the Act; if
  - the concentration of the notified chemical in plastic packaging for direct food contact exceeds 0.5% concentration;

or

(2) Under Section 64(2) of the Act; if
  - the function or use of the chemical has changed from a colourant for plastics, 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(S)
IMCD Australia Limited (ABN: 44 000 005 578)
Level 1, 372 Wellington Road
MULGRAVE VIC 3070

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

EXEMPT INFORMATION (SECTION 75 OF THE ACT)
Data items and details exempt from publication include: chemical name, other names, CAS number, molecular and structural formulae, molecular weight, analytical data, degree of purity, impurities, additives/adjuvants, import volume, identity of manufacturer and results of migration study.

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Schedule data requirements are varied for boiling point, vapour pressure, adsorption/desorption, dissociation constant, flashpoint, explosive properties and oxidising properties.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT
Previous permit (NICNAS)

NOTIFICATION IN OTHER COUNTRIES
Taiwan (2015)

2. IDENTITY OF CHEMICAL

MARKETING NAME
Paliotol Yellow K 1750

OTHER NAMES
Monoazo Pigment in Paliotol Yellow K 1750
Pigment Yellow 229

MOLECULAR WEIGHT
< 500 g/mol

ANALYTICAL DATA
Reference IR, UV, HPLC and MS spectra were provided.

3. COMPOSITION

DEGREE OF PURITY
> 95%

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20 °C AND 101.3 kPa: Yellow powder

Property	*Value*	*Data Source/Justification*
Melting Point	Decomposes without melting at 350 °C	Measured
Boiling Point	Not determined	Decomposes at 350 °C
Density	1,660 kg/m^3 at 20 °C	Measured
Vapour Pressure	Not determined	Expected to be low, based on melting point
Water Solubility	0.8×10^{-3} g/L at 23 °C	Measured

Property	*Value*	*Data Source/Justification*
Hydrolysis as a Function of pH	$t_{1/2}$ > 1 year at pH 4, 7, 9 and 50 °C	Measured
Partition Coefficient (n-octanol/water)	log Pow = -1.3 at 23 °C	Calculated from individual solubilities in water and in n-octanol
Adsorption/Desorption	Not determined	Expected to be immobile in soil based on very low log Pow
Dissociation Constant	Not determined	Contains an anionic group which can dissociate; however, overall, significant dissociation is not expected under environmental pH (4-9) due to low water solubility
Particle Size	Inhalable fraction (< 100 μm): 100% Respirable fraction (< 10 μm): 60.75%	Measured
Flash Point	Not determined	Solid
Flammability	Not highly flammable	Measured
Autoignition Temperature	> 400 °C	Measured
Explosive Properties	Not determined	Contains no functional groups that would imply explosive properties
Oxidising Properties	Not determined	Contains no functional groups that would imply oxidising properties

DISCUSSION OF PROPERTIES
For 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 into Australia neat as a solid powder.

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

TRANSPORTATION AND PACKAGING
The notified chemical will be imported in 20 kg fibreboard cartons with polyethylene inner lining. The notified chemical will be transported by road throughout Australia.

The formulated polymer masterbatch pellets containing the notified chemical at ≤ 15% concentration will be packaged in 15, 20 or 25 kg plastic bags for transport by road to customer sites.

USE
The notified chemical will be used as a colourant for plastics at ≤ 0.5% concentration, including in packaging film for food contact applications.

OPERATION DESCRIPTION

Masterbatch production
The notified chemical will be manually weighed and added to a blending vessel for mixing with other components. The resulting powdered mixture will then be automatically transferred to a feed hopper of an extruder from which it will be melted and extruded into pellets. Following cooling, the polymer masterbatch pellets containing the notified chemical at ≤ 15% concentration will be discharged through a closed transfer system for manual packaging into 15, 20 or 25 kg plastic bags for transport to customer sites.

Manufacture of plastic articles
At the customer sites, the polymer masterbatch pellets containing the notified chemical at ≤ 15% concentration will be manually added to a hopper and mixed with polymer and possibly other additives. The resulting mixture will then be melted and extruded or injection moulded into plastic articles. The finished plastics will contain the notified chemical at ≤ 0.5% concentration.

6. HUMAN HEALTH IMPLICATIONS

6.1. Exposure Assessment

6.1.1. Occupational Exposure

CATEGORY OF WORKERS

Category of Worker	*Exposure Duration (hours/day)*	*Exposure Frequency (days/year)*
Transport and storage	1	10-20
Process operators (masterbatch production and manufacture of plastic articles)	10-20	50-100

EXPOSURE DETAILS
Transport and storage
Transport and storage workers are not expected to be exposed to the notified chemical, except in the unlikely event of an accidental rupture of packaging.

Masterbatch production
Dermal, ocular and inhalation exposure to the neat notified chemical in powdered form may occur during weighing and transfer to the mixing vessel and feed hopper. The notifier states that exposure will be minimised through the use of a dust extractor during loading operations, and by the use of personal protective equipment (PPE) by process operators, including protective clothing, dust masks, eye protection and impervious gloves. Inhalation exposure to the notified chemical may also occur from emissions of the heated resin during extrusion. The notifier states that exposure will be minimised through the use of local exhaust ventilation and the largely enclosed, automated extrusion process.

Manufacture of plastic articles
Process operators will likely come into dermal contact with the polymer masterbatch pellets containing the notified chemical at ≤ 15% concentration. However worker exposure is not expected as the notified chemical will be encapsulated within the polymer masterbatch matrix and is not expected to be available for exposure. Inhalation exposure to the notified chemical may occur from emissions of the heated resin during extrusion or injection moulding. The notifier states that exposure will be minimised through the use of local exhaust ventilation and the largely enclosed, automated extrusion and injection moulding processes. Workers are also expected to wear PPE including protective clothing, eye protection and impervious gloves to minimise exposure.

Workers may come into contact with the finished plastics containing the notified chemical at ≤ 0.5% concentration. However once the notified chemical is incorporated into the finished plastic, it will be encapsulated within a polymer matrix and is not expected to be available for exposure.

6.1.2. Public Exposure
The notified chemical will be for industrial use only and will not be made available to the public. The public may come into dermal contact with the plastic articles containing the notified chemical at ≤ 0.5% concentration.

However once the notified chemical is incorporated into the finished plastic, it will be encapsulated within a polymer matrix and is not expected to be available for exposure.

The notified chemical will be used in packaging for direct food contact. The notifier has provided a migration study to determine the potential migration of the notified chemical and its impurities from low density polyethylene (LDPE) (containing the notified chemical at 0.55% concentration) using different food simulants (BASF, 2018). Tests were carried out for 2 hours at 100 °C followed by 22 hours, 94 hours and 238 hours at 40 °C according to FDA/CFSAN Guidance for Industry, Preparation of Premarket Submissions for Food Contact Substances: Chemistry Recommendations (April 2002/December 2007). The highest migration of the notified chemical of 40.21 ppb into food from LDPE was found into 10% ethanol after 2 hours at 100 °C followed by 238 hours at 40 °C. The impurities were all found to be below the level of quantification under all test conditions.

6.2. Human Health Effects Assessment

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

Endpoint	*Result and Assessment Conclusion*
Acute oral toxicity – rat	LD50 > 2,000 mg/kg bw; low toxicity
Acute dermal toxicity – rat	LD50 > 2,000 mg/kg bw; low toxicity
Acute inhalation toxicity – rat	LC50 > 4.651 mg/L/4 hour; low toxicity
Skin irritation – *in vitro* reconstructed human epidermis model (EpiSkin)	not a skin irritant
Skin irritation – rabbit	irritating
Eye irritation – rabbit	slightly irritating
Skin sensitisation – mouse local lymph node assay	no evidence of sensitisation at up to 25% concentration
Repeat dose oral toxicity – rat, 28 days	NOAEL = 1,000 mg/kg bw/day*
Mutagenicity – bacterial reverse mutation (including Prival and Mitchell modification for azo dyes)	non mutagenic
Genotoxicity – *in vitro* gene mutation test in Chinese hamster ovary cells	non clastogenic
Genotoxicity – *in vitro* chromosome aberration test in human lymphocytes	non clastogenic
Genotoxicity – *in vivo* mouse micronucleus test	non clastogenic
Reproductive and developmental toxicity – rat	NOAEL = 1,000 mg/kg bw/day*

*established by the study author

Toxicokinetics, Metabolism and Distribution

Given its relatively low molecular weight (< 500 g/mol), the notified chemical may be absorbed across the respiratory or gastrointestinal tract. Dermal absorption is expected to be limited given its low partition coefficient (log Pow = -1.3 at 23 °C) and low water solubility (0.8×10^{-3} g/L at 23 °C). However, bacterial skin microflora have been reported to be able to break down azo dyes into smaller species, which may be more readily absorbed, through azo reduction (SCCNFP, 2002).

The notified chemical has a very low water solubility (0.8 × 10-3 g/L at 23 °C) and contains a significant portion of particles in the respirable size range (< 10 µm), therefore lung overloading effects may occur if large amounts are inhaled.

Acute Toxicity

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

Irritation and Sensitisation

In an *in vitro* study using the reconstructed human epidermis model (EpiSkin), the notified chemical was determined not to be a skin irritant.

In a skin irritation study conducted in rabbits, the notified chemical was found to be irritating. Very slight to well defined erythema was observed in all animals which was resolved in 2/3 animals at the 24 hour and 7 day observation. Very slight erythema persisted in one animal to the end of the observation period (day 14), warranting the notified chemical to be classified as a Category 2 skin irritant according to the GHS criteria. The LLNA selecting 25% as the highest non-irritating concentration further supports this classification.

The notified chemical is slightly irritating to eyes based on a study conducted in rabbits. All animals showed conjunctival irritation (grade 1) which was resolved at the 72 hour observation.

The notified chemical was determined not to be a skin sensitiser in a mouse local lymph node assay (LLNA) at up to 25% concentration, with stimulation indices of 1.1, 1.8, and 0.9 at 6.25%, 12.5% and 25% concentration, respectively.

Repeated Dose Toxicity
In a repeated dose oral toxicity in rats with the notified chemical, the No Observed Adverse Effect Level (NOAEL) was established by the study authors as 1,000 mg/kg bw/day for systemic toxicity. However, there were statistically significant mean organ weight changes reported in treated animals at the highest dose (compared to the control groups) which did not resolve during the recovery period.

In a reproductive and developmental toxicity study in rats with the notified chemical, the NOAEL was established by the study authors as 1,000 mg/kg bw/day for parental and reproductive and developmental toxicity. The mean number of delivered pups for the high dose group were below the historical control range of 9.3-14.1 pups/dam due to a female that had 2 implantation sites with no live born pups. Mating partners of a low and a high dose male did not conceive; however, the respective males did not show relevant gross lesions. Sperm granuloma was observed in these males. The study authors did not regard this condition to be the cause of the infertility. The study authors also stated as no dose response was observed, sperm granuloma observed in 2 males is not considered to be treatment related, and indicated this effect is one of the most common changes observed in young adult rat epididymides (McInnes, 2012).

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

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

The notified chemical also tested negative in an *in vitro* gene mutation test in Chinese hamster ovary cells, in an *in vitro* chromosome aberration test in human lymphocytes and in an *in vivo* mouse micronucleus test. Bioavailability of the notified chemical in the *in vivo* study was confirmed by discoloured urine (yellowish).

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

Hazard Classification	*Hazard Statement*
Skin irritation (Category 2)	H315 – Causes skin irritation

6.3. Human Health Risk Characterisation

6.3.1. Occupational Health and Safety
Based on the available information, the notified chemical is a skin irritant and a slight eye irritant. A significant portion of the notified chemical as introduced in powder form is of a respirable particle size and lung overloading effects may occur if large amounts are inhaled.

Workers at risk of lung overloading and skin and eye irritating effects will be those handling the neat notified chemical as introduced. The notifier stated that dust extraction will occur during loading operations and PPE is worn by process operators (including protective clothing, dust masks, eye protection and impervious gloves) to minimise the risk.

Therefore, under the occupational settings described, the risk to the health of workers from use of the notified chemical is not considered to be unreasonable.

6.3.2. Public Health

The notified chemical is intended for use in industrial applications only. The public make come into dermal contact with plastics containing the notified chemical. However once the notified chemical is incorporated into the finished plastic, it will be encapsulated within a polymer matrix and is not expected to be available for exposure.

The public may be indirectly exposed to the notified chemical at ≤ 0.5% concentration through its use in packaging for direct food contact. A migration study provided by the notifier indicates that the notified chemical and its impurities are not expected to migrate from the plastic packaging to food at significant levels (see Section 6.1.2).

The public report of this assessment will be forwarded to Food Standards Australia and New Zealand (FSANZ) for their consideration.

Based on use of the notified chemical at ≤ 0.5% concentration in plastics, the risk to public health is not considered to be unreasonable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1. Environmental Exposure

RELEASE OF CHEMICAL AT SITE

The notified chemical will be imported into Australia neat as a powder and will be used as a colourant for plastics. As estimated by the notifier, up to 5% of the imported volume of the notified chemical may be lost during masterbatch production which will be disposed of to landfill, in accordance with local government regulations. The formulated polymer masterbatch pellets containing the notified chemical will be packaged in plastic bags for transport by road to customer sites. Accidental spills of the notified chemical during import, transport, storage or masterbatch production are expected to be collected for reuse or disposal to landfill, in accordance with local government regulations.

RELEASE OF CHEMICAL FROM USE

At the customer sites, the polymer masterbatch pellets containing the notified chemical will be manually added to a hopper and mixed with polymer and possibly other additives. The resulting mixture will be melted and extruded or injection moulded into plastic articles. As the notified chemical is incorporated into plastic articles, it will be immobilised in the polymer matrix and little release to the environment is expected during use. Any waste containing the notified chemical generated during plastic article production will be disposed of to landfill, in accordance with local government regulations.

RELEASE OF CHEMICAL FROM DISPOSAL

The notified chemical will share the fate of the plastic articles in which they have been incorporated. They may enter recycling streams, but they will ultimately end up in landfill at the end of their useful lives. Empty containers/bags containing residual notified chemical will also be disposed of to landfill.

7.1.2. Environmental Fate

Biodegradation studies conducted on the notified chemical show that it is not readily biodegradable (0% and 23% degradation over 28 days) and not inherently biodegradable (0% degradation over 28 days). For details of the biodegradation studies refer to Appendix C.

The majority of the notified chemical will share the fate of the plastic articles in which they have been incorporated. They may enter recycling streams, but they will ultimately end up in landfill at the end of their useful lives. A minor amount of the notified chemical may also be disposed of to landfill as collected spills and empty container residues. In landfill, the majority of the notified chemical will be encapulated within an inert polymer matrix and will be neither bioavailable nor mobile. The notified chemical is not expected to bioaccumulate based on its very low log Pow. The notified chemical is expected to eventually degrade via biotic and abiotic processes to form water and oxides of carbon, nitrogen and sulfur.

7.1.3. Predicted Environmental Concentration (PEC)

The predicted environmental concentration (PEC) has not been calculated since significant release of the notified chemical to the aquatic environment is not expected from the reported use pattern.

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 (Study 1)	96h LC50 > 100 mg/L (nominal) 96h LC50 > 105 mg/L (measured)	Not harmful to fish
Fish Toxicity (Study 2)	96h LC50 > 105 mg/L (nominal) 96h LC50 > 86.7 mg/L (measured)	Not harmful to fish up to its water solubility limit
Daphnia Toxicity	48h EC50 > 100 mg/L (nominal) 48h EC50 > 24.05 mg/L (measured)	Not harmful to aquatic invertebrates up to its water solubility limit
Algal Toxicity	72h EC50 > 10.3 mg/L (measured)	Not harmful to algae up to its water solubility limit

Based on the above ecotoxicological endpoints for the notified chemical, it is not expected to be harmful to aquatic life up to its solubility limit in water. 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 (PNEC)

A predicted no-effect concentration (PNEC) for the aquatic compartment has not been calculated as the notified chemical is not harmful to aquatic life up to its water solubility limit.

7.3. Environmental Risk Assessment

The Risk Quotient (Q = PEC/PNEC) has not been calculated as the notified chemical is not expected to be harmful to aquatic organisms up to its water solubility limit, and release of the notified chemical to the aquatic environment will be limited based on its reported use pattern. Therefore, based on the low hazard and the reported use pattern, the notified chemical is not expected to pose an unreasonable risk to the environment.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point Decomposes without melting at 350 °C

Method OECD TG 102 Melting Point/Melting Range
Test Facility Ciba (2008a)

Density 1,660 kg/m^3 at 20 °C

Method OECD TG 109 Density of Liquids and Solids
Remarks Determined using a pycnometer
Test Facility Ciba (2008b)

Water Solubility 0.8×10^{-3} g/L at 23 °C

Method OECD TG 105 Water Solubility
Remarks Flask Method; the test substance was analysed by UV/Vis spectrophotometer.
Test Facility Ciba (2009)

Hydrolysis as a Function of pH

Method OECD TG 111 Hydrolysis as a Function of pH

pH	*T (°C)*	*$t_{½}$ (year)*
4	50	>1
7	50	>1
9	50	>1

Remarks The test substance was analysed by HPLC. The hydrolysis rate of the test substance was 1.10% at pH 4, 1.81% at pH 7 and 0.32% at pH 9 over 5 days.
Test Facility GDCM (2014a)

Partition Coefficient (n-octanol/water) log Pow = -1.3 at 23 °C

Method ETAD Guideline ETAD-229, which in turn is based in part on the Water Solubility (shaken flask) OECD TG 105 method. The notified chemical was purified by sequential solvent washes (wash 1: methanol/toluene; wash 2: n-octanol; wash 3: methanol and wash 4: water). For each solvent, the notified chemical was stirred at room temperature for 2 hours, recovered on membrane filter and dried under vacuum. Then, 5 g of the purified notified chemical was dissolved in 60 mL of deionised water. The solution was stirred at 70 °C for 2 hours, and then at 23 °C for 72 hours. The resulting suspension was centrifuged and filtered once through a standard paper filter, twice through a 0.2 µm membrane filter and twice through a 0.025 µm membrane filter until a perfectly clear solution was achieved for analysis. The same procedure was also conducted for n-octanol, and log Pow was calculated from individual solubilities in water and in n-octanol.
Remarks Flask Method; the test substance was analysed by UV/Vis spectrophotometer.
Test Facility Ciba (2009)

Particle Size Inhalable fraction (< 100 µm): 100%
Respirable fraction (< 10 µm): 60.75%

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

Range (µm)	*Mass (%)*
< 100	100
< 10	60.75
< 5	52.85

Remarks	Determined using laser granulometry method
Test Facility	Ciba (2008c)

Flammability Not highly flammable

Method	EC Council Regulation No 440/2008 A.10 Flammability (Solids)
Test Facility	Currenta (2009a)

Autoignition Temperature > 400 °C

Method	EC Council Regulation No 440/2008 A.16 Relative Self-Ignition Temperature for Solids
Test Facility	Currenta (2009b)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute Oral Toxicity – Rat

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method (2001)
Species/Strain	Rat/WISTAR RjHan:WI
Vehicle	1% Aqueous carboxymethylcellulose
Remarks – Method	No protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Mortality*
1	3 F	2,000	0/3
2	3 F	2,000	0/3

LD50	> 2,000 mg/kg bw
Signs of Toxicity	No signs of toxicity were observed during the study period.
Effects in Organs	No abnormalities were observed during necroscopy.
Remarks – Results	All the treated animals showed expected bodyweight gain during the study period.
CONCLUSION	The notified chemical is of low acute toxicity via the oral route.
TEST FACILITY	BSL (2009a)

B.2. Acute Dermal Toxicity – Rat

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 402 Acute Dermal Toxicity (1987)
Species/Strain	Rat/WISTAR (Crl:WI (Han)
Vehicle	1% Aqueous carboxymethyl cellulose
Type of dressing	Occlusive
Remarks – Method	No protocol deviations.

RESULTS

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

LD50	> 2,000 mg/kg bw
Signs of Toxicity – Local	Clinical signs of toxicity included hunched posture, piloerection, chromodacryorrhoea (snout) and/or hypothermia on days 1 and/or 2. A female showed scales on days 8-14. All treated animals showed yellow staining on the treated area.
Signs of Toxicity – Systemic	No signs of systemic toxicity observed during the study.
Effects in Organs	No abnormalities were noted during necroscopy.
Remarks – Results	All the treated animals showed expected bodyweight gain during the study period.
CONCLUSION	The notified chemical is of low acute toxicity via the dermal route.
TEST FACILITY	NOTOX (2009a)

B.3. Acute Inhalation Toxicity – Rats

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 403 Acute Inhalation Toxicity (2009)
Species/Strain	Rats/WISTAR/RccHan:WIST
Vehicle	None
Method of Exposure	Nasal exposure
Exposure Period	4 hours
Physical Form	Solid aerosol (particulate).
Particle Size	Mass median aerodynamic diameter (MMAD): 4.4 μm (sample 1: 30 minutes after the beginning of exposure) and 2.8 μm (sample 2)
Remarks – Method	The test substance was de-agglomerated in a mixer for 10 seconds using 1% Aerosil® 200 (hydrophilic fused silica used to improve the fluidity of powders) and 1% Aerosil® R 972 (hydrophobic fused silica used to improve free flow and anti-caking of powders) before introduction into the dust generator.

RESULTS

Group	*Number and Sex of Animals*	*Concentration (units)*		*Mortality*
		Nominal	*Actual*	
1	5 M/5 F	70.9	4.651	0/10

LC50	> 4.651 mg/L/4 hours
Signs of Toxicity	Slight to moderate laboured respiration was observed during exposure (from hour 2) in all treated animals. Intermittent respiration was observed in all animals immediately after the end of exposure which persisted in 4 males and 2 females at the day 1 post-exposure observation and in 3 females at the day 4 post-exposure observation. All animals showed piloerection immediately after the end of exposure which persisted in a female up to the day 5 post-exposure observation. All animals showed test substance contaminated fur and yellow stain on fur immediately after the end of exposure until the end of observation (day 14).
Effects in Organs	No abnormalities were observed during necroscopy.
Remarks – Results	The mean bodyweights showed a decrease at the day 1 post-exposure observation but increased from the day 3 post-exposure observation.
CONCLUSION	The notified chemical is of low acute toxicity via inhalation.
TEST FACILITY	BASF (2014)

B.4. Skin Irritation – *In Vitro* Reconstructed Human Epidermis Model (EpiSkin)

TEST SUBSTANCE	Notified chemical
METHOD	ISO (10993)-10) Annex D: 2008 *In vitro* Tests for Skin Irritation (Draft). Similar to OECD TG 439 *In vitro* Skin Irritation: Reconstructed Human *Epidermis* Test Method
Vehicle	Distilled water
Remarks – Method	Reconstituted three-dimensional human skin (Episkin-SM™). Positive and negative controls were run in parallel with the test substance:

- Negative control: distilled water
- Positive control: 5% sodium dodecyl sulfate (SDS)

The MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, thiazolyl blue] assay was used to determine cell viability.

No significant protocol deviations.

RESULTS

Test Material	*Mean OD_{550} of Triplicate Tissues*	*Relative Mean Viability (%)*	*SD of Relative Mean Viability*
Negative control	0.942	100	2.4
Test substance	0.916	97.2	5.2
Positive control	0.024	2.5	0.7

OD = optical density; SD = standard deviation

Remarks – Results

The test substance was shown not to directly reduce MTT.

The relative mean tissue viability for the test substance as compared to the negative control was 97.2%. Given that the relative mean tissue viability for the test substance was > 50%, it is considered as a non-irritant.

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

CONCLUSION

The notified chemical was considered non-irritating to the skin under the conditions of the test.

Based on the mean tissue viability of > 50%, the notified chemical is not classified as a skin irritant according to the GHS criteria.

TEST FACILITY

BSL (2009b)

B.5. Skin Irritation – Rabbit

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 404 Acute Dermal Irritation/Corrosion (2002)

Species/Strain: Rabbit/New Zealand White
Number of Animals: 3 F
Vehicle: Moistened with deionised water
Observation Period: 14 days
Type of Dressing: Semi-occlusive
Remarks – Method: No protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Erythema/Eschar	0	2	1	2	> 14 days	1
Oedema	0	0	0	0	n/a	0

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

Remarks – Results

Very slight to well defined erythema was observed in all 3 animals which was resolved in 2 animals at the 24 hour and 7 day observation, respectively. Very slight erythema persisted in one animal to the end of the observation period (day 14).

CONCLUSION	The notified chemical is irritating to the skin.
TEST FACILITY	Bioassay (2013)

B.6. Eye Irritation – Rabbit

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion (2002)
Species/Strain	Rabbit/New Zealand White/Crl:KBL
Number of Animals	3 F
Observation Period	72 h
Remarks – Method	No significant protocol deviations.

RESULTS

Lesion	*Mean Score* Animal No.*			*Maximum Value*	*Maximum Duration of Any Effect*	*Maximum Value at End of Observation Period*
	1	2	3			
Conjunctiva – Redness	0.66	0.66	0.33	1	< 72 h	0
Conjunctiva – Chemosis	0.33	0	0	1	< 48 h	0
Conjunctiva – Discharge	Yes**	0	0	**	< 72 h	**
Corneal Opacity	0	0	0	0	n/a	0
Iridial Inflammation	0	0	0	0	n/a	0

* Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal, ** score has not been provided.

Remarks – Results	All animals showed conjunctival redness (grade 1) at the 1 hour observation which was resolved in 1 animal at the 48 hour observation and in the remaining 2 animals at the 72 hour observation. Chemosis (grade 1) was observed in 2 animals at the 1 hour observation which was resolved at the 24 hour observation in 1 animal and at the 48 observation in the second animal. All animals showed conjunctival discharge at the 1 hour observation which persisted in 1 animal up to the 48 hour observation. No further information provided about this symptom. No body weight gain or loss was observed in all three animals during the study.
CONCLUSION	The notified chemical is slightly irritating to the eye.
TEST FACILITY	BSL (2009c)

B.7. Skin Sensitisation – Local Lymph Node Assay (LLNA)

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 429 Skin Sensitisation: Local Lymph Node Assay (2002)
Species/Strain	Mouse/CBA/CaOlaHsd
Vehicle	Acetone and olive oil
Preliminary study	Yes
Positive control	Phenylenediamine (not conducted in parallel with the study)
Remarks – Method	A preliminary study with one animal was conducted using the test substance at 25%. Spontaneous activity, lethargy, recumbent position, convulsions, tremours, apnoe, asphyxia, vocalisation, diarrhoea, changes in the skin and fur, eyes and mucous membrane (salivation discharge) were observed. The study authors stated changes in skin and fur were also observed (no further information provided). Based on the results, the highest concentration selected for the main study was 25%.

No significant protocol deviations.

RESULTS

Concentration (% w/w)	*Number and Sex of Animals*	*Proliferative Response (DPM/lymph node)*	*Stimulation Index (test/control ratio)*
Test Substance			
0 (vehicle control)	5 F	799.1	-
6.25	5 F	866.2	1.1
12.5	5 F	1451.6	1.8
25	5 F	733.7	0.9
Negative Control			
Not stated	5 (sex not specified)	520.1	-
Positive control			
1	5 (sex not specified)	6738.7	13

Remarks – Results

No unscheduled mortalities or signs of systemic toxicity were observed during the study period.

The stimulation index was < 3 in all test groups, indicating a non-sensitising response.

Slight negative bodyweight gain (-1 to -2 g) was reported for an animal in the low dose group and in 2 animals in the high dose group.

CONCLUSION

There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical at up to 25% concentration.

TEST FACILITY

BSL (2009d)

B.8. Repeat Dose Oral Toxicity – Rat

TEST SUBSTANCE

Notified chemical

METHOD

OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents (2008)

Species/Strain: Rat/Crl:WI(Han)

Route of Administration: Oral – gavage

Exposure Information: Total exposure days: 28 days
Dose regimen: 7 days per week
Post-exposure observation period: 14 days

Vehicle: 1% Aqueous carboxymethylcellulose

Remarks – Method: In a dose range study, 3 female rats were orally administered with the test substance at 500 and 1,000 mg/kg bw/day for 5 days. No significant toxicological findings were observed.

RESULTS

Group	*Number and Sex of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	5 M/5 F	0	0/10
Low Dose	5 M/5 F	30	0/10
Mid Dose	5 M/5 F	300	0/10
High Dose	5 M/5 F	1,000	1/10
Control Recovery	5 M/5 F	0	0/10
High Dose Recovery	5 M/5 F	1,000	0/10

Mortality and Time to Death

A male from high dose group was euthanised *in extremis* on day 9 of the study. Clinical signs, such as hunched posture (on day 8), laboured respiration (on days 7 and 8) and piloerection (on day 9) were observed prior to being euthanised.

Clinical Observations

Hunched posture (on days 6-8 of treatment) and piloerection (on days 6-7 of treatment) was observed in a high dose female. One high dose male and 2 high dose females showed alopecia, scab and wound (observed only in the male) on neck and a control female showed alopecia on forelegs on day 7 recovery. Other incidental findings, such as exophthalmos and opacity were observed (on days 8-28 of treatment) in a high dose male. The study authors stated that these findings were considered to be incidental and are expected in rats of this age and strain under the conditions of the study.

Yellow faeces was observed in all mid dose (from day 6-28 of treatment in both sexes) and high dose (from day 3-28 of treatment in both sexes) animals and one recovery male on day 1 due to staining properties of the notified chemical.

A high dose female showed bodyweight loss in the second week of treatment. All other animals showed normal body weight gain during the study period.

No effect on food consumption or parameters of the functional observation battery were reported.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Although there were some statistically significant changes reported (e.g. basophile, neutrophil and lymphocyte counts, and chloride, albumin and creatinine levels), these were in rats at all doses and were within the range considered normal for rats of this strain and age.

Effects in Organs

Following statistically significant findings were reported with some changes still presenting at the end of the recovery period:

- Increase in absolute and relative mean seminal vesicle weights in low (50.3% and 52% increase in absolute and relative weights, respectively, compared to control males) and mid (59% and 57% increase in absolute and relative weights, respectively, compared to control males) dose males, and high dose recovery males (23% and 25% increase in absolute and relative weights, respectively, than control recovery males).
- Increase in absolute and relative mean spleen weights in low (37% and 32% increase in absolute and relative weights, respectively, compared to control females) and high (30% and 27% increase in absolute and relative weights, respectively, compared to control females) dose females. Increase in absolute spleen weight (25% increase compared to control females) in mid dose females.
- Increase in absolute and relative mean thyroid weights (52.6% and 40% increase in absolute and relative weights, respectively, compared to control recovery females) in high dose recovery females.
- Treatment related increase in relative mean liver weight (10% increase compared to control females) in high dose females.

Statistically not significant, but treatment related increase in relative epididymides weight in high dose males was also observed.

Minimal inflammation in heart, slight lymphoid hyperplasia in lungs, slight tubular basophilia and hyaline droplets, marked hemopoietic and slight lymphoid atrophy in spleen were observed in high dose males. Minimal inflammation in heart, minimal vascular mineralisation in lungs, marked tubular basophilia in kidneys, slight to moderate diestrus (in all doses) in uterus, moderate ectopic thymus in thyroid gland, moderate hemopoietic foci in spleen and marked diestrus in uterus were observed in high dose females. The study authors stated that these findings were incidental and/or were within the normal range of background alterations encountered in Wistar rats of this age and strain.

Green contents were observed in the gastrointestinal tract of several animals in both sexes at mid and high doses. Discolouration in the kidneys (in a high dose female) and mandibular nodes (in a mid dose female) were also observed. The study authors stated that such effects were attributed to oral administration of the coloured test substance and were not considered to be toxicologically relevant.

CONCLUSION
The NOAEL was established by the study authors as 1,000 mg/kg bw/day in this study.

TEST FACILITY NOTOX (2009b)

B.9. Genotoxicity – Bacteria (Prival and Mitchell modification)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test
Plate incorporation procedure (test 1) and pre incubation procedure (test 2).

Species/Strain *Salmonella typhimurium*: TA1535, TA1537, TA98, TA100,
Escherichia coli: WP2uvrA

Metabolic Activation System Test 1: S9 mix from phenobarbital/β-naphthoflavone induced rat liver
Test 2: S9 mixed from uninduced hamster liver

Concentration Range in Main Test a) With metabolic activation: 31.6, 100, 316, 1,000, 2,500 and 5,000 µg/plate
b) Without metabolic activation: 31.6, 100, 316, 1,000, 2,500 and 5,000 µg/plate

Vehicle Dimethyl sulfoxide (DMSO)

Remarks – Method Test 2 was performed with the modification according to Prival and Mitchell (1982) to allow for azo reduction.

A preliminary test at a concentration range of 3.16-5,000 µg/plate (with or without metabolic activation) was conducted on TA98 and TA100.

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

Negative controls: distilled water and DMSO
Positive control: With metabolic activation: 2-aminoanthracene

Without metabolic activation: sodium azide (TA1535 and TA100), 4-nitro-o-phenylene-diamine (TA1537 and TA98) and methyl methane sulfonate (WP2uvrA).

No protocol deviations.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 5,000	> 5,000	≥ 1,000	Negative
Test 2		> 5,000	≥ 2,500	Negative
Present				
Test 1	> 5,000	> 5,000	≥ 1,000	Negative
Test 2		> 5,000	≥ 2,500	Negative

Remarks – Results In test 1, without metabolic activation, the number of revertants in strain TA1535 was reduced down to a mutation factor of 0.5 at 100 µg/plate. As no dose-response was observed, this reduction was not considered to be biologically relevant by the study authors.

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

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

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

TEST FACILITY BSL (2009e)

B.10. Genotoxicity – *In Vitro* Gene Mutation Test in Chinese Hamster Ovary Cells

TEST SUBSTANCE Notified chemical

METHOD OECD TG 476 *In vitro* Mammalian Cell Gene Mutation Test (1997)
Species/Strain Chinese hamster
Cell Type/Cell Line Chinese hamster ovary (CHO) cell line
Metabolic Activation System S9 mix from phenobarbital/β-naphthoflavone induced rat liver
Vehicle Culture medium
Remarks – Method Negative control: culture medium
Positive control:
Without S9: ethyl methanesulfonate
With S9: methylcholanthrene

In a preliminary test, CHO cells were treated with test substance at 12.7 to 3,250.0 µg/mL for 4 hours with or without metabolic activation. An additional study without metabolic activation was conducted for 24 hours.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Expression Time*	*Selection Time*
Absent				
Test 1	10*, 20*, 40*, 80*, 1,700* and 3,400*	4 h	72 h	6-7 days
Test 2	10*, 20*, 40*, 80*, 1,700* and 3,400*	24 h	48 h	6-7 days
Present				
Test 1	10*, 20*, 40*, 80*, 1,700* and 3,400*	4 h	72 h	6-7 days
Test 2	10*, 20*, 40*, 80*, 160* and 320*	4 h	72 h	6-7 days

*Cultures selected for metaphase analysis.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 3,250.0	> 3,400	≥ 40	Negative
Test 2	≥ 3,250.0	> 3,400	≥ 40	Negative
Present				
Test 1	> 3,250.0	> 3,400	≥ 40	Negative
Test 2		> 3,400	≥ 40	Negative

Remarks – Results In the preliminary toxicity test up to 3,250 µg/mL, the test substance induced some evidence of toxicity (43.7% relative cloning efficiency) at the highest concentration tested (24 hour exposure, without metabolic activation).

Increased mutation frequency value relative to the vehicle control was noted in Test 1 with metabolic activation at 20, 40 and 80 µg/mL, and in Test 2, without metabolic activation at 10, 20, 40, 1,700 and 3,400 µg/mL and with metabolic activation at 10 and 80 µg/mL. However, as this increase was within the negative control historical value, this was judged by the study authors as biologically not significant.

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

CONCLUSION The notified chemical was not clastogenic to CHO cells treated *in vitro* under the conditions of the test.

TEST FACILITY BASF (2011a)

B.11. Genotoxicity – *In Vitro* Chromosome Aberration Test in Human Lymphocytes

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 *In vitro* Mammalian Chromosome Aberration Test (1997)
Species/Strain Human
Cell Type/Cell Line Peripheral lymphocytes
Metabolic Activation System S9 mix from phenobarbital/β-naphthoflavone induced rat liver
Vehicle Dimethyl sulfoxide (DMSO)
Remarks – Method Negative control: DMSO
Positive control: without metabolic activation: mitomycin
with metabolic activation: cyclophosphamide

In a preliminary dose range finding test, human peripheral lymphocytes were treated with the test substance at 1-100 µg/mL for 24 hours and 48 hours without metabolic activation and at 10-66 µg/mL for 3 hours with or without metabolic activation.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	10*, 33* and 66*	3 h	24 h
Test 2	10*, 33*, 66 and 100*	24 h	24 h
Test 3	10,* 33*, 66 and 100*	48 h	48 h
Present			
Test 1	10*, 33* and 66*	3 h	24 h
Test 2	10*, 33* and 66*	3 h	48 h

*Cultures selected for metaphase analysis

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/mL) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	> 66	> 66	≥ 66	Negative
Test 2	> 100	> 100	≥ 66	Negative
Test 3	> 100	> 100	≥ 66	Negative
Present				
Test 1	> 66	> 66	≥ 66	Negative
Test 2	-	> 66	≥ 66	Negative

Remarks – Results No statistically significant or biologically relevant increase in the number of cells with aberrations was observed at any concentration, with and without metabolic activation. There was also no statistically significant or biologically relevant increase in the numbers of polyploidy cells or cells with endo-reduplicated chromosomes.

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

CONCLUSION The notified chemical was not clastogenic to human lymphocytes treated *in vitro* under the conditions of the test.

TEST FACILITY NOTOX (2009c)

B.12. Genotoxicity – *In Vivo* Mouse Micronucleus Test

TEST SUBSTANCE Notified chemical

METHOD OECD TG 474 Mammalian Erythrocyte Micronucleus Test (1997)
Species/Strain Mice/Crl:NMRI
Route of Administration Oral – gavage
Vehicle Corn oil
Remarks – Method A preliminary study was conducted for dose selection. Both sexes were used in the preliminary study and only males were used in the main study in accordance with the guideline. No protocol deviations.

Group	*Number and Sex of Animals*	*Dose (mg/kg bw)*	*Sacrifice Time (hours)*
I (vehicle control)	5 M	0	24
II (vehicle control)	5 M	0	48
III (low dose)	5 M	500	24
IV (mid dose)	5 M	1,000	24
V (high dose)	5 M	2,000	24
VI (high dose)	5 M	2,000	48
VII (positive control, CP)	5 M	20	24

CP = cyclophosphamide

RESULTS
Doses Producing Toxicity No unscheduled mortalities were observed during the study.

The PCE/NCE ratio did not vary significantly between the test and vehicle control groups, indicating that bone marrow toxicity did not occur.
Genotoxic Effects No increase in the incidence of micronucleated polychromatic erythrocytes was observed in the test groups.
Remarks – Results The positive control showed the expected increase in micronucleated cells, confirming the validity of the test system. Bioavailability of the test substance was confirmed by discoloured urine (yellowish) observed in high dose animals.

CONCLUSION The notified chemical was not clastogenic under the conditions of this *in vivo* mouse micronucleus test.

TEST FACILITY BASF (2016)

B.1. Reproductive and developmental toxicity

TEST SUBSTANCE Notified chemical

METHOD OECD TG 421 Reproduction/Developmental Toxicity Screening Test (1995)
Species/Strain Rat/Wistar (Crl:WI(Han))
Route of Administration Oral – gavage
Exposure Information Exposure days:
males: 31 days
females: 57 days
Dose regimen: 7 days per week
Vehicle 1% Aqueous carboxymethylcellulose
Remarks - Method No significant protocol deviations.

RESULTS

Group	*Number of Animals*	*Dose (mg/kg bw/day)*	*Mortality*
Control	10M/10F	0	0/20

Low dose	10M/10F	100	0/20
Mid dose	10M/10F	300	0/20
High dose	10M/10F	1,000	1/20

Mortality and Time to Death

A high dose female was euthanised *in extremis* on gestation day 10. Clinical signs, such as discoloured faeces and urine, reduced faeces, piloerection and pale skin were observed on days 8-10. Severely reduced nutritional condition was also noted for this animal.

Discoloured (yellow) glandular stomach, yellow contents in jejunum, yellow deposition in lungs and thoracic cavity were observed at necropsy.

Slightly enlarged kidney was also observed at necroscopy. Histopathological examination of kidney showed multifocal nephritis (grade 3) and multifocal tubular mineralisation (grade 2). Diffused inflammation (grade 3) was observed in diaphragm and this correlated to gross lesion deposition. Multifocal inflammatory cell infiltrates (grade 3) was observed in lungs. The study authors stated these effects as incidental due to gavage trauma.

Effects on F0 generation

A high dose (sperm positive) and a low dose (sperm negative) female did not conceive. Another high dose female had 2 implantation sites and delivered only a single still born pup. A low dose, a mid dose and a high dose female delivered 2/11, 1/11 and 1/8 still born pups respectively.

The average implantation sites were 12.3 (control), 9.5 (low dose), 13.8 (mid dose) and 7.7 (high dose). The low and high dose implantation site values were lower than the historical control range of 10.6-14.4 implants/dam. The study authors stated that as the reduction of implantation site for low and high dose females was not statistically significant and not toxicologically significant.

The mean duration of gestation was 21.9 days for control group, 22 days for low and mid dose groups and 22.4 days for high dose group. Statistically significant increase (2.3% increase than control females) in mean duration of gestation was recorded for the high dose group. This increase is the same as the maximum value of historical control range. Statistically significant reduction (88%) in gestation index was observed in the high dose group which is slightly below the historical control range of 89-100%. The study authors stated that given that only 7 animals in this study were used for the index calculation, this effect is not considered to be toxicologically significant.

Mean number of delivered pups were 11.5, 10.2, 12.6 and 8.5 pups/dam for control, low, mid and high dose groups, respectively. Total number of delivered pups to animals exposed to mid dose group was comparatively higher than the control group (9% increase compared to control). This was due to higher number of implantation sites (138 and in control 123) but information on corpora lutea was not reported for the mid dose group. The mean number of delivered pups for the high dose group were below the historical control range of 9.3-14.1 pups/dam. The study authors stated this value was due to a female that had 2 implantation sites with no live born pups. This observation is therefore considered as incidental and not toxicologically relevant.

The post-implantation loss were 6.64% for control, 2.62% for low dose, 8.97% for mid dose and 10.71% for high dose groups.

The live birth indices for control, low, mid and high dose groups were 100%, 96.7%, 98.4% and 98.5%, respectively.

Histopathology

Yellow discolouration of the digestive tract was observed in all or most treated animals.

Slight degeneration in the testicular tubuli was observed in a high dose male. Given that only 1 animal had this symptom, the study authors stated this was spontaneous and not treatment related.

Mating partners of a low and a high dose male did not conceive; however, the respective males did not show relevant gross lesions. Sperm granuloma was observed in these males. The study authors did not regard this condition to be the cause of the infertility. The study authors also stated as no dose response was observed,

sperm granuloma observed in 2 males is not considered to be treatment related, and indicated this effect is one of the most common changes observed in young adult rat epididymides (McInnes, 2012).

Effects on Foetus

Pups of low dose parents showed dilated renal pelvis (2 males and 2 females) and dilated ureter (1 male). The study authors stated comparable or higher incidences of these findings were observed in historical control animals and not considered to be treatment related.

Remarks - Results

All clinical signs were reported as within the expected range for animals of this strain and age. No treatment-related statistically significant differences were observed on mean body weights, body weight changes and food consumption.

CONCLUSION

The NOAEL for parental and reproductive and developmental toxicity was established as 1,000 mg/kg bw/day in this study.

TEST FACILITY BASF (2015b)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready Biodegradability Study 1

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test
Inoculum	Activated sludge from a domestic STP
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Oxygen consumption by OxiTop system
Remarks – Method	No major deviations from the test guidelines were reported. The test substance was directly added to the test medium in the test bottles. A toxicity control was run.

RESULTS

Test Substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
0	0	0	0
14	0	14	89
28	0	28	91

Remarks – Results	All validity criteria for the test were satisfied. The test substance did not degrade after 28 days.
CONCLUSION	The test substance is not readily biodegradable.
TEST FACILITY	Currenta (2009c)

C.1.2. Ready Biodegradability Study 2

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 301 F Ready Biodegradability: Manometric Respirometry Test
Inoculum	Activated sludge from a STP
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Oxygen consumption by OxiTop system
Remarks – Method	No major deviations from the test guidelines were reported. The test substance was directly added to the test medium in the test bottles and ultra-sonicated for 10 minutes. A toxicity control was run.

RESULTS

Test Substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
0	0	0	0
14	13	14	88
28	23	28	-

Remarks – Results	Temperature during the test was 22.7 °C to 25.3 °C. There was 2 points of hourly temperature record which deviated from 22±1 °C. The authors concluded that based on the observed data, the temperature deviation did not affect the validity of the test. Other validity criteria for the test were satisfied. The degree of degradation of the test substance after 28 days was 23%.

CONCLUSION	The test substance is not readily biodegradable.
TEST FACILITY	GDCM (2013a)

C.1.3. Inherent Biodegradability

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 302C Inherent Biodegradability: Modified MITI Test (II)
Inoculum	Mixture of sewage, seawater and river water from 10 sites
Exposure Period	28 days
Auxiliary Solvent	None
Analytical Monitoring	Oxygen consumption by B1-2000 respirometer
Remarks – Method	No major deviations from the test guidelines were reported. The test substance was directly added to the test medium in the test bottles. The solution was ultra-sonicated for 10 minutes, and then stirred for 4 hours under the condition of sealing.

RESULTS

Test Substance		*Sodium benzoate*	
Day	*% Degradation*	*Day*	*% Degradation*
1	3	1	18
14	0	14	106
28	0	28	108

Remarks – Results	All validity criteria for the test were satisfied. The test substance did not degrade after 28 days.
CONCLUSION	The test substance is not inherently biodegradable.
TEST FACILITY	GDCM (2014b)

C.2. Ecotoxicological Investigations

C.2.1. Acute Toxicity to Fish Study 1

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test - Static EC Council Regulation No 440/2008 C.1 Acute Toxicity for Fish - Static
Species	*Danio rerio* (Zebrafish)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	UV/VIS Spectrometer
Remarks – Method	Based on results from a preliminary range finding test, a limit test was run with no major deviations from the test guidelines. A test concentration of 100 mg/L was prepared with test medium and stirred for 3 days before testing. The test water was sampled at the start and at the end of the experiment for analysis of the test substance.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*
Nominal	*Measured*		*96 h*
Control	Control	7	0
100	105	7	0

LC50	> 100 mg/L (nominal concentration) at 96 hours

	> 105 mg/L (measured concentration) at 96 hours
Remarks – Results	All validity criteria for the test were satisfied. Dissolved oxygen concentration during the test was ≥ 7.2 mg/L at 24 °C (≥ 86%, USGS, 2011). The test water samples were filtered, and both filtered and unfiltered samples were analysed. There was no appreciable difference in measured concentrations between filtered and unfiltered samples, indicating that all of the test substance was dissolved in the test water. All measured concentrations were within ± 20% of the nominal concentration.
CONCLUSION	The test substance is not harmful to fish.
TEST FACILITY	BASF (2011b)

C.2.2. Acute Toxicity to Fish Study 2

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 203 Fish, Acute Toxicity Test - Static
Species	*Brachydanio rerio* (Zebrafish)
Exposure Period	96 hours
Auxiliary Solvent	None
Water Hardness	124 mg $CaCO_3$/L
Analytical Monitoring	HPLC
Remarks – Method	Based on results from a preliminary range finding test, a limit test was run with no major deviations from the test guidelines. A test loading rate of 105 mg/L was prepared with test medium and stirred for 48 hours. The solution was then filtered and the filtered aqueous solution was used for testing. The test water was sampled at the start and at the end of the experiment for analysis of the test substance. A reference test with potassium dichromate was run.

RESULTS

Concentration (mg/L)		*Number of Fish*	*Mortality*
Nominal	*Measured*		*96 h*
Control	Control	10	0
105	86.7	10	0

LC50	> 105 mg/L (nominal concentration) at 96 hours > 86.7 mg/L (measured concentration) at 96 hours
Remarks – Results	All validity criteria for the test were satisfied. Dissolved oxygen during the test was ≥ 81%. All measured concentrations were within ± 20% of the nominal concentration. The 96h LC50 for fish exposed to potassium dichromate was within the range of expected responses.
CONCLUSION	The test substance is not harmful to fish up to its water solubility limit.
TEST FACILITY	GDCM (2013b)

C.2.3. 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	287 mg $CaCO_3$/L
Analytical Monitoring	HPLC - UV/VIS
Remarks – Method	Based on results from a preliminary range finding test, a limit test was run with no major deviations from the test guidelines. A solution of 105.5 mg/L test substance was prepared with test medium and ultra-sonicated for 1 hour. The solution was stirred for 24 hours before being filtered. 19 mL of the filtered aqueous solution were then taken and diluted with 1 mL dilution water containing 10 daphnids resulting in a final nominal test concentration of 100 mg/L. The test water was sampled at the start and at the end of the experiment for analysis of the test substance.

RESULTS

Concentration (mg/L)		*Number of D. magna*	*Number Immobilised*
Nominal	*Measured*		*48 h*
Control	Control	20	0
100	24.05	20	0

EC50	> 100 mg/L (nominal concentration) at 48 hours > 24.05 mg/L (measured concentration) at 48 hours
Remarks – Results	All validity criteria for the test were satisfied. Dissolved oxygen during the test was ≥ 93%. Measured results show the test substance concentration was stable during the test.

CONCLUSION The test substance is not harmful to aquatic invertebrates up to its water solubility limit.

TEST FACILITY Currenta (2009d)

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	*Desmodesmus subspicatus* (former name: *Scenedesmus subspicatus*)
Exposure Period	72 hours
Concentration Range	Calculated: 1.9, 3.7, 7.5, 15 mg/L Measured: 1.1, 2.4, 4.2, 10.3 mg/L
Auxiliary Solvent	None
Water Hardness	22.5 mg $CaCO_3$/L
Analytical Monitoring	HPLC - UV/VIS
Remarks – Method	Based on results from a preliminary range finding test, a limit test was run with no major deviations from the test guidelines. 125.3 mg of test substance were added to 1 litre of dilution water. The solution was ultra-sonicated for 1 hour and stirred for 24 hours before being filtered. The measured concentration of the test substance in the filtered aqueous solution was 18.7 mg/L. The calculated test concentrations were achieved by diluting this stock solution with dilution water, nutrient stock solution and algae.

RESULTS

Biomass		*Growth*	
ECr50	*NOEC*	*ECy50*	*NOEC*
(mg/L at 72 h)	*(mg/L)*	*(mg/L at 72 h)*	*(mg/L)*
> 10.3	≥ 10.3	> 10.3	≥ 10.3

Remarks – Results	All validity criteria for the test were satisfied. Measured results show the test substance concentration was stable during the test. The mean cell density in the control increased 135 times after 72 hours.
CONCLUSION	The test substance is not harmful to algae up to its water solubility limit.
TEST FACILITY	Currenta (2009e)

BIBLIOGRAPHY

BASF (2011a) [Notified Chemical] *In Vitro* Gene Mutation Test in CHO Cells (HPRT Locus Assay) (Study No: 50M0592/10M131, May, 2011). Ludwigshafen, Germany, BASF SE (Unpublished report submitted by the notifier).

BASF (2011b) [Notified Chemical] Acute Toxicity Study in the Zebrafish (Danio rerio) (Project No. 17F0592/10E101, September, 2011). Ludwigshafen, Germany, BASF SE (Unpublished report submitted by the notifier).

BASF (2014) [Notified Chemical] Acute Inhalation Toxicity Study in Wistar Rats 4-Hour Dust Exposure (Head-Nose Only) (Study No: 13/0592/10/175, February, 2014). Ludwigshafen, Germany, BASF SE (Unpublished report submitted by the notifier).

BASF (2015a) [Notified Chemical] Micronucleus Test in Bone Marrow Cells of the Mouse (Study No: 26M0592/10M215, March, 2016). Ludwigshafen, Germany, BASF SE (Unpublished report submitted by the notifier).

BASF (2015b) [Notified Chemical] Reproduction/Developmental Toxicity Screening Test in Wistar Rats Oral Administration (Gavage) (Study No: 80R0592/10R005, August, 2015). Ludwigshafen, Germany, BASF SE (Unpublished report submitted by the notifier).

BASF (2016) [Notified Chemical] Micronucleus Test in Bone Marrow Cells of the Mouse (Study No: 26M0592/10M215, March, 2016). Ludwigshafen, Germany, BASF SE (Unpublished report submitted by the notifier).

BASF (2018) Migration Report for Colorant Paliotol Yellow K 1750 (Study No: 17B05781, November, 2018). Ludwigshafen, Germany, BASF SE (Unpublished report submitted by the notifier).

Bioassay (2013) [Notified Chemical] Acute Dermal Irritation/Corrosion in Rabbits (Study No: 13-BF-DI118, December, 2013). Heidelberg, Germany, Bioassay GmbH (Unpublished report submitted by the notifier).

BSL (2009a) Acute Oral Toxicity, Acute Toxic Class Method with [Notified Chemical] (Study No: 084888, March, 2009). Planegg, Germany, BSL Bioservice Scientific Laboratories GmbH (Unpublished report submitted by the notifier).

BSL (2009b) *In Vitro* Skin Irritation: Human Skin Model Test with [Notified Chemical] (Study No: 084891, February, 2009). Planegg, Germany, BSL Bioservice Scientific Laboratories GmbH (Unpublished report submitted by the notifier).

BSL (2009c) Acute Eye Irritation/Corrosion with [Notified Chemical] (Study No: 084889, February, 2009). Planegg, Germany, BSL Bioservice Scientific Laboratories GmbH (Unpublished report submitted by the notifier).

BSL (2009d) Test for Sensitisation (Local Lymph Node Assay – LLNA) with [Notified Chemical] (Study No: 084890, March, 2009). Planegg, Germany, BSL Bioservice Scientific Laboratories GmbH (Unpublished report submitted by the notifier).

BSL (2009e) Reverse Mutation Assay using Bacteria (*Salmonella typhimurium* and *Escherichia coli*) – modified for azo compounds - with [Notified Chemical] (Study No: 084887, February, 2009). Planegg, Germany, BSL Bioservice Scientific Laboratories GmbH (Unpublished report submitted by the notifier).

Brown MA and DeVito SC (1993). Predicting Azo Dye Toxicity. *Critical Reviews in Environmental Science and Technology*. 23(3):249-324.

Ciba (2008a) Melting point of [Notified Chemical] (Study No. 49139, December, 2008). Basel, Switzerland, Ciba Inc (Unpublished report submitted by the notifier).

Ciba (2008b) Relative Density of [Notified Chemical] (Study No. 08/369, December, 2008). Basel, Switzerland, Ciba Inc (Unpublished report submitted by the notifier).

Ciba (2008c) Particle Size Distribution of [Notified Chemical] (Study No. 08/370, December, 2008). Basel, Switzerland, Ciba Inc (Unpublished report submitted by the notifier).

Ciba (2009) [Notified Chemical] n-Octanol and Water Solubility (Analyse No. 49139, March, 2009). Basel, Switzerland, Ciba Inc (Unpublished report submitted by the notifier).

Currenta (2009a) Determination of Safety-Relevant Data of [Notified Chemical] (Study No. 2009/00189, April, 2008). Leverkusen, Germany, Currenta GmbH & Co. OHG (Unpublished report submitted by the notifier).

Currenta (2009b) Determination of Safety-Relevant Data of [Notified Chemical] (Study No. 2009/00382, April, 2008). Leverkusen, Germany, Currenta GmbH & Co. OHG (Unpublished report submitted by the notifier).

Currenta (2009c) Biodegradation with [Notified Chemical] (Study No. 2009/0005/05, July, 2009). Leverkusen, Germany, Currenta GmbH & Co. OHG (Unpublished report submitted by the notifier).

Currenta (2009d) Daphnia sp., Acute Immobilisation Test with [Notified Chemical] (Study No. 2009/0005/02, August, 2009). Leverkusen, Germany, Currenta GmbH & Co. OHG (Unpublished report submitted by the notifier).

Currenta (2009e) Alga, Inhibition Test with [Notified Chemical] (Study No. 2009/0005/03, August, 2009). Leverkusen, Germany, Currenta GmbH & Co. OHG (Unpublished report submitted by the notifier).

GDCM (2013a) Ready Biodegradability: Manometric Respirometry Test of [Notified Chemical] (Study No. 2012ESG0238, May, 2013). Guangzhou, China, Guangdong Detection Center of Microbiology (Unpublished report submitted by the notifier).

GDCM (2013b) Acute Toxicity Test of [Notified Chemical] with Zebra Fish (*Brachydanio rerio*) (Study No. 2012ESG0236, May, 2013). Guangzhou, China, Guangdong Detection Center of Microbiology (Unpublished report submitted by the notifier).

GDCM (2014a) Hydrolysis as a Function of pH test of [Notified Chemical] (Study No. 2013ESG0203, May, 2014). Guangzhou, China, Guangdong Detection Center of Microbiology (Unpublished report submitted by the notifier).

GDCM (2014b) Inherent Biodegradability: Modified MITI Test (II) of [Notified Chemical] (Study No. 2013ESG0205, June, 2014). Guangzhou, China, Guangdong Detection Center of Microbiology (Unpublished report submitted by the notifier).

McInnes EF (2012) Background lesions in laboratory animals. A color atlas. Elsevier, Edinburgh, London, New York, Oxford, Philadelphia, St Louis, Sydney, Toronto.

NOTOX (2009a) Assessment of Acute Dermal Toxicity with [Notified Chemical] in the Rat (Study No: 490672, May, 2009). 's-Hertogenbosh, The Netherlands, NOTOX B.V. (Unpublished report submitted by the notifier).

NOTOX (2009b) Repeated Dose 28-Day Oral Toxicity Study with [Notified Chemical] by Daily Gavage in the Rat followed by a 14-Day Recovery Period (Study No: 490668, August, 2009). 's-Hertogenbosh, The Netherlands, NOTOX B.V. (Unpublished report submitted by the notifier).

NOTOX (2009c) Evaluation of the Ability of [Notified Chemical] to Induce Chromosome Aberrations in Cultured Peripheral Human Lymphocytes (with Repeat Experiment) (Study No: 490671, April, 2009). 's-Hertogenbosh, The Netherlands, NOTOX B.V. (Unpublished report submitted by the notifier).

Prival MJ and Mitchell VD (1982) Analysis of a method for testing azo dyes for mutagenic activity in Salmonella typhimurium in the presence of flavin mononucleotide and hamster liver S9. Mutat Res. 97(2): 103-16.

SCCNFP (2002) The Safety Review of the Use of Certain Azo-Dyes in Cosmetic Products. Opinion of the Scientific Committee on Cosmetic Products and Non-Food Products Intended for Consumers. Available at https://ec.europa.eu/health/archive/ph_risk/committees/sccp/documents/out155_en.pdf

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 >

USGS (2011) U.S. Geological Survey, Change to solubility equations for oxygen in water: Office of Water Quality Technical Memorandum 2011.03, accessed https://water.usgs.gov/software/DOTABLES/ v 3.5, 8 January 2018