File No: STD/1329

September 2009

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Y4002

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cwlth) (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 Ageing, 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, Water, Heritage and the Arts.

For the purposes of subsection 78(1) of the Act, this Full Public Report may be inspected at our NICNAS office by appointment only at 334-336 Illawarra Road, Marrickville NSW 2204.

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

Street Address:	334 - 336 Illawarra Road MARRICKVILLE NSW 2204, AUSTRALIA.
Postal Address:	GPO Box 58, SYDNEY NSW 2001, AUSTRALIA.
TEL:	+ 61 2 8577 8800
FAX	+ 61 2 8577 8888
Website:	www.nicnas.gov.au

Director
NICNAS

TABLE OF CONTENTS

FULL PUBLIC REPORT

Y4002

1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
DIC Australia Pty Ltd (ABN: 12 000 079 550)
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)
Data items and details claimed exempt from publication:
Chemical name; Other names; CAS number; Molecular formula; Structural formula; Molecular weight; Spectral data; Methods of detection and determination; Purity; Additives/adjuvants; Import volume; Identity of recipients

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
No variation to the schedule of data requirements is claimed.

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
Japan (2006), Korea (2006), China (2008)

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Y4002
Symuler Fast Yellow 4230 (containing 50% notified chemical)

OTHER NAME(S)
Yellow pigment

MOLECULAR WEIGHT
>500 Da

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

3. COMPOSITION

The notified chemical is a reaction mixture consisting of several structurally related azo components derived from 3,3'-dichlorobenzidine. The major component is present at >90% of the reaction mixture (an azo pigment).

DEGREE OF PURITY >99%

HAZARDOUS IMPURITIES/RESIDUAL MONOMERS None.
The notifier has indicated that there are no detectable levels of impurities of 3,3'-dichlorobenzidine (detection limit 10ppm).

NON HAZARDOUS IMPURITIES/RESIDUAL MONOMERS (>1% by weight) None

4. PHYSICAL AND CHEMICAL PROPERTIES

APPEARANCE AT 20°C AND 101.3 kPa: Yellow powder

Property	Value	Data Source/Justification
Melting Point	313°C (with reaction/decomposition during melting)	Measured
Boiling Point	>313°C	Measured
Density	1.40 x 10^3 kg/m^3 at 20°C	Measured
Vapour Pressure	<1.33 x 10^{-11} kPa at 20°C	Measured
Water Solubility	< 0.4 mg/L at 20°C	Measured
Hydrolysis as a Function of pH	Expected to be stable based on the structure.	Hydrolytic stability could not be measured because of low water solubility and the lack of a sufficiently sensitive analytical method.
Partition Coefficient (n-octanol/water)	log P_{ow} > 6.5 at 22°C	Measured (HPLC method)
Adsorption/Desorption	log K_{oc} > 5.6 at 35°C	Measured (HPLC method)
Dissociation Constant	Not expected to dissociate in the environmental pH range (4–9)	The dissociation constant could not be measured because of low water solubility and the lack of a sufficiently sensitive analytical method.
Particle Size	Inhalable fraction (<100 μm): ~97% Respirable fraction (<10 μm): 8.82% MMAD* = 34.324 μm	Measured
Flash Point	Not determined	Low vapour pressure solid
Solid Flammability	Not highly flammable	Measured
Flammability in Contact with Water	Not expected to be highly flammable in contact with water or damp air	Estimated based on structure
Pyrophoric Properties	Not expected to have pyrophoric properties	Estimated based on structure
Autoignition Temperature	Did not auto-ignite at temperatures below its melting point.	Measured
Explosive Properties	Not explosive	Measured
Oxidising Properties	Not expected to be oxidising	Estimated

* MMAD = Mass Median Aerodynamic Diameter

DISCUSSION OF PROPERTIES

The notified chemical has a very low solubility in water, as tabulated above, and in n-octanol (between 5 and 10 mg/L). Based on structural considerations, it is noted that the water solubility of some minor components of the notified chemical (each present at levels <10%) may be higher than that of the notified chemical itself (as the reaction mixture). For full details of tests on physical and chemical properties, please refer to Appendix A.

Reactivity

Under normal conditions the notified chemical does not react with water or air.

5. INTRODUCTION AND USE INFORMATION

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

The notified chemical will be imported at concentrations of 50-100% and in printing inks at concentrations of 4 - 8%.

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

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	1-3	3-10	3-10	10-30	30-100

PORT OF ENTRY
Sydney, Melbourne

TRANSPORTATION AND PACKAGING
The imported notified chemical will be transported by truck in kraft paper bags with a laminated liner (10kg) and in flexible containers (300kg). Printing inks will be transported in cans (18L), drums (200L) and plastic cartridges.

USE
The notified chemical will be used as a pigment in printing inks. It will be used to print paper or film substrates, including newspapers, magazines, catalogues, packaging materials, etc.

OPERATION DESCRIPTION
Formulation
The notified chemical (50 – 100% concentration) will be weighed either by counting the number of paper bags (known to contain 10kg per bag) or by manual weighing under local ventilation for quantities smaller than 10kg. It will then be transferred by manual emptying of the paper bags into a hopper that is fitted with a dust extractor (local ventilation equipment) together with other ingredients. Alternatively, for larger volumes of pigment (50-100% notified chemical) that are imported in flexible 300kg containers, the contents will be transferred into the hopper using automated procedures. From the hopper the material will then be pumped into an enclosed mixing tank. It will be mixed in a closed tank with other ingredients. At the completion of the mixing, the concentration of the notified chemical in the ink will be 4-8%. It will then be transferred to a kneader or beads mill for processing in a closed system. This transfer will be performed using a pump and hosing. During milling, quality control sampling will involve removing samples using a sampling valve. After milling, the processed ink containing the notified chemical (4-8%) will be transferred to packaging containers through a filter using a pump and hosing. Filters will be changed after this transfer and washed with organic solvents. Equipment will be cleaned by circulating organic solvent.

End use
Printing using inks containing the notified chemical (4-8%) will be performed using conventional offset printing processes that may also include heat-set drying. Generally, the ink containing the notified chemical (4-8%) will be transferred to the printing machine with a pump and through a filter (if necessary). Filters will be removed and washed with organic solvents. The printing process itself will be automated. For heat-set inks, the printed material will be briefly placed in a heatset oven that will typically be at temperatures of 120ºC (range of 100 - 130 ºC). Any ink remaining on the printing machine will be wiped off with a waste cloth soaked in organic solvents.

6. HUMAN HEALTH IMPLICATIONS

6.1 Exposure assessment

6.1.1 Occupational exposure

NUMBER AND CATEGORY OF WORKERS

Category of Worker	*Number*	*Exposure Duration*	*Exposure Frequency*
Printing ink formulators			
Mixing	2	30 minutes/day	80 times/year
Milling and packaging	2	2 hours/day	80 times/year
Printing ink users			
Charging	8	10 minutes/day	80 times/year

EXPOSURE DETAILS

Formulation

Inhalation exposure of workers to the notified chemical in powder form at concentrations of 50-100% may occur when weighing and transferring pigments to the mixing tank during ink formulation processes. The use of dust extractors and local ventilation systems is expected to collect most pigments containing the notified chemical that may be scattered during these processes. EASE modelling predicts dust exposure of 2-5 mg/m^3 (for powders consisting of 100% of the notified chemical), assuming that effective local exhaust ventilation is utilised. For a 70 kg worker, assuming an inhalation rate of 1.3 m^3/h, 4 hour exposure and that the notified chemical was all either absorbed or ingested via the mucociliary clearance mechanism, systemic exposure after inhalation is estimated to be 0.15-0.37 mg/kg bw/day. This estimate assumes that no respiratory protection is worn. However, the notifier has indicated that dust protective masks are expected to be worn during such procedures.

The process of weighing and transferring pigments to the mixing tank may also result in dermal and ocular exposure of workers to the notified chemical (50-100%). EASE modelling predicts 'very low' dermal exposure during such processes. Workers are also expected to wear personal protective equipment such as gloves, safety glasses, overalls, etc, that should further lower the potential for exposure.

Dermal and accidental ocular exposure of workers to the notified chemical at concentrations of 4-8% may occur during the occasional connecting and disconnecting of hoses from the mixing tank to the beads mill or from the beads mill to packaging. EASE modelling of the pipe disconnection, cleaning and quality control operations was performed to estimate dermal exposure of workers to the notified chemical. The following assumptions were used for these estimates: direct handling, non-dispersive use (only used by workers with knowledge of the processes and use of controls), and incidental contact level (assumed to be one event per day). The predicted dermal exposure to the notified chemical is up to 0.008 mg/cm^2/day. This is equivalent to up to 0.0048 mg/kg bw/day, based on dermal absorption of 10%, a surface area of 420 cm^2 (equivalent to the area of one hand or two half hands) and that the notified chemical is present at 8% concentration (EC, 2003). Such exposure is expected to be lowered by the use of personal protective equipment such as gloves, safety glasses and perhaps also protective masks.

Dermal and accidental ocular exposure of workers to the notified chemical at concentrations of 4-8% may also occur during quality control sampling, cleaning of removed filters and other equipment. Such exposure is expected to be lowered by the use of personal protective equipment such as gloves, safety glasses and perhaps also protective masks.

The total systemic exposure after both inhalation and dermal exposure is estimated to be 0.1548 - 0.3748 mg/kg bw/day. The lower end of this range is considered to be a more appropriate estimate of exposure when local exhaust ventilation is present and dermal protection is worn.

End use

Dermal and ocular exposure of workers to the notified chemical at concentrations of 4-8% may occur during transfer of inks to the printing machine, cleaning of filters and printing equipment, and contact with printed materials. Such exposure is expected to be lowered by the use of personal protective equipment such as gloves, safety glasses and, where necessary, protective masks. Also, during contact with the printed material, the ink will often be dry and thus the notified chemical should be trapped within a dried matrix and be unavailable for exposure. The notifier has also stated that for some end use applications the notified chemical will be covered by resin following its printing onto the substrate, which would minimise exposure.

6.1.2. Public exposure

The general public may make dermal contact with articles printed with the ink containing the notified chemical. However, once the ink has dried, the notified chemical will be trapped within the dried matrix and is not expected to be bioavailable. Whilst the potential for blooming and bleeding of the notified chemical from the substrate may exist, it is expected that the inks containing the notified chemical will be appropriately formulated with other ingredients and processed such as to minimise the tendency to bleed.

6.2. Human health effects assessment

The results from toxicological investigations conducted on the notified chemical are summarised in the table below.

Endpoint	*Result and Assessment Conclusion*
Rat, acute oral toxicity	low oral toxicity LD50 >2000 mg/kg bw
Rat, acute dermal toxicity	low dermal toxicity LD50 >2000 mg/kg bw
Rat, acute inhalation toxicity	harmful inhalation toxicity LC50 in range of 1.0 – 5.0 mg/L/4 hour
Rabbit, skin irritation	slightly irritating
Rabbit, eye irritation	non-irritating
Mouse, skin sensitisation – Local lymph node assay	no evidence of sensitisation
Rat, repeat dose oral toxicity – 28 days.	NOAEL ≥ 1000 mg/kg bw/day
Mutagenicity – bacterial reverse mutation	non mutagenic
Genotoxicity – in vitro mammalian chromosomal aberration test	non genotoxic

Note: there is a significant amount of toxicological information available on the major component of the notified chemical (an azo pigment that is structurally related to the minor components of the notified chemical and is derived from 3,3'-dichlorobenzidine). Where relevant, such data will be detailed in the below discussion, together with that on the notified chemical itself.

Toxicokinetics
The notified chemical has relatively high molecular weight (>500 Da), low water solubility (<0.4 mg/L) and high partition coefficient (log P >6.5). Some of the minor components of the notified chemical (<10% composition), while structurally related to the major component, have additional functional groups that may lead to higher water solubility of these components compared to the notified chemical itself. However, given that no solubility for the notified chemical was observed down to the level of detection in both the water solubility study and in the test medium of the ecotoxicity studies, the minor components are not expected to be significantly water soluble.

Based on its chemical properties (relatively high molecular weight, low water solubility and high partition coefficient), absorption of the notified chemical through the skin is not expected to be significant (European Commission 2003, NOTOX 2007g). However, the death of one male animal and clinical signs in surviving animals during the acute dermal toxicity study could not be excluded as being treatment related, and therefore suggest that some of the notified chemical may be absorbed following dermal exposure.

Systemic exposure following oral administration of the notified chemical is not expected to be significant as its absorption from the gastrointestinal tract is likely to be limited by its water solubility and molecular weight. Any uptake is likely to occur via micellular solubilisation, given its highly lipophilic nature and low water solubility. Increased liver weights in the oral repeated dose study may be indicative of absorption and metabolism. Yellow stool in the acute and repeated dose oral studies suggests that the main route of excretion is through the faeces, and therefore indicate that the majority of the notified chemical is not absorbed from the gastrointestinal tract.

Studies on the major component of the notified chemical have shown that it was not absorbed following oral ingestion or dermal administration in rats, with the majority of the dose being excreted in the faeces following oral administration, or remaining at the application site following dermal application (Exempt reference 2,6).

The vast majority of the notified chemical (~97%) is of inhalable (< 100 µm) particle size and could be inhaled into the upper respiratory tract. Some (~9%) is also of small enough particle size (<10 µm) to reach the lower respiratory tract (tracheobronchial and pulmonary regions). Larger particles of inhalable size are expected to deposit in the nasopharyngeal region and be cleared by coughing/sneezing or be swallowed. Due to the low water solubility of the notified chemical smaller particles lodging in the tracheobronchial region are likely to be cleared by the mucociliary mechanism and swallowed. However, inhaled respirable particulates of the notified chemical lodging in the pulmonary region may not be readily cleared due to its low water solubility. There may be some potential for absorption across the respiratory tract epithelium due to its lipophilic nature. In summary, higher concentrations of exposure may be expected to result in increased impairment of clearance mechanisms (European Commission 2003, NOTOX 2007g).

Azo compounds may also break down to their component amines. The azo linkage is the most labile portion of an azo colourant molecule, and it is readily enzymatically metabolised in mammals, including man (SCCNFP, 2002). Liver azo reductase enzymes reductively cleave the molecules into component amines. Some metabolism of azo colourants may also occur in the cells of the bladder wall, and during percutaneous absorption. Intestinal bacteria are also capable of catalysing reductive cleavage of the azo bond. Any cleavage of the notified chemical

that may occur in the gastrointestinal tract, though limited, will result in molecules with different physical and chemical properties than the notified chemical and are expected to be absorbed to a greater extent. The metabolites of the notified chemical are expected to be excreted via the bile or the urine (NOTOX 2007g).

Several studies on the major component of the notified chemical and structurally related chemicals have suggested that the degree of breakdown is low. Following oral administration to test animals these studies did not detect significant amounts of 3,3'-dichlorobenzidine in the urine of the animals (Exempt references 1,3). A rat inhalation study revealed no detectable levels of 3,3'-dichlorobenzidine in the urine or blood, suggesting that the chemical was not metabolically cleaved following inhalation (Exempt reference 3). Levels of 3,3'-dichlorobenzidine and monoacetyl-3,3'-dichlorobenzidine were below the detection limit in all urine samples obtained from textile workers who had been exposed to dichlorobenzidine based pigments (Exempt reference 3).

Acute toxicity.
The notified chemical was found to be of low acute oral and dermal toxicity in studies conducted on rats (LD50 > 2,000 mg/kg bw). During the acute dermal toxicity study, one male animal died and dark red discoloration was observed in its lungs, while other animals displayed clinical signs such as shallow respiration, ptosis and chromodacryorrhoea.

The notified chemical was determined to be of harmful acute inhalation toxicity (LC50 in the range of 1.0 – 5.0 mg/L/4hr) in a study where rats were exposed through the nose only to a solid/powder aerosol of the notified chemical. The notified chemical was tested at actual concentrations of 2.4 mg/L and 1.2 mg/L and the particles had a mass median aerodynamic diameter (MMAD) of 1.1 – 2.9 µm. Four of the five animals of each sex tested at the higher concentration were dead by the end of the exposure period, with observations in the survivors including slow breathing, laboured respiration, hunched posture and piloerection. All of the animals that died during exposure exhibited yellowish granular contents of the larynx. One of these males also showed yellowish granular contents in the stomach and many dark red foci in the lungs. The male survivor also showed many yellowish foci in the lungs. At the lower test concentration, there were a small number of observations of slow breathing and hunched posture. One female from this group was also observed to have many yellowish foci in the lungs at termination.

Irritation and Sensitisation.
The notified chemical was found to be slightly irritating to the skin of rabbits. Slight erythema was observed in all animals at the one hour observation, though it only persisted in one animal beyond this time (and was resolved by 72 hours). Such irritation was not sufficient to warrant hazard classification.

The notified chemical was found to be non-irritating to rabbit eyes.

The notified chemical was not a skin sensitiser when tested in a mouse local lymph node assay up to a concentration of 25% (maximum concentration that could technically be applied). Therefore, the notified chemical is unlikely to cause skin sensitisation in exposed humans. This is further supported by human patch testing that was performed with the major component of the notified chemical (Exempt reference 10). However, there is a report of skin sensitisation of the major component in humans (Exempt reference 1).

Repeat Dose Toxicity.
Effects observed during a 28 day oral repeat dose study in rats included soft stool, yellow staining of the body, and increased relative liver weights in females (considered to be adaptive effects) at the mid (250 mg/kg bw/day) and high (1000 mg/kg bw/day) dose levels. Based on the absence of any toxicological significant effects observed at the highest dose level, the NOAEL was considered to be ≥ 1000 mg/kg bw/day.

The effects of chronic inhalation exposure to the notified chemical was not studied. As mentioned above, impairment of lung clearance mechanisms may occur based on its low water solubility. In addition, the effects observed during the acute inhalation toxicity study suggest that the possibility of chronic respiratory effects cannot be ruled out.

Mutagenicity and Carcinogenicity.
Azo colourants are a concern for their potential induction of mutagenicity and carcinogenicity mainly through the aromatic amines that are present as impurities in the colourants, or that arise from their azo reduction in or outside of the body.

The notified chemical may be reductively cleaved to release one of the restricted aromatic amines specified in

the Appendix to EC Directive 76/769/EEC (EC, 2004), that is, 3,3'-dichlorobenzidine, CAS number 91-94-1, which is a category 2 carcinogen.

The notified chemical did not appear to contain impurities of component arylamines based on the HPLC information provided. The notifier also provided information indicating that 3,3'-dichlorobenzidine was not detectable (determination limit of 10ppm). As such, impurity levels are unlikely to contribute to carcinogenicity of the notified chemical.

The extent of azo reduction through metabolism is not known, however it is thought that this is likely to be lesser in pigments than in dyes due to reduced bioavailablity. It is noted that the EU and IARC lists benzidine-based azo dyes as being category 2 carcinogens (IARC 1987). However, this is not the case for 3,3'-dichlorobenzidine based azo pigments (Exempt reference 3).

However, the German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area (DFG, 1988) states that "*all azo colourants whose metabolism can liberate a carcinogenic aryl amine are suspected of having carcinogenic potential*." It is also mentioned that if there are indications that the colourant or its carcinogenic break down products are not bioavailable, the absence of risk needs to be proven either experimentally, substantiated by biomonitoring, or suitable animal experiments performed to rule out carcinogenic potential.

Amines may also be released during storage or processing. The USEPA considers dichlorobenzidine-based pigments to be of concern for their potential release of 3,3'-dichlorobenzidine when used at temperatures in excess of 200^{o}C (when release of 3,3'-dichlorobenzidine may occur) (USEPA 2002).

The notified chemical was not mutagenic in bacteria (under the conditions of the Ames test used), nor did it induce chromosomal aberrations in mammalian cells *in vitro*. In both studies, precipitation of the test substance was observed at several of the concentrations tested. An *in vivo* genotoxicity study was not provided.

The Ames test provided was not performed in accordance with the modified procedure suggested for azo compounds (Prival and Mitchell, 1982). Modified tests, such as that of Prival and Mitchell, utilise a reductive pre-incubation step (during which the azo colourant may be reduced to amine species) before the test is carried out to yield a greater detection of mutagenic azo species. It is recognised that the standard procedure is not sufficiently sensitive for azo compounds, likely due to their complex metabolism *in vivo* (Brown and DeVito 1993, referenced in Øllgaard *et al* 1998). Given this deficiency in the study performed on the notified chemical, the test result may not be strongly predictive of the mutagenicity of the notified chemical *in vivo*.

The major component of the notified chemical has been extensively tested for its genotoxicity. It was negative in several Ames tests (including some studies that involved prior reduction of the azo pigment) (Exempt references 4,9) and at least one chromosome aberration test (Exempt reference 8). However, one sister chromatid exchange test gave equivocal results (Exempt reference 8) and it was genotoxic in hepatocytes in a comet assay (Exempt reference 7). One study tested metabolites of the azo colourant and found them to be strongly mutagenic (Exempt reference 5). The lack of mutagenicity generally observed for the main component of the notified chemical has sometimes been attributed to its lack of solubility in the test medium (Exempt references 1,3,9).

Several in vivo tests have also been performed on the carcinogenicity of the major component of the notified chemical. All such studies gave negative results (Exempt reference 4).

In summary, although the notified chemical may potentially be metabolised to a known carcinogen *in vivo*, studies on the notified chemical itself, as well as its major component, indicate that it is not likely to be significantly bioavailable. In addition, in vivo carcinogenicity studies on the major component indicated that it was not an in vivo carcinogen. Overall, the data suggests that the notified chemical is not expected to be mutagenic or carcinogenic, however as very low exposures can cause these effects, the potential cannot be ruled out.

Health hazard classification

Based on the acute inhalation toxicity study the notified chemical is classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 2004).

Xn R20 Harmful by inhalation

6.3. Human health risk characterisation

6.3.1. Occupational health and safety

Respiratory effects

The notified chemical has been shown to be harmful by inhalation. The acute inhalation toxicity study resulted in an LC50 value in the range of 1 – 5 g/m^3/4hr (1 – 5 mg/L/4hr). The exposure conditions which would cause the equivalent toxic effects in humans (i.e. the LC50 value in humans) can be estimated from the formula below. As the primary toxic effects were respiratory and the majority of particles were inhalable the surface area of the respiratory tract was used as a normalising factor rather than body weight.

$$LC50(man) = LC50(rat) \times \frac{V_{min}\,(rat)}{V_{min}\,(man)} \times \frac{SA_{resp}\,(man)}{SA_{resp}\,(rat)} \times \frac{t_{exp}\,(rat)}{t_{exp}\,(man)} \times \frac{F_d\,(rat)}{F_d\,(man)}$$

$$= (1.0 \text{ to } 5.0)\text{ mg/L} \times \frac{0.090\text{ L/min}}{27\text{ L/min}} \times \frac{3424\text{ m}^2}{37.84\text{ m}^2} \times \frac{240\text{ min}}{240\text{ min}} \times \frac{0.04}{0.45}$$

$$= (0.027 \text{ to } 0.135)\text{ mg/L}$$

$$= (27 \text{ to } 135)\text{ mg/m}^3$$

Where : V_{min} = minute volume (value for rat from US EPA, 1994; value for human based on moderate activity level from EC TGD, 2003)
SA_{resp} = surface area of the respiratory tract (Values from US EPA, 1994)
t_{exp} = exposure duration (animal exposure duration from acute inhalation study, human exposure duration from maximum time indicated by notifier)
F_d = deposition fraction (Values for nose-breathing rat and mouth-breathing human (worst-case) from Wolff and Dorato, 1997)

The chronic effects after repeated inhalation exposure to the notified chemical were not investigated, and therefore an inhalation NOAEL can not be determined. Based on the effects seen in the acute inhalation study the notified chemical may pose a chronic respiratory hazard. Repeated inhalation of airborne dusts of the notified chemical, or inhalation of high airborne concentrations, may present some risk of lung overloading effects.

Inhalation exposure of workers to the notified chemical (concentrations up to 100%) may occur when handling the notified chemical in solid form during formulation of the inks. Some of this airborne powder is expected to be of respirable size. Inhalation exposure of workers was estimated to be 2 - 5 mg/m^3 in the presence of local exhaust ventilation. This value is approximately 5 - 70 fold less than the estimated LC50 value in humans (27-135 mg/m^3). This LC50 value is a worst case estimate as it takes into account a worst case particle deposition scenario. Since an inhalation NOAEL could not be determined the safety of the EASE estimated atmospheric concentration after repeated exposure could not be established. The inhalation risk to workers handling the powder is therefore expected to be significant.

The notifier has not specified the types of masks that will be worn by workers when formulating the notified chemical into inks. If particle filter masks capable of filtering out particles of respirable size are worn by formulation workers and cleaners and are used and fitted correctly, the exposure to the airborne notified chemical, and therefore the risk to formulation and cleaning workers, will be significantly reduced.

Systemic effects

Dermal and inhalation exposure are the main routes by which workers may be exposed to the notified chemical (at concentrations up to 100%) during formulation processes. EASE modelling predicted 'very low' levels of dermal exposure assuming that effective local exhaust ventilation was in place.

Dermal exposure to the notified chemical at concentrations up to 8% during pipe connection/disconnection, cleaning and quality control operations may occur and some of the notified chemical may be absorbed into the skin. It is noted that the use of PPE will act to further reduce exposure.

As the NOAEL value in a 28 day oral repeat dose toxicity study was determined to be greater than or equal to the highest tested dose (1000 mg/kg bw/day), significant systemic effects are not expected to occur as a result

of dermal/inhalation exposure to the notified chemical.

In summary, the risk of systemic effects associated with dermal/inhalation exposure to the notified chemical during formulation and end use is not considered to be unacceptable.

Mutagenicity/Carcinogenicity
Some potential for mutagenicity/carcinogenicity related to the carcinogenic metabolite, 3,3'-dichlorobenzidine, cannot be ruled out.

Whilst breakdown of the pigment through metabolism in the body is possible it is believed that for insoluble azo pigments such as the notified chemical this would be limited by the low solubility and thus low bioavailability of the notified chemical (Exempt reference 3). This is also supported by substantial testing on the main component (>90%) of the notified chemical, including a negative carcinogenicity test.

No component amines were identified as impurities in the notified chemical and the level of 3,3'-dichlorobenzidine is <10ppm.

The engineering controls in place and personal protective equipment expected to be used by workers during formulation and printing are expected to reduce exposure to a low level and consequently further minimise the risk of mutagenicity or carcinogenicity.

The use of heat may result in degradation of the notified chemical to 3,3'-dichlorobenzidine (a category 2 carcinogen), particularly at temperatures in excess of 200ºC (USEPA 2002). It is expected that the temperatures of heatset ovens used for application of heat-set inks will not exceed 200ºC and thus the notified chemical is unlikely to degrade to 3,3'-dichlorobenzidine during such processes. Thus the risk associated with end use of heat-set inks containing the notified chemical is unlikely to be significant.

Worker exposure to dried inks is likely to result in minimal exposure to the notified chemical, so the risk presented by the notified chemical in this scenario is not expected to be significant.

In summary, the risk of mutagenic/carcinogenic effects associated with worker exposure to the notified chemical is not considered to be unacceptable based on the use scenarios and controls in place to reduce exposure.

6.3.2. Public health
Potential for mutagenic or carcinogenic effects related to the carcinogenic metabolite of the notified chemical, 3,3´-dichlorobenzidine, cannot be ruled out.

The public will generally only have dermal exposure to dried inks containing the notified chemical, from which it is not expected to be significantly bioavailable. Therefore the risk to the public is not considered to be unacceptable.

7. ENVIRONMENTAL IMPLICATIONS

7.1. Environmental Exposure & Fate Assessment

7.1.1 Environmental Exposure

RELEASE OF CHEMICAL AT SITE
Minor releases are possible through spillage (0.3-0.6%), equipment cleaning (0.1-0.2%) and as container residues (0.1-0.2%). Combined annual releases may approach 1% of the imported quantity, or 1 tonne of the notified chemical.

RELEASE OF CHEMICAL FROM USE
The pigment applied to paper will not be exposed to the environment as it will be coated with resin as part of the printing process.

Most of the notified chemical that is applied via printing processes will share the fate of the paper, which may be either sent to landfill or recycled. Considering the very low water solubility, residues of the notified chemical that are removed during paper recycling are expected to partition to sludge. In landfill, the notified

chemical will undergo slow degradation processes via biotic and abiotic pathways, forming water and oxides of carbon and nitrogen.

RELEASE OF CHEMICAL FROM DISPOSAL
Formulation wastes will be collected and disposed of by thermal decomposition. Waste paper and sludge from paper recycling is expected to be disposed of to landfill.

7.1.2 Environmental fate

The notified chemical is expected to partition to soils and sediment, where it is likely to persist as it remained unchanged when tested for ready biodegradability. Reductive degradation may occur in anaerobic sediment, based on the structure, but could be very slow because of the very low water solubility. The notified chemical is not expected to bioaccumulate in fish based on the behaviour of analogue pigments identified by the notifier. While the analogue test reports have not been provided, the low bioconcentration factors (< 10) reported by the notifier are considered reliable based on the chemical structures of the notified chemical and the nominated analogues, and the properties of organic pigments. In particular, the notified chemical is expected to have a low potential for bioaccumulation because of its very limited affinity for the lipid phase of living organisms, as has been discussed in the screening assessment of Pigment Red 187 (Environment Canada and Health Canada, 2008). For the details of the environmental fate studies please refer to Appendix C.

7.1.3 Predicted Environmental Concentration (PEC)

It is neither necessary nor meaningful to determine a PEC as aquatic exposure is expected to remain very low when the notified chemical is used as proposed, and when printed paper is recycled.

7.2. Environmental effects assessment

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

Endpoint	*Result*	*Assessment Conclusion*
Fish Toxicity	LC50 > 100 mg/L	Not toxic to the limit of water solubility.
Daphnia Toxicity	EC50 > 1 mg/L	Not toxic to the limit of water solubility.
Algal Toxicity	EC50 > 0.1 mg/L	Not toxic to the limit of water solubility.
Inhibition of Bacterial Respiration	IC50 > 100 mg/L	Not harmful to bacterial respiration.

Aquatic toxicity testing was complicated by the very low water solubility of the test substance (determined to be less than the limit of detection (0.01 µg/L) in the test medium). Test organisms were exposed to dispersed suspensions. Harmful effects were seen in daphnids and algae at higher concentrations than those tabulated above, but were attributed to physical effects from undissolved material.

7.2.1 Predicted No-Effect Concentration

It is not possible to determine a PNEC as the measurement of aquatic toxicity is confounded by the low water solubility of the notified chemical.

7.3. Environmental risk assessment

No risk quotients (Q = PEC/PNEC) have been calculated since no significant release of the notified chemical to the aquatic environment is expected based on the reported use pattern.

The notified chemical is not considered to pose a risk to the environment based on its reported use pattern and the absence of aquatic toxicity at concentrations below the solubility limit.

8. CONCLUSIONS AND REGULATORY OBLIGATIONS

Hazard classification
Based on the available data the notified chemical is classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)]. The following risk phrases apply to the notified chemical:

Xn; R20 Harmful by inhalation

and

As a comparison only, the classification of the notified chemical using the Globally Harmonised System for the Classification and Labelling of Chemicals (GHS) (United Nations 2003) is presented below. This system is not mandated in Australia and carries no legal status but is presented for information purposes.

	Hazard category	***Hazard statement***
Acute toxicity	4	Harmful if inhaled

Human health risk assessment
Based on the proposed use scenarios and occupational controls in place to reduce exposure, the notified chemical is not considered to pose an unacceptable risk to the health of workers.

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

Environmental risk assessment
On the basis of the lack of toxicity to aquatic life at concentrations below the solubility limit and the reported use pattern, the notified chemical is not considered to pose a risk to the environment.

Recommendations

REGULATORY CONTROLS
Hazard Classification and Labelling

- Safe Work Australia should consider the following health hazard classification for the notified chemical:
  - Xn; R20 Harmful by inhalation

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - Conc ≥ 25%: R20

Health Surveillance

- As the notified chemical is classified as harmful by inhalation, employers should carry out health surveillance of workers.

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical as introduced in the powdered pigment:
  - Local exhaust ventilation wherever weighing and addition to mixers occurs

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced in the powdered pigment:
  - Avoid the formation of airborne dusts
  - Avoid skin contact
  - Regularly clean up any spills of the powdered pigment
  - Minimisation of the use of heat during handling/processing/use of the notified chemical

- Employers should implement the following safe work practices to minimise occupational exposure to the notified chemical in end use inks:

- Situations under which the notified chemical may be subject to temperatures in excess of 200ºC should be avoided, particularly when heat-setting inks containing the notified chemical.

- Measures should be taken to minimise the levels of 3,3'-dichlorobenzidine in the imported products containing the notified chemical.

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced in powdered pigment:
  - Respiratory protection sufficient for respirable particulates during processes where exposure to dust is likely
  - Gloves
  - Coveralls
  - Safety glasses

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical in printing inks:
  - Gloves
  - Coveralls
  - Safety glasses

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

- Atmospheric monitoring should be conducted by employers to measure workplace concentrations of the notified chemical during formulation processes.

- A copy of the MSDS should be easily accessible to employees.

- If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the *Approved Criteria for Classifying Hazardous Substances* [NOHSC:1008(2004)] workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Disposal

- The notified chemical should be disposed of to landfill.

Emergency procedures

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

Regulatory Obligations

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

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

(1) Under Section 64(1) of the Act; if
  - further information becomes available as to the mutagenic/carcinogenic potential of the notified chemical or its potential for breakdown

- the notified chemical is proposed to be used in printing inks for use by consumers

or

(2) Under Section 64(2) of the Act; if
- the function or use of the chemical has changed from a pigment used in printing inks, or is likely to change significantly;
- the amount of chemical being introduced has increased from 100 tonnes per annum, 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.

Material Safety Data Sheet

The MSDS of the notified chemical (and products containing the notified chemical) provided by the notifier were reviewed by NICNAS. The accuracy of the information on the MSDS remains the responsibility of the applicant.

APPENDIX A: PHYSICAL AND CHEMICAL PROPERTIES

Melting Point 313°C

Method	OECD TG 102 Melting Point/Melting Range. EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks	Differential scanning calorimetry (DSC) The observations indicated that the test substance had reacted and/or decomposed during melting.
Test Facility	NOTOX (2007a)

Boiling Point Not observed

Method	OECD TG 103 Boiling Point. EC Directive 92/69/EEC A.2 Boiling Temperature. Differential scanning calorimetry (DSC)
Remarks	Boiling was not observed below the temperature at which reaction and/or decomposition started.
Test Facility	NOTOX (2007a)

Density 1.40×10^3 kg/m^3 at 20°C

Method	OECD TG 109 Density of Liquids and Solids. EC Directive 92/69/EEC A.3 Relative Density. Gas comparison stereopycnometer
Test Facility	NOTOX (2007a)

Vapour Pressure $< 1.33 \times 10^{-11}$ kPa at 20°C

Method	OECD TG 104 Vapour Pressure. EC Directive 92/69/EEC A.4 Vapour Pressure. Isothermal thermogravimetric effusion method
Remarks	The vapour pressure of the test substance was found to be below the vapour pressure of benzo(ghi)perylene.
Test Facility	NOTOX (2007a)

Water Solubility < 0.4 mg/L at 20°C

Method	OECD TG 105 Water Solubility. EC Directive 92/69/EEC A.6 Water Solubility.
Remarks	Column Elution Method
Test Facility	NOTOX (2007a)

Hydrolysis as a Function of pH

The test could not be conducted because of very low water solubility and the lack of a sufficiently sensitive analytical method. The notified chemical is expected to be hydrolytically stable under environmental conditions, based on the structure and the very low water solubility.

Partition Coefficient (n-octanol/water) $\log P_{ow} > 6.5$ at 22°C

Method	OECD TG 117 Partition Coefficient (n-octanol/water). EC Directive 92/69/EEC A.8 Partition Coefficient.
Remarks	HPLC Method. The result should be treated with some caution, as the test guideline notes a preference for reference substances that are structurally related to the test substance, while the column was calibrated with a reference substance (DDT) that differs markedly in structure and is soluble in n-octanol. Preliminary testing found the solubility of the test substance in n-octanol to be very low (5-10 mg/L). It remained finely dispersed despite

centrifugation and filtration through a 0.2 µm membrane.
Test Facility NOTOX (2007a)

Adsorption/Desorption log K_{oc} > 5.6 at 35°C

Method OECD TG 121 Estimation of the Adsorption Coefficient (K_{oc}) on Soil and on Sewage Sludge using HPLC.
Remarks The retention time of the test substance was considerably longer than that of DDT even with gradient elution to 100% methanol.
Test Facility NOTOX (2007a)

Dissociation Constant

The test could not be conducted because of very low water solubility and the lack of a sufficiently sensitive analytical method. The notified chemical is not expected to dissociate under environmental conditions, based on the structure and the very low water solubility. Some of the minor components of the notified chemical contain functional groups that may dissociate under environmental conditions.

Particle Size

Method Laser diffraction particle size analysis (BS ISO 13320-1:1999)

Range (µm)	*Mass (%)*
<0.448	0
<1.002	0.86
<5.024	3.67
<10.00	8.82
<10.773	10
<20.00	29.57
<29.009	50
<65.187	90
<100.237	97.46
<355.656	100

Remarks Samples of the test substance were dispersed in methanol and five runs were performed to ensure repeatability of the results.
Mass Median Aerodynamic Diameter (MMAD) = 34.324 µm
Test Facility Chilworth (2007)

Solid Flammability Not highly flammable

Method EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks The test substance pile did not ignite but melted and turned into a charred residue.
Test Facility NOTOX (2007a)

Autoignition Temperature The test substance was not observed to auto-ignite at temperatures below its melting point.

Method EC Directive 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
Remarks The test substance did not self-ignite between 19°C and the melting temperature (i.e. 313°C).
Test Facility NOTOX (2007a)

Explosive Properties Not explosive

Method EC Directive 92/69/EEC A.14 Explosive Properties.
Remarks The notified chemical is not considered to be explosive as it was not thermally sensitive, shock sensitive or friction sensitive.
Test Facility NOTOX (2007a)

Oxidizing Properties Not oxidising

Method	Expert statement
Remarks	The molecular structure of the test substance suggests that it is unlikely to have oxidising properties.
Test Facility	NOTOX (2007a)

APPENDIX B: TOXICOLOGICAL INVESTIGATIONS

B.1. Acute toxicity – oral

TEST SUBSTANCE: Notified chemical

METHOD
- Species/Strain: Rat/CrJ:CD (SD) IGS
- Vehicle: Olive oil
- Remarks - Method: No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	1 F	300	0/1
2	1 F	2000	0/1
3	4 F	2000	0/4

- LD50: > 2000 mg/kg bw
- Signs of Toxicity: None
- Effects in Organs: None
- Remarks - Results: Yellowish stool was noted in all animals. This was considered to be caused by the staining properties of the substance.

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

TEST FACILITY: CERI (2005a)

B.2. Acute toxicity – dermal

TEST SUBSTANCE: Notified chemical

METHOD: OECD TG 402 Acute Dermal Toxicity – Limit Test.
EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal) – Limit Test.
- Species/Strain: Rat/Wistar strain, Crl:WI (outbred, SPF-Quality)
- Vehicle: Water (Milli-Q)
- Type of dressing: Occlusive
- Remarks - Method: No significant protocol deviations

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
1	5 Males	2000	1/5
2	5 Females	2000	0/5

- LD50: >2000 mg/kg bw
- Signs of Toxicity - Local: Yellow staining of treated skin was seen in the treated skin-area of all animals during the observation period. In addition one female showed a wound and scabs from Day 8 onwards.
- Signs of Toxicity - Systemic: Lethargy, hunched and/or flat posture, piloerection, shallow respiration, ptosis and/or chromodacryorrhoea were noted in all males between Days 1 and 4. The females showed chromodacryorrhoea between Days 1 and 4. One male was found dead on Day 2.
- Effects in Organs: The animal which was found dead on Day 2 showed dark red discolouration of the lungs.

	No abnormalities were found at macroscopic post mortem examination of the other animals.
Remarks - Results	The changes noted in body weight gain in males and females were within the range expected for rats used in this type of study and were therefore not considered indicative of toxicity. The death of the male animal was considered to be related to treatment with the test substance considering the clinical observations noted in this animal.
CONCLUSION	The notified chemical is of low toxicity via the dermal route.
TEST FACILITY	NOTOX (2007b)

B.3. Acute toxicity – inhalation

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 403 Acute Inhalation Toxicity. EC Directive 92/69/EEC B.2 Acute Toxicity (Inhalation).
Species/Strain	Rat: Crl:WI(Han)
Vehicle	None
Method of Exposure	Flow past nose-only inhalation chamber
Exposure Period	4 hours
Physical Form	Solid/Powder aerosol
Particle Size	The mean mass aerodynamic diameter (MMAD) and geometric standard deviation (GSD) were determined. The MMAD was in the range of 1.1 – 2.9 μm and the GSD was in the range of 3.6 - 6.6.
Remarks - Method	During exposure of Group 1, repeated clogging of the air mover by the test substance occurred, which resulted in lower exposure concentrations than intended.

RESULTS

Group	*Number and Sex of Animals*	*Particle size (MMAD (μm), GSD)*	*Concentration (mg/L)*		*Mortality*
			Nominal	*Actual*	
1	5 M / 5 F	2.9, 6.6	7.2	2.4	4 M / 4 F
2	5 M / 5 F	1st sample 1.1, 3.6 2nd sample 2.8 (GSD not determined)	15.5	1.2	0 M / 0 F

LC50	Within the range of 1.0 – 5.0 mg/L/ 4 hours
Signs of Toxicity	In Group 1, two males and four females were found dead following ~2.5hr exposure, one male after ~3 hr exposure, and one male was found dead 3hr after termination of exposure. The survivors of Group 1 exhibited slow breathing at approximately 2½ hours of exposure. The male survivor from Group 1 showed hunched posture and/or laboured respiration between Days 1 and 5 and the female survivor on Day 1. Piloerection was also observed in the same time frames. In Group 2 slow breathing was observed in one male and hunched posture in one female on Day 1. During the first week after exposure the surviving animals from Group 1 showed body weight loss and the animals in Group 2 showed slight body weight loss or reduced body weight gain.

	Yellow staining of the fur was noted in most of the animals during exposure and the observation period. Yellow faeces were observed in Group 2 animals on day 4 of observation. These findings were considered to be due to grooming of the fur and subsequent ingestion of the test substance.
Effects in Organs	Yellowish granular contents of the larynx were observed in all animals of Group 1 that died during the exposure period. One of these males also showed yellowish granular contents in the stomach and many dark red foci in the lungs. At termination, many yellowish foci were noted in the lungs of the male survivor from Group 1 and in one female from Group 2. The other surviving animals did not reveal any abnormalities.
Remarks - Results	LC50 considered to be in the range of 1 – 5 mg/L/4 hr.
CONCLUSION	The notified chemical is harmful via inhalation.
TEST FACILITY	NOTOX (2008)

B.4. Irritation – skin

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Directive 67/548/EEC B4 Acute Toxicity: Dermal Irritation/Corrosion
Species/Strain	Albino rabbit, New Zealand White, (SPF-Quality).
Number of Animals	3 males
Vehicle	The powdery test substance was moistened with watery ethanol (50% v/v), immediately before application, to ensure close contact with the animal's skin.
Observation Period	72 hours
Type of Dressing	Semi-occlusive.
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			
Erythema/Eschar	0.7	0	0	0.7	< 72 hr	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	Very slight erythema was observed in all animals at 1 hour, completely resolving in two animals within 24 hours and one animal within 72 hours. There were no deaths or test substance-related clinical signs or remarkable body weight changes during the study period.
CONCLUSION	The notified chemical is slightly irritating to the skin.
TEST FACILITY	NOTOX (2007c)

B.5. Irritation – eye

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Directive 67/548/EEC B.5 Acute Toxicity: Eye Irritation/Corrosion US EPA OPPTS 870.2400, Acute Eye Irritation.

	JMAFF, Notification No 8147
Species/Strain	Albino rabbit, New Zealand White, (SPF-Quality).
Number of Animals	3
Observation Period	72 hours
Remarks - Method	The test substance was ground to a powder before instillation. A solution of 2% fluorescein in water was used to wash the animals eyes at the end of the 24 hour observation.

RESULTS

Remarks - Results	Slight redness, chemosis and discharge were observed in all animals at 1 hour after instillation, and completely resolved within 24 hours.

CONCLUSION The notified chemical is non-irritating to the eye.

TEST FACILITY NOTOX (2007d)

B.6. Skin sensitisation – mouse local lymph node assay (LLNA)

TEST SUBSTANCE Notified chemical

METHOD OECD TG 429 Skin Sensitisation – Local Lymph Node Assay.
EC Directive 67/548/EEC B.42 Skin Sensitisation – Local Lymph Node Assay.

Species/Strain	Mouse/CBA strain, inbred, SPF-Quality
Vehicle	Acetone/Olive oil (4:1 v/v)
Remarks - Method	A preliminary study was conducted on concentrations up to 100% in order to select the highest test substance concentration to be used in the main study. No irritation was seen at any concentration. It was concluded that 25% concentration was the maximum concentration that could technically be applied. The animals were euthanased on Day 6.

RESULTS

Concentration (% w/w)	*Proliferative response (DPM/lymph node)*	*Stimulation Index (Test/Control Ratio)*
Test Substance		
0 (vehicle control)	208 ± 64	1.0
5%	191 ± 42	0.9 ± 0.3
10%	248 ± 58	1.2 ± 0.5
25%	265 ± 70	1.3 ± 0.5
Positive Control		
Vehicle Control	357 ± 61	1.0 ± 0.2
5% HCA	474 ± 85	1.3 ± 0.3
10% HCA	547 ± 48	1.5 ± 0.5
25% HCA	1980 ± 315	5.5 ± 1.3

Remarks - Results	Since there was no indication that the test substance could elicit an SI ≥ 3, it was established that the EC3 value (if any) exceeds 25%. There were no deaths or test substance-related clinical signs or bodyweight changes during the study.

CONCLUSION There was no evidence of induction of a lymphocyte proliferative response indicative of skin sensitisation to the notified chemical under the conditions of the study.

TEST FACILITY NOTOX (2007e)

B.7. Repeat dose toxicity

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 407 Repeated Dose 28-day Oral Toxicity Study in Rodents. EC Directive 96/54/EC B.7 Repeated Dose (28 Days) Toxicity (Oral).
Species/Strain	Rat/Crl:CD(SD)
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28days Dose regimen: 7 days per week Post-exposure observation period: 14 days
Vehicle	Olive oil
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw/day*	*Mortality*
I (control)	5 M / 5 F	0	0 / 10
II (low dose)	5 M / 5 F	50	0 / 10
III (mid dose)	5 M / 5 F	250	0 / 10
IV (high dose)	5 M / 5 F	1000	0 / 10
V (control recovery)	5 M / 5 F	0	0 / 10
VI (high dose recovery)	5 M / 5 F	1000	1 / 10

Mortality and Time to Death

No test substance related mortality occurred. One female died on Day 2 due to a technical dosing error.

Clinical Observations

Soft yellow stool was noted in females of the 250 and 1000 mg/kg groups and males of the 1000 mg/kg groups, though only on days 2 or 3. This was considered to be a treatment related effect.

Yellow stool was observed in the vast majority of test animals at all dose levels throughout the dosing period. This remained up until day 2 of the recovery period in all animals treated with 1000 mg/kg. In males of the 1000 mg/kg group, several animals were observed to have yellow staining of the whole body, around the anus, on the lower abdomen, and around the mouth and nose at various observation days. In some females at this treatment level, yellow staining of the whole body was also observed. These effects were considered to be treatment related, though they were not observed in recovery animals.

Yellow diarrhea was observed in some females of the 50 and 250 mg/kg group but this was not a dose related effect.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Haematology indicated elongation of PT and APTT in males of the 1000 mg/kg group, which was suspected to be due to effects on the blood coagulation system.

Effects in Organs

Relative liver weights were increased in females treated with 250 and 1000 mg/kg (11% and 11.6% increase, respectively). There were no associated histopathological findings. These effects were considered to be treatment related though not adverse in nature, given the lack of corresponding histopathological changes and the absence of similar observations during the recovery period.

Remarks – Results

There were other minor effects observed during the dosing period, however, these were either minor or did not show dose related trends and thus were not considered to be related to treatment with the test substance. There were some minor effects observed during the recovery period that were considered to be incidental as they were restricted to only a few animals and had not been observed at the completion of the dosing period.

CONCLUSION

The No Observed Adverse Effect Level (NOAEL) was determined to be ≥1000 mg/kg bw/day in this study.

TEST FACILITY CERI (2005b)

B.8. Genotoxicity – bacteria

TEST SUBSTANCE Notified chemical

METHOD OECD TG 471 Bacterial Reverse Mutation Test.
Pre incubation procedure
Species/Strain *S. typhimurium*: TA1535, TA1537, TA100, TA98.
E. coli: WP2 uvrA.
Metabolic Activation System S9 fraction from phenobarbital and 5,6-benzoflavone induced rat liver
Concentration Range in Main Test a) With metabolic activation: 39.1 – 1250 µg/plate
b) Without metabolic activation: 39.1 – 1250 µg/plate
Vehicle Acetone
Remarks - Method No significant protocol deviations

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	>5000	>1250	>313	Negative
Test 2	>5000	>1250	>313	Negative
Present				
Test 1	>5000	>1250	>313	Negative
Test 2	>5000	>1250	>313	Negative

Remarks - Results The negative and strain-specific positive control values were within the laboratory historical control data ranges indicating that the test conditions were adequate and that the metabolic activation system functioned properly.

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

TEST FACILITY CERI (2005c)

B.9. Genotoxicity – in vitro

TEST SUBSTANCE Notified chemical

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
Species/Strain Chinese hamster
Cell Type/Cell Line Chinese hamster lung fibroblasts (CHL/IU cells)
Metabolic Activation System Rat liver S9-mix induced by a combination of phenobarbital and 5,6-benzoflavone.
Vehicle 0.5 w/v% carboxymethyl cellulose sodium salt in distilled water
Remarks - Method No significant protocol deviations

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Present			
Test 1	19.5, 39.1, 78.1, 156, 313, 625*, 1250*, 2500* and 5000	6	24
Absent			
Test 1	19.5, 39.1, 78.1, 156, 313, 625*, 1250*, 2500* and 5000	6	24

Test 2	19.5, 39.1, 78.1, 156*, 313*, 625*, 1250, 2500 and 5000	24	24

*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*
Present				
Test 1	3300, 1900	1800	All	Negative
Absent				
Test 1	3900	>5000	All	Negative
Test 2	2100, 430	1000	All	Negative

Remarks - Results

Precipitation of the test substance tended to interfere with observation of the chromosomes at higher doses (>2500 µg/mL in the short term tests and >625 µg/mL in the continuous treatment test).

The frequency of cells with structural or numerical aberrations was not significantly increased following treatment with the test substance.

CONCLUSION

The notified chemical was not clastogenic to Chinese hamster lung fibroblasts treated in vitro under the conditions of the test.

TEST FACILITY

CERI (2005d)

APPENDIX C: ENVIRONMENTAL FATE AND ECOTOXICOLOGICAL INVESTIGATIONS

C.1. Environmental Fate

C.1.1. Ready biodegradability

TEST SUBSTANCE Notified chemical

METHOD OECD TG 301 C Ready Biodegradability: Modified MITI Test (I).
- Inoculum: Activated sludge
- Exposure Period: 28 days
- Auxiliary Solvent: None
- Analytical Monitoring: BOD, and HPLC for test substance
- Remarks - Method:

RESULTS

Test substance		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
7	0	7	55
14	-2	14	65
21	-2	21	68
28	-3	28	69

Remarks - Results: The test substance was recovered unchanged after the test.

CONCLUSION The notified chemical cannot be classed as readily biodegradable.

TEST FACILITY CERI (2005e)

C.1.2. Bioaccumulation

A bioaccumulation test was not conducted because of the lack of a sufficiently sensitive analytical method, but the notifier argues that the notified chemical is not expected to have potential for bioaccumulation because bioconcentration factors for analogue pigments are < 10. While supporting test reports were not presented, the notified chemical is not expected to bioconcentrate in fish because of its very limited affinity for the lipid phase of living organisms, as has been discussed in the screening assessment of Pigment Red 187 (Environment Canada and Health Canada, 2008).

C.2. Ecotoxicological Investigations

Aquatic toxicity testing was complicated by the extremely low solubility of the notified chemical, determined to be less than the limit of detection (0.01 μg/L) in the test medium.

C.2.1. Acute toxicity to fish

TEST SUBSTANCE Notified chemical

METHOD OECD TG 203 Fish, Acute Toxicity Test – semi-static
- Species: *Oryzias latipes* (Medaka)
- Exposure Period: 96 hours
- Auxiliary Solvent: None
- Water Hardness: 29.2 mg $CaCO_3$/L
- Analytical Monitoring: HPLC analysis, after purification by column chromatography (Sep-Pak PS-2 eluted with tetrahydrofuran containing 1% water and 1% hydrochloric acid).

Remarks – Method	A limit test only was conducted, using a dispersed suspension containing the test substance at a nominal 100 mg/L. The analytical data, expressed as the geometric mean, are likely to overestimate the dissolved concentration of the notified chemical in the test medium because of the use of tetrahydrofuran during sample purification.

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
0	0	10	0	0	0	0	0
100	12.2	10	0	0	0	0	0

LC50	> solubility in test medium
NOEC (or LOEC)	solubility in test medium
Remarks – Results	No sublethal effects were observed.

CONCLUSION	The notified chemical is not toxic to fish at concentrations up to the solubility limit.
TEST FACILITY	CERI (2005f)

C.2.2. Acute toxicity to aquatic invertebrates

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 202 Daphnia sp. Acute Immobilisation Test - static
Species	*Daphnia magna*
Exposure Period	48 hours
Auxiliary Solvent	None
Water Hardness	29.2 mg $CaCO_3$/L
Analytical Monitoring	HPLC analysis, after purification by column chromatography (Sep-Pak PS-2 eluted with tetrahydrofuran containing 1% water and 1% hydrochloric acid).
Remarks - Method	A limit test only was conducted, using a dispersed suspension containing the test substance at a nominal 1 mg/L. The analytical data, expressed as the geometric mean, are likely to overestimate the dissolved concentration of the notified chemical in the test medium because of the use of tetrahydrofuran during sample purification.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h*	*48 h*
0	0	20	0	0
1	0.27	20	0	0

LC50	> solubility in test medium
NOEC (or LOEC)	solubility in test medium
Remarks - Results	Daphnids showed reduced activity in preliminary testing at nominal concentrations of 10, 30 and 100 mg/L. This was attributed to undissolved material that adhered to the test organisms.
CONCLUSION	The notified chemical is not toxic to daphnids at concentrations up to the solubility limit.
TEST FACILITY	CERI (2005g)

C.2.3. Algal growth inhibition test

TEST SUBSTANCE	Notified chemical
METHOD	OECD TG 201 Alga, Growth Inhibition Test.
Species	*Pseudokirchneriella subcapitata*
Exposure Period	72 hours
Concentration Range	Nominal: 0.1 mg/L Actual: 0.025 mg/L
Auxiliary Solvent	None
Water Hardness	Typical algal culture medium (soft water).
Analytical Monitoring	HPLC analysis, after purification by column chromatography (Sep-Pak PS-2 eluted with tetrahydrofuran containing 1% water and 1% hydrochloric acid).
Remarks - Method	A limit test only was conducted, using a dispersed suspension containing the test substance at a nominal 0.1 mg/L. The analytical data are likely to overestimate the dissolved concentration of the notified chemical in the test medium because of the use of tetrahydrofuran during sample purification.

RESULTS

Biomass		*Growth*	
E_bC50 mg/L at 72 h	*NOEC ... mg/L*	*E_rC50> mg/L at 72 h*	*NOEC ... mg/L*
> 0.0114	0.0114	> 0.0114	0.0114

Remarks - Results	The results tabulated above are expressed as nominal concentrations. There was some inhibition (17%) of algal growth rate in preliminary testing at a nominal concentrations of 10, 30 and 100 mg/L. This was attributed to undissolved material
CONCLUSION	The notified chemical is not toxic to green algae at concentrations up to the solubility limit.
TEST FACILITY	CERI (2005h)

C.2.4. 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
Exposure Period	3 hours
Concentration Range	Nominal: 100 mg/L
Remarks – Method	Since the test substance was hardly soluble in water, test concentrations were prepared separately in Milli-RO water. Initial loading rates of 200 mg/L were magnetically stirred for at least 24 hours in test bottles (in duplicate). Subsequently, synthetic sewage feed and sludge were added resulting in final loading rates of 100 mg/L. Optimal contact between the test substance and test medium was ensured by continuous aeration and stirring.
RESULTS	
IC50	> 100 mg/L
NOEC	100 mg/L

Remarks – Results	Inhibition of respiration differed between the two replicate vessels (7 and 20%). This was probably due to the low water solubility. Inhibition did not reach 50% in either of the two bottles.
CONCLUSION	The notified chemical is not harmful to bacterial respiration
TEST FACILITY	NOTOX (2007f)

BIBLIOGRAPHY

Brown MA and DeVito SC (1993). Predicting azo dye toxicity, *Critical Reviews in Environmental Science and Technology,* 23(3): 249-324.

Chilworth (2007) Particle size analysis on a sample of Y4002. Project no 485191, August 2007, Chilworth Technology Limited, Southampton, UK (unpublished report provided by notifier).

DFG (1988) Deutsche Forschungsgemeinschaft. List of MAK and BAT Values 1988. VCH Publishers, Weinheim.

Environment Canada and Health Canada (2008) Screening Assessment for the Challenge: 4-[[5-[[[4-(Aminocarbonyl)phenyl]amino]carbonyl]-2-methoxyphenyl]azo]-N-(5-chloro-2,4-dimethoxyphenyl)-3-hydroxynaphthalene-2-carboxamide (Pigment Red 187) July 2008 (http://www.ec.gc.ca/substances/ese/eng/challenge/batch1/batch1_59487-23-9_en.pdf accessed 22 May 2009).

European Commission (2003) Technical Guidance Document on Risk Assessment in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC) No 1488/94 on Risk Assessment for Existing Substances and Directive 98/8/EC of the European Parliament and of the Council Concerning the Placing of Biocidal Products on the Market – Part I. Institute for Health and Consumer protection, European Chemicals Bureau, European Communities.

CERI (2005a) Acute oral toxicity study of Y4002 in rats. Study code: A16-0490, March 2005, Hita Laboratory Chemicals Evaluation and Research Institute, Oita, Japan (unpublished report provided by notifier).

CERI (2005b) Twenty-eight day repeated-dose oral toxicity study of Y4002 in rats. Study code: B11-0779, October 2005, Hita Laboratory Chemicals Evaluation and Research Institute, Oita, Japan (unpublished report provided by notifier).

CERI (2005c) Mutagenicity test of Y4002 using microorganisms. Study code: K01-3175, March 2005, Hita Laboratory Chemicals Evaluation and Research Institute, Oita, Japan (unpublished report provided by notifier).

CERI (2005d) Chromosomal aberration test of Y4002 using cultured mammalian cells. Study code: K06-1092, July 2005, Hita Laboratory Chemicals Evaluation and Research Institute, Oita, Japan (unpublished report provided by notifier).

CERI (2005e) Biodegradation Study of Y4002 by Microorganisms. Study no 14367, May 2005, Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan (unpublished report provided by notifier).

CERI (2005f) A 96-hour Acute Toxicity Study of Y4002 with Medaka. Study no 93567, September 2005, Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan (unpublished report provided by notifier).

CERI (2005g) A 48-hour Acute Immobilisation Study of Y4002 with *Daphnia magna*. Study no 93566, September 2005, Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan (unpublished report provided by notifier).

CERI (2005h) Algal Growth Inhibition Study of Y4002 with *Pseudokirchneriella subcapitata*. Study no 93565, September 2005, Kurume Laboratory, Chemicals Evaluation and Research Institute, Japan (unpublished report provided by notifier).

IARC (1987) International Agency for Research on Cancer. Benzidine-Based Dyes (Group 2A). Supplement 7, pg 125

NOTOX (2007a) Determination of Physico-Chemical Properties of Y4002. Project 485182, August 2007, NOTOX BV, DD's-Hertogenbosch, the Netherlands (unpublished report provided by notifier).

NOTOX (2007b) Assessment of acute dermal toxicity with Y4002 in the rat. Project 485199, October 2007, NOTOX BV, DD's-Hertogenbosch, the Netherlands (unpublished report provided by notifier).

NOTOX (2007c) Primary skin irritation/corrosion study with Y4002 in the rabbit (4-hour semi-occlusive application). Project 485200, October 2007, NOTOX BV, DD's-Hertogenbosch, the Netherlands (unpublished report provided by notifier).

NOTOX (2007d) Acute eye irritation/corrosion study with Y4002 in the rabbit. Project 485201, October 2007, NOTOX BV, DD's-Hertogenbosch, the Netherlands (unpublished report provided by notifier).

NOTOX (2007e) Assessment of contact hypersensitivity to Y4002 in the mouse (local lymph node assay). Project 485202, October 2007, NOTOX BV, DD's-Hertogenbosch, the Netherlands (unpublished report provided by notifier).

NOTOX (2007f) Activated Sludge Respiration Inhibition Test with Y4002. Project 485204, June 2007, NOTOX BV, DD's-Hertogenbosch, the Netherlands (unpublished report provided by notifier).

NOTOX (2007g) Toxicokinetic assessment of Y4002. Project 485203, October 2007, NOTOX BV, DD's-Hertogenbosch, the Netherlands (unpublished report provided by notifier).

NOTOX (2008) Assessment of acute inhalatory toxicity with Y4002 in the rat. Project 487877, August 2008, NOTOX BV, DD's-Hertogenbosch, the Netherlands (unpublished report provided by notifier).

NOHSC (1994) National Code of Practice for the Labelling of Workplace Substances [NOHSC:2012(1994)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

NOHSC (2003) National Code of Practice for the Preparation of Material Safety Data Sheets, 2nd edition [NOHSC:2011(2003)]. National Occupational Health and Safety Commission, Canberra, Australian Government Publishing Service.

NOHSC (2004) Approved Criteria for Classifying Hazardous Substances, 3rd edition [NOHSC:1008(2004)]. National Occupational Health and Safety Commission, Canberra, AusInfo.

Øllgaard H, Frost L, Galster J and Hansen OC (1998). Survey of azo-colorants in Denmark: Consumption, use, health and environmental aspects. Danish Technological Institute, Environment, Danish Environmental Protection Agency.

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. SCCNFP/0495/01 (prepared in the context of Directive 76/768/EEC).

United Nations (2003) Globally Harmonised System of Classification and Labelling of Chemicals (GHS). United Nations Economic Commission for Europe (UN/ECE), New York and Geneva.

US EPA (2002). TSCA New Chemicals Program (NCP) Chemical Categories. 1200 Pennsylvania Avenue, N.W, Washington, D.C. 20460 http://www.epa.gov/opptintr/newchems/pubs/cat02.htm.

US EPA (1994) Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry. Research Triangle Park, North Carolina. Environmental Criteria and Assessment Office, U.S. Environmental Protection Agency (Publication No. EPA/600/8-90/066F) pp 4-25 to 4-31

Wolff RK & Dorato MA (1997) Inhalation Toxicity Studies. In: Williams PD & Hottendorf GH ed. Comprehensive Toxicology. Oxford, Pergamon (Elsevier), Volume 2: Toxicological Testing and Evaluation, pp 279-282