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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME (NICNAS)

FULL PUBLIC REPORT

Chemical 2 in Lugafast Black AN

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 and Water Resources.

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.

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Director
NICNAS

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1. APPLICANT AND NOTIFICATION DETAILS

APPLICANT(S)
BASF Australia Ltd (ABN 62 008 437 867)
500 Princes Highway, Noble Park Vic 3174

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
- Structural Formula
- Molecular Formula
- Spectral data
- Purity
- Identity of toxic and hazardous impurities and % weight
- Identity of non-hazardous impurities and % weight
- Identity of additives/adjuvants and % weight
- Import volume
- Customer names and identity of sites

VARIATION OF DATA REQUIREMENTS (SECTION 24 OF THE ACT)
Variation to the schedule of data requirements is claimed as follows:

- Adsorption/Desorption
- Particle Size
- Flash point
- Acute Inhalation Toxicity
- Induction of germ Cell Damage
- Bioaccumulation

PREVIOUS NOTIFICATION IN AUSTRALIA BY APPLICANT(S)
None

NOTIFICATION IN OTHER COUNTRIES
USA, EU

2. IDENTITY OF CHEMICAL

MARKETING NAME(S)
Lugafast Black AN (Dye product containing approx 35-50% notified chemical) – related to STD/1224 (Chemical 1 in Lugafast Black AN)

METHODS OF DETECTION AND DETERMINATION

METHOD	UV-VIS, IR, HPLC, ^{1}H-NMR
Remarks	Spectrum data for the dye product Lugafast Black AN were provided.
TEST FACILITY	BASF (2004)

3. COMPOSITION

DEGREE OF PURITY
The notified chemical is imported as a component of the dye product Lugafast Black AN. The dye fraction of the product Lugafast Black AN is 60-80%.

4. INTRODUCTION AND USE INFORMATION

MODE OF INTRODUCTION OF NOTIFIED CHEMICAL (100%) OVER NEXT 5 YEARS
The notified chemical will not be manufactured in Australia. It will be imported as a component of a powdered product Lugafast Black AN (approx 35-50% notified chemical).

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

The tonnages are those reported for the product Lugafast Black AN.

Year	*1*	*2*	*3*	*4*	*5*
Tonnes	1-3	1-3	1-3	1-3	1-3

USE
Lugafast Black AN is used as a colorant to dye collagen materials such as wet white, wet blue and vegetable tanned leathers.

5. PROCESS AND RELEASE INFORMATION

5.1. Distribution, transport and storage

PORT OF ENTRY
Brisbane

IDENTITY OF RECIPIENTS
Leather dyeing facilities, with one identified in Queensland.

TRANSPORTATION AND PACKAGING
Lugafast Black AN will be imported in 25kg carton with polyethylene bag into Australia by sea as part of a mixed load of chemicals. It will be transported by road from wharf to a contracted warehouse in Brisbane before being distributed by road to leather processing facilities. Lugafast Black AN will not be reformulated or repackaged before the dyeing use.

5.2. Operation description

Dyeing process
At the leather dyeing facilities, the leather is dyed using an "Immersion Dyeing" procedure. Lugafast Black AN is transferred by folk lifts from a storage area to the operation area. It is then weighed out manually and transferred to a dye drum where an aqueous dyeing solution (dyestuff) that contains 4-10% of Lugafast Black AN (maximum 5% of the notified chemical) is made depending on the thickness of the leather. The "Immersion Dyeing" procedure is a two-step process which will occur inside the closed dye drum. Firstly, the dyestuff will be penetrated through the leather cross section for 30-60 minutes in a pH range of 5-8 which is achieved using sodium hydrogen carbonate. Afterwards, the dyestuff will be fixed to the collagen fibre at pH range 9-10 for 60 to 120 minutes via the addition of sodium carbonate. The fixation rate is stated to be between 90 and 98% under the alkaline conditions. The non-fixed and hydrolysed dyestuff will be washed out in a subsequent automated washing procedure. After the dyeing step, the leather will be retanned and fat-liquored to form end products (treated leather). The dye drums are rarely cleaned and are used only to dye black shades.

End uses
Leathers dyed with Lugafast Black AN are used primarily in high cost finished leather items such as automotive upholstery, automotive steering wheel covers, shoes/boots, and garments etc.

5.3. Occupational exposure

Number and Category of Workers

Category of Worker	*Number*	*Exposure Duration*	*Exposure Frequency*
Transport and Warehouse	2	2 hours/day	10 days/year
Dyehouse Labourer	2	0.25 hours/day	240 day/year

Exposure Details

It is unlikely that transport and storage workers come into contact with the notified chemical unless in the event of accident.

During the dyeing process, workers' exposure to the notified chemical via dermal, ocular and inhalation is possible, especially when manually weighing and transferring Lugafast Black AN into the dye drums. However, the potential exposure can be reduced by use of personal protection equipment and possible workplace engineering controls such as local exhaust ventilation. The exposure during drum dyeing is expected to be minimal as this process occurs in an enclosed and automated system. Workers' exposure is not expected after the dyeing process, as any un-reacted chemical remaining in the dyeing drum after the washing procedure would be minimal due to the chemical's high water solubility and instability to hydrolysis.

5.4. Release

RELEASE OF CHEMICAL AT SITE

Environmental exposure comes from three main routes:

1. Accidental spills. Lugafast Black AN containing the notified chemical should be contained physically and collected on an absorbent material. This is expected to account for less than 1% (< 30 kg per annum) and will be disposed of to authorised landfill.

2. Disposal of import containers with residual notified chemical. The notified chemical will be imported in 25 kg carton with polyethylene bag liners. It is expected that a maximum of 100 grams will remain in the polyethylene bag, thus the residual amount going to landfill would be <= 0.4%, which is equivalent to less than 12 kg per annum.

Waste produced from the dyeing process that uses the notified chemical. It is expected that due to the chemical's high water solubility and instability to hydrolysis that any un-reacted powder form of the chemical remaining in the dyeing drum would be minimal (<0.1%, ie. < 3 kg per annum). This is expected to be collected for disposal to authorised landfill or released to the sewer. The notifier has indicated that the fixation rates are between 90 and 98% under alkaline conditions for the dyeing of leather. However, no details of the fixation rate studies were provided. Therefore, it is assumed that ≥ 85% of the notified chemical will be fixed to the leather due to lack of supporting fixation rate data, meaning that at most 15% (< 450 kg per annum) will be treated in the on-site wastewater treatment plant then released to sewer in accordance with a permit provided by the local water authorities. The notified chemical is expected to be hydrolysed during the dyeing process and wastewater treatment processes.

RELEASE OF CHEMICAL FROM USE

Lugafast Black AN containing the notified chemical is used for dyeing leather. Leather products can be used in a wide variety of applications but no significant release of the product is expected during the use of the leather.

5.5. Disposal

The majority of the notified chemical will share the fate of the leather products in which it will be incorporated. It is expected that these products will be disposed of to landfill at the end of their useful lives.

The released notified chemical from the tanning site will be disposed of to authorised landfill or to sewer after treatment.

5.6. Public exposure

The imported Lugafast Black AN is intended for industrial use only. Therefore, public exposure to the notified chemical is unlikely except as a result of accidents during transportation. Public exposure via use of the leather products treated with Lugafast Black AN is not expected because after the dying process the notified chemical is irreversibly attached to the collagen fibres of the leather and is unlikely to be bioavailable.

6. PHYSICAL AND CHEMICAL PROPERTIES

The test substance is the dye product Lugafast Black AN, unless stated otherwise.

Appearance at 20ºC and 101.3 kPa Fine, black powder.

Melting Point/Freezing Point No melting temperature was observed between 30ºC and 400 ºC.

METHOD EC Directive 92/69/EEC A.1 Melting/Freezing Temperature.
Remarks Measured by Differential Scanning Calorimetry. Decomposition was observed within the temperature range of 160 to 180ºC. An exact decomposition temperature could not be determined.
TEST FACILITY BASF (2005a)

Boiling Point Cannot be determined

Remarks The vapour pressure is too low and the test substance starts to decompose at around 160ºC.
TEST FACILITY BASF (2005a)

Density $1.58 \pm 0.01 \times 10^3$ kg/m^3 at 20ºC

METHOD OECD TG 109 Density of Liquids and Solids.
Remarks Measured by the pycnometer method.
TEST FACILITY BASF (2005a)

Vapour Pressure $<10^{-7}$ kPa at 20ºC

METHOD EC Directive 92/69/EEC A.4 Vapour Pressure-Effusion Method.
Remarks In the temperature range used (20-50°C) for the vapour pressure measurements, the test substance was solid.
TEST FACILITY BASF (2005a)

Water Solubility 16.6 ± 0.9 g/L at 20ºC (for the notified chemical)

METHOD EC Directive 92/69/EEC A.6 Water Solubility.
Remarks The Flask Method
Analytical Method: HPLC system with UV/VIS detector
TEST FACILITY BASF (2005a)

Hydrolysis as a Function of pH

METHOD OECD TG 111 Hydrolysis as a Function of pH.

For the notified chemical

pH	*T (°C)*	*$t_{½}$ hours*
4	50	> 120
7	40	20*
9	50	"rapid"

*Tests at 50, 60 and 70°C showed rapid hydrolysis at pH 7. A value could not be extrapolated for 20°C for this pH.

TEST FACILITY BASF (2005a)

Partition Coefficient (n-octanol/water) log P_{ow} at 20°C = <-4.5 (estimated for the notified chemical)

METHOD EC Directive 92/69/EEC A.8 Partition Coefficient.
n-octanol solubility determined using OECD TG 105
Remarks Flask Method. As the solubility in n-octanol was too low for detection the log Pow was estimated from the single solubilities in n-octanol and in water. The solubility in n-octanol from the shake flask method was <0.5 mg/L. A modelled value was calculated as -3.59 (EPIWIN 2000).
TEST FACILITY BASF (2005a)

Adsorption/Desorption – screening test Not determined

METHOD OECD TG 121 Adsorption - Desorption Using a Batch Equilibrium Method.
Remarks This method was not suitable to determine the Koc of the test substance; as the notified chemical was not eluted from the column (UV/VIS detector). The notified chemical has a low Pow indicating little affinity for organic carbon but have several anionic sites which are likely to bind to cations in the soil.
TEST FACILITY BASF (2005a)

Adsorption on Activated Sludge Colour elimination after 24 hours by adsorption onto activated sludge: 86%. This is considered insufficient colour elimination as the 90% criterion was not met.

METHOD ISO 18749. Duplicate analyses containing 9 mg/L of test substance and 1 g/L inoculum were analysed for light adsorption at 460 nm at 3 and 24 hours. This was compared with light adsorption of the duplicate blanks. A physico-chemical test containing 9 mg/L of test substance but no inoculum was also run.
Remarks The physico-chemical adsorption was 13% after 24 hours.
TEST FACILITY BASF (2005k)

Dissociation Constant Not determined.

METHOD OECD TG 112 Dissociation Constants in Water.
Remarks The test substance has several dissociation constants and did not produce a single inflexion point on the titration curve when measured. The anionic and potentially cationic functional groups are expected to show typical acidity (pKa ~ -2 and 9, respectively).
TEST FACILITY BASF (2005a)

Surface Tension 69 mN/m at 20°C

METHOD In accordance with OECD TG 115 Surface Tension of Aqueous Solutions and EC Directive 92/69/EEC A.5 Surface Tension, using the ring tensiometer method.
Remarks Concentration: 0.1% aqueous preparation was completely dissolved. The dye is not surface active.
TEST FACILITY BASF (2005a)

Particle Size Not performed

REMARKS A dust value of 2 was determined according to a Cassella method which indicates that the test substance is a dust-free.
TEST FACILITY BASF (2002)

Flash Point Not performed

Remarks The test substance is a low volatility solid.

Flammability Limits The test substance is not considered to be highly flammable.

METHOD EC Directive 92/69/EEC A.10 Flammability (Solids).
Remarks A burning time of above 240s was measured.
TEST FACILITY BASF (2005c)

Autoignition Temperature 222ºC

METHOD 92/69/EEC A.16 Relative Self-Ignition Temperature for Solids.
TEST FACILITY BASF (2005c)

Explosive Properties The test substance is not considered to present a danger of explosion when submitted to the effect of flame (thermal sensitivity), impact or friction (mechanical sensitivity).

METHOD EC Directive 92/69/EEC A.14 Explosive Properties.
TEST FACILITY BASF (2005c)

Reactivity Not determined

Remarks The substance does not have any oxidising properties, therefore, dust explosion risk is low. However, the build up of fine dust can lead to a risk of dust explosions.

Oxidizing Properties Not considered to be an oxidising substance

METHOD EC Directive 92/69/EEC A.17 Oxidizing Properties (Solids).
Remarks The maximum burning rate of the test substance is lower than the maximum burning rate of a reference mixture.
TEST FACILITY BASF (2005c)

7. TOXICOLOGICAL INVESTIGATIONS

Endpoint and Result	*Assessment Conclusion*
Rat, acute oral	low toxicity, LD50 >2000 mg/kg bw
Rat, acute dermal	low toxicity, LD50 >2000 mg/kg bw
Rat, acute inhalation	Not performed
Rabbit, skin irritation	non-irritating
Rabbit, eye irritation	Severe ocular lesion
Mouse, skin sensitisation - LLNA	no evidence of sensitisation
Rat, gavage repeat dose toxicity – 28 days	NOAEL 100 mg/kg bw/day (Male) NOAEL 300 mg/kg bw/day (Female)
Genotoxicity - bacterial reverse mutation	mutagenic
Genotoxicity – in vitro chromosome aberration assay	non genotoxic
Genotoxicity – in vivo mouse micronucleus test	non genotoxic
Genotoxicity – in vivo unscheduled DNA synthesis test	non genotoxic

7.1. Acute toxicity – oral

TEST SUBSTANCE Lugafast Black AN

METHOD OECD TG 423 Acute Oral Toxicity – Acute Toxic Class Method.
EC Directive 2004/73/EC B.1tris Acute Oral Toxicity – Acute Toxic Class Method.

Species/Strain	Rat/Wistar/HanBrl:WIST (SPF), Females
Vehicle	0.5% Carboxymethylcellulose-solution in doubly distilled water
Remarks - Method	No significant protocol deviations.

RESULTS

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Mortality*
I	3	2000	0
II	3	2000	0

LD50	>2000 mg/kg bw
Signs of Toxicity	No mortality occurred. No clinical signs and findings were observed. All animals showed expected gains in bodyweight over the study period.
Effects in Organs	No macroscopic pathological abnormalities were noted.

CONCLUSION The test substance is of low toxicity via the oral route.

TEST FACILITY BASF (2004)

7.2. Acute toxicity – dermal

TEST SUBSTANCE Lugafast Black AN

METHOD OECD TG 402 Acute Dermal Toxicity.
EC Directive 92/69/EEC B.3 Acute Toxicity (Dermal).

Species/Strain	Rat/Wistar/HanBrl:WIST (SPF), Females
Vehicle	0.5% CMC-solution in doubly distilled water
Type of dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations.

RESULTS

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

LD50	>2000 mg/kg bw
Signs of Toxicity - Local	No skin effects were noted.
Signs of Toxicity - Systemic	No mortality occurred. No systemic clinical observations were noted. All animals showed expected weight gains during the study period.
Effects in Organs	No macroscopic pathological abnormalities were noted.
CONCLUSION	The test substance is of low toxicity via the dermal route.
TEST FACILITY	BASF (2005)

7.3. Irritation – skin

TEST SUBSTANCE	Lugafast Black AN
METHOD	OECD TG 404 Acute Dermal Irritation/Corrosion. EC Directive 2004/73/EC B.4 Acute Toxicity - Skin Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White A 1077 INRA (SPF)
Number of Animals	3 (2 males and 1 female)
Vehicle	Moistened with doubly distilled water
Observation Period	7 days
Type of Dressing	Semi-occlusive
Remarks - Method	No significant protocol deviations. The observation on one animal was discontinued at 72 hours after removal of the patch because of free of findings. A preliminary in vitro corrosivity study using an EpiDerm human skin model was conducted. No information on the methodology was available. The result indicated that the notified chemical is not corrosive to the skin.
RESULTS	Moderate erythema was observed in all animals within 1 hour but was reversible within 24 hours after removal of the patch. Residual of the test substance were noted in two animals up to 1 hour after removal of the patch and a slight grey discolouration at parts of the application area in these two animals were observed which disappeared within 7 days.
CONCLUSION	The test substance is non-irritating to the skin.
TEST FACILITY	BASF (2005e)

7.4. Irritation – eye

TEST SUBSTANCE	Lugafast Black AN
METHOD	OECD TG 405 Acute Eye Irritation/Corrosion. EC Directive 2004/73/EC B.5 Acute Toxicity - Eye Irritation/Corrosion.
Species/Strain	Rabbit/New Zealand White A 1077 INRA (SPF)
Number of Animals	3 (2 males and 1 female)
Observation Period	21 days
Remarks - Method	No significant protocol deviations. A preliminary HET-CAM test (an alternative method to study the potential of serious damage to the eyes/mucous membranes in incubated hen eggs) was provided. No information on the methodology was available. The result indicated that the notified chemical did not produce

changes indicative for severe eye damage.

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	1	0.33	0.33	2	<72 hours	0
Conjunctiva: chemosis	0.33	0	0	1	<48 hours	0
Conjunctiva: discharge	0	0	0	2	<24 hours	0
Corneal opacity	0	0	0	0	NA	0
Iridial inflammation	0	0	0	0	NA	0

*Calculated on the basis of the scores at 24, 48, and 72 hours for EACH animal.
NA, not applicable.

Remarks - Results: No reactions/effects were observed in cornea and iris throughout the study. Slight or moderate conjunctival redness, slight conjunctival chemosis, and slight or moderate discharge were noticed in all animals within 48 hours after application. These ocular reactions were reversible within 72 hours at latest after application. Injected scleral vessels in a circumscribed area or circular were noted within 24 hours after application. Also the test substance caused a black discoloration of parts of the conjunctival and the nictitating membrane in all animals, and small part of sclera in two animals. The discolouration was not reversible within the study period (21 days).

CONCLUSION: Based on the irritation scores the test substance is slightly irritating to the eye, however, as the test substance caused irreversible colouration of the eyes, the test substance is considered to cause severe ocular lesions.

TEST FACILITY: BASF (2005f)

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

TEST SUBSTANCE: Lugafast Black AN

METHOD: OECD TG 429 Skin Sensitisation: Local Lymph Node Assay.
EC Directive B.42 Skin Sensitisation - Local Lymph Node Assay

Species/Strain: Mouse/CBA/CaOlaHsd
Vehicle: Acetone/olive oil
Remarks - Method: The vehicle was chosen due to its good homogeneity and applicability. A 30% suspension was the highest concentration of the test substance preparation suitable for application. Therefore, higher concentrations were not tested. The evaluation of the result was based on changes (Index of test group/control group) in lymph node weight, cell counts, and ear weights rather than proliferative response (as DPM/lymph node) and Stimulation Index. A concurrent positive control with a known sensitiser was not included into this study. The results of the most recent positive control study was provided using Alpha-hexylcinnamaldehyde conducted by the same laboratory.

RESULTS

Concentration (% w/w)	*Lymph Node Weight Index*	*Cell Count Index*	*Ear Weight Index*
Test Substance			
0 (vehicle control)	1.00	1.00	1.00
3	0.93	1.03	0.97

10	1.02	1.14	1.04
30	1.04	1.17	1.03
Positive Control			
0 (vehicle control)	1.00	1.00	1.00
1	1.08	1.25	1.07*
3	1.21*	1.56*	1.14**
10	1.73**	2.58**	1.14**

* p ≤0.05, ** p ≤0.01

Remarks - Results: The expected body weight gain was generally observed in the course of the study.

No local and obvious systemic toxicity and abnormalities were observed during the study.

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

TEST FACILITY: BASF (2005g)

7.6. Repeat dose toxicity

TEST SUBSTANCE: Lugafast Black AN

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	Wistar Rats
Route of Administration	Oral – gavage
Exposure Information	Total exposure days: 28 days Dose regimen: 7 days per week Post-exposure observation period: 2 Weeks
Vehicle	Double Distilled Water
Remarks – Method	No significant protocol deviations.

RESULTS

Dose mg/kg bw/day	*Number and Sex of Animals*	*Mortality*
0	10/sex	0
100	5/sex	0
300	5/sex	0
1000	10/sex	0
0	10/sex	0
1000	10/sex	0

Mortality and Time to Death
No animal died prematurely during the present study.

Clinical Observations
All treated animals showed ‘faeces discoloured dark’ from day 2 until the end of the administration period. In addition, the rats of each sex at 1000 mg/kg/day showed ‘urine discoloured dark’ from day 13 throughout the entire phase of administration. These findings were assessed as substance related but rather due to the physical property of the test substance (solid/black) than reflecting a real toxicologically relevant or adverse finding.

During the recovery period, the above mentioned findings were only observable until day 36. During the last few days of the 2 week recovery period no abnormal effects were reported. Therefore, these treatment related effects were reversible.

Anogenital region smeared with urine (slight to moderate) was observed in 2 female rats at 1000 mg/kg/day.

Only one of the two rats showed 'anogenital region smeared with faeces slight'. The same rat showed 'anogenital region smeared with urine' at the end of the recovery period, but less pronounced compared to the administration period. The above mentioned findings were considered as related to the test material and revealed signs of general systemic toxicity but were assessed to reversible.

In 1000 mg/kg/day female test group there was a statistically significant increased water consumption during the entire administration period. This finding was related to the treatment with the test material and was reversible as seen during the recovery period.

No significant changes were found in food consumptions, body weight, food efficiency and functional observations.

Laboratory Findings – Clinical Chemistry, Haematology, Urinalysis

Clinical Chemistry

Administration Period

Significantly increased aspartate aminotransferase activities were noted in the serum of males and females at 1000 mg/kg/day. Increased alanine aminotransferase activities were also recorded in the females treated at 1000 mg/kg/day.

Recovery Period

The increases in alanine and aspartate aminotransferase activities in animals at 1000 mg/kg/day were normalized following cessation of test material administration.

Blood Chemistry – Administration Period

Blood chemistry examinations revealed increased inorganic phosphate and calcium concentrations in the serum of rats at 1000 mg/kg/day. Higher potassium, urea, total bilirubin and magnesium levels were also found in the peripheral blood of males at 1000 mg/kg/day. Moreover, inorganic phosphate concentrations were increased in the males of 300 mg/kg/day group. No treatment-related changes were seen in the other blood chemistry parameters of the treated animals.

Blood Chemistry – Recovery Period

During the treatment-free recovery period all changes in blood chemistry parameters returned to normal.

Haematology

Administration Period

At the end of the administration period significantly increased white blood cell counts were found in the peripheral blood of animals at 1000 mg/kg/day. In the differential blood count the increased in leukocytes correlate with increases in lymphocytes. No treatment related effects were seen in the other haematological parameters of both sexes.

Recovery Period

After cessation of exposure the increases in leukocytes and lymphocytes in females treated with 1000 mg/kg/day were reversible within a recovery period of 2 weeks. However, male rats treated with 1000 mg/kg/day showed increases in white blood cell counts and lymphocytes at the end of the recovery period.

Urinalyses

Administration Period

At the end of the administration period urine specimens of males and females at 1000 mg/kg/day were discoloured from light brown to dark brown. With reagent test strips, increased blood was also detected in the urine specimens of animals of both sexes at 1000 mg/kg/day and higher protein levels were found in the urine samples of females at 1000 mg/kg/day. Moreover, microscopic examinations of the urine sediments revealed increased number of granular and/or epithelial cell casts in the 1000 mg/kg/day males and a higher quantity of transitional epithelial cells in the urines of the 1000 mg/kg/day females.

Recovery Period

All effects seen in the urine specimens of the male and female animals were reversible following cessation of test material administration.

Effects in Organs

Organ Weight

Main Group
No significant absolute organ weight changes were observed. There were statistically significant increases in relative organ weights of heart, kidney and liver in females at 1000 mg/kg/day, brain in males at 100 and 300 mg/kg/day, and kidneys and liver in males at 1000 mg/kg/day. These increases were considered to be incidental due to lack of histomorphological changes.

Recover Group
The statistically significant increased relative liver weight in males at 1000 mg/kg/day recovery group is regarded as incidental in the absence of histomorphological changes.

Gross Lesions
The gross lesions observed in the gastrointestinal tract (discoloration of contents, grey-brown) of 1000 mg/kg/day male and female animals in the main group is attributed to the test material colour (black)

The dark brown and grey brown discoloration of skeletal muscle and kidneys respectively, in all male and female animals at 1000 mg/kg/day in recovery group are considered to be treatment related effects.

Histopathology
A multifocal, minimal to slight hyaline degeneration with lymphohistiocytic infiltrations of skeletal muscle in 3/5 females at 1000 mg/kg/day in the main group were observed. Additionally, a minimal to slight yellow-brown granular intracytoplasmic pigment storage in macrophages and myocytes was detected in all 1000 mg/kg/day animals of the main and recovery groups, whereby an increased number of pigment-laden macrophages in affected areas were found.

The kidney of several 1000 mg/kg/day animals of the main and recovery groups showed minimal to slight yellow-brown pigment storage in proximal tubular epithelial cells without further morphological alterations. Similar pigment storage was also found in the cardiac muscle of all male and female animals at 1000 mg/kg/day in the main and recovery groups. Diffusely distributed in the cytoplasm of the cardiac myocytes, the pigment storage was recorded to a minimal to slight degree, whereby further morphological alterations were missing.

For kidney and skeletal muscle the 'Perls staining' and the 'PAS reaction' revealed a negative result , whereas the 'Schmori staining' showed a positive reaction.

A minimal increase of extramedullary hematopoiesis was detected in the spleen of some treated animals, which is considered to be an incidental observation.

All other findings noted were either single observations, or similar in severity to control rats.

Remarks – Results
Mild signs of general systemic toxicity were observed. Skeletal muscle was identified as the target tissue, due to the hyaline degeneration in females at 1000 mg/kg/day. Regarding the discolouration of urine and faeces as well as several organs and tissues, these findings were caused by the physical property of the test material (black) and did not reflect a real toxicologically relevant or adverse finding. Most substance related findings were either reversible within the recovery period or less pronounced compared to the treatment phase, at least in part.

CONCLUSION
The No Observed Adverse Effect Level (NOAEL) was established as 100mg/kg bw/day (male) and 300mg/kg bw/day (female) in this study, based on mild signs of general systemic toxicity (increased inorganic phosphate in males at 300 mg/kg/day, corresponding to mild impairment of renal function, and hyaline degeneration in 1000 mg/kg/day females).

TEST FACILITY BASF (2005h)

7.7. Genotoxicity – bacteria

TEST SUBSTANCE Lugafast Black AN

METHOD	OECD TG 471 Bacterial Reverse Mutation Test. EC Directive 2000/32/EC B.13/14 Mutagenicity – Reverse Mutation Test using Bacteria. Plate incorporation procedure and Pre incubation procedure
Species/Strain	*S. typhimurium*: TA98, TA100, TA1535, TA1537 *E. coli*: WP2uvrA
Metabolic Activation System	Plate incorporation procedure - Aroclor-induced rat liver S9 mix Pre incubation procedure - Uninduced hamster liver S9 mix
Concentration Range in Main Test	<u>Test 1 - Plate incorporation procedure in all strains</u> a) With metabolic activation: 0, 28, 140, 700, 3500, 7000 µg/plate b) Without metabolic activation: 0, 28, 140, 700, 3500, 7000 µg/plate <u>Test 2 - Pre incubation procedure in all strains</u> a) With metabolic activation: 0, 28, 140, 700, 3500, 7000 µg/plate b) Without metabolic activation: 0, 28, 140, 700, 3500, 7000 µg/plate <u>Test 3 - Pre incubation procedure in some strains</u> With metabolic activation only: – 0, 10, 100, 200, 600, 800, 1000 µg/plate (TA 98 only) – 0, 100, 200, 400, 600, 800, 1000 µg/plate (TA100, TA 1537)
Vehicle	Water
Remarks - Method	No significant protocol deviation. Historical negative controls (both sterility control and vehicle) and positive controls using a number of test substances were provided.

RESULTS

Metabolic Activation	*Test Substance Concentration (µg/plate) Resulting in:*			
	Cytotoxicity in Preliminary Test	*Cytotoxicity in Main Test*	*Precipitation*	*Genotoxic Effect*
Absent				
Test 1	NA	>7000 µg/plate	>7000 µg/plate	Negative
Test 2	NA	>7000 µg/plate	>7000 µg/plate	Negative
Test 3	NA	NA	NA	NA
Present				
Test 1	NA	>7000 µg/plate	>7000 µg/plate	Negative
Test 2	NA	≥28 µg/plate	>7000 µg/plate	Positive
Test 3	NA	≥10 µg/plate (TA98) ≥100 µg/plate (TA 1537)	>7000 µg/plate	Positive

NA, not applicable.

Remarks - Results

No genotoxic effects were observed in the Plate Incorporation Procedure test with and without metabolic activation and the Pre Incubation Procedure test without metabolic activation in all test strains.

In the Pre Incubation Procedure test with metabolic activation:

- TA 100: slight increases in the number of revertants from 28 µg/plate onward with a significant response at 140 µg/plate (2.8 time higher than control) in Test 2 and 400 µg/plate in Test 3.
- TA 1537: mutagenicity was observed from 100 µg/plate onward with peaks at 700 µg/plate in Test 2 and at 400 µg/plate in Test 3.
- TA 98: increase in the number of mutant colonies from 10 µg/plate onward with a maximum at 140 to 700 µg/plate.

- No genotoxic effects were observed in TA 1535 and *E. coli*: WP2uvrA.

CONCLUSION The notified chemical was mutagenic to bacteria in the Pre Incubation Procedure test under the conditions of the test.

TEST FACILITY BASF (2005i)

7.8. Genotoxicity – in vitro

TEST SUBSTANCE Lugafast Black AN

METHOD OECD TG 473 In vitro Mammalian Chromosome Aberration Test.
EC Directive 2000/32/EC B.10 Mutagenicity - In vitro Mammalian Chromosome Aberration Test.

Species/Strain	Chinese hamster
Cell Type/Cell Line	V79 cell line
Metabolic Activation System	S9 mix
Vehicle	Water
Remarks - Method	No significant protocol deviation. Rang-finding cytotoxicity tests were conducted with cultures exposed for 4 hours and 18-hour sampling time to doses ranging from 1 to 5000 µg/mL. Based on cytotoxicity and the assessments of the slides, doses of 62.5 to 1000 µg/mL with and without S9 mix were selected. Historical negative and positive control data were provided.

Metabolic Activation	*Test Substance Concentration (µg/mL)*	*Exposure Period*	*Harvest Time*
Absent			
Test 1	0*, 62.5, 125*, 250*, 500*, 750, 1000	4 h	24 h
Test 2a	0*, 62.5, 125*, 250*, 500*, 750, 1000	18 h	36 h
Test 2b	0*, 250, 500, 750*, 1000	18 h	46 h
Present			
Test 1	0*, 62.5, 125, 250*, 500*, 750*, 1000	4 h	24 h
Test 2	0*, 62.5, 125*, 250*, 500*, 750, 1000	4 h	32 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	>250	>750	Not reported	Negative
Test 2a	>250	>1000	Not reported	Negative
Test 2b	Not tested	>500	Not reported	Negative
Present				
Test 1	>500	>750	Not reported	Negative
Test 2	Not tested	>250	Not reported	Negative

Remarks - Results The test substance did not cause relevant increases in the number of structural chromosomal aberrations including and excluding gaps, either with or without the metabolic activation system.

No increase in the number of cells containing numerical chromosomal aberrations was observed.

Cytotoxicity (slight occasionally observed suppression of the mitotic

activity and dose-related growth inhibition under all experimental conditions) was observed in both the range-finding and the main tests.

CONCLUSION The notified chemical was not clastogenic to V79 cell line of Chinese hamster treated in vitro under the conditions of the test.

TEST FACILITY BASF (2005j)

7.9. Genotoxicity – in vivo

TEST SUBSTANCE Lugafast Black AN

METHOD OECD TG 474 Mammalian Erythrocyte Micronucleus Test.
EC Directive 2000/32/EC B.12 Mutagenicity - Mammalian Erythrocyte Micronucleus Test.

Species/Strain	Mice/Crl:NMRI
Route of Administration	Single oral administration (gavage)
Vehicle	Aqueous CMC formulation
Remarks - Method	No significant protocol deviation.

Dose mg/kg bw	*Group*	*Number and Sex of Animals*	*Sacrifice Time hours*
0	I (vehicle control)	5 males	24
500	II (low dose)	5 males	24
1000	III (mid dose)	5 males	24
2000	IV (high dose)	5 males	24
0	V (vehicle control)	5 males	48
2000	VI (high dose)	5 males	48
20 (positive control)*	VII	5 males	24
0.15 (positive control)**	VIII	5 males	24

*cyclophosphamide; **vincristine

RESULTS

Doses Producing Toxicity	>2000 mg/kg bw
Genotoxic Effects	No increase in the number of polychromatic erythrocytes were reported.
Remarks - Results	One animal at 1000 mg/kg bw died after gavage error. All animals had urine and faeces coloured by the test substance 4 hours after the administration at all doses, except at dose of 500 mg/kg bw (observed 1 day after administration).

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

TEST FACILITY BASF (2006)

7.10 Genotoxicity – in vivo

TEST SUBSTANCE Lugafast Black AN

METHOD OECD TG 486 Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo.
EC Directive 2000/32/EC B.39 Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells *In vivo*

Species/Strain	Wistar Han-rats (Crl:WI)
Route of Administration	Oral – gavage
Vehicle	Corn oil
Remarks - Method	No significant protocol deviation.

Group	*Number and Sex of Animals*	*Dose mg/kg bw*	*Sacrifice Time hours*
I (vehicle control)	3 males/group	0	3 and 14 hours
II (low dose)	3 males/group	1000	3 and 14 hours
III (high dose)	3 males/group	2000	3 and 14 hours
V (positive control*)	3 males/group	50	3 and 14 hours

* 2-acetylaminofluorene C.

RESULTS

Doses Producing Toxicity	No cell toxicity was observed at the highest dose tested.
Genotoxic Effects	No relevant increase in the mean number of net nuclear grain counts was observed either 3 hours or 14 hours after single oral administration of the test substance. Significant increased DNA repair activity was found in the positive control group.
Remarks - Results	No any signs of toxicity or symptoms were observed during the study.
CONCLUSION	The notified chemical was not clastogenic under the conditions of this in vivo UDS Test.
TEST FACILITY	BASF (2007)

8. ENVIRONMENT

8.1. Environmental fate

8.1.1. Ready biodegradability

TEST SUBSTANCE	Lugafast Black AN
METHOD	OECD TG 301 B Ready Biodegradability: CO_2 Evolution Test.
Inoculum	Sewage sludge
Exposure Period	28 days
Auxiliary Solvent	None specified
Analytical Monitoring	Titrimetric analysis of trapped inorganic carbon.
Remarks - Method	Duplicate analyses of Theoretical Oxygen Demand (ThOD) were performed on the test substance by subjecting it to activated sludge from a wastewater treatment plant, treating municipal sewage (Mannheim/Baden). Duplicate blanks were run, as well as an inhibition control containing both the test substance and the reference substance (aniline). A further reference substance (aniline) was also run.

RESULTS

*Test substance**		*Aniline*	
Day	*% Degradation*	*Day*	*% Degradation*
3	1	3	12
10	2	10	65
14	3	14	68
21	6	21	74
28	12	28	78

* Average of results

Remarks - Results	The CO_2 trapped by the secondary flask was measured on day 29 and added to the day 28 value. The amount of CO_2 produced by the inhibitory control exceeded the amount of the reference substance and therefore the test substance was considered non-inhibitory to sludge micro-organisms. The reference substance showed adequate degradation and the blanks showed adequate CO_2 production.
CONCLUSION	The test substance is not readily bio-degradable.
TEST FACILITY	BASF (2005k)

8.1.2. Bioaccumulation

Considering the chemical structure, the low log Pow and high water solubility, bio-accumulation is not expected.

8.2. Ecotoxicological investigations

8.2.1. Acute toxicity to fish

TEST SUBSTANCE	Lugafast Black AN
METHOD	In accordance with OECD TG 203 Fish, Acute Toxicity Test and EC Directive 92/69/EEC C.1 Acute Toxicity for Fish - static.
Species	Zebrafish *(Danio rerio)*
Exposure Period	96 h
Auxiliary Solvent	None specified

Water Hardness	100 mg $CaCO_3$/L
Analytical Monitoring	Daily observation; UV/VIS photometry.
Remarks – Method	A range finding test using 10 Zebra Fish of approx. 2 months old (2.7-3.2 cm body length, 0.14-0.22 g body weight) were subjected to the test substance and a control. pH 7.8 – 8.2 Temperature 24°C Dissolved oxygen 8.1 – 8.5 mg/L Photoperiod 16 h light, 8 h dark Temperature 24±1°C

RESULTS

Concentration mg/L		*Number of Fish*	*Mortality*				
Nominal	*Actual*		*1 h*	*24 h*	*48 h*	*72 h*	*96 h*
(Control) 0	0	10	0	0	0	0	0
120	102	10	0	0	0	0	0

LC50	> 102 mg/L at 96 hours
NOEC	102 mg/L at 96 hours
Remarks – Results	The value of 102 mg/L is based on the mean of the analytically determined concentrations at the beginning and the end of the test. The recovery for the chemical was 99 and 72% of the nominal amount at the beginning and end of the test respectively. The fish did not show any signs of abnormal behaviour. The solutions were clear but black.

CONCLUSION The notified chemical is practically non-toxic to Zebra Fish *(Danio rerio).*

TEST FACILITY BASF (2005l)

8.2.2. Acute/chronic toxicity to aquatic invertebrates

TEST SUBSTANCE Lugafast Black AN

METHOD In accordance with OECD TG 202 Daphnia sp. Acute Immobilisation Test and Reproduction Test and EC Directive 92/69/EEC C.2 Acute Toxicity for Daphnia – semi-static.

Species	*Daphnia magna* STRAUS
Exposure Period	48 hours
Auxiliary Solvent	None specified
Water Hardness	220 – 320 mg expressed as $CaCO_3$/L; Ca:Mg molar ratio ~ 4:1
Analytical Monitoring	Visual observation
Remarks - Method	Twenty daphnids (4 replicates of 5 daphnia) were subjected to each test concentration and a control. The test solutions were renewed after 24 hours. Temperature: 20.3-20.7°C, pH range: 7.8 – 8.1 dissolved oxygen concentration: 8.7 –9.3 mg/L Photoperiod a 16 h light, 8 h dark.

RESULTS

Concentration mg/L		*Number of D. magna*	*Number Immobilised*	
Nominal	*Actual*		*24 h [acute]*	*48 h [acute]*
0 (Control)		20	0	0
6.25		20	0	1
12.5		20	0	0
25		20	0	3
50		20	0	1
100		20	1	9

LC50 >100 mg/L at 48 hours

LOEC 12.5 mg/L at 48 hours

Remarks - Results The recovery of the notified chemical was 91-104% at 0, 24 hours old and new and at 48 hours. Whilst not stated it is assumed that the solutions were as for the fish test.

CONCLUSION The notified chemical practically non-toxic to *Daphnia magna*.

TEST FACILITY BASF (2005m)

8.2.3. Algal growth inhibition test

TEST SUBSTANCE Lugafast Black AN

METHOD In accordance with OECD TG 201 Alga, Growth Inhibition Test and EC Directive 92/69/EEC C.3 Algal Inhibition Test.

Species Green algae (*Desmodesmus subspicatus*)

Exposure Period 72 hours

Concentration Range Nominal: 0.32-100 mg/L
Actual: 0.11-93.4 mg/L

Auxiliary Solvent None specified

Water Hardness Not specified

Analytical Monitoring Cell counter

Remarks - Method Three replicates of ~ 1×10^4 cells/mL per test concentration and six replicates for the control were used. A further test was conducted using 1×10^4 cells/ mL in algal test growth media only, but under reduced light intensities by the filter effect of the coloured test substance at the test concentrations.
Temperature: 21°C and 25°C.
Light intensity 60-120 $\mu Em^{-2}.s^{-1}$

RESULTS

Biomass		*Growth*	
Nominal EbC50 mg/L at 72 h	*Nominal EbC50 mg/L at 72 h filter effect only*	*Nominal ErC50 mg/L at 72 h*	*Nominal ErC50 mg/L at 72 h filter effect only*
7.43	12.2	21.4	29.0

Remarks - Results The initial recovery of the nominal test concentrations was between 84-95% and 34-80% at the termination of the test. A difference was observed in the shape of the algal cells between the 10, 32 and 100 mg/L test concentrations and the control. A NOEC for the biomass and growth rate for the notified chemical when in direct contact with the alga was calculated to be 1.0 and 3.2 mg/L. The growth inhibition of the test substance was similar to the growth inhibition of algae in test water but under reduced light intensities. As the former were slightly lower, the

observed inhibition effect was likely to be mainly due to the indirect effect of light filtering. Any effect would therefore only be algistatic and not algicidal.

CONCLUSION The notified chemical showed some toxicity to algae but is likely to also inhibit algal growth by light filtering.

TEST FACILITY BASF (2005n)

8.2.4. Inhibition of microbial activity

TEST SUBSTANCE Lugafast Black AN

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

Inoculum	Activated sludge
Exposure Period	3 hours
Concentration Range	Nominal: 10-1000 mg/L Actual: Not determined
Remarks – Method	No significant protocol deviations. Activated sludge from laboratory wastewater plant treating municipal sewage was subjected to test concentrations of the notified substance of 10, 100, 500 and 1000 mg/L. A reference substance (1,3 dichlorophenol) was also run. Temperature 20±2°C.

RESULTS

IC50	> 1000 mg/L
IC20	>1000 mg/L
Remarks – Results	The test substance showed 19 % inhibition of respiration rate of activated sludge at 1000 mg/L. The reference substance had an EC50 of ~ 15 mg/L, thus validating the test.

CONCLUSION The notified chemical is not inhibitory to micro-organisms.

TEST FACILITY BASF (2005o)

9. RISK ASSESSMENT

9.1. Environment

9.1.1. Environment – exposure assessment

The majority of Lugafast Black AN containing the notified chemical will be used in dyeing (tanning) of collagen material, especially leather. During the dyeing process most of the notified chemical will be hydrolysed and fixed to the collagen (leather) material. Environmental exposure from release from collagen (leather) material is expected to be minimal and disperse. At the end of the leather goods' useful lives they will be disposed of to landfill. During manufacture of leather goods approximately 1.5% (< 45 kg per annum) will be released as solid waste. This will be disposed of by authorised landfill. It is expected that 85% will be fixed to the leather, leaving approximately 15% (< 450 kg per annum) to be treated in the on-site wastewater treatment plant with subsequent release to sewer. Sewage sludge containing the notified chemical is expected to be landfilled or possibly incinerated. The notified chemical is expected to be hydrolysed during the dyeing process and wastewater treatment processes. In landfill the notified chemical is expected to bind to cationic sites in soil. It will degrade mainly by abiotic processes (hydrolysis), with further degradation including biotic processes to produce landfill gases, such as hydrogen sulphide, ammonia, methane, chlorine compounds; oxides of carbon, nitrogen and sulphur; and water vapour.

9.1.2. Environment – effects assessment

A predicted no effect concentration (PNEC) is calculated using the lowest robust endpoint for toxicity and applying a safety factor. Little or no ecotoxicity was shown in any of the tests; a physical effect of light reduction effected algal growth and biomass and there seems to be a toxicity component attributable to the dye. The value for the PNEC is calculated by dividing the lowest robust toxicity value (ErC50 for algae) by a safety factor of 100 (as toxicity results are available for three trophic levels). The resulting PNEC is 214 µg/L which takes into account the light reduction effect.

9.1.3. Environment – risk characterisation

The risk to the environment will be from release of the notified chemical to the aquatic environment during the dyeing process. It appears that it will be mainly used at a single country dyehouse. If a worst case scenario is assumed where all the waste chemical (<450 kg per annum) is released over 260 working days to one small inland sewage treatment plant (STP) with a capacity of 5 ML per day, then a predicted environmental concentration (PEC) of 346 µg/L is calculated at sewage outfall. This is 1.62 times the PNEC, which shows an unacceptable risk.

Import amount	3000 kg per annum
Amount released to sewer assuming 85% fixation	450 kg per annum
Daily release assuming 260 working days	1.73 kg per day
Concentration at outfall assuming 5 ML per day STP	346 µg/L
Q (PEC of 346 µg/L ÷ PNEC of 214 µg/L)	1.62

However, this does not take into account the adsorption of the notified chemical to sewage sludge. In a 24 hour period the adsorption was found to be ~86%. Assuming that the residence time for wastewater in an STP is 8 hours, then adsorption can be estimated as 50% (three half lives of 8 hours will result in 87.5% elimination). When adsorption to sewage sludge is taken into account, the PEC at sewage outfall becomes 173 µg/L. The resulting Q (PEC÷PNEC) is 0.81. This shows an acceptable risk to the aquatic environment. The actual PEC is likely to be even lower as the notified chemical is expected to hydrolyse before it reaches sewage outfall. Furthermore as the main effect is likely to be due to light reduction, and it will therefore be expected to be algistatic and not algicidal.

9.2. Human health

9.2.1. Occupational health and safety – exposure assessment

During the dyeing process, workers may be exposed to the notified chemical via dermal, ocular and inhalation when manually weighing and during transferring Lugafast Black AN into the dye drums. The estimated dermal exposure to Lugafast Black AN powder is 90 to 900 mg/day, based on the EASE model using following inputs: direct handling, intermittent contact, non-dispersive use, and an average exposed surface areas of 1800 cm^2 for forearms and hands, and assuming the notified chemical is present at a concentration of up to 50%. Therefore, for a 70 kg worker and a 10% dermal absorption factor (given its high molecular weight), systemic exposure is estimated to be 0.13 to 1.29 mg/kg bw/day. The dye product containing the notified chemical is a non-volatile dust free powder, therefore, inhalation exposure is expected to be low. In addition, use of personal protective equipment and typical workplace engineering controls such as local exhaust ventilation will further reduce the potential exposure.

The exposure during other drum dyeing processes is expected to be minimal due to either automated enclosed system or minimal un-reacted chemical remaining in the dyeing drum.

9.2.2. Public health – exposure assessment

The imported Lugafast Black AN is for industrial use only. Therefore, public exposure to the notified chemical is only possible via unlikely accidental leakage during transportation. Public exposure to the notified chemical via use of the leather products treated with Lugafast Black AN is considered to be negligible because the notified chemical is unlikely to be bioavailable after the dying process. Potential public exposure via leaching of the notified chemical from leather products treated with Lugafast Black AN is also unlikely due to low likelihood of leather product having direct skin contact and being washed or processed during its use lifespan.

9.2.3. Human health – effects assessment

Toxicokinetics, metabolism and distribution:

Many azo dyes are unlikely to be absorbed through the skin because of their size and polarity (Øllgaard *et al* 1998). Smaller species may undergo percutaneous absorption, dependent on their properties. There was no evidence from the toxicological investigations to suggest that the notified chemical was absorbed following dermal exposure. However, bacterial skin microflora have been speculated to be able to break down azo dyes into smaller species through azo reduction, which may be more readily absorbed (SCCNFP, 2002).

Highly sulfonated azo dyes are generally poorly absorbed from the intestine after oral intake. However, cleavage of the azo linkage takes place in the gastrointestinal tract through the action of azo reductases. The sulfonated aromatic amines are rapidly absorbed. Given the discoloured dark urine and faeces and dark brown discolouration of the kidney and skeletal muscle observed in the repeated dose oral toxicity study (BASF, 2005h), it is clear that the notified chemical can be absorbed from the gastrointestinal tract following oral exposure.

The notified chemical is likely to have only limited potential for broad physiological distribution once absorbed, due to its polarity. Ultimately, the metabolites of azo dyes are excreted in the urine and bile (Brown and DeVito, 1993, referenced in Øllgaard *et al*, 1998). Supporting this prediction for the notified chemical is the black-stained urine and faeces that were observed in the oral gavage study (BASF, 2005h).

General toxicity:

The imported product Lugafast Black AN containing the notified chemical was of low acute oral and dermal toxicity in rats (LD50 >2,000 mg/kg bw). Although it is in powder form, an acute inhalation study was not conducted because a calculated dust value indicates that it is dust-free, plus worker exposure to the powder form will be limited as handling is expected to be controlled.

It is not irritating to the skin and only cause mild conjunctival redness and chemosis which is reversible. However, it caused irreversible black discolouration of the parts of the conjunctival and the nictitating membrane in all animals, and small part of sclera in two of the three test animals. Therefore, Lugafast Black AN containing the notified chemical is considered to cause severe ocular lesions which are highly likely attributed by the dye fraction containing her

notified chemical.

Lugafast Black AN containing the notified chemical was not a skin sensitiser, as shown in a mouse local lymph node assay. Relatively few azo dyes have been demonstrated to be skin sensitisers (Øllgaard *et al* 1998). Therefore, the notified chemical is unlikely to cause skin sensitisation in exposed humans.

The NOAEL in a 28-day oral repeat dose study in rats was 100 mg/kg bw/day for males and 300 mg/kg bw/day for females on the basis of the treatment related changes observed in the kidney and skeletal muscle, respectively, at the higher doses.

Mutagenicity:
Lugafast Black AN containing the notified chemical was found to be mutagenic in bacteria (only in Pre Incubation procedure with metabolic activation), performed using a standard test method according to OECD Test Guideline 471. It did not induce chromosomal aberrations in mammalian cells *in vitro* and both mouse micronucleus and unscheduled DNA synthesis test in vivo showed negative results. This genotoxicity profile does not rule out the notified chemical as a possible mutagen. However, in general, the degree of correlation between mutagenicity study results and the carcinogenicity of azo dyes as a class is poor, likely due to their complex metabolism *in vivo* (Brown and DeVito, 1993, referenced in Øllgaard *et al* 1998).

Azo dyes are a concern for their potential induction of mutagenicity and carcinogenicity. The azo linkage is the most labile portion of an azo dye molecule, and it is readily enzymatically metabolised in mammals, including man (SCCNFP, 2002). Liver azo reductase enzymes reductively cleave the molecule into component amines. Some metabolism may also occur in the cells of the bladder wall and during percutaneous absorption. Anaerobic intestinal bacteria are also capable of reductive cleavage of the azo linkage.

The aromatic amines that arise from the azo reduction of azo dyes are thought to be activated through their *N*-oxidation by cytochrome P450 isozymes (SCCNFP, 2002). These *N*-hydroxylarylamines may be further glucuronated (activated) or acetylated (inactivated), which may influence their mutagenicity (Bartsch, 1981). Under acidic pH, they form reactive nitrenium ions that can alkylate bases in DNA, particularly the nucleophilic centres in guanine. This mechanism is thought to contribute to the carcinogenicity of many azo dyes.

The notified chemical is not expected to be reductively cleaved to release any of the restricted aromatic amines specified in either the Appendix to EC Directive 76/769/EEC (EC, 2004) or the annexes of EU SCCNFP/0495/01 (SCCNFP, 2002). However, the notified chemical can be broken by azo reduction into arylamine species which is thought to be a mutagenicity-enhancing moiety when present in azo dyes (Chung and Cerniglia, 1992). Therefore, this reduction-product arylamine species may contribute to the potential of Lugafast Black AN containing the notified chemical to be mutagenic in bacteria.

In addition, azo dyes are renowned for their content of impurities, particularly for the presence of component arylamines (SCCNFP, 2002; Øllgaard *et al* 1998). Such impurities are thought to contribute to their carcinogenicity, as these species may be more readily absorbed, and activated as carcinogens. The notifier indicates that the dye fraction containing the notified chemical contains several by products. The identity of the contaminant is unknown, but it may be an aromatic amine.

Based on the currently available data, the notified chemical is classified as a hazardous substance in accordance with the *Approved Criteria for Classifying Hazardous Substances* (NOHSC 2004).

9.2.4. Occupational health and safety – risk characterisation

Potential risks arising from acute exposure

The primary route for potential exposure to the notified chemical is dermal. However, given the low dermal toxicity of the notified chemical and the lack of skin irritating and sensitising effects, together with the use of personal protective equipment (PPE) by workers, a low risk of these effects in the workplace is indicated.

Powders of the dye product containing the notified chemical may pose a risk of slight eye irritation to workers. Given the use of appropriate PPE and its nature of dust-free, this risk is minimal. However, due to the possible effect of irreversible discolouration of the eye, proper eye

protection should be worn at all time during handing dye powders and aqueous solutions containing <5% the notified chemical.

The dye product containing the notified chemical is a non-volatile dust free powder. Standard recommendations applied to avoid the formation of dusts during use and local exhaust ventilation are typically used during weighing and addition to mixing vessels. Rest of dyeing procedures involve dye solution, so workers are unlikely to be at risk of toxicity from the inhalation of the notified chemical. Workplaces should have local exhaust ventilation in place and workers should wear respiratory protection equipment when handling the powder containing the notified chemical.

Potential risks arising from repeated exposure

Based on the lowest NOAEL of 100 mg/kg bw/day, derived from a 28-day rat oral study and the reasonable worst-case worker systemic exposure estimation during formulation, the margin of exposure (MOE) is calculated as 77 to 769 (100 ÷ 1.29 to 0.13). MOEs greater than or equal to 100 are considered acceptable to account for intra- and inter-species differences. Although the lowest MOE is below 100, the risk of systemic effects based on the conservative modelled data is considered acceptable for workers who handle Lugafast Black AN powder containing up to 50% of the notified chemical during formulation, given that this is the worst-case scenario estimation plus workplace control measures are typically in place. Protection from dermal exposure will be recommended.

9.2.5. Public health – risk characterisation

Members of the public will only come into contact with dyed articles containing the notified chemical; so their exposure will be primarily dermal. In dyed leather, the notified chemical is irreversibly attached to the collagen fibres of the leather and is unlikely to be bioavailable. Therefore, no significant risks are expected to members of the public.

10. CONCLUSIONS – ASSESSMENT LEVEL OF CONCERN FOR THE ENVIRONMENT AND HUMANS

10.1. Hazard classification

Based on the available data the notified chemical is classified as hazardous under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC 2004). The classification and labelling details are:

R41 Risk of serious damage to eyes

and

As a comparison only, the classification of 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***
Environment	Acute 3	Harmful to aquatic life
Human Health	1	Causes severe eye damage

10.2. Environmental risk assessment

On the basis of the PEC/NOEC ratio:

The chemical is not considered to pose a risk to the environment based on its reported use pattern.

10.3. Human health risk assessment

10.3.1. Occupational health and safety

There is Low Concern to occupational health and safety under the conditions of the occupational settings described.

10.3.2. Public health

There is No Significant Concern to public health when used in the proposed manner.

11. MATERIAL SAFETY DATA SHEET

11.1. Material Safety Data Sheet

The MSDS of the product containing the notified chemical provided by the notifier was in accordance with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC 2003). It is published here as a matter of public record. The accuracy of the information on the MSDS remains the responsibility of the applicant.

11.2. Label

The label for the product containing the notified chemical provided by the notifier was in accordance with the *National Code of Practice for the Labelling of Workplace Substances* (NOHSC 1994). The accuracy of the information on the label remains the responsibility of the applicant.

12. RECOMMENDATIONS

REGULATORY CONTROLS
Hazard Classification and Labelling

- The Office of the ASCC, Department of Employment and Workplace Relations (DEWR), should consider the following health hazard classification for the notified chemical:

Risk phrases:

- R41 Risk of serious damage to eyes

Safety phrases:

- S25 Avoid contact with eyes
- S39 Wear eye/face protection

- Use the following risk phrases for products/mixtures containing the notified chemical:
  - 5% ≤ conc <10%: R36, irritating to eye
  - ≥10%: R41, Risk of serious damage to eyes

CONTROL MEASURES
Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical in the product Lugafast Black AN:
  - Local exhaust ventilation when powder form of the product is handled.

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical in the product Lugafast Black AN:
  - Avoid contact with eyes
  - Avoid contact with the skin
  - Avoid inhaling the powder during handling

- Employers should ensure appropriate eye, skin and respiratory protections are used by workers to minimise occupational exposure to the notified chemical in the product

Lugafast Black AN:

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

- 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 2004), workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Environment

- The notified chemical should be disposed of by authorised landfill or incineration.
- Spills or accidental release of the notified chemical should be handled by physical collection; avoid dust formation. If necessary use suitable dust binding material. Place in suitable containers for disposal or re-use to the extent practicable.

12.1. Secondary notification

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

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

(1) Under Section 64(2) of the Act:
   - if any of the circumstances listed in the subsection arise.

The Director will then decide whether secondary notification is required.

No additional secondary notification conditions are stipulated.

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