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**NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME**

**FULL PUBLIC REPORT**

# Disazo Blue AE 3510

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Director
Chemicals Notification and Assessment

# FULL PUBLIC REPORT

## Disazo Blue AE 3510

## 1. APPLICANT

Ciba-Geigy Australia Ltd, 140 Bungaree Rd, Pendle Hill NSW 2145

## 2. IDENTITY OF THE CHEMICAL

Based on the nature of the chemical and the data provided, Disazo Blue AE 3510 is considered to be non-hazardous. Therefore, the chemical name, CAS No, molecular formula, structural formula, molecular weight, method of detection and determination and spectral data have been exempted from publication in the Full Public Report and the Summary Report.

| | |
|---|---|
| **Other names:** | Disazo Blue AE 351040'408/A |
| **Trade name:** | Cibacron Navy F-R (commercial product containing 60% Disazo Blue AE 3510) |

## 3. PHYSICAL AND CHEMICAL PROPERTIES

| | |
|---|---|
| **Appearance at 20°C and 101.3 kPa:** | blue powder |
| **Odour:** | none |
| **Melting Point:** | none below 300°C |
| **Density:** | 1790 kg/$m^3$ |
| **Surface Tension:** | 57 mN/m at 1 g/L |
| **Vapour Pressure:** | negligible (based on the high molecular weight of the chemical) |
| **Water Solubility:** | > 412 g/L at 20°C |
| **Fat Solubility:** | < 0.07 mg/100g fat at 37°C |

| | |
|---|---|
| **Partition Co-efficient (n-octanol/water)** log $P_{O/W}$: | -10.5 |
| **Hydrolysis as a function of pH:** | at pH 4 and 7: stable at 50°C half life time > 1 year at 25°CpH 9: 83.2% hydrolysed after 5 days at 50°C half life time < 1 year at 25°C |
| **Dissociation Constant:** | $-SO_3-$: -2.5 > pKa > -3.0$NH_2$: pKa ~ 3.6OH: pKa ~ 8.8 (calculated) |
| **Flammability:** | not flammable |
| **Autoignition Temperature:** | 215°C |
| **Explosive Properties:** | no shock, friction or thermal sensitivity shown |
| **Reactivity/Stability:** | no known oxidising properties |
| **Particle size distribution:** | 95% > 20 µm: 82 µm (width) |

**Comments on physico-chemical data:**

Adsorption/Desorption data were not provided. Due to the chemicals low water solubility and low partition coefficient, no significant amounts of the notified chemical should enter the compartment of the environment which could result in contamination of soil.

## 4. PURITY OF THE CHEMICAL

The degree of purity as well as the identity of all impurities and additives/adjuvants have been exempted from publication. The following hazardous impurity, however, is present at levels above the cut off concentration for classifying the chemical as a hazardous substance (1) and therefore has been included for publication in the Full Public Report. A number of unknown impurities are also present in Disazo Blue AE 3510.

**Toxic or hazardous impurity:**

| | | |
|---|---|---|
| . | **Chemical name:** | sodium sulfate |
| | **CAS No.:** | 7757-82-6 |
| | **Weight percentage:** | >5 |
| | **Toxic properties:** | mildly toxic by ingestion; experimental teratogen; experimental reproductive effects; mouse oral $LD_{50}$ 5989 mg/kg (2) |

## 5. INDUSTRIAL USE

Disazo Blue AE 3510 will be imported in the dyestuff Cibacron Navy F-R. It will be sold for the purpose of colouring of cellulosic textiles by the cold pad-batch or exhaust dyeing methods. The pad-steam method is not expected to be used. The estimated quantity of the notified chemical to be imported into Australia has been exempted from publication.

## 6. OCCUPATIONAL EXPOSURE

The notified chemical will be imported and road transported by accredited carriers to Ciba-Geigy warehouses. The powder product will arrive in fibreboard boxes with sealed polyethylene liners (30 kg/box). For purposes of supplying samples or material for mill trials, it may occasionally be necessary for the product to be repacked at the warehouses. Less than 100 kg will be repacked (manually scooped from the original container to 5 kg tins) by a maximum of two workers on 10 days/year for 15-20 minutes/day. The notifier states that workers will wear personal protective equipment and the procedure will be carried out in a "downdraught" booth.

From the Ciba-Geigy warehouses Cibacron Navy F-R will be transported by road to industrial dyehouses throughout Australia. Exposure during transportation will result only during accidental spills or mishandling.

A total of 48 employees will be potentially exposed to the notified chemical, both in liquid and powder form. Batch weighers (~2) will be involved in weighing out the powder and adding it to a sealable blending vessel. For each batch weigher, weighing operations are expected to take no more than 1 hour, 56

days/year and are usually conducted under local exhaust ventillation or with the use of respiratory protection. Other workers, such as plant operators (~3) and laboratory technicians (~1) will have limited exposure to the notified chemical in powder form.

Once a solution of the dye has been formed, the potential for exposure will be significantly reduced as contact with the liquid is unlikely. After dyeing has occurred, 8 - 35% of the dye (depending on the fixation method) and other additives are washed away and the cellulosic fibre dried. These steps are carried out within the blending vessel and the dye becomes permanently fixed to cellulosic textiles.

## 7. PUBLIC EXPOSURE

There is low potential for public exposure to Disazo Blue AE 3510 during transport and distribution.

At the dyehouse, the commercial product will be weighed out in a dispensary and incorporated in a dyebath. Application of the dyestuff by cold pad-batch or exhaust dyeing methods is not expected to result in significant public exposure.

Disposal of waste notified chemical will be limited to traces remaining from the clean-up of any spill, trace residues in empty packaging and discharges to dyehouse effluent systems. The material safety data sheet for Cibacron Navy F-R indicates that waste product and contaminated packing material be sent to secure landfill or disposed by high temperature incineration. Approximately 50% of notified chemical discharged into the dyehouse effluent system is expected to be retained in the sediment of the effluent system. There is low potential for public exposure resulting from disposal of the notified chemical.

The notified chemical will be used to dye fabrics (100% cotton and cotton blends) for clothing and other products. Public exposure to the dye is expected to be negligible as the dye does not 'bleed' from cotton fabrics and therefore, transfer of dye to the skin is not expected to occur.

## 8. ENVIRONMENTAL EXPOSURE

### . Release

The notified chemical will be used to colour cellulosic textiles by the cold batch or exhaust dyeing methods. It is a reactive dye that becomes chemically bound to cellulosic fibres via ester or amide links between hydroxyl or amino groups on the fibre and the active vinylic bromide. The dye is a limited variation on dyes already in use in Australia (e.g. Disazo Navy TZ 2646) and it is claimed that the padding fixation characteristic should reduce the quantity of dye released to the environment.

The notifier indicates that the substance exhibits a 65% level of fixation on substrates dyed by exhaust and a 92% level of fixation when using the pad cold-batch method. The remainder will be discharged with wastewater to effluent treatment plants or sewer, depending on local requirements.

The notifier has indicated that levels of the notified dye in the receiving waters will typically range between 0.76 and 19 ppb.

### . Fate

The bulk of the dye will become chemically bound to fibre and in this state is not expected to impact on the environment.

Unfixed residues from dying operations will enter the aquatic environment following discharge from textile mills and subsequent treatment, during which they may be removed through degradation (chemical or biological) or sorption to sludge. In view of the hydrolytic stability, it is likely that significant quantities will remain in the aquatic compartment, in spite of the relative alkalinity of the sewerage system. Furthermore, reactive dyes in general have been found not to adsorb to sludge in model systems (3).

Turning to specific tests on Disazo Blue AE 3510, ready biodegradation was not observed (0% in 28 days), when the dye was tested using activated sludge from a domestic sewage plant according to OECD Test Guideline 301A.

Residues that survive treatment will enter freshwater or marine environments in solution. Azo dyes are generally stable under aerobic conditions, but are susceptible to reductive degradation

under the anaerobic conditions characteristic of sediment (4). Also, highly sulphonated *bis*(azo) dyes have been shown to sorb to sediment (5). Degradation of such dyes in sediment water systems proceeded with a half-life of 2-16 days. Accordingly, no significant increase in dissolved concentrations over time is predicted, while residues bound to sediment are expected to undergo reductive degradation. However, the hydrophilic Disazo Blue AE 3510 and its sulphonated metabolites are expected to have a low affinity for soil and sediment because of its high water solubility (>412 g/L) and very low partition coefficient (log $P_{O/W}$ = -10.5), and thus should remain in the aquatic compartment.

The bioaccumulation potential of Disazo Blue AE 3510 was not investigated because of the very low partition coefficient (log $P_{O/W}$ = -10.5) and lipid solubility (< 0.07 mg/100 g). Hydrophilic dyes with log $P_{O/W}$ < 3 have been shown not to bioaccumulate (5).

## 9. EVALUATION OF TOXICOLOGICAL DATA

### 9.1 Acute Toxicity

Table 1 Summary of the acute toxicity of Disazo Blue AE 3510

| Test | Species | Outcome | Reference |
|---|---|---|---|
| Oral | Rat | $LD_{50}$ >2000 mg/kg | 6 |
| Dermal | Rat | $LD_{50}$ >2000 mg/kg | 7 |
| Skin Irritation | Rabbit | non-irritant | 8 |
| Eye Irritation | Rabbit | slight irritant | 9 |
| Skin sensitisation | Guinea-Pig | sensitiser | 10 |

#### 9.1.1 Oral Toxicity (6)

This study was conducted in accordance with OECD guideline No: 401 (11).

Disazo Blue AE 3510 was administered to 10 Wistar Han. rats (5 male and 5 female) by oral gavage, at a single dose of 2000 mg/kg

in bi-distilled water (10 ml/animal). Clinical observations were made over a 15-day period. No deaths occurred during the observation period. All rats were sacrificed on day 15 and necropsy performed. Bodyweight gains of the treated animals were unaffected by treatment and no clinical signs were noted. Necropsy on sacrificed animals revealed no significant macroscopic legions.

Results of this study indicate an acute oral $LD_{50}$ of >2000 kg/mg in rats of both sexes for Disazo Blue AE 3510.

**9.1.2 Dermal Toxicity (7)**

This study was conducted in accordance with OECD guideline No: 402 (12).

A single dose of 4 ml Disazo Blue AE 3510 in bi-distilled water at 2000 mg/kg was applied to the clipped backs of 10 Wistar Han. rats (5 male and 5 female) and covered with a semi-occlusive dressing. Twenty-four hours later the dressing was removed and the test site washed with lukewarm tap water and dried with paper towelling. Clinical observations were made at 24 hours and over a 15 day period. No deaths occured during the observation period. All rats were sacrificed on day 15 and necropsy performed. Bodyweight gains of the treated animals were unaffected. Scales were observed on the backs of all animals intermittently throughout the study. Necrosis was reported in one female rat on day 11 through 15, and slight erythema in two male rats at about day 5. The test site of all animals was stained blue by the test compound. Necropsy on sacrificed animals revealed no significant macroscopic legions.

Results of this study indicate an acute dermal $LD_{50}$ of >2000 kg/mg in rats of both sexes for Disazo Blue AE 3510.

**9.1.3 Skin Irritation (8)**

This study was conducted in accordance with OECD guideline No: 404 (13).

A single dose of 0.5 g Disazo Blue AE 3510 (moistened with bi-distilled water) was applied by semi-occlusive application to the closely-clipped dorsa of 3 New Zealand white rabbits (1 male and 2 females). Four hours later the dressings were removed and the

test site washed with lukewarm tap water. Skin reactions were assessed 1, 24, 48 and 72 hours after dressing removal. No clinical symptoms or mortality were observed in the animals during the 72 hour observation period. Very slight erythema was observed in all animals at the 1 hour observation, which persisted in 2 of these animals through to 24 hours. Very slight oedema was observed in 2 animals at 1 hour observation only. No corrosive effects occurred on the skin of any of the animals. It should be noted that the test site of all animals was stained blue by the test compound pigment, indicating that some effects may have been masked.

Results of this study indicate that Disazo Blue AE 3510 is not a skin irritant in rabbits.

### 9.1.4 Eye Irritation (9)

This study was conducted in accordance with OECD guideline No: 405 (14).

A single dose of 0.1 g of Disazo Blue AE 3510 was instilled in the conjuctival sac of the left eye of each of 3 New Zealand white rabbits (1 male, 2 females). The right eye served as the control. The eyes were examined 1, 24, 48 and 72 hours as well as 7, 14, and 21 days after treatment. The test site of all animals was stained blue by the test compound pigment for the entire test period. Slight corneal opacity was observed at 1 hour for all animals, persisting to 48 hours in one animal. Injected conjunctival blood vessels were reported in one animal at 1 hour through to 48 hours and in another at 48 hours through to 14 days. Conjuctival chemosis was observed in one animal at 1 and 24 hours, and in another at 1 hour only. No corrosion was observed. The staining by the test article however may have masked any iridic irritation or further conjunctival irritation. No deaths occurred and no clinical symptoms were observed during the study. Necropsy was not performed on these animals.

The results of this study suggest that Disazo Blue AE 3510 is a slight eye irritant in rabbits.

### 9.1.5 Skin Sensitisation (10)

This study was conducted in accordance with OECD guideline No: 406 (15).

The Magnusson-Kligman Maximisation Test was used. Test animals were albino guinea-pigs.

**Pretest**

Two female guinea-pigs were injected intracutaneously with 0.1 ml of 1, 3 and 5% Disazo Blue AE 3510 in physiological saline and the skin reactions assessed 24 hours later.

Another four female guinea-pigs were treated topically (on shaved flanks) with 5, 10, 15 and 25% Disazo Blue AE 3510 in vaselinum album. The sites were occluded for 24 hours and then depilated to remove blue staining. Skin reactions were assessed after patch removal and 24 hours later.

Based on the results of the above studies, no primary irritation concentration was determined. An induction dose of 5% w/v Disazo Blue AE 3510 in physiological saline and a challenge dose of 25% Disazo Blue AE 3510 in vaselinum album were chosen for the main study.

**Induction**

On day one, 20 test animals (all female) were injected intradermally (on either side of a 4 x 6 cm clipped area of the dorsal scapula) with 0.1 ml Freund's Complete Adjuvant diluted 50:50 with physiological saline (50:50 FCA), 5% w/v Disazo Blue AE 3510 in physiological saline and 5% w/v Disazo Blue AE 3510 in 50:50 FCA. Similar injections were made in the control animals (10 female) however test material was excluded.

On day 7, all animals were induced with 10% sodium lauryl sulfate (SLS) in petroleum oil massaged into the skin. The following day, a filter paper patch covered with a thin film of Disazo Blue AE 3510 (25% in vaselinum album) was placed over the injection sites of the test animals and covered with a dressing for 48 hours. Control animals were treated as above with the omission of Disazo Blue AE 3510. Skin reactions were assessed by the Draize method 24 and 48 hours after patch removal.

**First challenge**

Two weeks after the epidermal induction application, a filter paper patch coated with 25% Disazo Blue AE 3510 in vaselinum album was placed on the shaved right flank of both test and

control animals. The left flank was treated with vaselinum album alone. The patches were occluded for 24 hours after which the test sites were depilated to remove blue staining caused by the test material. Sensitisation reactions were assessed 24 and 48 hours after patch removal.

At 24 hours the 25% Disazo Blue AE 3510 caused positive erythema in 3/10 of the control and 7/20 test animals. At 48 hours, reactions were observed in 3/10 control and 5/20 test animals. No reactions were observed with vaselinum album alone in either group.

**Second challenge**

Further challenge applications were made to all animals two weeks after the first challenge. This time the patches were reversed, 25% Disazo Blue AE 3510 in vaselinum album on the left flank and vaselinum album alone on the right. An additional application of 15% Disazo Blue AE 3510 in vaselinum album was made to the posterior right flank.

Twenty-four and 48 hours after the second challenge application, positive erythema was observed in 7/20 test animals treated with 25% Disazo Blue AE 3510 and 2/20 treated with 15% Disazo Blue AE 3510. No reactions were observed with vaselinum album alone.

The results of this study suggest that Disazo Blue AE 3510 is a skin sensitiser in guinea-pigs. However it was described as having a weak allergic tendency.

## 9.2 Repeated Dose Toxicity

A 5-day oral toxicity range finding study (16) was conducted in accordance with OECD guideline No: 401 (11). Disazo Blue AE 3510 (in bi-distilled water) was administered daily by gavage to Wistar Han. rats at 0 (control group), 200 or 1000 mg/kg for 5 days. Six rats (3 of each sex) were used per dose group. The animals were observed for mortality and clinical signs of toxicity during the treatment period and necropsy performed at the termination of the study. No deaths, body weight changes or ophthalmic changes were observed during the study. All treated animals showed discoloured faeces (dark-blue) on days 4 and 5 of the study. Necropsy revealed bluish discolouration of the mucosa of various organs of the digestive tract and the kidneys in 5 animals treated with the high dose. The remaining animal in this

group showed staining of the kidneys only. Absolute liver weights was significantly lower than controls in females treated with the low dose.

A 28-day oral toxicity study (17) was conducted in accordance with OECD guideline No: 407 (18). The dose levels used in this study were based on the 5-day oral toxicity range finding study above as well as data from the acute oral toxicity study mentioned earlier (6). Disazo Blue AE 3510 (in bi-distilled water) was administered daily by gavage to Wistar Han. rats at 0 (control group), 50, 200 or 1000 mg/kg for 28 days. A total of 40 rats were used for the toxicity test (5 of each sex/dose). An additional 20 rats (5 of each sex at the 0 and 1000 mg/kg doses) were observed for a further 15 days without exposure in a recovery test.

Viability, mortality and clinical signs of toxicity were recorded daily. At the end of the treatment and recovery periods (4 and 6 weeks respectively), blood and urine samples were collected for clinical investigations, while ophthalmoscopic examinations and scheduled necropsy were also performed.

No deaths occurred prior to necropsy and there were no treatment related body weight or ophthalmic changes during the study. Animals treated with Disazo Blue AE 3510 showed blue discolouring of the faeces from day 18 of the treatment period to day 4 of the recovery period.

Haematology revealed a slight increase in methaemoglobin in one rat treated with the highest dose, however this was not supported by histopathology and may have been due to spectral interference by the blue dye. No treatment related changes in serum biochemistries were apparent at the termination of either the treatment or recovery stage of the study.

Urinalysis revealed no toxicologically significant changes in urinary protein, glucose, ketones or bilirubin levels.

Pathology conducted at 4 and 6 weeks revealed a slight increase in relative liver and kidney weights of both male and female rats treated with 1000 mg/kg. All treated females showed a decrease in absolute and relative spleen weights at 4 weeks, however this effect was not dose-dependent. Male rats treated with 1000 mg/kg had decreased absolute and relative adrenal weights after the

recovery period, while female rats showed an increase in absolute brain weights. Blue staining was observed in most organs.

Microscopic examination showed the storage of a brown or blue pigment in kidneys, mucosa of the stomach, duodenum, jejunum, ileum, cecum, colon, rectum and alveolar macrophages of the lungs; but no evidence of tissue damage.

Under the conditions of this study Disazo Blue AE 3510 exhibited low toxicity in the rat after 28-day oral administration. Based on organ weight changes the target organs for toxicity were liver and kidney.

## 9.3 Genotoxicity

### 9.3.1 *Salmonella typhimurium* Reverse Mutation Assay (19)

This study was conducted in accordance with OECD guideline No: 471 (20).

Disazo Blue AE 3510 was tested in a *Salmonella typhimurium* reverse mutation assay using the plate incorporation procedure in the test strains TA 98, TA 100, TA 1535, TA 1537 and 1538, with or without metabolic activation.

Three experiments were conducted, each in triplicate. All strains were tested with Disazo Blue AE 3510 at concentrations of 0, 10, 100, 333.3, 1000 or 5000 µg/plate (experiment 1), and 0, 10, 100, 1000, 2500 and 5000 µg/plate (experiment 2). TA 100 was also tested at concentrations 0, 1000, 2000, 3000, 4000 or 5000 µg/plate (experiment 3). The reference mutagens sodium azide (TA 100 and TA 1535; - S9), 4-nitro-o-phenylene-diamine (TA 98, TA 1537 and TA 1538; - S9) and 2-aminoanthracene (all strains; + S9) were used as positive controls.

A dose-dependent increase in the revertant colony number was observed in strain TA 100 (1.6 x negative control) in the absence of metabolic activation, and in TA 1538 (2.8 x negative control) in the presence of metabolic activation.

Under the experimental conditions reported, Disazo Blue AE 3510 induced point mutations *in vitro* by base pair changes and frameshifts in the genome of the strains TA 100 and TA 1538. A test substance is considered to be positive in this assay if it induces a 2.5-fold increase in the number of histidine

revertants. Therefore Disazo Blue AE 3510 is considered to be weakly mutagenic in this assay.

### 9.3.2 Chromosome Aberrations in V79 Chinese Hamster Cells (21)

This study was conducted in accordance with OECD guideline No: 473 (22).

Experiments were conducted in duplicate with a 4 h treatment interval. V79 Cells were exposed to Disazo Blue AE 3510 with and without exogenous metabolic activation. Concentrations of 0.0, 0.1, 0.3, 1.0 or 10.0 µg/ml were incubated with S9 mix and harvested at 18 hours (all concentrations) or 28 hours (10.0 µg/ml only). Cultures without S9 mix were treated with 0, 100, 300 or 1000 µg/ml and harvested at 18 hours (all concentrations) and 28 hours (300 and 1000 µg/ml only). Cultures were also treated with appropriate reference mutagens (ethylmethanesulfonate in the presence of S9 or cyclophosphamide without). Stained chromosome preparations were examined for chromosomal aberrations (100 metaphases per treatment group).

In the absence of metabolic activation Disazo Blue AE 3510 at 300 µg/ml produced a significant (~8-fold) increase in cells with structural aberrations at the 28 hour treatment interval. Cells treated with 1000 µl/ml were difficult to score due to cell toxicity and poor metaphase quality. The positive controls showed marked increases in the number of cells with structural aberrations.

Under the conditions of this test Disazo Blue AE 3510 is clastogenic *in vitro*.

### 9.3.3 Micronucleus Assay in the Bone Marrow Cells of the Mouse (23)

This study was conducted largely in accordance with OECD guideline No: 474 (24).

Three groups of 10 test animals (5 male and 5 female NMRI mice) were given a single dose of 5000 mg/kg Disazo Blue AE 3510 (20 ml/kg in distilled water). Control animals were given vehicle alone. Bone marrow cells were collected for micronuclei analysis from each group at either 24, 48 or 72 hours after treatment.

The reference mutagen cyclophosphamide was used as a positive control.

One thousand polychromatic erythrocytes (PCE) were scored per animal, and the number of micronucleated PCEs recorded. The frequency of micronucleated cells was expressed as percent of total PCEs scored per animal. Cytotoxic effects were described by the ratio of normochromatic erythrocytes (NCE)/1000 PCE for each animal. This measure deviates from OECD guideline No: 474 which recommends a ratio of PCE/NCE as an indicator of cytotoxicity.

A slight cytotoxic response to Disazo Blue AE 3510 was shown in treated animals at the above dose, however no significant enhancement of micronuclei frequency noted at any time point. Cyclophosphamide produced a significant increase in the micronuclei frequency.

The results of this study indicate that Disazo Blue AE 3510 does not cause chromosomal damage *in vivo*.

### 9.3.4 Unscheduled DNA Synthesis in rat hepatocytes (25)

Two groups of male Wistar/WU rats were pretreated with Disazo Blue AE 3510 at an oral dose of either 100 or 1000 mg/kg (10 ml/kg in distilled water) and sacrificed at 16. Animals dosed with vehicle alone served as negative controls, while others treated with the reference mutagen 2-acetylaminofluorene (2-AAF) served as positive controls. An additional group was given 1000 mg/kg Disazo Blue AE 3510 and treated for 4 hours. Three rats were used per group.

Hepatocytes isolated from each animal by liver perfusion were used to establish primary cultures. For each animal, three cultures were incubated for 4 hours with tritium labelled thymidine ($^{3}$HTdR) for the estimation of unscheduled DNA synthesis (UDS). Two additional cultures were used to determine cytotoxicity and attachment efficiency of the cells.

The test compound produced no toxic reactions in the animals and no significant effects on the viability and attachment of cells cultured from these animals.

Hepatocytes from Disazo Blue AE 3510 treated animals revealed no increase in radiolabel incorporation as compared to negative

controls. Cultures prepared from 2-AAF treated animals showed significant increases in nuclear and whole cell incorporation of labelled thymidine.

The results of this study indicate that Disazo Blue AE 3510 does not induce DNA-damage *in vivo* leading to repair synthesis under the conditions of this assay.

### 9.4 Overall Assessment of Toxicological Data

Animal tests suggest that Disazo Blue AE 3510 has low acute oral and dermal toxicity (rat $LD_{50}$s >2000 mg/kg) and is not a skin irritant. It is a slight eye irritant, and the skin sensitisation study showed some indication that allergic responses may be possible. After 28 days of oral administration with Disazo Blue AE 3510 the liver and kidney were shown to be the target organs of toxicity. No other significant effects were noted.

Genotoxicity studies indicate that the chemical causes point mutations in *Salmonella typhimurium* and is clastogenic in V79 Chinese Hamster cells, but showed no clastogenic effects *in vivo* in the mouse, or DNA damage in an *in vivo* unscheduled DNA synthesis assay in rats. As there were no positive *in vivo* genotoxicity data Disazo Blue AE 3510 could not be classified as a mutagen.

## 10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

Ecotoxicity studies were conducted using technical grade Disazo Blue AE 3510 and the following results were provided by the notifier:

| Test | Species | Result |
|---|---|---|
| 96 h acute | Zebrafish ppm (*Brachydanio rerio*) | $LC_{50}$ = 264 |
| 48 h immobilisation | *Daphnia magna* | $EC_{50}$ = 421 mg/L |
| 96 h static | *Scenedesmus subspicatus* | LOEL = 4 mg/L<br>NOEC = 2 mg/L |
| 3 h respiration inhibition | Activated sludge (mixed bacterial culture) | $IC_{50}$ > 100 mg/L |

The above results show the dye to be practically nontoxic to fish and daphnids. This is consistent with the water solubility and high molecular weight of the substance. The reproduction part of the *Daphnia* acute immobilisation test was not performed because the company reports that the dense colouration of the test media inhibits reproduction and would invalidate the result. Based on the acute result, chronic effects would not be expected at environmental concentrations.

The company also reports that the relatively high toxicity to algae may be due to reduced light intensity or a change in light quality in the coloured test medium. Therefore, the result should be treated with caution, though it should be noted that the algal species tested is considered by the US Environmental Protection Agency to be insensitive (26).

The influence of Disazo Blue AE 3510 on respiration and nitrifying ability of activated sludge was tested under aerobic conditions according to OECD Test Method 209. A concentration of 100 mg/L exerted no inhibition over bacterial respiration processes.

## 11. ASSESSMENT OF ENVIRONMENTAL HAZARD

As noted previously up to 35% of the dye is not fixed in the exhaust dyeing process and significant quantities will be discharged into effluents. The notifier has calculated that the worst case scenario predicted environmental concentration (PEC) is 19 ppb when the application is by exhaust (65% fixation) and the effluent is diluted by a factor of 10 in the receiving waters at Devonport. Higher levels may be approached in a country dyehouse, on the mainland, during drought conditions.

This calculation is based on the internationally acceptable assumption that 50% of the dyestuff is retained in sludge in the biological effluent treatment works. However, Disazo Blue AE 3510 is expected to have a low affinity for soil and sediment because of its high water solubility (>412 g/L) and very low partition coefficient (log $P_{O/W}$ = -10.5). Assuming that no dyestuff is retained in sludge in the biological effluent treatment works then the worst case PEC is calculated to be 38 ppb dye in effluent discharged from Devonport. Based on this most extreme scenario, the PEC of 0.038 ppm is still well below the NOEL of 2 ppm for the algal test species *S. subspicatus*, and

is at least two orders of magnitude below the LOEL level of 4 ppm. Further, it should be noted that the inhibition effect may be a function of decreased light intensity or change in light quality reaching the algae in the coloured media. In any event, the dye's high solubility suggests that once released to the waterways dilution would be expected to swiftly reduce the environmental concentration to undectable levels.

Using the cold batch method 92% of the dye is expected to be adsorbed to the fibres. Assuming that 50% of the dyestuff is retained in sludge the PEC is calculated to range between 0.18 ppb and 4.4 ppb. If no dyestuff is retained in sludge then the worst case PEC is calculated to be 8.8 ppb.

The substance is practically nontoxic to aquatic fauna, and is not expected to accumulate in sediment or to bioaccumulate.

## 12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Disazo Blue AE 3510 is stable at room temperature, is not flammable and has negligible vapour pressure. Due to the chemicals high molecular weight, it is considered that absorption through biological membranes would be minimal.
Disazo Blue AE 3510 powder has a particle size distribution where 95% of particles are >20µm and therefore not respirable. The notifier states that the commercial product, Cibacron Navy F-R, is formulated to contain anti-dusting properties to minimize inhalational exposure. As other reactive dyes have been linked with respiratory sensitisation, and toxicological studies on Disazo Blue AE 3510 show possible allergic responses in skin, inhalation of Disazo Blue AE 3510 powder is a concern and should be prevented.

Dermal contact with the chemical has shown no significant systemic toxicity or skin irritation. However skin sensitization is possible. Skin contact to both the powder and products containing it should also be prevented.

Genotoxicity studies showed a weak mutagenic response to Blue AE 3510 in the *Salmonella typhimurium* reverse mutation assay, as well as significant clastogenicity *in vitro* in mammalian cells. However, the results of two *in vivo* genotoxicity studies indicate that Disazo Blue AE 3510 is neither clastogenic in the mouse nor

capable of inducing DNA damage in rats. Given the potency of the responses *in vitro* and the negative *in vivo* results, the notified chemical could not be classified as a mutagen.

Due to low occupational exposure under normal use conditions, with appropriate control measures and/or precautions to minimise contact, the notified chemical is not expected to present any significant health or safety hazard to workers.

There is low potential for public exposure to the notified chemical. The large proportion of impurities present in the notified chemical are not expected to be associated with the final products treated with the dye, and therefore, do not constitute a toxicological hazard to the public. Therefore, there should be negligible risk to public safety.

## 13. RECOMMENDATIONS

To minimise occupational exposure to Disazo Blue AE 3510 the following guidelines and precautions should be observed:

- If engineering controls and work practices are insufficient to reduce exposure to dye solutions containing Disazo Blue AE 3510 to a safe level, the following personal protective equipment should be used:
  - respiratory protection conforming to AS 1715 (27) and AS 1716 (28);
  - chemical-type goggles conforming to Australian Standards 1336 (29) and 1337 (30);
  - impervious gloves conforming to Australian Standard 2161 (31); and
  - protective clothing conforming to Australian Standards 3765.1 (32) or 3765.2 (33).
- Good work practices should be implemented to avoid generation of dust.
- Spills should be cleaned up promptly.
- Good personal hygiene practices, such as washing of hands prior to eating food, should be observed.

. A copy of the MSDS for products containing the notified chemical should be easily accessible to all employees.

To minimise environmental exposure to Disazo Blue AE 3510 the following guidelines and precautions should be observed:

. The exhaust method of dye fixation should be restricted to dyehouses located in the coastal city locations.

. The notifier should continue to evaluate the effectiveness of using the Colfloc RD process as a means of reducing the amount of coloured effluent being released to the environment, and keep the Department of the Environment, Sport, and Territories informed of the results.

## 14. MATERIAL SAFETY DATA SHEET

The Material Safety Data Sheet (MSDS) for Cibacron Navy F-R (Attachment 1), containing Disazo Blue AE 3510, was provided in Worksafe Australia format (34). This MSDS was provided by Ciba-Geigy Australia Ltd as part of their notification statement. It is reproduced here as a matter of public record. The accuracy of this information remains the responsibility of Ciba-Geigy Australia Ltd.

## 15. REQUIREMENTS FOR SECONDARY NOTIFICATION

Under the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act), secondary notification of Disazo Blue AE 3510 shall be required if any of the circumstances stipulated under subsection 64(2) of the Act arise. No other specific conditions are prescribed.

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