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**NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION
AND ASSESSMENT SCHEME**

FULL PUBLIC REPORT

BASILEN BLUE E-RTN

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Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT**BASILEB BLUE E-RTN****1. APPLICANT**

BASF Australia Ltd, 500 Princes Highway, Noble Park, Victoria 3174

2. IDENTITY OF THE CHEMICAL

Based on the nature of the chemical and the data provided, Basilen Blue E-RTN is not considered to be hazardous. Therefore, the details of chemical name, CAS number, molecular formula, spectral data and exact purity, have been exempted from publication in the Full Public Report

Other name(s): Reaktivblau 1463
 Reactive Blue 1463

Trade name: BASILEN BLUE E-RTN

Method of detection and determination:

UV/visible spectroscopy and Fourier transformed infra red spectroscopy (FTIR).

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa: Blue granulated powder

Odour: Not known

Melting Point: > 300°C

Boiling Point: Not applicable

Density: 1840 kg/m³

Vapour Pressure: Not determined

Water Solubility: 95 g/L at 24°C

Fat Solubility: <0.002 mg/kg

Hydrolysis: Not determined

Partition Co-efficient

log P_{ow}: < -4.3 @ 24°C

Hydrolysis as a function of pH: Not determined

Adsorption/Desorption:	Not determined
Dissociation Constant pKa:	Not determined
Surface Tension:	70.1 mN/m
Flash Point:	Not determined
Flammability Limits:	Not flammable
Combustion Products:	Not determined
Explosive Properties:	Not determined
Pyrolysis Products:	Not determined
Decomposition Temperature:	Not determined
Decomposition Products:	Not determined
Autoignition Temperature:	270°C
Reactivity/Stability:	Stable under ambient conditions; not oxidising
Particle size distribution:	77 µm (average) 16% > 115 µm 84% > 35 µm 2% < 10 µm

. Comments on the physico-chemical properties

Reference to OECD or EC test guidelines only are available, rather than detailed test reports. However, the above properties are consistent with those expected for a high molecular weight penta sulfonic acid salt.

5. INDUSTRIAL USE

The notified chemical in BASILEN BLUE E-RTN will be imported into Australia and used in the textile industry only as a dye for colouring cellulosic fibres and blends with other fabrics.

The notified chemical was listed in the European Listing of New Chemical Substances (ELINCS) in 1992. It has also been registered in the USA, Austria (<1 tonne), Japan (<1 tonne), Switzerland, Canada (Transitional substance) and Korea.

6. OCCUPATIONAL EXPOSURE

The estimated import of Basilen Blue E-RTN will be less than one tonne per annum in the first two years increasing to 10 tonnes per annum over the following three years. The estimated distribution of the notified chemical in Australia is as follows:

New South Wales 30%; Victoria 40%; South Australia 10%; Tasmania 10%; and other states and territories 10%.

The notified chemical will be shipped into Australia in 25 kg high density polyethylene drums. Any one of three drivers and any one of six storemen will be involved in transport of the notified chemical from the wharf to storage and dyeing sites. The dyeing is carried out in a closed vessel containing the 0.2% dye solution prepared by dilution of a stock dye solution pumped from a storage tank. The stock

solution (2%) is prepared by mechanically mixing with water. For conditions of maximum use ie. at all sites and for 48 weeks of the year the maximum exposure has been estimated as follows: 30 storemen and 110 operators will be exposed to the notified chemical during weighing and preparation of the dye in 20 plants throughout Australia. Each storeman will be exposed to the notified chemical for 12 hours per year at the rate of 15 minutes per week for 48 weeks. Each operator will be exposed to the notified chemical for 16 hours per year, if the weighing of the powder is the principal activity or 2 hours and 40 minutes per year if the solution preparation is rotated between 6 operators. Four laboratory workers will also be exposed to the notified chemical only at the testing site of the notified chemical.

7. PUBLIC EXPOSURE

As Basilen Blue E-RTN will only be sold to the dyeing industry, the potential for public exposure to the notified chemical during dyeing operations is minimal, but once incorporated into fabrics, there is a potential to come into contact with the notified chemical. However, the dyeing process fixes the dye to the fabric (by covalently bonding to cellulose) and it is claimed that the process is wetfast and the dye is not removed by contact with moist skin or water. Overall, there should be minimal public exposure.

8. ENVIRONMENTAL EXPOSURE

. Release

The dye is to be used to colour cellulosic textiles in commercial dye houses only. It will be chemically bound by triazine links between the hydroxyl group of the cellulose fibres and the displaceable chlorine of the notified chemical.

The notifier has indicated that the dye has a 85-90% level of fixation on the fibres. This level of fixation is within the normal range for reactive dyes. The remainder will be discharged into the sewer.

Spills of granulated powder that may occur during transport or handling will be cleaned up according to the procedure in the MSDS and consigned to a secure landfill or incinerated.

. Fate

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

The unfixed residues from dyeing operations will enter the aquatic environment after discharge from the textile mills and subsequent treatment at sewage treatment plants. As a result of the dye's low K_{ow} and hydrolytic stability, it is likely that significant quantities will remain in the aquatic phase. Furthermore, reactive dyes have been found not to strongly adsorb to sludge (1) in model systems.

The dye was not tested for its biodegradability but based on its chemical structure it is not expected to show any appreciable degradation.

Residues that survive treatment in the sewage plant, which is likely, will enter either freshwater or marine environments in solution. The dye is expected to be stable to aerobic conditions but azo dyes are susceptible to reductive degradation under anaerobic conditions, characteristic of sediments (2). The half life of this degradation was found to be between 2 and 16 days for several sulfonic azo dyes (3), thus no significant increase in concentration overtime is expected. One possible route for the dye to enter the sediments is by precipitation of its calcium salts, as several calcium salts of sulfonic dyes are known to be insoluble (3) at modest concentrations. However, apart from this possibility, the hydrophilic nature of Basilen Blue E-RTN should limit the affinity for soil and sediment and thus the dye should remain mainly in the aquatic compartment.

The bioaccumulation potential of the dye was not investigated due to its very low partition coefficient ($\log P_{ow} < -4.3$), as allowed by the *Act*. This, together with the high water solubility and low fat solubility, indicate that bioaccumulation should not occur.

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

Table 1 Summary of the acute toxicity of Basilen Blue E-RTN

Test	Species	Outcome	Reference
Oral	rats	LD ₅₀ >2000 mg/kg	(4)
Dermal	rats	LD ₅₀ >2000 mg/kg	(6)
Skin irritation	rabbit	non-irritant	(8)
Eye irritation	rabbit	slight irritant	(11)
Skin sensitisation	guinea pig	weak sensitiser	(13)

9.1.1 Oral Toxicity (4)

This study was carried out according to OECD Guidelines for Testing of Chemicals No: 401 (5).

A single dose of 2200 mg/kg of Basilen Blue E-RTN in double distilled water was administered by gavage to Wistar rats (5/sex). The animals were observed at 1 and 4 hours after dosing and subsequently once daily for 14 days. No deaths were noted during the study. All animals showed the expected gain in body weight over the study period. No abnormalities were noted at necropsy.

The results of this study indicate an oral LD₅₀ of >2000 mg/kg for Basilen Blue E-RTN in male and female rats.

9.1.2 Dermal Toxicity (6)

This study was carried out in accordance with OECD Guidelines for Testing of Chemicals No: 402 (7).

A single dose of 2000 mg/kg of Basilen Blue E-RTN was administered by semi-occlusive application to the shaved skin of Wistar rats (5/sex) for 24 hours. The animals were observed for 14 days after removal of the bandage. No deaths were noted during the study. Male rats showed the expected gain in body weight during the study, while, female rats showed a gain in body weight only after the seventh day. No evidence of systemic toxicity or skin irritation was noted. No abnormalities were noted at necropsy.

The results of this study indicate a dermal LD₅₀ of >2000 mg/kg for Basilen Blue E-RTN in male and female rats.

9.1.3 Acute Inhalation Toxicity

Based on the notifiers experience with similar dyestuffs, production quality control of the commercial product as a granulated preparation which has a particle distribution size where 84% is greater than 35 micron and 2% is less than 10 micron and a low potential for inhalation exposure, ie small quantities handled, an acute inhalation study was not carried out.

9.1.3 Skin Irritation (8)

This study was carried out in accordance with OECD Guidelines for Testing of Chemicals No: 404 (9).

A single dose of 0.5 g of Basilen Blue E-RTN moistened with water was administered by occlusive application to the clipped flank of three male Vienna White rabbits for four hours. The site of application was examined approximately 60 minutes and 1, 2 and 3 days after removal of the dressing. Skin reactions were assessed according to Draize (10). There were no signs of erythema or oedema in any of the animals.

The results of this study indicate that Basilen Blue E-RTN is a non-irritant to the skin of rabbit.

9.1.4 Eye Irritation (11)

This study was carried out in accordance with OECD Guidelines for Testing of Chemicals No: 405 (12).

Three Vienna White male rabbits were used in the study. Initially, a single dose of 69 mg of Basilen Blue E-RTN was instilled into the conjunctival sac of the right eye of each rabbit. The other eye which remained untreated, served as the control. Immediately after instillation of the test substance, the initial pain reaction of the rabbit was assessed using a six point scale. Ocular reactions were assessed according to Draize (10) after 1 hour and 1, 2, 3, 8 and 15 days post-treatment.

Slight conjunctival redness and chemosis were observed in all animals one hour post-treatment and slight conjunctival redness persisted in one animal up to day 3 and in 2 animals up to day 8. Chemosis was observed in all animals up to day 3. On day 15 all treated eyes appeared normal.

The results of this study indicate that Basilen Blue E-RTN is a slight eye irritant in rabbits.

9.1.5 Skin Sensitisation (13)

This study was carried out according to the OECD Guidelines for Testing of Chemicals No:406 (14).

The maximisation test was used to assess skin sensitisation potential of Basilen Blue E-RTN. Skin reactions were assessed according to a four-point scale. There is no mention that the sensitivity of the strain of guinea pig used in this study was periodically tested with a known skin sensitiser such as 2,4-dinitrobenzene.

The following concentrations for induction and the challenge were selected from a preliminary study.

Intradermal induction:	5% test substance in 0.9% aqueous NaCl solution in Freund's adjuvant/0.9% aqueous NaCl-solution (1:1). 0.9% aqueous NaCl solution.
Percutaneous induction:	50% test substance in distilled water
1st challenge:	25% test substance in distilled water
2nd challenge:	25% test substance in distilled water

Induction

Forty female albino Pirbright White, guinea pigs (20 test and 20 control) were used.

Two injections each of: 1) 0.1 ml Freund's Complete Adjuvant with 1:1, 0.9% aqueous NaCl solution 2) 0.1 ml of 5% w/v suspension of the test substance in 0.9% aqueous NaCl solution; and 3) 0.1 ml Freund's Adjuvant, 1:1, 5% w/v suspension of the test substance in 0.9% aqueous NaCl solution, was made on each side of the mid-line of the test animals. One week later, a single dose of 50% w/w of the test substance in distilled water was administered by occlusive application to the shoulder area of each test animal for 48 hours. Twenty-four hours after the removal of the dressing, the application sites were examined for reactions. Control animals were similarly treated but without the test substance.

Challenge

Two weeks after induction the test and one control group animal were challenged with a single dose of 25% w/v of the test substance in distilled water by occlusive application for 24 hours on the shoulder area of the intradermal application. One week later the test group and both control groups were challenged in a similar manner to the first.

One control group animal and two test group animals were found dead on day nine, ten and twelve respectively. Twenty-four hours after the first challenge, one test animal exhibited distinct erythema and after the second challenge another test animal exhibited slight erythema. However, the level of erythema could not be determined due to staining of the skin by the coloured test substance. No adverse skin reactions were noted in the two control groups.

The results of this study indicate that Basilen Blue E-RTN to be a weak skin sensitiser in guinea pigs at the concentration tested.

9.2 28-Day Repeated Dose Toxicity (15)

This study was carried out according to OECD Guidelines for Testing of Chemicals No: 407 (16).

Basilen Blue E-RTN in distilled water was administered by gavage once daily to groups Wistar rats (5/sex) at dose levels of 100, 300 or 1000 mg/kg/day over 28 days. The control group received only distilled water. Two other groups were treated with the high dose or distilled water.

Food consumption and body weight gain in test animals were comparable with that seen in controls.

There were no clinical chemistry and haematology changes observed in the study.

At necropsy the relative weights of the kidneys (relative to the body weight) of females of the 2 higher groups showed significant increase. However, one of the other groups that was given the highest dose also showed a significant increase in the absolute kidney weight after the recovery period. There were no treatment-related macroscopic abnormalities and histopathological changes observed in the study.

The results of this study indicate the 'no adverse effect level' for Basilen Blue E-RTN is > 1000 mg/kg.

9.3 Genotoxicity

9.3.1 Salmonella typhimurium Reverse Mutation Assay (17)

This study was carried out according to OECD Guidelines for Testing of Chemicals No: 471 (18).

Basilen Blue E-RTN at dose levels of 5000, 2000, 500, 100, or 20 µg/plate was tested for gene mutation using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 both in the presence or absence of metabolic activation (S9-mix). The experiment was also repeated using Basilen Blue E-RTN at dose levels of 5000, 2500, 500, 100, or 20 µg/plate. Positive controls used were N-

methyl-N'-nitro-N-nitrosoguanidine, 9-aminoacridine, 4-nitro-O-phenylenediamine (without S-9 mix) and 2-aminoanthracene (with S-9 mix). Distilled water was used as the diluent for the test substance and as the negative control.

In both experiments the test substance did not induce statistically significant dose-related increases in the number of revertant colonies in *Salmonella typhimurium* strains both in the presence or absence of S-9 mix. The positive controls induced the expected increases in all strains tested.

The results of this study indicate that Basilen Blue E-RTN is not mutagenic in *Salmonella typhimurium* assay..

9.3.2 Chromosome Aberration Essay (19)

This study was carried out according to the *OECD Guidelines for Testing Chemicals No 473(20)*.

Cultured human lymphocytes were incubated with Basilen Blue E-RTN for a four hour period with or without metabolic activation. Mitotic activity was arrested by the addition of colchicine to the culture three hours before the end of the incubation. Ethylmethanesulfonate 0.72 mg/ml without metabolic activation and cyclophosphamide 60 µg/ml with metabolic activation were used as positive controls. The dose levels of Basilen Blue E-RTN selected for the metaphase analysis were 0.1, 1.0 and 2.5 mg/ml in the absence of S-9 mix and from 0.1, 0.3, 2.5 and 5.0 mg/ml in its presence. Cells were fixed, stained and examined for chromosomal aberrations.

No statistically significant increases in the proportion of aberrant cells were observed when Basilen Blue E-RTN was incubated with or without S-9 mix. Both positive controls caused large increases in the number of aberrant cells.

The results of this study indicate that Basilen Blue E-RTN is not clastogenic towards cultured human lymphocytes.

9.4 Overall Assessment of Toxicological Data

Basilen Blue E-RTN has low acute oral and dermal toxicity in rats. It is a slight eye irritant. It is not a skin irritant in rabbits but a weak skin sensitizer in guinea pigs. A 28-day repeated dose study showed no treatment-related effects at doses of up to 1000 mg/kg/day. Basilen Blue E-RTN was found to be non-mutagenic in the *Salmonella typhimurium* reverse mutation assay and non-clastogenic in the cultured human lymphocytes assay.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

Ecotoxicity tests were performed using technical grade Basilen Blue E-RTN dye and the results (table 1) were provided by the notifier. No precipitates or other irregularities were noted in these tests and the concentrations were measured (UV/Vis spectroscopy) at the start and end for the fish studies. The *Daphnia* studies used the penta sodium salt and nominal concentrations were used to calculate the EC₅₀. These tests were performed in accordance with standard OECD test methods and at facilities complying with OECD principles of GLP.

Species	Test	Result
Zebra fish, <i>Brachydanio rerio</i> (21)	96 hour acute TG 203	NOEC >100 mg.L ⁻¹ LC ₅₀ >100 mg.L ⁻¹
<i>Daphnia</i> , <i>Daphnia magna</i> (22)	48 hour immobilisation TG 202	NOEC = 250 mg.L ⁻¹ EC ₅₀ = 450 mg.L ⁻¹

Table 1

Toxicity tests on algae were not performed, but toxic effects are not expected because of the dye's high water solubility and large molecular weight. It is evident from the fish and daphnia tests that the copper in the dye is not biologically available and therefore not likely to effect algae (copper salts are known to be highly toxic to aquatic organisms and algae).

As indicated above, 85-90% of the dye is fixed in the exhaust dyeing process, thus 15% of the dye used could be discharged into effluents of the dyehouses where it is used. The notifier has calculated the concentration of discharge for two model dyehouses, one in a regional centre (Type A) and the other city based (Type B). These were chosen to represent a range of situations. The calculations presented by the company are as follows:

Effluent concentration Type A = 0.25 ppm
 Type B = 0.49 ppm

Ocean discharge from city (10:1 dilution) = 0.25 ppb

The dye is not expected to accumulate in the sediment nor bioaccumulate.

Exposure by inhalation is a possible route of exposure during the use of the notified chemical in powdered form. There is no acute inhalation data from an animal study as the notifier stated that the particle size distribution demonstrated that respirable particle concentration is low and consequently did

not warrant such a study. With conformation to the NOHSC exposure standard for nuisance dusts of 10 mg/m³ inspirable (24) and recommended handling and use procedures, risks to workers via an inhalation route should be minimal.

Basilen Blue E-RTN has a low partition coefficient, is not flammable and is non-reactive under normal use conditions.

The principal source of exposure will occur during the handling of the granulated powder during weighing and preparation of the dye. However, given the toxicological profile of the notified chemical and with the appropriate engineering controls and safe work practices in place exposure to the notified chemical should be minimal under normal use..

There is low potential for public exposure to the notified chemical. Therefore, there should be negligible risk to public safety.

13. RECOMMENDATIONS

To minimise occupational exposure to Basilen Blue E-RTN the following guidelines and precautions should be observed:

- . the work place should be well ventilated and local exhaust ventilation should be used during weighing;
- . good work practices to avoid generation of dust, if there is any likelihood of dust generation a face mask should be worn;
- . airborne dust levels should be kept as low as possible; and
- . if engineering controls and work practices are insufficient to reduce exposure to a safe level, the following personal protective equipment which complies with Australian Standards should be worn such as respiratory protection devices (AS 1715-1991 (25), AS 1716-1992 (26)), safety spectacles (AS 1336-1982 (27), AS 1337-1982 (28)) gloves (AS 2161-1978 (29) and overalls; and
- . a copy of the Material Safety Data Sheet (MSDS) should be easily accessible to all employees.

14. MATERIAL SAFETY DATA SHEET

The MSDS for Basilen Blue E-RTN (Attachment 1) was provided in Worksafe Australia format (30). This MSDS was provided by BASF 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 BASF Australia Ltd.

15. REQUIREMENTS FOR SECONDARY NOTIFICATION

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

16. **REFERENCES**

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