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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

Direct Blue FC 57087

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Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT

Direct Blue FC 57087

1. APPLICANT

Clariant (Australia) Pty Ltd of 675-685 Warrigal Road CHADSTONE VIC 3148 has submitted a limited notification statement in support of their application for an assessment certificate for Direct Blue FC 57087.

2. IDENTITY OF THE CHEMICAL

Direct Blue FC 57087 is not considered to be hazardous based on the nature of the chemical and the data provided. Therefore the chemical name, CAS number, molecular and structural formulae and spectral data have been exempted from publication in the Full Public Report and the Summary Report.

Trade Name:	Optisal Royal Blue 3RL (contains 28% of the notified chemical)
Molecular Weight:	1 302

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa:	fine blue granules
Melting Point:	> 300°C (EEC method A1-capillary method, liquid bath)
Density:	1 861 kg/m^3 at 22°C
Vapour Pressure:	not provided
Water Solubility:	not determinable (see comments below)
Partition Co-efficient (n-octanol/water):	log K_{ow} < -4.0
Hydrolysis as a Function of pH:	not degradable (see comments below)

Adsorption/Desorption:	not provided
Dissociation Constant:	not provided
Fat Solubility:	not provided
Flash Point:	not available
Flammability Limits:	not highly flammable (the reaction ended after 15 seconds within the start segment)
Autoignition Temperature:	> 400°C
Explosive Properties:	not explosive
Reactivity/Stability:	not oxidising

Comments on Physico-Chemical Properties

Tests were performed at facilities complying with OECD Principles of Good Laboratory Practice.

No melting point was detected below 300°C.

Vapour pressure was not determined. It is however expected to be negligible, noting the dye is a high molecular weight, organic pentasodium/lithium salt and has a very high melting point.

The water solubility was not able to be determined using the methods employed by the notifier (EEC Method A6: HPLC). It is claimed that the notified chemical is a substantive “dye”. These are known to form colloidal solutions rather than “real” solutions. Investigation showed that the solution of the dyestuff is a rough disperse distribution (colloidal solution) in water, rather than a “true” solution in the sense of the EEC Guideline A6 (Water Solubility). The diameters of particle aggregations were measured to be 200 to 500 nm. However, water solubility of the notified chemical in the ecotoxicological tests is claimed as less than 30 g/L. The notifier claims that the low analytical concentrations attained in these tests are attributed to microdisperse flocculations which could not be perceived by the eye. These flocculations decreased during the ecotoxicological studies.

Preliminary testing revealed that at 50°C, no degradation of the notified chemical was observed at pH 4, 7 and 9. Hence, it has a hydrolysis half-life period longer than one year at 25°C at pH 4, 7 and 9.

The results obtained by the preliminary partitioning experiment showed that log K_{OW}, determined to be less than -4.0, lies outside the range determinable by the flask shaking method and no further testing was performed.

Adsorption/desorption data were not provided. High water “solubility” and an expected low partition coefficient would normally indicate low affinity for soil or sediment. It is expected the notified chemical to be relatively mobile in groundwater. It is likely to bind to positively charged substances such as clay particles, though binding of the chemical to organic matter is unlikely (1).

The notifier expects that the notified chemical will have a high degree of dissociation in water. The notified chemical contains sulfonic acid functionalities that will be expected to remain completely dissociated under typical environmental conditions.

The notified chemical is highly surface active at a concentration of approximately 1 g/L. By definition, a chemical has surface activity when the surface tension is less than 60 mN/m (2).

4. PURITY OF THE CHEMICAL

Purity: 58.9% (lower-58%, upper-70%)

Toxic or Hazardous Components:

Chemical name: methanol

CAS No.: 67-56-1

Weight percentage: 1.2%

Toxic properties: toxic by inhalation and if swallowed (3)

listed on the *List of Designated Hazardous Substances* with the lowest cut-off concentration of 3.0% (3)

Worksafe Australia exposure standard:

time weighted average (TWA) 262 mg/m^3; short term exposure limit (STEL) 328 mg/m^3 (4)

Non-Hazardous Impurities (> 1%):

Name	*CAS Number*	*% Weight*
N-methyltaurin	-	2.3%
Emulsifier	-	1.0%
sodium chloride	7647-14-5	13.9%
water	7732-18-5	4.4%

Additives/Adjuvants: None.

5. USE, VOLUME AND FORMULATION

The notified chemical is to be used as a textile dye for cellulosic fabrics. It will be imported in 25 kg polyethylene-lined cardboard boxes at a rate less than 1 000 kg per year for the first five years. It will be imported into Australia as a granulated formulation; this formulation contains the notified chemical at a concentration of 28% and is known as Direct Blue FC 57087. The product may be repacked into smaller plastic containers at the notifier's warehouse.

6. OCCUPATIONAL EXPOSURE

The imported formulation will be imported in boxes that will either be distributed to the notifier's customers or repackaged. Repackaging will be infrequent, accounting for less than 5% of the chemical. This will be undertaken at the notifier's premises and will be undertaken by a storeman.

Use of the dye will entail removal of the dye from the containers by scoop. It is then weighed into a mixing tank and dissolved by mechanical stirring. The dye will be added at a concentration of 1% based on the weight of fabric to be dyed. The dye solution is pumped into a closed dye bath. The notifier indicates a fixation rate of 85%, resulting in a high dilution rate in waste water from the dyeing procedure. Some dye baths will have exhaust ventilation others will not. There will be some manual handling and therefore potential exposure to the dye solution on textiles in the dyebath. Up to 5 dyehouses will use the dye containing the notified chemical with one storeman and one operator in each potentially exposed to the notified chemical. Potential exposure will be for periods of approximately one hour during weighing and for unspecified periods during other operations such as removal of dyed textiles from dyebaths.

Occupational exposure is most likely to occur during weighing of the granulated formulation and during handling of textiles from the dyebath. As the formulation is granulated actual exposure during weighing will be reduced. The likely exposure pathway during this process is inhalational and possible eye contact from any mobilised dust. The most significant exposure pathway when handling the dyed textile will be dermal.

7. PUBLIC EXPOSURE

8. ENVIRONMENTAL EXPOSURE

Release

The bulk of the dye will become chemically fixed to the cellulosic textiles, and in this state is not expected to impact on the environment. After application to fabrics, the dye becomes chemical bound to the substrate. The notifier claims that the

The major environmental exposure to dye will come from effluent discharge from dyehouses and waste water treatment systems. Other releases will be limited to traces remaining from repacking operations and clean-up of any spills, and from trace residues in empty packaging. Empty cardboard boxes will be crushed and sent to landfill. Empty plastic pails may be retained for general use by the dyehouse, or sent to landfill. The notifier claims that the packages should be effectively empty when they are disposed of.

All clean up of spills and disposal of empty packaging should be carried out according to the Material Safety Data Sheet (MSDS).

Fate

The dye normally released in water as effluent from the dyehouse is expected to be the major environmental exposure. The dye may either partition to sediment or stay in the aqueous compartment. Any dye that binds to the sludge during the waste treatment process would be disposed of through incineration or landfill. Incineration is the preferred option because of the potential high mobility of the material. Incineration of the dye will produce oxides of carbon, nitrogen and sulfur, together with sodium/lithium salts in the ash and a small amount of hydrogen chloride. Disposal by landfill should be at a secured site so the risk of leaching to the water table is significantly reduced. Residues that persist after sewage treatment will enter aquatic environments in solution.

The dye was found to be not readily biodegradable (expressed as percentage elimination of dissolved organic carbon, biodegradation amounted to 12% at the end of the 28-day exposure to micro-organisms from a domestic sewage treatment plant) in the Modified OECD Screening Test for ready biodegradability (EEC 79/831 C4-B). No inhibition on the activity of the bacteria was observed in this test, which is consistent with the findings of the Activated Sludge - Respiration Inhibition Test (see Environmental Effects Section below). The dye's inherent biodegradability was not measured.

Although the dye is not readily biodegradable, the potential for bioaccumulation is low due to the expected low partition coefficient (log K_{OW} < -4.0) and high "water solubility" of the substance. Also, biological membranes are not permeable to chemicals of very large molecular size. Therefore, bioaccumulation of the notified polymer is not expected (5,6).

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

Summary of the acute toxicity of Direct Blue FC 57087

Test	*Species*	*Outcome*	*Reference*
acute oral toxicity	rat	LD_{50} > 2 000 mg/kg	7
acute dermal toxicity	rat	LD_{50} > 2 000 mg/kg	8
skin irritation	rabbit	not an irritant	9
eye irritation	rabbit	slight irritant	10
skin sensitisation	guinea pig	not a sensitiser	11

9.1.1 Oral Toxicity (7)

Species/strain:	rat/Wistar
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	by oral gavage; vehicle: 2% carboxymethylcellulose
Clinical observations:	blue discolouration of the feces
Mortality:	none
Morphological findings:	none
Test method:	OECD Guidelines for Testing of Chemicals (12)
LD_{50}:	> 2 000 mg/kg
Result:	the notified chemical was of low oral toxicity in rats

9.1.2 Dermal Toxicity (8)

Species/strain:	rat/Wistar
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	notified chemical in 2%

	carboxymethylcellulose under occlusive dressing for 24 hours
Clinical observations:	a slight inflammation at the application in all animals until forth day of observation
Mortality:	none
Morphological findings:	none
Test method:	OECD Guidelines for Testing of Chemicals (12)
LD_{50}:	> 2 000 mg/kg
Result:	the notified chemical was of low acute dermal toxicity in rats

9.1.3 Skin Irritation (9)

Species/strain:	rabbit/New Zealand White
Number/sex of animals:	3 males
Observation period:	72 hours
Method of administration:	0.5 g of the notified chemical, moistened with water, was applied under occlusive dressing for 4 hours
Test method:	OECD Guidelines for Testing of Chemicals(12)
Comments:	evaluation of erythema of the skin was not possible in two animals due to the intense colouration of the test substance; no other inflammatory signs (eschar and oedema formation) were observed within the observation period of seven days;
Result:	the notified chemical was not a skin irritant in rabbits; no erythema or oedema were observed at 1, 24, 48 or 72 hours post-treatment in the third animal

9.1.4 Eye Irritation (10)

Species/strain:	rabbit/New Zealand white
Number/sex of animals:	3 males

Observation period:	72 hours
Method of administration:	0.1 g of the notified chemical was placed in the conjunctival sac of the left eye of each rabbit
Test method:	OECD Guidelines for Testing of Chemicals (12)
Comments:	one hour after the application the evaluation of the eyes and conjunctivae was only partly possible due to the colouration of the test substance; in two animals slight inflammatory reactions of the mucous membranes were observed which proved to be reversible within 48 and 72 hours respectively
Result:	the notified chemical was a slight eye irritant in rabbits

9.1.6 Skin Sensitisation (11)

Species/strain:	guinea pig/Dunkin-Hartley
Number of animals:	10/sex (test group), 5/sex (control group)
Induction procedure:	three pairs of intradermal injections (0.1 mL) in the scapular region: - Freund's complete adjuvant (FCA), 1:1 with tap water - the notified chemical, diluted to 5% with tap water - the notified chemical at 5%, emulsified in a 1:1 mixture of FCA and tap water; one week after the injections, the same region was treated with 20% notified chemical in vaseline under occlusive dressing for approximately 48 hours
Challenge procedure:	two weeks after the topical induction, challenge the left flank of each animal was treated with 20% notified chemical under occlusive dressing for 24 hours; no re-challenge was performed

Challenge (and re-challenge) outcome:

	Test animals		Control animals	
Challenge/ rechallenge concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
25%	0/20**	0/20	0/10	0/10

* time after patch removal
** number of animals exhibiting positive response

Test method: OECD Guidelines for Testing of Chemicals (12)

Result: the notified chemical was not a skin sensitiser in guinea pigs

9.2 Repeated Dose Toxicity (13)

Species/strain: rat/Wistar

Number/sex of animals: 30/sex(5/sex/dose group)

Method of administration: gavage; the vehicle was demineralised water

Dose/Study duration:: the test substance was administered daily for a period of 30 days:

control: 0 mg/kg/day
low dose: 60 mg/kg/day
mid dose 250 mg/kg/day
high dose: 1 000 mg/kg/day

all animals were sacrificed at the end of the treatment period

the vehicle was administered daily for a period of 30 days

control 0 mg/kg/day
high dose 1 000 mg/kg/day

Clinical observations: none

Clinical chemistry/Haematology no treatment-related changes

Histopathology: no treatment-related changes except low grade inflammatory infiltrations of the submucosa of the stomach in males at 1 000 mg/kg dose

Test method:	OECD Guidelines for Testing of Chemicals (12)
Result:	no specific organ toxicity was noted in rabbits when the notified chemical was administered orally at 1 000 mg/kg/day for 28 days; no indications of systemic toxicity were noted in the clinical laboratory investigations

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* Reverse Mutation Assay (14)

Strains:	TA 98, TA 100, TA 1535, TA 1537 and
Concentration range:	16 - 5 000 μg/plate
Test method:	OECD Guidelines for Testing of Chemicals (12)
Result:	the notified chemical was not mutagenic in *S. typhimurium* in the presence or absence of metabolic activation provided by rat liver S9 fraction

9.3.2 Micronucleus Assay in the Bone Marrow Cells of the Mouse (15)

Species/strain:	mouse/NMRI KFM
Number and sex of animals:	5/sex/treatment group
Doses:	10 mg/ kg
Method of administration:	by gavage - vehicle: distilled water
Test method:	OECD Guidelines for Testing of Chemicals (10)
Result:	no significant increase in the frequency of micronucleated polychromatic erythrocytes was detected at 16, 24 or 48 hours post-treatment

9.4 Overall Assessment of Toxicological Data

The notified chemical has a low oral and dermal toxicity in rats with respective LD_{50} values in excess of 2 000 mg/kg. It was not a skin irritant but a slight eye irritant in rabbits.

In a guinea pig skin sensitisation study there was no evidence of sensitisation.

In a 28-day feeding study in rats there was no statistically significant treatment related effects or organ toxicity apart from a low grade inflammatory effects observed in the submucosa in males at a dose rate of 1 000 mg/kg/day. No mutagenicity was observed in bacteria and no clastogenicity was observed in the bone marrow cells of the mouse.

On the basis of the submitted data, the notified chemical would not be classified as hazardous according to Worksafe Australia's Approved Criteria for Classifying Hazardous Substances (Approved Criteria) in relation acute lethal effects (skin, eye), irritant effects (skin, eye), sensitising effects (skin) or severe effects after repeated or prolonged exposure (oral route).

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

Ecotoxicological data is not required for chemicals with import volumes less than 1 tonne per year according to the Act. However, the notifier supplied following ecotoxicity studies (Table 1). The tests were performed in compliance with OECD/EEC Test Methods and according to OECD Principles of Good Laboratory Practices.

Table 1: **Ecotoxicity test results**

Test	*Species*	*Results (Measured)*
Acute Toxicity (Semi-Static Test) (EEC Method C1)	Zebra Fish (*Brachydanio rerio*)	96 h NOEC $\geq$ 60.3 mg/L
Acute Toxicity - Immobilisation Test (Static Test) (EEC Method C2)	Water Flea (*Daphnia magna*)	48 h NOEC = 47.6 mg/L
Growth Inhibition - Growth (μ) & Biomass (b) (OECD TG 201)	Green Algae (*Scenedesmus subspicatus*)	72 h E_bC_{50} = 15 mg/L 72 h $E_\mu C_{50}$ = 39 mg/L
Respiration Inhibition (OECD 209)	Activated Sludge - Aerobic Waste Water Bacteria	30 min EC_{50} > 9380 mg/L

Test media were strongly coloured by the test substance. Therefore, the signs and symptoms (the response) of the fish during testing could not be determined. However, based on the NOEC value, it is likely that the notified chemical is practically non-toxic to the zebra fish. An EC_{50} value could not be determined for the water flea. However, based on the determined NOEC, it is possible that the notified chemical is slightly to practically non-toxic to the water flea.

The notifier claims on the basis of further investigations, *ie* a modified algal growth inhibition test for dyestuffs, the toxicity may be exclusively attributed to the light

absorption in the coloured test solution (algistatic effects). However, the modified test report was not supplied as a part of the notification package.

Environment Australia notes that since the test solution is intensely coloured deleterious effects can be caused by the interception of light (shading effect) necessary for algal growth. However, it should be noted that for environmental purposes, growth inhibition, whether due to chemical or physical factors, is still of relevance. Algistatic effects may still lead to an undesirable environmental impact if exposure is continuous. Therefore, based on the determined EC_{50} values for algae, the notified chemical can be classified as slightly, bordering on moderately toxic, taking into account the known insensitivity of the algal species (5).

The notified chemical showed practically no toxic effects to the respiration rate of aerobic waste water bacteria in the respiration test, with a 30 min EC_{50} greater than 9380 mg/L. Increased toxicity may be observed with increased time.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The environmental hazard from the dye, when fixed to the cellulosic fibre, is rated as negligible. The notifier has specified that a limited number of dyehouses (five) will be using the notified dye, thus the environmental hazard has been determined for both a metropolitan and country based dyehouses. The Predicted Environmental Concentration (PEC) is estimated below in Table 2.

These calculations assume that no dye is removed in treatment of the different waste effluents and represent the worst case scenario for dyehouses, *ie* sewage treatment plants provide the lowest dilution. The typical use of dye per day amount was supplied by the notifier and is claimed to be the expected maximum useable for any one day.

Table 2: **Predicted Environmental Concentrations (PEC)**

Calculation Factor	*City Dyehouse*	*Country Dyehouse*
Maximum use of dyestuff expected per day	10 kg	10 kg
Weight of Active Lost - fixation rate 85%	1.5 kg	1.5 kg
Total dyehouse effluent	4 ML/day	2 ML/day
Influent concentration	0.375 mg/L[1]	0.75 mg/L
Dilution factor in sewage treatment plant	1:100	1:2
Conc. balance in effluent from sewage treatment plant	3.75 μg/L	0.375 mg/L
Dilution factor in receiving waters	1:10 (ocean)	1:2 (river)
PEC in receiving waters	0.375 μg/L (0.38 ppb)	0.1875 mg/L (0.19 ppm)
Safety factor for exposure to most sensitive aquatic organism, Algae (72 h E_bC_{50} 15 mg/L)	40000	80

It has been assumed in the calculations that no removal of the dye would take place during the wastewater treatment process. However, some of the dye may be removed due to the adsorption of the dye to the organic sludge and possible complexation of the dye (1). Therefore, the actual concentration in receiving waters is likely to be even lower than that calculated. In any event, once the dyestuff is released to the waterways, dilution would be expected to swiftly reduce the environmental concentration.
These calculations show that the exposure to fish, daphnia and algae is at levels unlikely to cause any significant effect. Dye concentrations less than 1 ppm can give rise to intensely coloured effluent that is unacceptable to waste water authorities (6,7). Therefore, at higher release rates, there is still unlikely to be any significant effect on algae.

The only other source of environmental contamination is from accidental spills and disposal of packaging. The MSDS is adequate to limit the environmental exposure and therefore limit the environmental effects.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

The notified chemical has a molecular weight greater than 1 000 and high enough to significantly limit transmission across biological membranes. In addition the water solubility and low fat solubility and resultant low log partition coefficient indicate a reduced potential for bioaccumulation. The notifier indicates that the notified chemical has a negligible vapour pressure. The chemical will be imported as a 28% component of a granulated formulation. This will result in a lower level of dust and therefore a reduced potential for inhalational exposure when handling the formulation.

The notified chemical contains the impurity methanol, this has an atmospheric exposure standard specified in Worksafe Australia's Exposure Standards for Atmospheric Contaminants in the Occupational Environment () of TWA 200 ppm (262 mg/m^3) and STEL 250 ppm (328 mg/m^3). However, the level of methanol is below the level where the formulation would require a hazardous classification according to Worksafe Australia's *List of Designated Hazardous Substance* ().

The notified chemical has a low oral and dermal toxicity when tested with rats. A repeat dose study indicated no statistically significant treatment related effects and organ toxicity at a dose level of 1 000 mg/kg/day. The chemical was not a skin irritant but was a slight irritant to rabbit eye. It was not a skin sensitiser in a study using guinea pigs.

Occupational exposure will be greatest during handling of the dye formulation and dyed textiles in the dye baths. Dermal exposure during handling of wet textiles is possible as is inhalational exposure during weighing and addition of the formulation to the mixing tank. The latter will be limited by the granulated form of the formulation. The toxicological profile and mode of use of the notified chemical indicate that significant risks through occupational exposure to the notified chemical are unlikely. The potential for eye irritation is the most significant concern, the use of suitable eye protection during handling of the dye formulation and where there is the potential for splashing from the dye bath will reduce this.

13. RECOMMENDATIONS

To minimise occupational exposure to Direct Blue FC 57087 the following guidelines and precautions should be observed:

- Safety goggles should be selected and fitted in accordance with Australian Standard (AS) 1336 (21) to comply with Australian/New Zealand Standard (AS/NZS) 1337 (22);
- Industrial clothing should conform to the specifications detailed in AS 2919 (23);
- Impermeable gloves or mittens should conform to AS 2161 (24);

- All occupational footwear should conform to AS/NZS 2210 (25);
- Spillage of the notified chemical should be avoided, spillages should be cleaned up promptly with absorbents which should then be put into containers for disposal;
- Good personal hygiene should be practised to minimise the potential for ingestion;
- A copy of the MSDS should be easily accessible to employees.

14. MATERIAL SAFETY DATA SHEET

The MSDS for the imported formulation containing the notified chemical was provided in accordance with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (26).

This MSDS was provided by the applicant as part of the notification statement. It is reproduced here as a matter of public record. The accuracy of this information remains the responsibility of the applicant.

15. REQUIREMENTS FOR SECONDARY NOTIFICATION

Under the Act, secondary notification of the notified chemical 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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2. European Economic Community (EEC), EEC Directive 92/69, Annex V, Part A*, Methods for the determination of physico-chemical properties*, A.5 "Surface Tension" EEC Publication No. L383. December 1992.
3. National Occupational Health and Safety Commission 1994, *List of Designated Hazardous Substances* [NOHSC:10005(1994)], Government Publishing Service Publ., Canberra.
4. Australian National Occupational Health and Safety Commission 1995, 'Adopted National Exposure Standards for Atmospheric Contaminants in the Occupational Environment', [NOHSC:1003(1995)], in *Exposure Standards for Atmospheric Contaminants in the Occupational Environment: Guidance Note and National Exposure Standards*, Australian Government Publishing Service Publ., Canberra.

5. Anliker R, Moser P & Poppinger D (1988). "Bioaccumulation of dyestuffs and organic pigments in fish. Relationships to hydrophobicity and steric factors". *Chemosphere* 17(8): 1631-1644.

6. Gobas FAPC, Opperhuizen A & Hutzinger O (1986). "Bioconcentration of hydrophobic chemicals in fish: relationship with membrane permeation". *Environmental Toxicology and Chemistry* 5: 637-646.

7. Ullmann, L. 1983. *Acute oral toxicity study with Blue N-RM 2114 in rats*. Report No. 016672, Research and Consulting Company Ltd, Itingen, Switzerland.

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9. Ullmann, L. 1983. *Primary skin irritation following a single 4-hour occlusive application with Blue N-RM 2114 in rabbits*. Report No. 016683, Research and Consulting Company Ltd, Itingen, Switzerland.

10. Ullmann, L. 1983. *Primary eye irritation after single application with Blue N-RM 2114 in the rabbit*. Report No. 025244, Research and Consulting Company Ltd, Itingen, Switzerland.

11. Ullmann, L. 1983. *Test for delayed hypersensitivity in the albino guinea pig with Blue N-RM 2114*. Report No. 016661, Research and Consulting Company Ltd, Itingen, Switzerland.

12. Organisation for Economic Co-operation and Development, *OECD Guidelines for Testing of Chemicals*, OECD, Paris.

13. Ullmann, L. 1983. *28-day dermal toxicity study with Blue N-RM 2114 in rabbits*. Report No. 016514, Research and Consulting Company Ltd, Itingen, Switzerland.

14. Guenard, J. 1984. *Salmonella/mammalian-microsome mutagenicity test with Blue N-RM 2114*. Report No. 016637, Research and Consulting Company Ltd, Itingen, Switzerland.

15. Guenard, J. 1983. *Mouse micronucleus assay with Blue N-RM 2114*. Report No. 016648, Research and Consulting Company Ltd, Itingen, Switzerland.

Attachment 1

The Draize Scale for evaluation of skin reactions is as follows:

Erythema Formation	*Rating*	*Oedema Formation*	*Rating*
No erythema	0	No oedema	0
Very slight erythema (barely perceptible)	1	Very slight oedema (barely perceptible)	1
Well-defined erythema	2	Slight oedema (edges of area well-defined by definite raising	2
Moderate to severe erythema	3	Moderate oedema (raised approx. 1 mm)	3
Severe erythema (beet redness)	4	Severe oedema (raised more than 1 mm and extending beyond area of exposure)	4

The Draize scale for evaluation of eye reactions is as follows:

CORNEA

Opacity	*Rating*	*Area of Cornea involved*	*Rating*
No opacity	0 none	25% or less (not zero)	1
Diffuse area, details of iris clearly visible	1 slight	25% to 50%	2
Easily visible translucent areas, details of iris slightly obscure	2 mild	50% to 75%	3
Opalescent areas, no details of iris visible, size of pupil barely discernible	3 moderate	Greater than 75%	4
Opaque, iris invisible	4 severe		

CONJUNCTIVAE

Redness	*Rating*	*Chemosis*	*Rating*	*Discharge*	*Rating*
Vessels normal	0 none	No swelling	0 none	No discharge	0 none
Vessels definitely injected above normal	1 slight	Any swelling above normal	1 slight	Any amount different from normal	1 slight
More diffuse, deeper crimson red with individual vessels not easily discernible	2 mod.	Obvious swelling with partial eversion of lids	2 mild	Discharge with moistening of lids and adjacent hairs	2 mod.
Diffuse beefy red	3 severe	Swelling with lids half-closed	3 mod.	Discharge with moistening of lids and hairs and considerable area around eye	3 severe
		Swelling with lids half-closed to completely closed	4 severe		

IRIS

Values	*Rating*
Normal	0 none
Folds above normal, congestion, swelling, circumcorneal injection, iris reacts to light	1 slight
No reaction to light, haemorrhage, gross destruction	2 severe