File No: EX/30 (NA/755

September 2001

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

1H-pyrazole-1-ethanol, 4,5-diamino-, sulfate (1:1) salt

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For enquiries please contact the Administration Section at:

Street Address: 92 -94 Parramatta Rd CAMPERDOWN NSW 2050, AUSTRALIA
Postal Address: GPO Box 58, SYDNEY NSW 2001, AUSTRALIA
Telephone: (61) (02) 9577 9514 FAX (61) (02) 9577 9465

Director
Chemicals Notification and Assessment

September 2001

EX 30 (NA/755)

FULL PUBLIC REPORT

1H-Pyrazole-1-ethanol, 4,5-diamino-, sulfate (1:1) salt

1. APPLICANT

First Applicant

Cosmetic Products (Wella) Pty Ltd of 1 Wella Way, SOMERSBY NSW has submitted a limited notification statement in support of their application for an assessment certificate for 1H-Pyrazole-1-ethanol, 4,5-diamino-, sulfate (1:1) salt.

The Assessment Report for 1H-Pyrazole-1-ethanol, 4,5-diamino-, sulfate (1:1) salt is identified by the sequence number NA/755.

Second Applicant

Since granting of the above mentioned Assessment Certificate, Schwarzkopf Pty Ltd (ABN: 21 000 076 782) of 20 Rodborough Road, Frenchs Forest NSW 2086 has submitted a notification statement in support of their application for an extension of the Assessment Certificate for 1H-Pyrazole-1-ethanol, 4,5-diamino-, sulfate (1:1) salt.

Cosmetic Products (Wella) Pty Ltd has agreed to this extension.

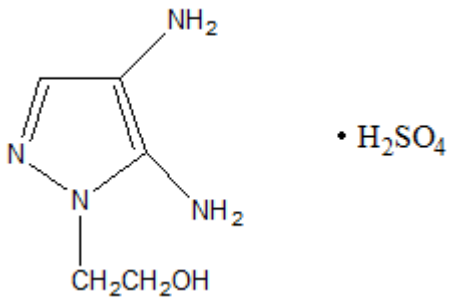
The new information in this current application supplied by Schwarzkopf Pty Ltd affects primarily the occupational exposure, public exposure and environmental sections of NA/755. The chemical will be imported as a final product in 50mL heavy wall glass containers. The concentration of the notified chemical in the hair dye products will be 0.9%. There is a decrease in the import volume of the notified chemical in the application for an extension of the original certificate.

2. IDENTITY OF THE CHEMICAL

The notifier did not request the identity of the chemical or other related information to be exempted from publication in the Full Public Report and the Summary Report.

Chemical Name: 1H-Pyrazole-1-ethanol, 4,5-diamino-, sulfate (1:1) salt

Chemical Abstracts Service (CAS) Registry No.: 155601-30-2

Other Names:	4,5-Diamino-1-(2-hydroxyethyl)-1H-pyrazole sulfate; 1-Hydroxy 4,5-diamino pyrazole sulfate
Marketing Name:	*Cosmetic Products (Wella) Pty Ltd* Pyrazole DHE Dye Concentrate (0-80%) *Schwarzkopf Pty Ltd* NAPRO LIVE Permanent Colour
Molecular Formula:	$C_5H_{10}N_4O.H_2O_4S$
Structural Formula:	
Molecular Weight:	240.23
Method of Detection and Determination:	Infrared (IR) spectroscopy
Spectral Data:	IR absorbance peaks were observed at: 3 100, 2 640, 1 670, 1 635, 1 350, 1 300, 1 110, 980, 880, 750, 710 and 630 cm^{-1}

Comments on Chemical Identity

The notifier has provided an IR spectrum for the identification of the chemical.

3. PHYSICAL AND CHEMICAL PROPERTIES

All of the physicochemical properties listed below were measured using the notified chemical.

Appearance at 20°C and 101.3 kPa:	White to pink powder
Melting Point:	174.7°C
Density:	1.87 kg/m^3 at 20°C
Vapour Pressure:	3 x 10^{-9} kPa at 25°C
Water Solubility:	666 g/L at 25°C

Particle Size:	Not measured
Partition Co-efficient (n-octanol/water):	Log P_{ow} = -1.75 $\pm$ 0.01
Hydrolysis as a Function of pH:	Not applicable (see comments below)
Adsorption/Desorption:	Log K_{oc} = 1.29; estimated
Dissociation Constant:	Not applicable (see comments below)
Flash Point:	Not applicable
Flammability Limits:	Not flammable
Autoignition Temperature:	No self-ignition <400°C
Explosive Properties:	Not explosive
Reactivity/Stability:	Not reactive

Comments on Physico-Chemical Properties

Tests were performed according to EEC/OECD test guidelines at facilities complying with OECD Principles of Good Laboratory Practice. Test reports for melting point, specific gravity, vapour pressure, water solubility, partition coefficient and adsorption/were provided by the notifier.

Melting point was determined via the 92/69/EEC, A.1 guideline, capillary method. This test entailed the filling of capillary tubes to the desired height, placing them into a heating block then incrementally increasing the temperature. Six replicates were done. The melting point was found to be 174.7 ± 0.2ºC. DSC-scan was also done to determine if the chemical decomposed or boiled. It was found that exothermal decomposition began at approximately 200ºC.

The specific gravity/relative density was determined using the 92/69/EEC, A.3 guideline, relative density, pycnometer method. A sample of the chemical was weighed into a pre-weighed pycnometer. Once the pycnometer had reached 20ºC it was reweighed. The relative density of the chemical was then calculated via the difference in weight of the pycnometer with and without sample and decane, and weight of decane. This test was repeated three times.

The vapour pressure was determined using the 92/69/EEC, A.4 guideline. In this method the vapour pressure is determined over a range of temperatures. A known amount of sample is placed in a cell and degassed in a vacuum overnight at room temperature. As the cell is heated, the amount of material leaving the cell via known size apertures is determined under vacuum at required time intervals. The vapour pressure was initially measured at 25.2ºC. It was determined that at 25ºC the vapour pressure was very low at approximately $3x10^{-8}$ hPa

($3x10^{-9}$ kPa).

The water solubility was determined using the 92/69/EEC, A.6 guideline. A preliminary test then a flask test were conducted. The measurement was done at the desired test temperature without any preliminary heating. The flasks were stirred to bring the contents to equilibrium. The maximum concentration was reached soon after the equilibrium process began. With a water solubility of 666 g/L, this chemical is highly soluble in water.

The determination of hydrolysis is not applicable, as the chemical does not have any hydrolysable groups.

The partition coefficient was determined using the 92/69/EEC, A.8 guideline. The computer model KOWWIN was used to obtain a preliminary estimation of the partition coefficient, whereupon a laboratory test was set-up with duplicates of three test concentrations. Each vial of solution was mixed by overhead-rotation for 30 minutes at 25°C, after which it was allowed stand until each phase was clear. The calculated log P_{ow} for the chemical was –1.75±0.01 which indicates that the chemical is hydrophilic.

A HPLC test method was used to estimate the adsorption/desorption of the chemical. The elution time of the test solution was compared to the elution time of reference substances. The log K_{oc} was estimated to be 1.29.

It was claimed by the notifier that there are no dissociable groups. However, there may be some dissociation of the fully ionised amino groups at lower pH.

4. PURITY OF THE CHEMICAL

Degree of Purity: 99.5% (range 99-100%)

Hazardous Impurities: None known

Non-hazardous Impurities (> 1% by weight): None known

Additives/Adjuvants: None

5. USE, VOLUME AND FORMULATION

Cosmetic Products (Wella) Pty Ltd
The notified chemical will not be manufactured in Australia, but will be imported in pre-weighed vacuum-sealed plastic bags as a component of dye mixture in powder form. The bags consist of two layers of polyethylene and are laminated with aluminium. Each bag represents an individual batch and an individual color shade when incorporated into the finished product. In some shades (or bags) the notified chemical comprises up to 80% by weight of the dye mixture, whereas in others (blonde shades) the notified chemical concentration is zero.

Approximately 500 kg/year will be imported over five years.

The notified chemical will be used in the formulation of hair dye products in the range from

0% (blonde shades) to a maximum of 4.5% (dark shades). The concentration of the notified chemical in the dye mixture depends on the colour being formulated as it produces a reddish-brown colouration when dissolved and subsequently oxidised with color developer at time of use.

Variations to this assessment specific to the Schwarzkopf Pty Ltd extension

In the application for extension, the notified chemical is used as a component of hair dye products. It will be imported in a final product in 50mL heavy wall glass containers. It is intended for use initially for the consumer market but it may also be used later in products designed for use in professional hair salons. The concentration of the notified chemical in the imported product is 0.9%.

Approximately 200 kg per year will be imported over the next five years.

6. OCCUPATIONAL EXPOSURE

Transport and storage: 2-3 hours/day, 10 days/year
The imported dye mixture bags containing the notified chemical are transported in sealed plastic drums of 100-120 kg capacity. Drums will be transported from the dockside by road directly to the notifier's site, where they will be stored in a chemical warehouse prior to being used at the same site. Waterside workers, transport drivers, warehouse and retail workers would only be exposed to the notified chemical in the event of a spill from a transport or handling incident. The nature of the packaging used for transport minimises the likelihood of accidental release or loss of the chemical. Three to six waterside workers and transport drivers and 2-3 warehouse workers will be involved in transport and storage operations.

Reformulation

Plant operators: 8 hours/day, 150 days/year
The notified chemical will be formulated into viscous liquid or cream hair-colouring end product. The dye is formulated as follows. Bags will be cut open next to the mixing vessel and the contents directly poured manually into blending vessels of 100 kg capacity filled with hot water. The vessel is closed immediately and mixing and dissolution of the dye would take place. The solution would then be pumped to the main blending vessel (250 kg) where the dissolved dyes would be incorporated with other ingredients. This will then be pumped via an automatic filling line to a multi-head filling machine for packaging into tubes (60 g) or bottles (50 mL).

Worker exposure to dust may occur as the bags are opened and the powdered chemical is poured into the mixing vessel and during disposal of empty used bags. Skin contact, eye contact and inhalation are the main routes of exposure to the dusty product. The particle size of the dye mixture in powder form was not provided by the notifier, however, the Material Safety Data Sheet (MSDS) implies that dust may be generated during handling of the powder.

Dermal exposure to the notified chemical at 0-4.5% in solution may occur as the operators connect/disconnect containers to transfer lines, during clean up operations and maintenance of equipment. Inhalation exposure to the mixed dye is considered negligible because the

chemical is not volatile, and any aerosol formation during mixing would be controlled through the use of enclosed systems. In addition, as the chemical is formulated into a viscous liquid or cream product, aerosol generation is expected to be low.

The notifier stated that formulation takes place in a bunded area and that operators are required to wear impervious gloves, coveralls, respirators with P3 filter (as recommended in the MSDS) and eye protection during connection and disconnection of containers to transfer lines and during cleaning and maintenance of equipment. Also, general and local exhaust ventilation is in place at all points of transfer of materials between mixing vessels and filling stations.

Overall, exposure to the notified chemical is controlled through the use of engineering controls such as enclosed lines and vessels and local exhaust ventilation and the provision and wearing of personal protective equipment.

Quality and control staff: 4 hours/day, 150 days/year
Sampling and quality control (QC) testing of the raw material and the final formulations will be conducted. Equipment used in these procedures includes sampling and testing equipment for spectroscopy and determination of physicochemical properties such as pH and viscosity. Exposure to the notified chemical may occur via inhalation of dust and aerosols, or by skin and/or eye contact, but is expected to be low given the small quantities handled, the engineering control measures in place and the use of personal protective equipment such as laboratory coats and safety glasses. Approximately 6-12 plant operators will be involved in formulation and QC sampling and testing operations.

End Use

The finished viscous liquid formula (in bottles) is intended as a single application treatment and is aimed at home user market. The cream product (in tubes) is intended to extend to several applications and is for the hair salon market. Both products will require dilution (50:50) with a developer prior to use. At hair salons the product will be further diluted and mixed with other dyes.

Hair salons: one hour/day, 200 days/year
The notifier indicated that the cream dye formulation will be sold to > 1000 hair salons around Australia. Hairdressing staff will empty the tube or bottle, mix with other dye formulations and apply the product containing the notified chemical to hair. Following application, the dye material will be washed from hair. Staff will be exposed to the notified chemical during preparation and application of the dyeing material. Skin and eye contact are the most likely routes of exposure to the notified chemical. The product label states that impermeable gloves should be used.

Variations to this assessment specific to the Schwarzkopf Pty Ltd extension
The notifier has indicated that the chemical may be used in products designed for professional hair salons. Typically, protective gloves (provided in the pack) would be worn when mixing and using.

7. PUBLIC EXPOSURE

The notifier estimated that the dye viscous liquid product will be sold to > 10 000 retail customers around Australia. The public will be exposed to the notified chemical at up to 4.5% in hair dye products at a maximum usage rate of approximately 50 mL hair dye, once a month. Individuals at home will follow the instructions provided by the supplier. Also, it is indicated that the product packaging will contain disposable gloves. Given that impermeable gloves will be used when handling the product and the low concentration (≤ 4.5%) of the notified chemical in the hair dye products, exposure is expected to be very low.

Variation to this assessment specific to the Schwarzkopf Pty Ltd extension
The public will be exposed to the notified chemical at up to 0.9% in hair dye products. Exposure will be limited by the intermittent nature of application (once every 4 weeks) and the commercial success of the hair dye. Exposure will primarily occur via the dermal route, with the possibility of accidental ocular and oral exposure. It is expected that during transport and storage, exposure of the general public to the notified chemical will be minimal, except in the event of an accidental spill.

8. ENVIRONMENTAL EXPOSURE

Release

Reformulation Site
The residue in the individual import bags is estimated to be 0.1% of the contents. If each bag holds 5 kg, 0.005 kg of dye will remain in the bag after emptying. Assuming the concentration of the notified chemical is 80%, the amount of notified chemical in the bag residue is 4 g, which equates to a maximum of 500 g annually. The bags are not washed. A licensed waste disposal contractor disposes of the bags, with residue, and containers. It is assumed that these will be taken to landfill.

The notifier has indicated that there will be 150 batches made in a year, ie 3 per week. It is estimated that for each batch there will be approximately 2 kg of product left in the process equipment. All equipment washwater is sent to the on-site treatment plant. The maximum concentration in the final product is 4.5%; therefore the maximum amount of notified chemical reaching the wastewater treatment plant per batch is 0.09 kg (90 g). This means that annually up to 13.5 kg of notified chemical will enter the on-site wastewater treatment plant from process equipment cleaning. The treatment plant consists of a 100 000 L averaging tank, a solids separator, a grease remover, automatic pH adjustment and a dissolved air flotation tank. The treated effluent, which is likely to contain the waste notified chemical, then enters the sewer.

All process areas are bunded so that any spill will be contained and sent to the on-site wastewater treatment plant. The notifier has not indicated how much material will be lost via this route. Since the imported material is contained in small packages, a 1% loss due to spills is assumed. Annually, this equates to 5 kg of imported material, or a maximum of 4 kg of notified chemical (the maximum concentration of notified chemical in the imported powder is 80%). It should be noted that the concentration of notified chemical in the bags would range from 0 to 80%. So the estimated maximum 4 kg is conservative, and the actual amount is likely to be much less.

User Sites
Once the dye has been applied to hair and allowed to develop, the dye solution is rinsed from

the hair into the sewer. The majority of the dye may be bound to the hair. However, as the notifier has not provided an indication of the percentage uptake of the dye by the hair, it has been assumed that all the dye (containing the notified chemical) will end up in the sewer following rinsing. When a 50 mL bottle is used (generally at home) 2.25 mL (approx 2.25 g) of notified chemical will enter the sewer. For a 60 g tube (salon use) 2.7 g of notified chemical enters the sewer.

The notifier has not indicated how much of the product will be left in the user's container after emptying. This is estimated to be 2%; ie in a 50 mL bottle 0.045 g and in a 60 g tube 0.054 g of notified chemical will remain in the container. These containers will then be disposed of with the general garbage and end up in a landfill.

Fate

During reformulation, a summary of estimated maximum annual amount of waste notified chemical is:

spills, 1%	4 kg
bag residue, 0.1%	0.5 kg
washwater	13.5 kg
TOTAL	19.0 kg

All of this waste is unreacted chemical. However, only the bag residue goes to landfill while the rest is disposed of via sewer.

During end-use mixing of the notified chemical with the other dye components, a reaction will take place during which the chemical will be consumed in the formation of the hair dye. Once applied and allowed to cure for up to 30 minutes, presumably the majority of the dye will become bound to the hair.

The notifier has provided the results of a biodegradation test in an aerobic aqueous media. The biodegradation was determined by the measurement of CO_2 produced after 2 mg/L of the notified chemical inoculated in a culture medium was stored in the dark at 21°C for 28 days. The results indicated that 33.3% of the chemical had degraded over this time, and therefore it is not readily biodegradable.

With a log P_{ow} of –1.75, it is unlikely that the chemical will bioaccumulate.

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

Summary of the acute toxicity of Pyrazole DHE; also referred to as DA 010894.

Test	*Species*	*Outcome*	*Reference*

Acute oral toxicity	Rat	LD_{50} > 2 000 mg/kg	(Klein 1996a)
Skin irritation	rabbit	Slight irritant	(Klein 1996b)
Skin irritation	Human	Non-irritating	(Articus 1998)
Eye irritation (undiluted powder)	rabbit	Severe irritant	(Klein 1996c)
Eye irritation (5% solution in water)	rabbit	Non-irritant	(Ott 1996)
Skin sensitisation (maximisation test)	Guinea pig	Extreme sensitiser	(Kocsis and Bornatowicz 1995a)
Skin sensitisation (Büehler test)	Guinea pig	Non-sensitising	(Kocsis and Bornatowicz 1995b)

9.1.1 Oral Toxicity (Klein 1996a)

Species/strain:	Rat/Sprague-Dawley (Him:OFA)
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	10 mL/ kg bw administered by gavage; test substance was dissolved in deionised water.
Test method:	OECD TG 401; limit test
Mortality:	None
Clinical observations:	No adverse effect on bodyweight gain during the course of the study was noted; all animals had orange coloured urine within one day following administration of the test substance. This was reportedly caused by renally excreted test substance.
Morphological findings:	All females were normal at necropsy; one male had enlarged mesenteric lymph nodes and a grey-white covering on the spleen capsule. The report indicated that these signs are known to occur spontaneously in the strain of rats used.
Comment:	A preliminary range finding study using 200 mg and 2 000 mg/kg bw was conducted using four animals (2/sex). Animals dosed with 2 000 mg/kg bw in the preliminary study were included in the main study. No toxic effects of the test substance were noted during the course of the preliminary/main studies and at post mortem.
LD_{50}:	> 2 000 mg/kg

Result: the notified chemical was of very low acute oral toxicity in rats

9.1.4 Skin Irritation in rabbits (Klein 1996b)

Species/strain: Rabbit/new Zealand White

Number/sex of animals: 3 females

Observation period: 3 days

Method of administration: 0.5 g of test substance moistened with 0.5 mL deionised water applied to an area (~ 6 cm^2) of shorn intact skin (median on the dorsal thoracal region) and held under semi-occlusive dressing. After 4 hours, the dressing was removed and residual test substance was removed with wet cellulose tissue.

Test method: OECD TG 404

Draize scores (Draize 1959):

Skin reaction/ Animal				
	Observation Time (hours)			
Erythema	***1***	***24***	***48***	***72***
1	1 [a]	1	1	0
2	1	1	0	0
3	1	1	0	0
Oedema				
1	0	1	0	0
2	0	1	0	0
3	0	1	0	0

[a] see Attachment 1 for Draize scales

Comment: No general toxic signs were seen in any of the animals. All areas treated with the test substance were normal before application. The control areas were normal at each observation time.

Result: The notified chemical was a slight irritant to the skin of rabbits.

9.1.4 Skin Irritation in humans (Articus 1998)

Species/strain: Human

Number/sex of animals: 18 male/33 female

Observation period: 48 hours

Method of administration:	20 μL of test substance applied to the back of volunteers for 24 hours under semi-occlusive conditions using an epicutaneous patch test system.
Test method:	Cosmetic product test guidelines for the assessment of human skin compatibility (COLIPA, 1995).
Comment:	Positive control (4% SDS) and vehicle control (water) were also included in the patch test study. First visual evaluation was performed 15 min after test substance removal, followed by another evaluation 48 hours later. At 24 and 48 hours, skin reactions in all 51 volunteers were scored as "0" (no apparent cutaneous involvement). Orange-red staining was observed at the test site in two volunteers, which interfered with the evaluation of skin reactions.
Result:	The notified chemical was non-irritating to the skin of humans.

9.1.5.1 Eye Irritation with neat notified test material (Klein 1996c)

Species/strain:	Rabbit/New Zealand white
Number/sex of animals:	3 females
Observation period:	21 days
Method of administration:	Approximately 0.1 mL (95-100 mg) of test substance was applied to the conjunctival sac of the right eye; the left eye served as a control.
Test method:	OECD TG 405

Draize scores (Draize 1959) of un-irrigated eyes:

	Time after instillation													
Animal	***1 day***		***2 days***		***3 days***		***6 days***		***8 days***		***10 days***		***21 days***	
Cornea	***o***	***a***	***o***	***a***	***o***	***a***	***o***	***a***	***o***	***a***	***o***	***a***	***o***	***a***
1	1[1]	4	2	4	1	4	1	1	0	0	0	0	0	0
2	1	4	1	4	1	4	1	3	1	2	1	1	1	0
3	1	4	2	4	2	4	1	3	1	2	0	0	0	0
Iris														

1	1		1		1		0		0		0		0	
2	1		1		1		1		0		0		0	
3	1		1		1		1		1		0		0	

Conjunctiva	***r***	***c***	***r***	***c***	***r***	***c***	***r***	***c***	***r***	***c***	***r***	***c***	***r***	***c***
1	3	2	3	2	3	1	2	0	1	0	0	0	0	0
2	3	3	3	3	3	3	2	2	2	2	2	1	2	2
3	2	2	3	3	2	2	2	2	1	1	1	1	0	0

[1] see Attachment 1 for Draize scales
o = opacity a = area r = redness c = chemosis

Mean scores (24, 48, 72 hours observation):

Animal	***Corneal opacity***	***Iridial inflammation***	***Conjunctival redness***	***Conjunctival chemosis***
1	1.3	1	3	1.7
2	1	1	3	3
3	1.7	1	2.7	2.3

Comment: All animals had irritation to eyes according to all parameters. In one animal, redness and chemosis persisted over 21 days.

Result: The notified chemical was severely irritating to the eyes of rabbits. There was a risk of serious eye damage due to the persistence of conjunctival redness and chemosis in one animal at 21 days.

9.1.5.2 Eye Irritation with 5% notified test material in water (Ott 1996)

Species/strain: Rabbit/New Zealand white

Number/sex of animals: 3 females

Observation period: 3 days

Method of administration: 0.1 mL of a 5% solution of test substance in water was applied to the conjunctival sac of the right eye; the left eye served as a control.

Test method: EC Guideline 92/69, method B.5.

Draize scores (Draize 1959) of un-irrigated eyes:

	Time after instillation		
Animal	***1 day***	***2 days***	***3 days***
Cornea	***o***	***o***	***o***

1	0		0		0	
2	0		0		0	
3	0		0		0	
Iris						
1	0		0		0	
2	0		0		0	
3	0		0		0	
Conjunctiva	***r***	***c***	***r***	***c***	***r***	***c***
1	0	0	0	0	0	0
2	1	0	1	0	0	0
3	0	0	0	0	0	0

[1] see Attachment 1 for Draize scales
o = opacity a = area r = redness c = chemosis

Comment: No general toxic effects were observed following administration of the test substance.

Result: The notified chemical, when applied as a 5% solution in water, was non-irritating to the eyes of rabbits.

9.1.6.1 Skin Sensitisation – Magnusson and Kligman Maximisation Test (Kocsis and Bornatowicz 1995a)

Species/strain: Hartley guinea pig/Crl:HA)BR

Number of animals: 10 test females, 5 control females

Test method: EC-guideline 92/69, B.6.

Pre-test

Intradermal (i.d.) 0.1 mL of 0.01, 0.1, 1 and 10% w/v dilutions of the test substance in physiological saline were injected intradermally within an area of approx. 2 cm x 4 cm in the interscapular region;

Epicutaneous application filter paper covered with the test substance in white petrolatum at 0.5, 2.5, 10 and 40% w/v dilutions were applied to the area of intradermal injections under occlusive dressing for 24 hours.

Results of pretest:

i.d. induction:	Very slight to severe erythema and/or oedema was observed in 3/3 animals after 24 and/or 48 hours following application of the test substance at 10%; 2/3 animals had very slight to well defined erythema at 1%. No skin reactions were observed in any animals at 0.1% and 0.01%. Accordingly, the concentration of the test substance selected for *i.d.* induction in the main study was 1% w/v;
epicutaneous exposure:	Pink staining was observed at the test sites, which interfered with assessment of skin reactions. Thus, histopathological examination was conducted. No adverse skin reactions were observed at any of the concentrations used for epicutaneous application in all three animals tested. Accordingly, 40% w/v was selected for epicutaneous induction and challenge exposure in the main study.
Main study	
test group: day 0	three pairs of intradermal injections (0.1 mL) in the interscapular region: 1- Freund's complete adjuvant (FCA), 1+1 (v/v) in physiological saline; 2- the test substance, diluted to 1% w/v in physiological saline; 3- the test substance diluted to 1% w/v in physiological saline emulsified in 1+1 (v/v) FCA.
day 6	pre-treatment with a formulation of SDS, 10% (w/w) (~ 0.6 g/animal) in white petrolatum.
day 7	filter paper covered with the test substance 40% (w/v) in white petrolatum (~ 0.6 g of test substance and 0.5 g of white petrolatum) was applied to the treated area and held under occlusive dressing for 48 hours.
control group:	treated similarly to test animals omitting the test substance from the intradermal injections and epicutaneous application.
Skin reactions following, Intradermal (i.d.) and Epicutaneous Induction:	
i.d. induction:	Local irritation was observed at the sites treated with FCA

(1 and 3) in all animals on the day following injections. Local erythema became more severe and led to ulceration, which did not heal until the end of the study.

Test substance injection site (2) revealed very slight to severe erythema in 9/10 animals, 24 hours following induction exposure. Control group animals revealed no irritation at this site.

epicutaneous induction: Skin ulceration obscured the reading of reactions following epicutaneous induction. All animals had severe erythema, oedema and eschars (score 3), which were attributed to FCA.

Challenge procedure:

day 21 filter paper covered with the test substance 40% (w/v) in white petrolatum (~ 0.6 g test substance and 0.5 g vehicle/animal), or with white petrolatum only, was applied to sites on the left and right flank respectively, and held under occlusive dressing; after 24 hours the test sites were cleaned and examined.

Challenge outcome:

	Test animals		***Control animals***	
Challenge concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
40%	10**/10	10/10	0/5	0/5

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

Comments: All animals survived till the end of study. No abnormal behaviour or clinical signs were detected.

The test substance-treated sites were stained orange-red, which obscured the scoring of erythema. Visual and histopathological examinations were combined to assess skin reactions.

No positive skin reactions were observed in the control animals, except for eschars at the edges of the test substance treated site in one animal.

Visually, 1/10 test group animals revealed well-defined erythema at the vehicle application site, whereas very slight to severe erythema and/or oedema were observed in 10/10 animals of the test group.

Histopathologically, test group animals had numerous

lesions, including hyperkeratosis, parakeratosis, vesicle formation, acanthosis, spongiosis, pustule formation, inflammation, oedema, vascular dilatation, and lymphohisitocytic infiltration. No skin reactions were observed in negative control animals.

Result: The test material was extremely sensitising to the skin of guinea pigs.

9.1.6.2 Skin Sensitisation – Büehler test (Kocsis and Bornatowicz 1995b)

Species/strain: Hartley guinea pig/Crl:HA)BR

Number of animals: 20 test substance females; 10 negative control females; 10 positive control females.

Dose preparation Test substance: 40% (w/w) formulation was prepared with white petrolatum for induction and challenge. About 0.6 g/animal of test substance was applied.

Positive control: 10% (w/w) 1,4-phenylenediamine was prepared with white petrolatum for induction, and 2% (w/w) for challenge; 0.6-0.7 g/animal and 0.6 g/animal, respectively.

filter paper covered with the test substance, positive control or vehicle was applied as follows:

test group:
day 0 Epicutaneous induction: test substance administered to the left flanks of animals for 6 hours under occlusive dressing. Procedure was repeated twice, on days 7 and 14.

day 28 Challenge: test substance was applied to the posterior right flanks of animals and the vehicle to the anterior right flanks for 6 hours as above. Dressing was removed and sites cleaned and examined for skin reactions

The positive and vehicle controls were applied in the same manner.

Test method: OECD TG 406; EC-Guideline 92/96 B.6.

Challenge outcome, test substance:

	Test animals		***Control animals***	
Challenge concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***

40%	0**/20	0/20	0/10	-

- * time after patch removal
- ** number of animals exhibiting positive response
- - not examined

Challenge outcome, positive control:

	Test animals		***Control animals***	
Challenge concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
2%	5**/5	5/5	0/5	-

- * time after patch removal
- ** number of animals exhibiting positive response
- - not examined

Comment: After the second induction, the test substance produced very slight erythema in 3/20 animals; after the third induction very slight erythema was seen in 4/20 animals. No adverse skin reactions were seen in any of the controls.

After challenge, no animal had a "positive skin reaction" with either test material or negative control.

The positive control group performed as expected.

Result: The notified chemical was non-sensitising to the skin of guinea pigs.

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* Reverse Mutation Assay (Faller 1994)

Strains: TA 98, TA 100, TA 1535, TA 1537, TA 1538

Concentration range: Two independent experiments were conducted at the following concentrations:

Experiment 1:
1.0, 10, 100, 1 000, 5 000 μg/plate; with/without S9 mix

Experiment 2:
30, 100, 300, 1 000, 3 000 μg/plate; with/without S9 mix

Positive controls:
Sodium azide (1 μg/plate), TA1535 and TA100;
9-aminoacridine (50 μg/plate), TA1537;
2-nitrofluorene (10 μg/plate), TA1538 and (3 μg/plate) TA98;
without S9-mix;

	and 2-aminoanthracene (2.5 μg/plate), TA1535, TA1537 and TA1538; 2-aminofluorene (1 μg/plate) TA98, and (10 μg/plate) TA100; with S9-mix
Metabolic activation:	liver fraction (S9 mix) from rats induced with Aroclor 1254.
Test method:	OECD TG 471; EEC-Directive 79/831, Annex V, Part B, B14- plate incorporation method.
Comment:	No evidence of mutagenicity was observed at any concentration of the test substance. There were some growth-inhibiting effects with TA 1537 and TA 98 in the absence of metabolic activation (- S9) and TA 100 in the presence of metabolic activation (+ S9), at the highest concentrations (3000/5000 μg/plate). All positive controls used in the study confirmed the sensitivity of the strains and the efficacy of the S9-mix. Colony counts in the vehicle controls were within historical limits.
Result:	Under the experimental conditions reported, the test substance was not considered mutagenic to the bacterial strains tested in the presence or absence of metabolic activation.

9.3.2 Micronucleus Assay in the Bone Marrow Cells of the Mouse (King 1995a)

Species/strain:	Mouse/NMRI
Number and sex of animals:	5/sex 24 hours exposure: vehicle control and test substance dose groups; 5/sex 48 hours exposure: positive control and test substance groups.
Doses:	A dose volume of 20 mL/kg bw at the following concentrations: test substance- 500, 1 000, 2 000 mg/kg bw; vehicle control- 4% gum arabic; positive control- 9,10-dimethyl-1,2-benzanthracene dissolved in olive oil - 50 mg/kg bw.
Method of administration:	Oral (gavage)
Test method:	Not stated

Comment:	After dosing, animals in all groups treated with test substance showed reduced motility in a dose-related manner. The ratio of polychromatic to normochromatic erythrocytes was decreased in the male test groups of the highest dosage. The test substance did not induce any increase in the frequency of micronucleated polychromatic erythrocytes (MN) above the negative control level. Both negative and positive control frequencies of MN were within laboratory limits. There was no evidence of bone marrow cytotoxicity up to the highest dose of test material.
Result:	The test substance was considered non-genotoxic in the *in vivo* mouse micronucleus assay.

9.3.3. Chromosome aberration assay in human peripheral blood lymphocytes *in vitro* (King 1995b)

Cells:	Human Peripheral Lymphocytes
Metabolic activation system:	liver fraction (S9 mix) from rats pretreated with Aroclor 1254
Dosing schedule:	The test substance was prepared fresh for each assay. Duplicate cultures were used to test each concentration, with or without metabolic activation (S9), in two independent experiments. <u>without S9</u>, 10, 300, 1 000 µg/mL; treatment/harvest time: 24 hours; Positive control: Mitomycin C, 0.5 µg/mL, dissolved in Ham's F-10 medium. <u>with S9</u>: 300, 1 000, 3 000 µg/mL; treatment/harvest time: 3/24 hours, Positive control: Cyclophosphamide, 25 µg/mL, dissolved in sterile distilled water. The vehicle control was Ham's F-10 medium used to dissolve the test substance.
Test method:	Not stated
Comment:	At the highest concentration, the test substance resulted in ~ 70% and 20% cytotoxicity in the absence and presence of

S9 mix, respectively.

The test substance induced a slight but statistically significant increase in the number of aberrant metaphases in the absence of S9 mix compared with solvent controls. No increased rates of aberrations were observed in the presence of S9 mix.

All solvent control cultures had frequencies of chromosome aberrations within the expected range. Positive controls induced statistically significant increases in the incidence of aberrant cells and the activity of the S9 fraction was found to be satisfactory.

Result: The test substance was considered clastogenic under the conditions of the chromosomal aberration assay only in the absence of S9.

9.4 Overall Assessment of Toxicological Data

The notified chemical was of very low acute oral toxicity in rats with oral LD_{50} >2000 mg/kg bw. The notified chemical was a slight skin irritant in rabbits but non-irritant in human volunteers. In its pure form, the notified chemical was a severe irritant to the eyes of the rabbit with potential risk of serious eye damage, but a non-irritant when applied as a 5% aqueous solution. The notified chemical was extremely sensitising to the skin of guinea pigs in the Maximisation test but non-sensitising in the Büehler test.

The notified chemical was considered non-mutagenic to the bacterial strains tested and non-genotoxic in an *in vivo* mouse micronucleus assay. However, it was considered to be clastogenic *in vitro* in a chromosomal aberration assay only in the absence of metabolic activation provided by S9 mix.

The notified chemical is classified as a hazardous substance according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (National Occupational Health and Safety Commission 1999b) based on the findings of the persistent conjunctival effects in an eye irritation study, and the potential for skin sensitisation observed in an adjuvant type test. The overall classification is Irritant (Xi) and the risk phrase R41- Risk of Serious Damage to Eyes and R43- May Cause Sensitisation by Skin Contact, are assigned.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

The notifier provided the following aquatic toxicity results. The tests were carried out according to OECD Test Methods.

Species	*Test*	*Concentrations[a] (mg/L)*	*Result (mg/L)*
Zebrafish (*Brachydanio rerio*)	96 hours acute	0, 100 and 130	LC50 > 130
Water Flea (*Daphnia magna*)	48 hours acute	0, 10, 18, 32, 56 and 100	EC50 =11.12
Algae (*Selenastrum capricornutum*)	72 hours growth inhibition	1.0, 1.8, 3.2, 5.6 and 10	E_rC_{50} = 10.0 E_bC_{50} = 5.33 NOEC[b] = 1.8

[a] nominal concentration

[b] NOEC - no observable effect concentration

Zebrafish *(Brachydanio rerio)* were used in a 96 hour semi-static acute toxicity study for the notified chemical. The study was set up using seven fish per 5 L beaker of test solution. The nominal concentrations of notified chemical were 0, 100 and 130 mg/L. Due to a drop in the nominal concentration (a 20% drop in the concentration after 24 hours was observed), the test solution was changed every 24 hours, thus becoming a semi-static study. Observations were made at the start of the experiment then at every 24 hours period. The observations included mortality, visible abnormalities (eg appearance and behaviour), oxygen, temperature and pH. No visible abnormalities or mortalities were observed over the period of the study at any concentration. Therefore, the LC_{50} was determined to be higher than the nominal concentration of 130 mg/L. This indicates that the notified chemical is practically non-toxic to fish.

Daphnia was used in a 48 hour semi-static acute toxicity study for the notified chemical. The study was set up using 20 animals per concentration distributed into 4 groups of 5 animals in glass beakers. The nominal concentrations of notified chemical were 0, 10, 18, 32, 56 and 100 mg/L. Observations were made at the start of the experiment then at every 24 hours period. The observations included immobility, oxygen, temperature and pH. Due to a drop in the nominal concentration (there was a 20% drop in the concentration after 24 hours), the concentration of the solution in each beaker was checked every 24 hours. At the end of study the concentrations ranged from 6.1 to 56.5 mg/L (approximately a 40% drop).

No immobilisation was observed in concentrations below 6.1 mg/L, while 100% immobilisation was observed in concentrations above 56.5 mg/L. The calculated EC_{50} at 24 hours was 30.0 mg/L and at 48 hours was 11.1 mg/L. These results indicate that the chemical is slightly toxic to daphnia but as toxicity increases sharply between 24 and 48 hours, it could easily be moderately toxic if exposed for longer as a plateau has not been reached.

Algae (*Selenastrum capricornutum*) was used in a 72 hours growth inhibition study for the notified chemical. The study was set up using glass flasks with initial algae cell concentration of 10^4 cells/mL, with cell counts being done every 24 hours. The nominal concentrations of notified chemical were 0, 1, 1.8, 3.2, 5.6 and 10 mg/L. The pH was checked at the start and finish of the study. The modified Probit method set up by the Flemish Institute for Technology Research (VITO) was used to calculate the E_bC_{50}, E_rC_{50} and statistical limits. The calculated values are given below:

	24 hours	48 hours	72 hours
E_bC_{50} (mg/L)	2.04	2.43	5.33
E_rC_{50} (mg/L)	4.23	>10	>10
NOEC (B) (mg/L)	1.8	<1	<1
NOEC (R) (mg/L)	3.2	1	1.8

The large decrease in algal toxicity observed at 48–72 hours may be due to the degradation of the test solution and algae subsequently recovering. However there were similar dramatic increases in cell count in the control. This may indicate a natural process occurring in regards to food availability or natural by-product production. These results indicate that the chemical is moderately toxic to algae.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The notified chemical is expected to pose a low environmental hazard if used as specified by the notifier.

The annual amount of waste notified chemical produced during the reformulation is 19.0 kg. The majority of this waste will enter the sewer. The remainder will likely be disposed of to landfill (ie in bags/containers), where is likely to leach out. The empty end-use containers will generally go to landfill, equating to about 10 kg of unreacted notified chemical waste annually. All these inputs into the environment are likely to be at very low concentrations and in a very diffuse manner.

As the notifier has not provided the percentage uptake of the dye by the hair it has been presumed that all the dye (containing the notified chemical) will end up in the sewer. The use of the hair dye would be dispersed over Australia, so a PEC for the notified chemical could be calculated as follows:

Amount of notified chemical imported per year, subsequently entering sewer	500 kg
Population of Australia	18 million
Amount of water used per person per day	150 L
Number of days in a year	365
Estimated PEC	0.00005 mg/L (0.05 ppb)

The scenario where a whole formulation batch is dropped and subsequently enters the sewer would represent a worst case PEC. The resultant PEC is:

Quantity of notified chemical entering the on-site treatment plant	4.5 kg
Volume of water handled by the treatment plant	40 000 L
Sewer concentration	112.5 mg/L
Amount of effluent handled daily by MTP	250 ML
Dilution in receiving water	1:10
Worst case daily PEC	0.045 mg/L (0.045 ppm)

The ecotoxicity studies indicated that the most sensitive species to the notified chemical is algae (E_bC_{50} = 5.33 mg/L). All of the PECs calculated are several orders of magnitude below the calculated E_bC_{50} level for algae, therefore the use as proposed poses a low hazard.

Variation to this assessment specific to the Schwarzkopf Pty Ltd extension
The following calculations assume that the new chemical will be used nationwide and that it is released to the sewer system. It is also assumed that 150 L of sewerage are generated each day by each person.

Import rate	200kg/annum
Release rate	200kg/annum
Population (national)	19×10^6
Volume of sewerage per annum	1×10^{12} L/annum
Mean concentration of sewerage	0.2 μg L^{-1} (0.2ppb)

On release to receiving waters (after treatment at the sewage treatment plant), it is usually assumed that the effluent is diluted by a factor of 10. This gives a final PEC in receiving of 0.02 μg L^{-1} (0.02 ppb).

However, it should be noted that no removal through other mechanisms has been considered in the above calculations. Biodegradation of the chemical or adsorption to sediment will lower the above PEC estimates to the extent these processes occur.

Conclusion
The notified chemical is not likely to present a hazard to the environment when it is stored, transported and used in the proposed manner.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

The notified chemical was of very low acute oral toxicity in rats. It was a slight skin irritant in rabbits but non-irritant in human volunteers. In its pure form, the notified chemical caused severe damage to the eyes of the rabbit, but was non-irritant when applied as a 5% aqueous solution. The notified chemical was extremely sensitising to the skin of guinea pigs in the Maximisation test but non-sensitising in the Büehler test.

The notified chemical was considered non-mutagenic to the bacterial strains tested and non-genotoxic in an *in vivo* mouse micronucleus assay. However, it was clastogenic *in vitro* in a chromosomal aberration assay only in the absence of metabolic activation.

The notified chemical is classified as a hazardous substance according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (National Occupational Health and Safety Commission 1999b) based on the findings of the persistent conjunctival effects in an eye irritation study, and the potential for skin sensitisation observed in an adjuvant type test. The overall classification is Irritant (Xi) and the risk phrase R41- Risk of Serious Damage to Eyes and R43- May Cause Sensitisation by Skin Contact, are assigned.

The imported product and formulated end-use products will require the appropriate risk phrases under hazardous substances regulations.

Occupational Health and Safety

Transport and Storage

Exposure to the notified chemical is not expected during transport or storage as long as the packaging remains intact. Exposure after a spill would be controlled by use of the recommended practices for spillage clean up given in the MSDS supplied by the notifier. The risk of adverse health effects for transport and storage workers is considered low.

Formulation
The greatest potential for exposure to the notified chemical is during opening, emptying and mixing of the powdered chemical and during disposal of empty used bags. There exists potential for exposure by inhalation and/or skin and eye contact with dust particles with the associated health effects of skin sensitisation and eye irritation. The notified chemical comprises up to 80% of the imported dye powder for dark dye shades. At concentrations of $\geq$ 1% chemical, Pyrazole DHE is a hazardous substance. There may also be potential for genotoxic effects as revealed in a chromosome aberration study. Given the low molecular weight (240) of the notified chemical, significant absorption through the skin cannot be excluded.

Given this risk of adverse health effects during this stage of the formulation process, local exhaust ventilation and dust extraction need to be maintained over mixing areas to capture dust and aerosols at source, and minimise exposure to airborne particulates generated from the notified chemical and any other ingredients. The wearing of an air purifying dust respirator (with P3 particulate filter) and other protective equipment throughout the formulation process, such as impervious gloves, overalls and eye protection, is needed. The NOHSC exposure standard for inspirable dust will need to be adhered to in the workplace.

Exposure to the chemical at 0-4.5% may occur after dilution, when mixing with other hair dye ingredients, during connection/disconnection of containers to transfer lines and during cleaning and maintenance of equipment. Inhalation exposure is not expected as any aerosols would be within enclosed automated operation systems. Skin and/or eye contact will be the main routes of exposure. As the notified chemical is hazardous at $\geq$ 1%, a risk of skin sensitisation, and possibly eye irritation, exists during formulation and packaging, particularly with the darker shades which contain the higher concentrations. The prompt clean up of spills and the wearing of impervious gloves, overalls and chemical splash goggles are needed to reduce these risks when handling the dye solutions.

Exposure to dusts and aerosols may also occur during laboratory testing, however, given the smaller quantities handled, the potential for skin sensitisation and eye irritancy is reduced. Local exhaust ventilation and the routine wearing of laboratory coats, impervious gloves and safety glasses would be expected to further reduce these risks.

Measures should also be implemented in the disposal of the notified chemical to ensure that exposure is avoided.

End use
Workers in hairdressing salons could handle this chemical on a frequent basis. The product concentration of 0-4.5% is diluted in use, to a maximum of 0-2.25%. At 2.25%, the chemical is still a hazardous substance (skin sensitiser) and hairdressers will need to wear gloves when making up, applying and rinsing off this chemical.

Public Health

The public will be exposed to the notified chemical at up to 4.5% in hair dye products. At this concentration, it is expected that the products will not cause skin or eye irritation, but may cause skin sensitisation in certain susceptible individuals. Exposure of individuals, in the at home use, to the notified chemical should be controlled by following the instructions supplied on product labels. Exposure is expected to be low and for short periods only; thus, it is considered that Pyrazole DHE will not pose a significant hazard to public health when used in the proposed manner.

Variations to this assessment specific to the Schwarzkopf Pty Ltd extension
Public exposure to hair dye products containing the notified chemical is likely to be intermittent (based on the use pattern), and widespread (sold to the public and limited only by the commercial success of the products). When used, the colour gel containing the notified chemical (≤1.0%) will be diluted 4:5 with developer, leading to maximal exposure concentrations of ≤0.5%. At these concentrations, it is expected that the products will not cause skin or eye irritation, but may cause skin sensitisation in certain susceptible individuals. Use of hair dye products strictly in accordance with the product information, should minimise the potential skin sensitisation hazard. There will be minimal public exposure from transport and storage.

13. RECOMMENDATIONS

1. Pyrazole DHE is a skin sensitiser; workers handling it or products containing it will need to be strictly protected against skin contact. To minimise occupational exposure to Pyrazole DHE the following guidelines and precautions should be observed:

- Safety goggles should be selected and fitted in accordance with Australian Standard (AS) 1336 (Standards Australia 1994) to comply with Australian/New Zealand Standard (AS/NZS) 1337 (Standards Australia/Standards New Zealand 1992); industrial clothing should conform to the specifications detailed in AS 2919 (Standards Australia 1987) and AS 3765.1 (Standards Australia 1990); impermeable gloves or mittens should conform to AS 2161.2 (Standards Australia/Standards New Zealand 1998); all occupational footwear should conform to AS/NZS 2210 (Standards Australia/Standards New Zealand 1994); respiratory protection should conform to AS/NZS 1715 (Standards Australia/Standards New Zealand 1994), and AS 1716 (Standards Australia/Standards New Zealand 1994);

- Local exhaust ventilation should conform to AS 1668.2(Standards Australia 1994);

- Dust levels in the workplace should be maintained below the NOHSC exposure standard for nuisance dusts, 10 mg/m^3 (TWA) measured as inspirable fraction (National Occupational Health and Safety Commission 1995). Employers are responsible for ensuring the exposure standard is not exceeded;

- Spillage of the notified chemical should be avoided. Spillages should be cleaned up promptly and put into containers for disposal;

- Good personal hygiene should be practised to minimise the potential for ingestion; and

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

2. If the conditions of use are varied, then greater exposure to the public may occur. In such circumstances, further information may be required to assess the hazards to public health.

3. The notified chemical may be recommended to the National Occupational Health and Safety Commission for consideration for inclusion in the NOHSC List of Designated Hazardous Substances.

14. MATERIAL SAFETY DATA SHEET

The MSDS for the notified chemical was provided in a format consistent with the *National Code of Practice for the Preparation of Material Safety Data Sheets* (National Occupational Health and Safety Commission 1994).

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 may be required if any of the circumstances stipulated under section 64 of the Act arise. Secondary notification will be required if the method of use changes in such a way as to greatly increase the environmental exposure of the notified chemical, particularly to natural waters, or if additional information becomes available on adverse environmental effects of the chemical.

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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