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January 2002

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

Substance S176939

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Director

Chemicals Notification and Assessment

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1. APPLICANT

Toxikos Pty Ltd of 293 Waverly Road MALVERN EAST VIC 3145 (ABN 30 095 051 791) has submitted a limited notification statement in support of their application for an assessment certificate for Substance S176939.

2. IDENTITY OF THE CHEMICAL

The chemical name, CAS number, molecular and structural formulae, molecular weight, spectral data and composition of the chemical have been exempted from publication in the Full Public Report and the Summary Report.

3. PHYSICAL AND CHEMICAL PROPERTIES

The physico-chemical parameters were determined in accordance with OECD Principles of Good Laboratory Practice, EC Directives, and UK Good Laboratory Practice Regulations Test Guidelines.

Appearance at 20°C & 101.3 kPa:	Yellow powder
Melting Point:	>320°C
Specific Gravity:	1.58
Vapour Pressure:	Not determined. The notified chemical is unlikely to have a significant vapour pressure based on its chemical structure.
Water Solubility:	1600 mg/L at 25°C
Partition Co-efficient (n-octanol/water):	log P_{ow} = 1.5 at 38°C
Hydrolysis as a Function of pH:	Hydrolysis at pH 4.0, 7.0 and 9.0 = <10% after 5 days at 50°C.
Adsorption/Desorption:	Not determined (see comments below).

Dissociation Constant:	Not determined (see comments below).
Particle Size:	Not determined.
Flash Point:	Not applicable – substance is a solid at room temperature.
Flammability:	Non-flammable. Combustible.
Autoignition Temperature:	Does not spontaneously ignite at ambient temperature.
Explosive Properties:	Non-explosive.
Reactivity/Stability:	Stable. There was no significant active ingredient degradation, which indicates and ambient shelf life of at least 2 years.
Surface Tension:	69.8mN at 25°C

3.1 Comments on Physico-Chemical Properties

The vapour pressure of the notified chemical was not determined for this notification. However based on its structure (salt form), the notified chemical is not expected to be volatile.

Due to difficulties with obtaining clear solutions, the water solubility of the notified chemical was determined using a stirring method (Zeneca UK Ltd 1999a). The notified chemical (1 or 2 g) was added in to 50, 65 or 75 mL of distilled water. The suspensions were incubated with stirring in a water bath for either 24, 48 or 72 h at 30ºC. After these times the samples were equilibrated at 25ºC for 24 h, filtered and analysed. This method indicated the water solubility of the notified chemical is 1600 mg/L.

The partition coefficient was determined by the shake-flask method, EC Method A8 (Zeneca UK Ltd 1999a). The partition coefficient obtained indicates that notified chemical is relatively hydrophilic.

The hydrolytic stability of the notified chemical was determined using Method C7 of Commission Directive 92/69/EEC (Zeneca UK Ltd 1999a). The notified chemical has an amide linkage that may undergo hydrolysis under extreme pH conditions. However, the hydrolytic stability tests conducted indicate that the notified chemical exhibited less than 10 % hydrolysis after 5 days at 50°C.

The surface tension was determined using the method detailed in Method A3 of Commission Directive 92/69/EEC (Zeneca UK Ltd 1999a). The notified chemical is not classified as being surface active.

The notifier indicates that no adsorption/desorption tests were conducted for the notified chemical. The notified chemical is relatively water soluble, as a consequence of its anionic nature, it is expected to associate with the soil matrix and sediments and as such will be immobile in soil.

No dissociation constant tests were conducted for the notified chemical. The notified chemical contains a fully ionised sulfonate group, which is expected to remain so in the environmental pH range (4-9) due to its strong acidity.

4. PURITY OF THE CHEMICAL

Degree of Purity: 95%

Maximum Content of Residual Monomers:

Chemical Name	*CAS No.*	*Weight %*
Calcium carbonate		0.7
Water		3.7
Unidentified impurities		≤0.1

Additives/Adjuvants: None known

5. USE, VOLUME AND FORMULATION

The notified chemical will not be manufactured, formulated or repackaged in Australia. It will be imported in 100 – 200 g pre-packed sealed toner cartridges.

The notified chemical will be used as a crystal growth inhibitor in toner for laser printers or photocopiers. The formulated toner contains a maximum of 2% notified chemical.

The import volume of the notified chemical is expected to be less than 1 tonne/year for the first 5 years.

6. OCCUPATIONAL EXPOSURE

Office workers and printer maintenance workers may be intermittently exposed to the notified chemical contained in the ink cartridge when replacing the spent ink cartridge, and during repair maintenance and cleaning of printers or photocopiers. Maintenance workers for printers or photocopiers may potentially come in contact with the notified chemical more often than office workers. Exposure would be principally by skin contamination, however, inhalation exposure could also occur, particularly if spillage occurs. Particle size data were not provided, however, toners are usually fine powders, with a significant proportion of particles in the respirable fraction (less than 10 μm). Exposure is expected to be controlled through the design of the ink cartridges and the printing and photocopier machines. Printer and photocopier maintenance personnel often wear cotton disposable gloves. Pre-packed ink cartridges are sealed and worker exposure to the ink is minimised by the use of the replacement procedures recommended by the manufacturer.

Waterside, warehouse and transport workers are unlikely to be exposed to the notified chemical unless the packaging is breached.

Contact with paper printed with printing inks containing the notified chemical is unlikely to result in dermal exposure, as it will be bound in the structure of the paper.

7. PUBLIC EXPOSURE

The notified chemical will not be manufactured, formulated or repackaged in Australia. The public may be exposed to the notified chemical following a transport accident involving the breakage of cartridges and release of toner. Members of the public may experience dermal contact through spillage of toner during the changing of printing cartridges. The most likely form of contact is dermal, but eye or inhalation exposure is also possible. Such contact is likely to be infrequent and of a transient nature. The potential for public exposure to toner containing the notified chemical is minimal. During copying or printing the toner will be transferred to the paper and firmly fixed to it by heat. Contact with the notified chemical on printed paper will therefore be unlikely.

8. ENVIRONMENTAL EXPOSURE

8.1 Release

Release of the toner containing the notified chemical to the environment is not expected under normal use as the cartridge is designed to prevent leakage. However, if leakage does occur, the toner will be contained and presumably disposed of in landfill. Environmental exposure will result from the disposal of printed paper and discarded cartridges as well as the possibility of accidental leakage of the cartridges during use. Toner residues contained in the empty cartridges are expected to be about 1% of the import volume and to remain within these containers, although release could occur from deterioration of the cartridge. The total import volume of the notified chemical will ultimately be disposed of in either landfill or be incinerated or recycled with paper.

8.2 Fate

Some waste paper may be disposed of directly to landfill with the notified chemical strongly bound to the paper. It is anticipated that prolonged residence in an active landfill environment would eventually degrade the notified chemical. Incineration of waste paper will destroy the compound with the generation of water vapour and oxides of carbon.

In addition to landfill, some of the toner printed on paper will enter the paper recycling process. During such processes, waste paper is repulped using a variety of alkaline, dispersing and wetting agents, water emulsifiable organic solvents and bleaches. These agents enhance fibre separation, ink detachment from the fibres, pulp brightness and the whiteness of paper. De-inking wastes are expected to go to trade waste sewers. Trade sources estimate the washing process will recover 30-60% of the total amount of toner and therefore at least 30% of the notified chemical in the recycled paper will be disposed of with

sludge in landfill.

A biodegradation study was conducted according to OECD TG 301F – Ready Biodegradability; Manometric Respirometry (Zeneca UK Ltd 1999b). Activated sludge, obtained from Buckland Sewage Treatment Plant in Devon, was mixed with the test substance or standard material (sodium acetate). The study was carried out at 22°C. The sodium acetate standard attained 70% biodegradation after 28 days, indicating the test conditions were valid. After 28 days, the biodegradation of the test substance was determined to be less than 5% and as such was not considered to be readily biodegradable under the conditions of the test.

The notified chemical is not expected to bioaccumulate due to its moderate partition coefficient and limited release to water (Connell 1990).

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

9.1.1 Oral Toxicity (Noakes, 1999a)

Species/strain:	Rat/Alpk:AP_fSD
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	A single oral dose of 2000 mg/kg notified chemical in corn oil was given by gavage.
Test method:	OECD TG 401
Mortality:	None
Clinical observations:	Yellow stained faeces in all animals for up to 3 days and yellow stained fur and tail in male animals for up to 6 days.
Morphological findings:	No abnormality detected.
Comment:	All animals showed initial weight loss and recovered for the remainder of the study.
LD_{50}*:*	>2000 mg/kg
Result:	the notified chemical was of very low acute oral toxicity in rats

9.1.2 Skin Irritation (Noakes, 1999b)

Species/strain:	Rabbit/New Zealand White

Number/sex of animals: 3/female

Observation period: 3 days

Method of administration: A single dose of approximately 500 mg notified chemical (moistened with 0.5 mL deionised water) was applied to the left flank under occlusive dressing for 4 hours.

Test method: OECD TG 404

Comment: The application site was stained yellow by the test substance throughout the study. However, the staining did not prevent the assessment of irritation.

Very slight erythema was seen in one animal approximately one hour after application. No other signs of irritation effects were observed.

Result: the notified chemical was very slightly irritating to the skin of rabbits

9.1.3 Eye Irritation (Noakes, 1999c)

Species/strain: Rabbit/New Zealand White

Number/sex of animals: 3/female

Observation period: 3 days

Method of administration: A single dose (approximately 100 mg notified chemical) was applied into the conjunctival sac of the left eye of each animal. The eyes remained unwashed. The untreated eye served as a control.

Test method: OECD TG 405

Draize scores of unirrigated eyes:

	Time after Instillation											
Animal	***1 hour***			***24 hours***			***48 hours***			***72 hours***		
Cornea	*No corneal effects noted*											
Iris												
1	1			0			0			0		
2	0			0			0			0		
3	0			0			0			0		
Conjunctiva	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***

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

[1] see Attachment 1 for Draize scales. r = redness c = chemosis d = discharge

Comment: No staining was evident in all animals when fluorescein dye was applied to the eyes.

Yellow staining of the periorbital area from the test substance was observed in all animals throughout the study (3 days) and one animal had convoluted eyelids. The reversibility of yellow staining was not determined due to the short observation period (3 days).

All signs of irritation were resolved after 72 hours of application.

Result: the notified chemical was slightly irritating to the eyes of rabbits

9.1.4 Skin Sensitisation (Noakes, 1999d)

Species/strain: Guinea pigs/Dunkin Hartley

Number of animals: Control group: 10
Test group: 20

Induction procedure:

test group:
day 1 Intradermal Induction
Three intradermal injections (0.05-0.01 mL) on each side of the mid line:

- Freud's Complete Adjuvant (FCA) 1:1 in corn oil
- 3% w/v preparation of the test substance in corn oil
- 3% w/v preparation of the test substance in a 1:1 mixture of FCA and corn oil

day 6 Local Irritation:
Animals pre-treated with 10% w/v preparation of sodium lauryl sulphate in paraffin wax at the induction site in order to provoke a mild inflammatory response.

day 7 Topical induction

A 48-hour occluded application of 79% w/v test substance in corn oil to the test area.

control group: Treated similarly to the test animals using corn oil in intradermal injections and topical applications instead of the

notified chemical.

Challenge procedure:

day 21 — Occluded applications of patches containing 79% w/v, 50%w/v, 25% w/v and 10% w/v notified chemical in corn oil on the left and right flank of each animal for 24 hours.

Test method: OECD TG 406, Magnusson and Kligman Maximisation Test

Challenge outcome:

	Test animals		***Control animals***	
Challenge concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
10%[#]	**0/20	0/20	0/10	0/10
25%[#]	0/20	0/20	0/10	0/10
50%[##]	0/20	0/20	0/10	0/10
79%[##]	0/20	0/20	0/10	0/10

* time after patch removal
** number of animals exhibiting positive response
[#] not tested due to yellow staining
[##] restricted numbers tested due to yellow staining

Comment: The test substance stained the application site yellow of all test and control animals preventing full assessment of erythema. Therefore, histological examination was conducted on skin samples from the application sites of all animals treated with 79% and 50% w/v preparations.

Minimal to slight changes (acanthosis, inflammatory cell infiltration or parakeratosis/surface debris) on application site were observed in test animals treated with 79% and 50% w/v preparations and in control animals, indicating an irritant response. Therefore, the study did not adequately test for a sensitising effect.

Result: the notified chemical was not sensitising to the skin of guinea pigs under the conditions of the test.

9.2 Genotoxicity

***Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay (Callander, 1999)**

Strains: *Salmonella typhimurium*: TA1535, TA1537, TA98 and TA100
Escherichia coli: WP2P and WP2P *uvrA*

Metabolic activation:	Liver S9 fractions from rats pre-treated with combined phenobarbital and β-naphthoflavone in corn oil solution.
Concentration range:	0-5000 μg/plate of test substance in deionised water Each concentration was tested in triplicate, with or without metabolic activation with S9, in two independent experiments. Appropriate strain specific control reference substances were used.
Test method:	OECD TG 471 and 472
Comment:	There were no significant, reproducible increases in the number of revertant colonies in the presence and absence of metabolic activation at any test concentration. Concurrent positive controls induced marked increases in the number of revertant colonies and the activity of the S9 fraction was found to be satisfactory.
Result:	The notified chemical was non mutagenic under the conditions of the test

9.3 Overall Assessment of Toxicological Data

The notified chemical was of very low acute oral toxicity (LD50 >2000 mg/kg) in rats. It was not a skin irritant but a slight eye irritant to rabbits. Yellow staining occurred in the eye irritation study. However, its reversibility could not be determined as the observation period was limited to 3 days. Evidence of skin sensitisation potential was not observed in guinea pigs in an adjuvant study. Yellow staining from the test substance hampered the sensitisation study.

The notified chemical was not mutagenic in the bacterial strains tested.

Based on the information available, the notified chemical is not classified as a hazardous substance in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999).

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

The notifier has provided a summary of the toxicity report for the notified chemical to Daphnia magna.

Test	***Species***	***Results***

Acute toxicity	*Daphnia magna*	48 h EC_{50} = 0.77 mg/L

The immobilisation tests with *Daphnia* (Zeneca UK Ltd 1999c) were performed in quadruplicate using 5 daphnids per flask and observations were made after 24 and 48 hours. The tests were conducted at the nominal concentration of 50 mg/L which was prepared by adding an appropriate amount of the notified chemical to 1 L of dilution water. Analysis of the test solution after 48 h indicated the concentration of the notified chemical of 0.75 mg/L. The solubility of the notified chemical observed in this test is considerably lower than in distilled water. The notifier indicates that the aqueous testing medium contains ionised phosphates, nitrates and chlorides together with vitamins and trace elements, which could account for the observed differences in solubility figures. Another possibility is that the notified chemical associates with calcium ions and forms an insoluble complex. After 48 h, no immobilised daphnids were observed at any test vessel. The 48-hour EC_{50} for the notified chemical to *Daphnia magna* is greater than the notified chemical's limit of solubility.

The ecotoxicity data indicates the notified chemical is not likely to be toxic to daphnia up to the limit of its water solubility.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The notified chemical will enter environmental compartments indirectly by disposal of waste paper (for recycling, to landfill or for incineration) and by direct release from discarded printer cartridges at landfill sites. Based on the import volume, method of packaging and low concentration of the notified chemical in the toner, release of the notified chemical to the environment is expected to be low but widespread. Waste from the recycling process includes sludge which is dried and disposed of to landfill, and very little of the notified chemical is expected to partition to the supernatant water which is released to the sewer.

Abiotic or slow biotic processes would be largely responsible for the degradation of the notified chemical as it was not found to be readily biodegradable. As a consequence of its relatively low water solubility and anionic character, the notified chemical is likely to be immobilised through adsorption onto soil particles and sediments.

The notified chemical is not toxic to daphnia up to the limit of its water solubility and releases to the sewer will be low because very little of the notified chemical is expected to be released to water. Furthermore, the substance is not expected to bioaccumulate due to its moderate partition coefficient and limited release to water.

On the basis of the available information, the overall environmental hazard of the notified chemical is expected to be low.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Hazard Assessment
Based on the toxicological data provided, the notified chemical was of very low acute toxicity. It was not a skin irritant and not a skin sensitiser. It was a slight eye irritant, where

yellow staining of the eye was observed in rabbits.

The notified chemical was not mutagenic in the bacterial strains tested.

The notified chemical would not be classified as a hazardous substance according to NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999) based on the toxicological data provided.

The MSDS for a typical toner containing the notified chemical warns against contact with skin and eyes and inhalation of the fine powder.

Occupational Health and Safety
Exposure to printing inks containing the notified chemical during transport of pre-packed cartridges should not result in exposure except in the event of accidental spillage.

The notified chemical will be imported in pre-packed cartridges at a maximum of 2%. Dermal and inhalation exposure of office workers to the notified chemical will potentially occur when replacing spent cartridges and clearing paper jams from the printer or photocopier. However, the design of the cartridges is such that exposure to the notified chemical should be low.

Dermal and inhalation exposure of maintenance workers to the notified chemical is possible during routine maintenance but is expected to be low due to the low concentration of the notified chemical in the toner. Overall, the risk of adverse health effects arising from exposure to the notified chemical is low due to its expected low toxicity, low concentration in toner and low potential for exposure. Nevertheless, due to the probable fine nature of the toner, skin, eye and respiratory exposure should be avoided. The national exposure standard for nuisance dusts is 10 mg/m3 TWA [NOHSC, 1995]. Australia has no exposure standard for respirable dust, however, the ACGIH TLV of 3 mg/m3 TWA is recommended [ACGIH, 2001]. Due to their frequent exposure to inks, maintenance personnel should wear cotton or disposable gloves.

It is concluded that the health risk to workers involved in transport, storage, and disposal of the notified chemical in this application is low.

Public Health
S176939 is present at a low concentration in a toner powder which is encased in a cartridge. The potential for public contact with the toner powder is most likely limited to an infrequent and transient dermal contact during the preparation of a cartridge for use or disposal. Due to the low toxicity of S176939 and its low concentration in the toner powder, it is considered that the notified chemical will not pose a significant hazard to public health when used in the proposed manner.

13. RECOMMENDATIONS

Control Measures

Occupational Health and Safety

No special precautions are required for the notified chemical when used at low quantities in inkjet printer cartridges. However, in the interests of good occupational health and safety, the following guidelines and precautions should be observed for use of toners containing the notified chemical:

- Avoid contact with skin and eyes
- Avoid generation of dust
- Service personnel should wear cotton or disposable gloves when servicing printers.

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

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

If products and mixtures containing the notified chemical are classified as hazardous to health in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances*, workplace practices and control procedures consistent with provisions of State and Territory hazardous substances legislation must be in operation.

Secondary notification

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

(1) Under subsection 64(1) of the Act:
- if the import volume increases above 1 tonne per year, the notifier should provide soil adsorption/desorption studies for the notified chemical to clarify the chemical's mobility potential in soils.

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

The Director will then decide whether secondary notification is required.

14. MATERIAL SAFETY DATA SHEET

The MSDS for the notified chemical and toner provided by the notifier were in accordance with the NOHSC *National Code of Practice for the Preparation of Material Safety Data Sheets* (NOHSC, 1994a). They are published here as part of the assessment report. The accuracy of the information on the MSDS remains the responsibility of the applicant.

15. REFERENCES

ACGIH, (2001); The American Conference of Governmental Industrial Hygienists (ACGIH): Threshold Limit Values for Chemical Substances and Physical Agents and Biological Indices 2001; ACGIH Cincinnati, Ohio.

Callander RD (1999). Substance S176939: Bacterial Mutation Assay in S. Typhimurium and E. Coli. Central Toxicology Laboratory, Report No. CTL/P/6182, Cheshire, UK.

Connell, D.W. (1990). General Characteristics of Organic Compounds Which Exhibit Bioaccumulation. In: Bioaccumulation of Xenobiotic Compounds. CRC Press, Boca Raton, USA, pp. 47-57.

Federal Office of Road and Safety, Australian code for the Trnasport of Dangerous Goods by Road and Rail (1998). Australian Dangerous Goods Code, 6th Edition, Australian Government Publishing Service, Canberra.

National Occupational Health and Safety Commission (1994a) National Code of Practice for the Preparation of Material Safety Data Sheets [NOHSC:2011(1994)]. Australian Government Publishing Service, Canberra.

National Occupational Health and Safety Commission (1994b) National Code of Practice for the Labelling of Workplace Substances [NOHSC:2012(1994)]. Australian Government Publishing Service, Canberra.

National Occupational Health and Safety Commission (1999) Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(1999)]. Australian Government Publishing Service, Canberra.

National Occupational Health and Safety Commission (1995): Exposure Standards for Atmospheric Contaminants in the Occupational Environment, [NOHSC:1003(1995)]. Australian Government Publishing Service, Canberra.

Noakes JP (1999a). Substance S176939: Fixed Dose Acute Oral Toxicity Study in Rats. Central Toxicology Laboratory, Report No. CTL/P/6256, Cheshire, UK.

Noakes JP (1999b). Substance S176939: Skin Irritation Study in Rabbits. Central Toxicology Laboratory, Report No. CTL/P/6257, Cheshire, UK.

Noakes JP (1999c). Substance S176939: Eye Irritation Study in Rabbits. Central Toxicology Laboratory, Report No. CTL/P/6258, Cheshire, UK.

Noakes JP (1999d). Substance S176939: Skin Sensitisation Study in Guinea pigs. Central Toxicology Laboratory, Report No. CTL/P/6259, Cheshire, UK.

Zeneca UK Ltd (1999a) 9000287: S176939, Determination of Physical-Chemical Properties for Product Notification, Manchester, UK, (unpublished report submitted by Toxikos Ltd).

Zeneca UK Ltd (1999b) BL6583/B: S176939 Determination of 28 Day biodegradability; Manchester, UK, (unpublished report submitted by Toxikos Ltd).

Zeneca UK Ltd (1999c) BL6602/B: S176939 Determination of Acute Toxicity, Manchester, UK, (unpublished report submitted by Toxikos Ltd).

Attachment 1

The Draize Scale (Draize, 1959) 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 (Draize *et al*., 1944) 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

Draize, J. H., Woodward, G., Calvery, H. O. (1944) Methods for the Study of Irritation and Toxicity of Substances Applied Topically to the Skin and Mucous Membranes, J. Pharmacol. Exp. Ther. 82 : 377-390.

Draize J. H. (1959) Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. Association of Food and Drug Officials of the US, 49 : 2-56.