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November 2001

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

CR-600

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Director
Chemicals Notification and Assessment

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1. APPLICANT

Sola Optical Australia (ACN 007 719 708) of Sherriffs Road LONSDALE SA 5160 has submitted a standard notification statement in support of their application for an assessment certificate for CR-600.

2. IDENTITY OF THE CHEMICAL

The chemical name, CAS number, molecular and structural formulae, molecular weight, spectral data, impurities, details of exact use and import volume have been exempted from publication in the Full Public Report and the Summary Report.

Other Names:	OM600 Experimental Comonomer Blend Polymerizable Monomer Mix Carbonate Ester
Marketing Name:	CR-600; CR-607 (containing 73% CR-600 and 27% of another component used for the manufacture of ophthalmic lenses).
Method of Detection and Determination:	Infra-red (IR) spectroscopy, UV/Visual (UV/Vis) spectroscopy, Gel Permeation Chromatography, ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectroscopy, LC-MS.
Spectral Data:	IR, NMR, UV/Vis and LC-MS spectra was provided.

3. PHYSICAL AND CHEMICAL PROPERTIES

The notified chemical is comprised of two components identified below as “component 1” and “component 2”. All the physico-chemical tests were conducted on the standard grade chemical.

Appearance at 20°C & 101.3 kPa:	Clear, colourless, slightly viscous liquid.
Boiling Point:	Decomposed prior to boiling from approximately 190°C.

Specific Gravity:	1130 kg/m^3 at 20°C
Vapour Pressure:	Component 1 = 4.8 × 10^{-6} kPa at 25°C Component 2 = 6.9 × 10^{-8} kPa at 25°C
Water Solubility:	Component 1 = 0.839 g/L at 20°C, pH 5.1 Component 2 = 0.055 g/L at 20°C, pH 5.1
Partition Co-efficient (n-octanol/water):	Component 1: log Pow = 1.69 at 30°C Component 2: log Pow = 2.64 to > 6.2 at 30°C
Hydrolysis as a Function of pH:	Component 1: $T_{1/2}$ at pH 4.0 > 8760 hours at 25°C $T_{1/2}$ at pH 7.0 = 6792 hours at 25°C $T_{1/2}$ at pH 9.0 < 24 hours at 25°C Component 2: Assessment of hydrolytic stability was not carried out for this component as it had limited solubility in water (the test was not suitable).
Adsorption/Desorption:	Component 1: logKoc = 2.21 at 30°C. Component 2: logKoc = 2.83 to greater than 5.63 at 30°C.
Particle Size:	Not applicable for liquids.
Dissociation Constant:	Not determined
Surface tension:	54.4 mN/m at 21°C
Flash Point:	159°C (closed cup).
Flammability Limits:	Not determined.
Autoignition Temperature:	396°C
Explosive Properties:	Predicted to be non-explosive.
Reactivity/Stability:	The substance is predicted to be non-oxidising on the basis of structure.

3.1 Comments on Physico-Chemical Properties

All tests were performed by Safepharm Laboratories Ltd (2001a), including determination of vapour pressure (Safepharm Laboratories Ltd; 2001b, 2001c).

The vapour pressures of the two components were determined using a vapour pressure

balance and Method A4 of Commission Directive 92/69/EEC. Linear regression analysis was used to calculate vapour pressures at 25°C. The low values determined indicate that the notified chemical is very slightly volatile.

The water solubilities for both components were determined using the flask method detailed in Method A6 of Commission Directive 92/69/EEC. A known quantity of the notified chemical (~1.8 g) was added to glass double distilled water (400 mL), shaken for approximately 3 h at 30°C and equilibrated for at least 24 h at 20°C. The concentration of each of the test substance components was determined by gas chromatography (GC) and gel permeation chromatography (GPC). The both components of the notified chemical are classified as being moderately soluble (Mensinck, 1995).

The hydrolytic stability of component 1 of the notified chemical was determined using Method C7 of Commission Directive 92/69/EEC. The hydrolytic stability test conducted indicates that this component is slightly hydrolysing at pH 4 and 7 and very rapidly hydrolysing at pH 9 at 25°C. The hydrolytic stability of the second component of the notified chemical was not assessed due to its low solubility in water. However, it contains ester and carbonate linkages and based on the above, the latter could be expected to hydrolyse under basic conditions (> pH 9), though possibly at a slower rate.

The partition coefficients were determined using the HPLC method detailed in Method A8 of Commission Directive 92/69/EEC and the estimates provided for the adsorption coefficient were obtained by the HPLC method detailed in the OECD draft guideline. The values of log P and log Koc for component 1 are indicative of a substance which is relatively hydrophilic and mobile in soil while the values of log P and log Koc for the component 2 are indicative of a substance which is relatively hydrophobic and immobile in soil. The single values for the partition and adsorption coefficient for the component 1 of the notified chemical appear to reflect a purer compound (namely n = 1) or that all the oligomers are eluted together.

The surface tension of the notified chemical was determined using OECD TG 115. Substances having a surface tension below 60 mN/m are considered to be surface active and those with values below 40 mN/m are considered to have significant surface activity. The value obtained for surface tension for the notified chemical indicates that it is slightly surface active.

A dissociation constant was not determined for the notified chemical because neither component contains any groups which will dissociate in water.

4. PURITY OF THE CHEMICAL

Degree of Purity: > 90%

Hazardous Impurities: One hazardous impurity at a concentration of < 6%: moderate acute oral toxicity, skin irritant, experimental reproductive effects (http://www.tomescps.com)

Non-hazardous Impurities (> 1% by weight):

Chemical name:	Cross polymerisation product between components 1 & 2
Weight percentage:	5-10%
Additives/Adjuvants:	1 substance, maximum concentration of 0.01% (w/w). The imported formulation contains the hazardous impurity mentioned earlier in this section at a concentration of 27%.

5. USE, VOLUME AND FORMULATION

The notified chemical will be used in the manufacture of ophthalmic plastic lenses. It will be imported for the first three years only in an amount of less than 100 tonnes per annum.

6. OCCUPATIONAL EXPOSURE

Transport and storage

The notified chemical will be imported in 200 kg steel drums by sea and will be transported from the dockside by road to the notifier's warehouse for storage and use. Waterside, transport and storage workers would only be exposed to the notified chemical in the event of a spill. Cargo unloaders will use a fork lift to unload the containers. Fork lifts will also be used at the notifier's warehouse site to unload trucks and transfer steel drums to storage. The nature of the packaging used for transport minimises the likelihood of release or loss of the chemical in incidents. It is anticipated that 3 – 6 transport and storage workers will be involved for 1 hour/day, 241 days/year).

Lens manufacture

Laboratory staff (1 person, 3 hours/day, 241 days/year), process workers (26 – 28, 1.5 hours/day, 241 days/year) and a maintenance worker (1 hour/day, 241 days/year) will be involved in the manufacturing process.

Normally the notified chemical is not subjected to analysis on arrival but if necessary up to 200 mL may be sampled and subjected to analysis. Laboratory workers wear wrap around safety spectacles, cotton laboratory coats, natural rubber gloves and totally enclosed footwear to prevent exposure to the notified chemical. Laboratory staff are responsible for formulating the preparation used for lens manufacture which involves pumping the notified chemical into a 200 L holding vessel, addition of initiator and mould release agent, mixing and sampling 20 mL for analysis. In addition to personal protective equipment to control exposure, various other engineering controls are employed. A dedicated pump is used to transfer the notified chemical to the holding vessel and the transfer line of reinforced polyethylene is permanently connected to the pump. The connection to the holding vessel is a brass coupling of a unique type which avoids contamination of other substances. Cleaning of the pump and transfer line is not frequent as residues of the notified chemical will not cure and the equipment is in daily use.

Transfer of the notified chemical to the mixing vessel occurs through a dedicated transfer line which is permanently connected. The discharge end is a stainless steel coupling and shut-off valve and incorporates a 4.5 micron filter. Cleaning of the holding vessel and transfer line is not a frequent operation for the same reasons as mentioned above. Mixing of the lens formulation is carried out in an air conditioned room using a reverse cycle air conditioner with 45% fresh air make-up.

The low vapour pressure of the notified chemical precludes inhalation exposure. Dermal exposure is controlled by the use of engineering controls and personal protective equipment.

Following mixing, the vessels are transferred on wheels to the filling area where process workers complete the process. These workers wear wrap around safety spectacles, latex gloves with cotton under-gloves, cotton laboratory coats, cotton aprons and totally enclosed footwear to control exposure. Semi-automatic filling machines are used to fill the glass moulds to minimise exposure. The mould assemblies include a thermoplastic gasket and operators load the empty assemblies onto the filling machine and remove the filled assemblies to trays for curing at up to 80°C. Operators may be exposed to the notified chemical during filling and loading/unloading of mould assemblies from the filling machine (because waste liquid resin is generated at this stage), and when loading mould assemblies into ovens for curing. Exposure would mainly occur via the skin and/or eyes, with less potential for inhalation given the physico-chemical properties of the notified chemical and the use of general exhaust systems. Exposure to odours and vapours generated during the curing operation and at high temperatures is expected to be low, given that curing ovens are located in a remote area of the workplace with exhaust ventilation.

After removal of the gasket, the assemblies, which are still in one piece, are cleaned in automatic equipment to remove traces of uncured material on exterior surfaces. After curing of the notified polymer there is not expected to be any exposure of workers to the notified chemical in the finished products.

The single maintenance worker is responsible for repairs to the production equipment and will wear wrap around safety spectacles, cotton boiler suits and totally enclosed footwear.

Any waste formulation is cured by heating in drums and sent to landfill. The pumps, lines and vessels are cleaned with acetone which is collected in 200 L steel drums and sent to an approved disposal contractor for incineration.

The notifier estimates that workers may collectively be exposed to approximately 3 kg of uncured monomer per day.

7. PUBLIC EXPOSURE

Exposure of the public as a result of reformulation, transport and disposal of products made using the notified chemical is assessed as being negligible. The public may make dermal contact with eyewear products made using the notified chemical. However, public exposure to the notified chemical is unlikely since it is expected to be entirely consumed during the manufacture of lenses and any residual that may be present will be an integral part of a cured polymer matrix and unlikely to be bioavailable.

8. ENVIRONMENTAL EXPOSURE

8.1 Release

The notifier estimates that less than 2.5 tonnes of lens monomer waste containing the notified chemical will generated each year. These wastes will result from unused lens material and spills during lens monomer manufacture. The notifier indicates that there is potential for this amount to be reduced by approximately 30% through the recycling of unused lens monomer preparation. This waste will be collected in 200 L steel drums, heated to initiate polymerisation and disposed of in landfill.

The notifier further estimates that less than 170 kg per annum of the notified chemical will be released from the cleaning of mould assemblies. The wastewater from this process will be sent to a four-compartment sedimentation tank that is maintained at a pH of approximately 8.5-9. The sediment will be separated and transferred to 200 L steel drums and transported by road to a licensed waste disposal contractor. At this site, the liquid wastes will be neutralised and the solids removed and presumably recovered and disposed of in landfill. The liquid stream from the sedimentation tank will be released into the sewer.

Empty import drums will be sent to a drum recycling company where they will be rinsed with a caustic solution, and the resulting solution sent to a licensed waste disposal contractor for treatment and disposal as described above.

The mixing vessels, filling lines, conveyor belts and exterior surfaces of equipment will be periodically cleaned with acetone. The notifier estimates that up to 1570 L of waste acetone containing the notified chemical will be collected and disposed of by incineration.

8.2 Fate

The majority of the notified chemical will be incorporated in ophthalmic plastic lenses for eyewear. During manufacture, the moulded lenses are heat cured which initiates the polymerisation to form a high molecular weight polymer matrix. Therefore, once incorporated into the lens, the notified chemical is expected to be immobile and pose little risk to the environment.

The majority of wastes containing the notified chemical generated during manufacture and from spills will be heat cured prior to disposal in landfill. Exposure to heat forms a high molecular weight polymer matrix, therefore, the notified chemical is expected to be immobile and pose little risk to the environment.

Liquid wastes containing the notified chemical resulting from equipment cleaning will be incinerated by licensed waste disposal contractors and are expected to produce water vapour and oxides of nitrogen and carbon.

A small amount of the notified chemical will enter the sewer as a result of lens mould cleaning. However, prior to release the wastewater containing the notified chemical is passed through a four compartment sedimentation tank (residence time of 3 h) which is maintained at a pH of approximately 8.5-9. Component 1 of the notified chemical is classified as being very rapidly hydrolysing at pH 9 (see section 3 above for the physico-chemical properties of

the two components) and it and component 2 of the notified chemical have a low water solubility. Therefore, a large proportion of the notified chemical will either hydrolyse or precipitate and be removed in sludge to landfill. Of the small amount that is released into the sewer, the low Koc and Pow values for component 1 of the notified chemical indicate that it will dilute and disperse in the aquatic compartment, eventually partitioning to sediment. The moderately high Koc and Pow values for component 2 of the notified chemical indicate that it will associate with sediments when introduced into the aquatic compartment.

A biodegradation study was conducted using the notified chemical according to OECD TG 301B – Ready Biodegradability; CO_2 Evolution Test (Safepharm Laboratories Ltd, 2000d). The activated sludge, obtained from Severn Trent Water Plc sewage treatment plant in Derbyshire, was mixed with the test substance or standard material (sodium benzoate) at a final concentration of 10 mg/L. The biodegradation of sodium benzoate was 84% after 28 days, indicating the test conditions were valid. After 28 days at 21°C, the biodegradation of the test substance was determined to be 83% and satisfied the 10-day window validation criterion, whereby 60% degradation must be attained within 10 days of the degradation rate exceeding 10%. This indicates the notified chemical can be considered to be readily biodegradable in aerobic environments. Therefore, sludge containing the notified chemical placed into landfill would degrade through biological and abiotic processes to produce carbon dioxide, methane and water.

The notified chemical readily biodegrades and there will be limited environmental exposure and as such it should not bioaccumulate (Connell, 1990).

9. EVALUATION OF TOXICOLOGICAL DATA

All toxicological studies were conducted on the notified chemical.

9.1 Acute Toxicity

Summary of the acute toxicity of CR-600

Test	***Species***	***Outcome***	Reference
acute oral toxicity	rat	300 mg/kg < LD50 < 500 mg/kg	(Safepharm Laboratories Ltd, 2001e)
acute dermal toxicity	rat	LD50 > 2000 mg/kg	(Safepharm Laboratories Ltd, 2001f)
skin irritation	rabbit	not irritating	(Safepharm Laboratories Ltd, 2001g)
eye irritation	rabbit	slightly irritating	(Safepharm Laboratories Ltd, 2001h)
skin sensitisation	guinea pig	not sensitising	(Safepharm Laboratories Ltd, 2001i)

9.1.1 Oral Toxicity (Safepharm Laboratories Ltd, 2001e)

Species/strain:	rat/Sprague-Dawley.
Number/sex of animals:	3 females/dose group.
Observation period:	14 days.
Method of administration:	Oral (gavage); vehicle: arachis oil; notified chemical administered to 3 animals at 2000 mg/kg, then 3/sex at 200 mg/kg.
Test method:	OECD TG 423
Mortality:	Animals treated with 2000 mg/kg were found dead on the day of dosing or one day after the day of dosing. No deaths occurred at 200 mg/kg.
Clinical observations:	Signs of systemic toxicity at 2000 mg/kg were hunched posture, lethargy, ataxia, pilo-erection, ptosis, decreased respiratory rate, laboured respiration and increased lachrymation. No signs of toxicity were noted in animals treated with 200 mg/kg.
Morphological findings:	Abnormalities noted at necropsy of animals that died during the study (ie dosage of 2000 mg/kg) were haemorrhagic lungs, dark liver or patchy pallor of the liver, dark kidneys, haemorrhage and/or sloughing of the gastric mucosa, sloughing of the non-glandular region of the stomach and slight haemorrhage of the small and large intestines.
LD_{50}:	Estimated between 300 and 500 mg/kg according to the OECD Acute Toxic Class classification system.
Result:	The notified chemical was estimated to be of moderate acute oral toxicity in rats.

9.1.2 Dermal Toxicity (Safepharm Laboratories Ltd, 2001f)

Species/strain:	rat/Sprague-Dawley.
Number/sex of animals:	5/sex.
Observation period:	14 days.
Method of administration:	24-hour semi-occluded dressing on undiluted test substance at 2000 mg/kg.
Test method:	OECD TG 402
Mortality:	None.

Clinical observations: No signs of systemic toxicity.

Morphological findings: No abnormalities.

Draize scores: No irritation noted.

LD_{50}: > 2000 mg/kg.

Result: The notified chemical was of low dermal toxicity in rats.

9.1.3 Inhalation Toxicity

No data provided.

9.1.4 Skin Irritation (Safepharm Laboratories Ltd, 2001g)

Species/strain: rabbit/New Zealand White (NZW).

Number/sex of animals: 3 males.

Observation period: 72 hours

Method of administration: Single 4-hour semi-occluded application of 0.5 mL of the notified chemical to intact skin.

Test method: OECD TG 404

Comment: Neither erythema nor oedema was observed in any animal at 1, 24, 48 or 72 hours after patch removal.

Result: The notified chemical was not irritating to the skin of rabbits.

9.1.5 Eye Irritation (Safepharm Laboratories Ltd, 2001h)

Species/strain: rabbit/NZW.

Number/sex of animals: 3 males.

Observation period: 72 hours.

Method of administration: 0.1 mL of the notified chemical was instilled into the conjunctival sac of the right eye of each animal. The left eye served as control.

Test method: OECD TG 405

Comment: No corneal or iridal effects were seen. Slight conjunctival redness was observed in all animals at 1 hour post-instillation which persisted in one animal to 24 hours post-

instillation. One animal also exhibited slight discharge at 1 hour post-instillation.

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

9.1.6 Skin Sensitisation (Safepharm Laboratories Ltd, 2001i)

Species/strain: guinea pig/Dunkin Hartley.

Number of animals: 20 test; 10 control.

Induction procedure:

test group:

day 1 Pairs of intradermal injections (0.1 mL) to the scapular region as follows:

- Freund's Complete Adjuvant (FCA), 1:1 in water;
- notified chemical 5% (v/v) in arachis oil;
- notified chemical 5% (v/v) in FCA, 1:1 in distilled water.

day 8 100% notified chemical under occlusive patch for 48 hours.

control group: Treated similarly to the test animals except that the test substance was omitted from the intradermal injections and the topical application.

Challenge procedure:

day 22 Topical application on the flank of the notified chemical, 50% and 25% (v/v) in arachis oil for 24 hours under occlusive dressing. Control animals treated similarly except that the test substance was omitted from the topical application.

Test method: OECD TG 406

Challenge outcome:

Challenge concentration	• *Test animals* • *24 hours**	• *48 hours**	• *Control animals* • *24 hours*	• *48 hours*
25%	0/20**	0/19	0/10	0/9
50%	0/20	0/19	0/10	0/9

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

Comment: One test animal was found dead on day 25 and one control animal was killed for humane reasons on the same day. Discrete or patchy erythema in 2 animals at 50% concentration at the 24 hour reading. Reactions subsided at the 48 hour observation and, therefore, were not attributed to sensitisation.

Result: The notified chemical was not sensitising to the skin of guinea pigs.

9.2 Repeated Dose Toxicity (Safepharm Laboratories Ltd, 2001j)

Species/strain: rat/Sprague-Dawley.

Number/sex of animals: 5/sex/dose group.

Method of administration: Oral (gavage); vehicle: PEG 400. Administered on 28 consecutive days.

Dose/Study duration: 0, 3, 15 and 150 mg/kg/day for 28 days. Two recovery groups (control and 150 mg/kg/day) were allowed a 14-day recovery period from day 28.

Test method: OECD TG 407

Clinical observations

Clinical signs indicative of administration of an irritant test substance were observed in the high dose group from day 5 onward and included: increased salivation of short duration, noisy respiration and wet fur together with associated red/brown staining of the external body fur. Also seen were hunched posture and less frequently, pallor of the extremities, pilo-erection, tiptoe gait and yellow staining on the cage tray liners. All clinical signs regressed over the 14-day recovery period.

In the mid-dose animals, increased salivation of short duration was seen sporadically with hunched posture mainly in females from day 14 onwards. Noisy respiration was observed in one female on day 7 only.

Behavioural assessment confirmed the clinical signs described above and no treatment-related effects relative to functional performance tests and sensory reactivity tests were observed.

A slight reduction in bodyweight gain in high dose animals was observed in week 1 accompanied by a reduction in dietary intake. Water consumption was unaffected by treatment.

Clinical chemistry/Haematology/Urinalysis

Clinical chemistry

High dose animals exhibited increases in levels of aspartate and alanine aminotransferases (ASAT and ALAT, respectively) but the increases were not statistically significant. These were accompanied in females only by significant increases in levels of gamma glutamyl transpeptidase (γGT) and cholesterol. Reduced albumin was observed in high and mid dose males and total protein in mid dose males only.

Haematology

No treatment-related effects.

Urinalysis

No treatment-related effects.

Macroscopic findings/Organ weights

Macroscopic findings

High dose animals exhibited yellow discolouration of the liver and thickening of the non-glandular region of the stomach. Recovery high dose animals exhibited an accentuated lobular pattern of the liver.

Organ weights

Both absolute and relative liver weights were increased in high dose animals but were similar to controls in the recovery animals.

Histopathology

Treatment-related changes in high dose animals were limited to gastric changes such as minimal to moderate hyperkeratosis and minimal to mild acanthosis and hepatic changes involving an altered pattern of hepatocyte cytoplasm-centriacinar glycogen vacuolation associated with minimal or mild bile duct hyperplasia with possibly a slight incidence of hepatocytic focal coagulation necrosis.

Minimal gastric changes were present in 1/10 animals at the end of the recovery period. Hepatic changes remained in 3/10 recovery animals. In these animals the centriacinar glycogen pattern had regressed but 2 animals displayed mild pigmentation of Kuppfer cells and associated bile duct hyperplasia.

Comment

Clinical signs were suggestive of oral administration of an irritant substance. Thickening of the non-glandular region of the stomach and hyperkeratosis and acanthosis supported this view. An effect of the test substance on the liver was suggested by effects in high dose animals: increased ALAT and ASAT accompanied by increased γGT and cholesterol in females, reduced albumin in males (also together with lower total protein, in mid dose

males), yellow discolouration, increased absolute and relative liver weights, centriacinar glycogen vacuolation associated with minimal or mild bile duct hyperplasia and possibly a slight incidence of hepatocytic focal coagulation necrosis.

Result

The No Observed Effect Level (NOEL) for the notified chemical was considered to be 3 mg/kg/day.

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay (Safepharm Laboratories Ltd, 2001k)

Strains:	*S. typhimurium* strains TA 1535, TA 1537, TA 98 and TA 100 and *E. coli* strain WP2*uvr*A.
Metabolic activation:	Sprague-Dawley rat liver microsomal fraction (S9); induction: phenobarbitone/β-naphthoflavone.
Concentration range:	- S9: 0, 50 150, 500, 1500 and 5000 microgram/plate +S9: 0, 5 (TA 100 and TA 1535 only), 15 (not WP2*uvr*A), 50, 150, 500 and 5000 (TA 1535 and WP2*uvr*A only.
Test method:	OECD TG 471
Comment:	The test substance caused a visible reduction in the growth of the background lawn and/or a decrease in the number of back mutant colonies in the *Salmonella* strains at and above 1500 microgram/plate in the presence of S9 fraction. No toxicity was observed in the *Salmonella* strains in the absence of S9 or in the *E. coli* strains either with or without S9. The oily precipitate present at 5000 microgram/plate did not prevent scoring of mutant colonies. The negative controls (dimethylsulfoxide) were within normal limits and the positive controls demonstrated the sensitivity of the test system. No increases in the induced mutation frequency were observed in any strain at any dose level in the absence or presence of S9 fraction.
Result:	The notified chemical was non mutagenic under the conditions of the test.

9.3.2 Chromosomal Aberration Assay in human lymphocytes (Safepharm Laboratories Ltd, 2001m)

Cells: Peripheral blood human lymphocytes.

Metabolic activation system: Sprague-Dawley rat liver microsomal fraction (S9); induction: phenobarbitone/β-naphthoflavone.

Dosing schedule:

• *Metabolic Activation*	• ***Experiment Number***	• *Test concentration (μg/mL)*	• *Controls*
-S9	1	treatment time = 4 hours, harvest time = 24 hours; doses = 0*, 156.25, 312.5, 625, 1250*, 2500* and 5000* microgram/mL	Positive: mitomycin C, 0.4 microgram/mL
	2	treatment time = harvest time = 24 hours; doses = 0*, 39.07, 78.13, 156.25, 312.5, 625*, 1250* and 2500* microgram/mL	Positive: mitomycin C, 0.2 microgram/mL
		treatment time = harvest time = 48 hours; doses = 0, 78.13, 156.25, 312.5*, 625*, 1250* and 2500* microgram/mL	Positive: mitomycin C, 0.1 microgram/mL Negative: dimethylsulfoxide
+S9	1	treatment time = 4 hours, harvest time = 24 hours; doses = 0*, 156.25, 312.5, 625, 1250*, 2500* and 5000* microgram/mL	Positive: cyclophosphamide, 12.5 microgram/mL Negative: dimethylsulfoxide
	2	treatment time = 6 hours, harvest time = 24 hours; 0*, 156.25, 312.5, 625, 1250*, 2500* and 5000* microgram/mL	

* - cultures selected for metaphase analysis

Test method: OECD TG 473

Comment: In experiment 1 a precipitate was observed at the end of the treatment period at and above 625 microgram/mL in the absence of S9 and at all dose levels in the presence of S9; 50% or greater reduction in the mitotic index was observed at 5000 microgram/mL in the absence or presence of S9. The test material did not induce statistically significant increases in the frequency of cells with aberrations with or

without S9.

In experiment 2, in the 24-hour continuous exposure group there were scorable metaphases up to 2500 microgram/mL and in the 48-hour continuous exposure group there were scorable metaphases up to 1250 microgram/mL. There was an approximate 50% growth inhibition at both 1250 and 2500 microgram/mL in the 24-hour treatment group with a precipitate at 2500 microgram/mL In the 48-hour group the mitotic index appeared to be slightly reduced at 1250 microgram/mL but there was an unexpectedly low value for the control so that the effect of treatment was likely to be greater than calculated. No statistically significant increases the frequency of cells with aberrations were observed with or without S9.

Result: The notified chemical was non clastogenic under the conditions of the test.

9.4 Overall Assessment of Toxicological Data

The notified chemical was estimated to be of moderate acute oral toxicity in rats (LD50 between 300 and 500 mg/kg) and of low acute dermal toxicity in rats. The notified chemical was not irritating to rabbit skin, was a slight eye irritant in rabbits and was not a skin sensitiser in guinea pigs. In a 28-day repeated dose oral toxicity study the target organ was identified as the liver from clinical chemistry, macroscopic findings, organ weights and microscopic findings. The NOEL was judged to be 3 mg/kg/day but the effects at 15 mg/kg/day were indicative of serious damage to health. In genotoxicity tests the notified chemical was neither mutagenic in bacteria nor clastogenic in human lymphocytes in vitro.

The notified chemical is considered to be a hazardous substance in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999) and is assigned the risk phrases R48/22: danger of serious damage to health by prolonged exposure if swallowed and R22: harmful if swallowed.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

Full test reports on the ecotoxicity studies for the notified chemical were provided by the notifier.

Test	*Species*	*Results*
Acute Toxicity OECD TG 203	Rainbow Trout *Oncorhynchus mykiss*	LL_{50} (96 h) = 1.3 mg/L WAF NOEC (96 h) = 1.0 mg/L WAF

Acute Immobilisation	Water Flea	EL_{50} (48 h) = 12 mg/L WAF
OECD TG 202	*Daphnia magna*	NOEC (48 h) = 5.6 mg/L WAF
Growth Inhibition	Algae	E_bL_{50} (96 h) = 24 mg/L WAF
OECD TG 201	*Pseudokirchneriella subcapitata*	E_rL_{50} (96 h) > 50 mg/L WAF
		NOEC (96 h) = 3.13 mg/L WAF
Inhibitory Effect	Activated Sewerage Sludge	EC_{50} (3 h) = 74 mg/L
OECD TG 209		NOEC (48 h) = 32 mg/L

* NOEC - no observable effect concentration

The fish, daphnia and algal ecotoxicity tests were performed on the Water Accommodated Fraction (WAF) of the notified chemical. The WAF was prepared by adding an amount of the notified chemical to water to give the required loading rate and the resulting solution was then stirred for 23 hours. The mixture was then allowed to stand for 1 hour prior to the removal of the aqueous phase by siphon. Dispersed test material was observed in the WAFs but these were used with out further treatment.

The tests on fish (Safepharm Laboratories Ltd, 2001n) were performed using a semi-static methodology in which test preparations were renewed daily to ensure that concentrations of test material were maintained near nominal and to prevent the accumulation of nitrogenous wastes. Observations were performed at 3, 6, 24, 48, 72 and 96 hours. The test was performed using ten specimen fish per loading rate at a temperature of 14ºC. The tests were conducted using a siphoned water accommodation fraction (WAF) of the test substance made up at nominal concentrations of 1, 1.8, 3.2, 5.6 and 10 mg/L. Analysis based on component 1 of the siphoned WAF after 24 h showed measured concentrations to range from 0.66–9.34 mg/L. The results of the definitive study showed that after 96 h, 100% mortality was observed at test concentrations above 1 mg/L siphoned WAF. The 96-hour LL_{50} for the notified chemical to *Oncorhynchus mykiss* is 1.3 mg/L.

The immobilisation tests with daphnia (Safepharm Laboratories Ltd, 2001o) were also performed under semi-static conditions with observations performed at 24 and 48 hours. The test was performed in duplicate using 10 daphnids per flask at a temperature of 21ºC. The tests were conducted using a siphoned water accommodation fraction (WAF) of the test substance made up at nominal concentrations of 1.0, 1.8, 3.2, 5.6, 10, 18, 32, 56 and 100 mg/L. Analysis based on component 2 of the siphoned WAF after 48 h showed measured concentrations to range from 0.575–90.35 mg/L. After 48 h, no immobilised daphnids were observed in the test vessels with less than 5.6 mg/L siphoned WAF, 50% immobilisation at a test concentration of 10 mg/L siphoned WAF and 100% mortality was observed at test concentrations above 18 mg/L siphoned WAF. The 48-hour EL_{50} for the notified chemical to *Daphnia magna* is 12 mg/L.

Algae were exposed to the test substance at nominal concentrations of 3.13, 6.25, 12.5, 25 and 50 mg/L for 96 h at 24°C under constant illumination and shaking (Safepharm Laboratories Ltd, 2001p). No abnormalities were detected in any of the replicate test samples. Both biomass and growth rate of *Pseudokirchneriella subcapitata* was adversely affected by the test substance, giving a 96 h E_bC_{50} of 24 mg/L, E_rC_{50} of greater than 50 mg/L and NOEC of 3.13 mg/L. Analysis based on the component 1 of the WAF after 96 h showed measured concentrations ranging from below the limits of quantification to 18.9 mg/L.

The activated sludge study was conducted using sludge obtained from Severn Trent Water Plc sewage treatment plant in Derbyshire (Safepharm Laboratories Ltd, 2001q). Based on the

results of the range finding studies, the definitive study was conducted on nominal concentrations of 10, 18, 32, 56 and 100 mg/L. Amounts of test material (5, 9, 16, 38 and 50 mg) were added to water and sonicated for approximately 30 min. Synthetic sewerage, activated sludge and water were added to give the required concentrations of test substance. The reference material used in the study was 3,5-dichlorophenol. When compared to the control, activated sludge at the nominal concentrations of 10, 18 and 32 mg/L after 3 h displayed increases in respiration of 1, 7, 13%, respectively. Activated sludge at the nominal concentrations of 56 and 100 mg/L after 3 h displayed 30 and 72% inhibition, respectively. The 3-hour EC_{50} for the notified chemical to activated sludge is 74 mg/L and the NOEC is 32 mg/L.

The authors suggested that the discrepancies between the measured concentrations and the nominal concentrations of the test substance can be attributed in part to the instability of component 1 under test conditions.

The ecotoxicity data indicate the notified chemical is slightly toxic to daphnia, algae and activated sludge up to the limit of its water solubility, but appears to be moderately to highly toxic to fish based on measured concentrations.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The majority of the notified chemical (greater than 98%) will be incorporated in ophthalmic plastic lenses for eyewear. During manufacture, the moulded lenses are heat cured which initiates polymerisation to form a high molecular weight polymer matrix. Therefore, once incorporated into the lens, the notified chemical is expected to pose little risk to the environment it is expected to be entirely consumed during the manufacture of lenses, and any residue that may be present should be contained within the cured polymer matrix and should not be bioavailable.

The majority of wastes containing the notified chemical generated during manufacture and from spills will be heat cured prior to disposal in landfill. Exposure to heat forms a high molecular weight polymer matrix, therefore, the notified chemical is expected to be immobile and pose little risk to the environment.

Liquid wastes containing the notified chemical resulting from equipment cleaning will be incinerated by licensed waste disposal contractors and are expected to produce water vapour and oxides of nitrogen and carbon.

Uncured wastes containing the notified chemical disposed of in landfill would be degraded through biological and abiotic processes to produce carbon dioxide, methane and water.

A small amount of the notified chemical (up to 170 kg) has the potential to enter the sewer as a result of lens mould cleaning. Prior to release it is expected that a large proportion of the notified chemical will either hydrolyse or precipitate and be removed in sludge to landfill. Of the small amount that is released into the sewer, the Koc and Pow values for the notified chemical suggest that both components will eventually partition to sediment when introduced into the aquatic compartment. The quantity of the notified chemical released into the sewer will be low and subsequent treatment at municipal wastewater treatment plants would further reduce its concentration in the aquatic compartment.

The ecotoxicity data indicate the notified chemical is slightly toxic to daphnia, algae and activated sludge up to the limit of its water solubility, but appears to be moderately to highly toxic to fish based on measured concentrations. However, release to the aquatic compartment will be low.

The notified chemical readily biodegrades and there will be limited environmental exposure and as such it should not bioaccumulate (Connell, 1990).

Therefore, the environmental exposure and overall environmental hazard from the notified chemical is expected to be low.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Hazard assessment

The notified chemical was estimated to be of moderate acute oral toxicity in rats (LD50 between 300 and 500 mg/kg) and of low acute dermal toxicity in rats. The notified chemical was not irritating to rabbit skin, was a slight eye irritant in rabbits and was not a skin sensitiser in guinea pigs. In a 28-day repeated dose oral toxicity study the target organ was identified as the liver from clinical chemistry, macroscopic findings, organ weights and microscopic findings. The NOEL was judged to be 3 mg/kg/day but the effects at 15 mg/kg/day were indicative of serious damage to health. In genotoxicity tests the notified chemical was neither mutagenic in bacteria nor clastogenic in human lymphocytes in vitro.

The notified chemical is considered to be a hazardous substance in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999) and is assigned the risk phrases R48/22: danger of serious damage to health by prolonged exposure if swallowed and R22: harmful if swallowed.

The formulation to be imported contains 27% of a chemical with structurally similarity to the notified chemical which may render the formulation irritating to skin and eyes. This component also is of moderate acute oral toxicity in rats and exhibits experimental reproductive effects in rabbits.

Occupational health and safety

The notified chemical will be imported in 200 kg steel drums by sea and will be transported from the dockside by road to the notifier's warehouse for storage and use. Waterside, transport and storage workers would only be exposed to the notified chemical in the event of a spill. The nature of the packaging used for transport minimises the likelihood of release or loss of the chemical in incidents and the risk of acute or chronic toxic effects to transport and storage workers is considered to be low.

Normally the notified chemical is not subjected to analysis on arrival but if necessary up to 200 mL may be sampled and subjected to analysis. Laboratory workers wear wrap around safety spectacles, cotton laboratory coats, natural rubber gloves and totally enclosed footwear

to prevent exposure to the notified chemical. Laboratory staff are responsible for formulating the preparation used for lens manufacture which involves pumping the notified chemical into a 200 L holding vessel, addition of initiator and mould release agent, mixing and sampling 20 mL for analysis. In addition to personal protective equipment to control exposure, various engineering controls are employed including dedicated transfer equipment and couplings designed to limit spills. Cleaning of the pump and transfer line is not frequent as residues of the notified chemical will not cure and the equipment is in daily use. Transfer of the notified chemical to the mixing vessel occurs through a dedicated transfer line which is permanently connected. The discharge end is a stainless steel coupling and shut-off valve and incorporates a 4.5 micron filter. Cleaning of the holding vessel and transfer line is not a frequent operation for the same reasons as mentioned above. Mixing of the lens formulation is carried out in an air conditioned room using a reverse cycle air conditioner with 45% fresh air make-up. The low vapour pressure of the notified chemical precludes inhalation exposure. Dermal exposure is controlled by the use of engineering controls and personal protective equipment. Given the above engineering control and work practices and the use of personal protective equipment, the risk of toxic effects to laboratory staff is considered to be low as is the risk of irritant effects from the imported formulation.

Following mixing, the vessels are transferred on wheels to the filling area where process workers complete the process. These workers wear wrap around safety spectacles, latex gloves with cotton under-gloves, cotton laboratory coats, cotton aprons and totally enclosed footwear to control exposure. Semi-automatic filling machines are used to fill glass moulds to minimise exposure. The mould assemblies include a thermoplastic gasket and operators load the empty assemblies onto the filling machine and remove the filled assemblies to trays for curing. Operators may be exposed to the notified chemical during filling and loading/unloading of mould assemblies from the filling machine (because waste liquid resin is generated at this stage), and when loading mould assemblies into ovens for curing. Exposure would mainly occur via the skin and/or eyes, with less potential for inhalation given the physico-chemical properties of the notified chemical and the use of general exhaust systems. Exposure to odours and vapours generated during the curing operation and at high temperatures is expected to be low, given that curing ovens are equipped with local exhaust ventilation. After removal of the gasket, the assemblies, which are still in one piece, are cleaned in automatic equipment to remove traces of uncured material on exterior surfaces. After curing of the notified polymer there is not expected to be any exposure of workers to the notified chemical in the finished products. The risk of irritant and toxic effects to process workers should be low given the semi-enclosed filling system and the use of personal protective equipment.

The single maintenance worker responsible for repairs to the production equipment will wear wrap around safety spectacles, cotton boiler suits and totally enclosed footwear and the risk of irritant and toxic effects should be low.

Any waste formulation is cured by heating in drums and sent to landfill. The pumps, lines and vessels are cleaned with acetone which is collected in 200 L steel drums and sent to an approved disposal contractor for incineration. Process workers will be protected against exposure as described above and the risk of irritant and toxic effects while disposing of the notified chemical should be low.

Public Health

Exposure of the public as a result of reformulation, transport and disposal of the product containing the notified chemical is assessed as being negligible. Although members of the public may make dermal contact with optical lenses made using the notified chemical, the risk to public health is considered to be minimal since public exposure to the notified chemical is unlikely.

13. RECOMMENDATIONS

Regulatory controls

- The NOHSC Chemicals Standards Sub-committee should consider the following health hazard classification for the notified chemical:
  - R48/22: danger of serious damage to health by prolonged exposure if swallowed and R22: harmful if swallowed

Control Measures

Occupational Health and Safety

- Employers should implement the following engineering controls to minimise occupational exposure to the notified chemical as introduced:
  - pumps, couplings and vessels should be designed to minimise spillage
  - good general and local exhaust ventilation should be employed during mixing

- Employers should implement the following safe work practices to minimise occupational exposure during handling of the notified chemical as introduced:
  - Spillage of the chemical should be avoided; any spillage should be cleaned up mechanically and placed into drums for heat curing prior to disposal

- Employers should ensure that the following personal protective equipment is used by workers to minimise occupational exposure to the notified chemical as introduced:
  - safety glasses, protective clothing, protective footwear and gloves

  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.

13.1 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 Section 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.

No additional secondary notification conditions are stipulated.

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* (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. REFERENCES

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.

Mensinck, B. J. W. G. et al. (1995) Manual for summarising and evaluating the environmental aspects of pesticides, National Institute of Public Health and Environmental Protection, Bilthoven, The Netherlands. Report no. 679101022.

National Occupational Health and Safety Commission (1994) 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 (1999) Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(1994)]. Australian Government Publishing Service, Canberra.

Safepharm Laboratories (2001a) SPL Project Number 1234/088: Determination of General Physico-chemical Properties, Safepharm Laboratories Ltd, Derby, UK, (unpublished report submitted by SOLA Optical).

Safepharm Laboratories (2001b) SPL Project Number 1234/105: Determination of Vapour Pressure, Safepharm Laboratories Ltd, Derby, UK, (unpublished report submitted by SOLA Optical).

Safepharm Laboratories (2001c) SPL Project Number 1234/106: Determination of Vapour Pressure, Safepharm Laboratories Ltd, Derby, UK, (unpublished report submitted by SOLA Optical).

Safepharm Laboratories (2001d) SPL Project Number 1234/102: Assessment of ready biodegradability; CO_2 Evolution Test, Safepharm Laboratories Ltd, Derby, UK, (unpublished report submitted by SOLA Optical).

Safepharm Laboratories (2001e) SPL Project Number 1234/091: OM600: Acute Oral Toxicity in the Rat – Acute Toxic Class Method, Safepharm Laboratories Ltd, Derby, UK, (unpublished report submitted by SOLA Optical).

Safepharm Laboratories (2001f) SPL Project Number 1234/092: OM600: Acute Dermal Toxicity (Limit Test) in the Rat, Safepharm Laboratories Ltd, Derby, UK, (unpublished report submitted by SOLA Optical).

Safepharm Laboratories (2001g) SPL Project Number 1234/093: OM600: Acute Dermal Irritation in the Rabbit, Safepharm Laboratories Ltd, Derby, UK, (unpublished report submitted by SOLA Optical).

Safepharm Laboratories (2001h) SPL Project Number 1234/094: OM600: Acute Eye Irritation in the Rabbit, Safepharm Laboratories Ltd, Derby, UK, (unpublished report submitted by SOLA Optical).

Safepharm Laboratories (2001i) SPL Project Number 1234/095: OM600: Skin Sensitisation in the Guinea Pig – Magnus and Kligman Maximisation Method, Safepharm Laboratories Ltd, Derby, UK, (unpublished report submitted by SOLA Optical).

Safepharm Laboratories (2001j) SPL Project Number 1234/096: OM600: Twenty-Eight Day Repeated Dose Oral (Gavage) Toxicity Study in the Rat, Safepharm Laboratories Ltd, Derby, UK, (unpublished report submitted by SOLA Optical).

Safepharm Laboratories (2001k) SPL Project Number 1234/097: OM600: Reverse Mutation Assay "Ames Test" using *Salmonella typhimurium* and *Escherichia coli*, Safepharm Laboratories Ltd, Derby, UK, (unpublished report submitted by SOLA Optical).

Safepharm Laboratories (2001m) SPL Project Number 1234/098: OM600: Chromosome Aberration Test in Human Lymphocytes in vitro, Safepharm Laboratories Ltd, Derby, UK, (unpublished report submitted by SOLA Optical)

Safepharm Laboratories (2001n) SPL Project Number 1234/099: Acute Toxicity to Rainbow Trout, Safepharm Laboratories Ltd, Derby, UK, (unpublished report submitted by SOLA Optical).

Safepharm Laboratories (2001o) SPL Project Number 1234/100: Acute Toxicity to *Daphnia Magna*, Safepharm Laboratories Ltd, Derby, UK, (unpublished report submitted by SOLA Optical).

Safepharm Laboratories (2001p) SPL Project Number 1234/101: Algal Inhibition Test, Safepharm Laboratories Ltd, Derby, UK, (unpublished report submitted by SOLA Optical).

Safepharm Laboratories (2001q) SPL Project Number 1234/103: Assessment of the Inhibitory Effect on the Respiration of Activated Sludge, Safepharm Laboratories Ltd, Derby, UK, (unpublished report submitted by SOLA Optical).

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.