File No: NA/902

June 2001

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

Indoclear

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Director
Chemicals Notification and Assessment

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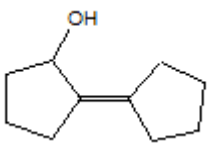
1. APPLICANT

Quest International Australia Pty Ltd of 6 Brighton Street, Smithfield, NSW 2164 (ABN 41 078 584 184) has submitted a standard notification statement in support of their application for an assessment certificate for **Indoclear**.

The notifier has not claimed any information to be exempted from publication in the Full Public Report.

2. IDENTITY OF THE CHEMICAL

Chemical Name:	Cyclopentanol, 2-cyclopentylidine
Chemical Abstracts Service (CAS) Registry No.:	6261-30-9
Other Names:	[Bicyclopentyliden]-2-ol; Angelol; QRM 1764
Marketing Name:	Indoclear
Molecular Formula:	$C_{10}H_{16}O$
Structural Formula:	
Molecular Weight:	152 g/mol
Method of Detection and Determination:	Indoclear can be detected and characterised using Nuclear Magnetic Resonance (NMR), Ultraviolet (UV), Infrared (IR) spectroscopy and Gas Chromatography (GC).

Spectral Data:	NMR, UV, IR and GC spectra were supplied by the notifier.

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C & 101.3 kPa:	White waxy solid
Relative Density :	1.1128 at 20°C
Particle Size:	Not determined due to the waxy nature of the solid.
Melting Point :	596-606°C
Boiling Point:	779°C at 100.61-101.02 kPa
Vapour Pressure:	0.2×10^{-3} kPa at 25°C
Water Solubility:	466 mg/L at 20°C
Partition Co-efficient (n-octanol/water):	1.22×10^{3}, $\log_{10} P_{ow} = 3.09$
Hydrolysis as a Function of pH:	$T_{1/2}$ at pH 4.0 = <1 day, at 25ºC $T_{1/2}$ at pH 7.0 = 4.16 days, at 25ºC $T_{1/2}$ at pH 9.0 = > 1 year, at 25ºC
Adsorption/Desorption:	217, $\log_{10} K_{oc} = 2.34$
Dissociation Constant:	Not determined, material does not dissociate in water
Flash Point:	Not determined
Flammability Limits:	Combustible
Autoignition Temperature:	230 +/- 5 °C
Explosive Properties:	Not explosive
Reactivity/Stability:	No oxidising properties, stable under normal conditions

3.1 Comments on Physico-Chemical Properties

All tests were performed by Safepharm Laboratories Ltd (2000a, b).

The vapour pressure provided was determined using a vapour pressure balance and Method A4 of Commission Directive 92/69/EEC. Linear regression analysis was used to calculate vapour pressure at 25°C. The low value determined indicates that the notified chemical is classified as being very slightly volatile (Mensink 1995).

The water solubility was determined using the flask method detailed in Method A6 of Commission Directive 92/69/EEC. The notified chemical is classified as being moderately soluble which is consistent with its predominantly hydrocarbon structure.

The hydrolytic stability of the notified chemical was determined using Method C7 of Commission Directive 92/69/EEC. The hydrolytic stability tests conducted indicate that the notified chemical is very rapidly hydrolysing at pH 4, fairly hydrolysing at pH 7 and slightly hydrolysing at pH 9 at 25°C. The notified chemical does not contain functional groups capable of undergoing hydrolysis. Therefore, depletion of the test substance is most likely due to an acid catalysed rearrangement.

The partition coefficient was determined using the HPLC method detailed in Method A8 of Commission Directive 92/69/EEC. The moderate water solubility is consistent with the high log P_{ow}, indicating a high affinity for the organic component of soils and sediments. This is confirmed by the high log K_{oc} determined by the HPLC method. The notified chemical is classified as being hydrophobic and moderately mobile in soil.

Although no dissociation tests were conducted, the notified chemical is unlikely to undergo dissociation in the environmental pH range of 4 to 9 as no acidic or basic groups are present.

The surface tension of 0.511 g/L solution was determined using a White Electrical Institute interfacial tension balance and a procedure based on the ISO 304 ring method. The result indicates the notified chemical may be considered to be surface active.

4. PURITY OF THE CHEMICAL

Degree of Purity: > 95 %, typically 96.5 %

Hazardous Impurities: None

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

Additives/Adjuvants: None

5. USE, VOLUME AND FORMULATION

Indoclear is an aroma-chemical that is blended with other such chemicals to produce fragrances. The fragrances are in turn used in a wide variety of personal and household products including washing products, household cleaners and air fresheners. The content of Indoclear in the fragrance will vary depending on the final product. Typical content will be 0.005%, with a maximum concentration of 1% being used in fragrances intended for use in air fresheners and solid soaps.

Indoclear will not be manufactured in Australia, but will be imported to one site at a rate of up to 1 tonne per annum for the first 5 years. It will be imported in liquid form at a maximum concentration of 1% as part of a fragrance compound. Import will be in 200 litre steel kegs

that are lacquer or polyethylene lined. The notified chemical will be distributed to a number of customers for incorporation into consumer products.

6. OCCUPATIONAL EXPOSURE

The 200 litre kegs are imported on forklift pallets and are stored initially at one site before shipment to consumer product manufacturers. Kegs are opened manually. Contents are likely to be transferred to holding, filling or reaction vessels using suction pumps. All workers handling the drums of fragrances containing Indoclear and who are present during transfer operations should wear suitable gloves, eye protection and protective clothing.

The liquid fragrances containing Indoclear will be added to other consumer product ingredients in either open or closed vessels. Most processing will be carried out in closed, highly automated systems. If open vessels are used, adequate ventilation is required to ensure that any vapours are removed. The personal protective equipment (PPE) described for transfer workers also applies to those employed in the mixing operations when open vessels are being used. Subsequent filling and packaging operations are usually highly automated and the potential for exposure is minimal.

The kegs are washed and reused after use. The PPE described for transfer and mixing activities also apply to cleaning operations.

Overall, it is anticipated that between 5 and 20 workers may be exposed potentially to fragrances containing Indoclear during warehouse, production line, cleaning and sampling or analysis tasks. Exposure via all three routes is anticipated to be minimal and irregular. Dermal exposure, at a maximum concentration of 1% in fragrances may also occur during accidental splashing. Cleaning operations are likely to involve the highest exposures. It is also considered unlikely that any single worker in Australia will be exposed to Indoclear in a fragrance for more than 1 hour per day, over 50-300 days per year.

7. PUBLIC EXPOSURE

Personal and household products containing up to 1% of the notified chemical (ie. air fresheners consisting solely of the imported fragrance compound) will be sold to the public. Consequently, public exposure to the notified chemical is likely to be high.

8. ENVIRONMENTAL EXPOSURE

8.1 Release

Spills may occur during transport and use but expected losses would be minimal at less than 1% (approximately 10 kg annually). It is likely that any spilt material would ultimately be disposed of to landfill.

Less than 1% of the compounded fragrance would be left as residue in the import containers after emptying. This equates to 10 kg annually. It is likely that the containers will be rinsed and the rinsate added into the production mix or flushed into the sewer. The cleaned

containers would then be returned to the importer. Release to the environment during reformulation and cleaning processes are expected to be small as closed, automated systems are used. Approximately 1% or up to 10 kg of the notified chemical is expected to be released in this fashion. Wastes from these processes will be disposed of to landfill or the sewer.

Approximately 1% of the contents will remain in end-user containers. This represents up to 10 kg of Indoclear annually that would go into domestic rubbish and ultimately landfill.

Approximately 97% (970 kg) of the imported Indoclear via the use of household, laundry and personal cleaning products will eventually end up in the sewer. A small amount of the notified chemical will also be incorporated into air fresheners. This will be the main release into air as partitioning from water is not expected, based on the Simple Treat Model calculations (see Section 8.2).

8.2 Fate

Up to 2% (20 kg) of the imported Indoclear may end up in landfill. Since the chemical has a moderate water solubility (466 mg/L) and is likely to be moderately mobile in soil (log K_{oc} = 2.34), it may leach but in a very dispersed manner.

The notifier has provided the results of a ready biodegradation test in an aerobic aqueous media following OECD TG 301B (Safepharm Laboratories Ltd 2000d). The biodegradation was determined by the measurement of CO_2 produced after the culture medium inoculated with sufficient notified chemical to give 10 mg carbon/L had been stored in the dark at 21ºC for 28 days. Sodium benzoate was used as the standard material. The results indicated that only 14% of the chemical had degraded over this time, while the degradation result for the sodium benzoate was 85%. The results indicate that Indoclear is not readily biodegradable.

The notified chemical will be released eventually into the environment, and the majority could be expected to be discharged into sewerage systems. The proportions of chemical which reach sewage treatment plants (ie is not volatilised or otherwise destroyed during passage to the plant), and partition into the different environmental compartments may be estimated using the Simple Treat Model (EEC Technical Guidance Document, 1996). These estimates based on the chemical having a calculated Henry's constant of 6.52×10^{-2} $Pa.m^3.mole^{-1}$ using measured vapour pressure and water solubility, a log P_{ow} of 3.09 and not being biodegradable, indicate that the chemical would partition into the air, water and sewer sludge compartments as follows –

Air	0%
Water	93%
Sewage Sludge	7%

The new chemical is hydrophobic and surface active. When released into the sewer system some may remain associated with the organic component of the particulate matter in raw sewage, and eventually become incorporated into sediments. It would slowly degrade through biological and abiotic processes to water, carbon dioxide and methane. The majority may remain in the aquatic compartment where, according to the hydrolysis properties, it will readily degrade only in neutral and acidic solution. Hydrolysis in the sewer will be limited due to normally alkaline conditions.

In the atmosphere, it is likely that the notified chemical will be degraded through reaction with hydroxyl radicals through both addition to the double bond and hydrogen abstraction from the hydroxyl group. A calculation (AOPWIN 2001) indicated that in the troposphere the notified chemical would react in this manner with an estimated rate constant of 121.3 x 10^{-12} cm^3/molecule/sec. Rate constants of this order indicate rapid degradation, and assuming the mean atmospheric concentration of hydroxyl radicals is 5 × 10^5 radicals/cm^3 (Calamari, 1993), leads to an atmospheric half-life of 1.058 hours. Consequently, any notified chemical that does not volatilise is not expected to persist in the atmosphere.

Residual chemical disposed of into landfill with empty containers or with residual solids derived from water treatment at the production facilities is also expected to remain adsorbed to soil/sediment particles, and become slowly destroyed by similar mechanisms to those operating in sediments. Incineration of the material would produce water vapour and oxides of carbon.

Although much of the chemical will enter the water compartment, its modest partition coefficient and moderate water solubility indicate low potential for bioaccumulation (Connell, 1990).

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

Summary of the acute toxicity of Indoclear

Test	*Species*	*Outcome*	*Reference*
acute oral toxicity	rat	LD50 > 2000 mg/kg	Driscoll, 1999a
acute dermal toxicity	rat	LD50 > 2000 mg/kg	Driscoll, 1999b
skin irritation	rabbit	Irritant	Driscoll, 1999c
eye irritation	rabbit	Severe irritant	Driscoll, 1999d
skin sensitisation	guinea pig	Non-sensitiser	Driscoll, 1999e

9.1.1 Oral Toxicity (Driscoll, 1999a)

Species/strain: Rat / Sprague-Dawley

Number/sex of animals: 3 males and 3 females

Observation period: 14 days

Method of administration: 2000 mg/kg in arachis oil BP, by gavage

Test method: OECD TG 423 (Acute Toxic Class)

Mortality: None

Clinical observations:	Ataxia, hunched posture and lethargy were seen in all animals within ½ hour of dosing, but were reversible by day 5 in females and 3 in males. Decreased respiratory rate and/or laboured breathing were also noted in animals from 1 or 2 hours until day 2 in females and day 1 in males. All females also showed red/brown staining around the snout and mouth on days 1 and 2. Diarrhoea, prostration, piloerection as well as red/brown staining around snout, mouth or eyes were also intermittently seen in at least one of each of the males, up to day 1. One male also appeared comatose on days 1 and 2. No signs of toxicity were seen from days 3 and 5 onwards in males and females, respectively.
Morphological findings:	No treatment-related macroscopic findings were observed
LD_{50}:	> 2000 mg/kg
Result:	The notified chemical is of very low acute oral toxicity in rats

9.1.2 Dermal Toxicity (Driscoll, 1999b)

Species/strain:	Rat / Sprague-Dawley
Number/sex of animals:	5 males and 5 females
Observation period:	14 days
Method of administration:	2000 mg/kg, moistened with water and held against shaven intact skin, under semi-occlusive dressing, for 24 hours
Test method:	OECD TG 402
Mortality:	none
Clinical observations:	No signs of local or systemic toxicity were observed
Morphological findings:	No treatment-related macroscopic findings were observed
LD_{50}:	> 2000 mg/kg
Result:	The notified chemical was of low dermal toxicity in rats

9.1.3 Inhalation Toxicity

No data were submitted.

9.1.4 Skin Irritation (Driscoll, 1999c)

Species/strain: Rabbit / New Zealand White

Number/sex of animals: 1 female and 2 males

Observation period: 1, 24, 48 and 72 hours, and days 7 and 14

Method of administration: 0.5g, moistened with distilled water, applied to shaven intact skin, under semi-occlusive dressing for 4 hours

Test method: OECD TG 404

Draize scores:

Time after treatment (days)	***Animal #***		
	1	***2***	***3***
Erythema			
1	2	2	2
2	2	2	2
3	2	2	2
Oedema			
1	1	1	1
2	1	1	1
3	1	1	1

[a] see Attachment 1 for Draize scales

Comment: In addition to the scores above, well-defined erythema (grade 2) in all, slight oedema (grade 2) in one and very slight oedema (grade 1) in two animals were observed at 1 hour. Loss of elasticity was observed at 72 hours, moderate desquamation at day 7 and slight desquamation at day 14 were also noted at all treatment sites.
Mean scores for erythema = 2 in each animal.
Mean scores for oedema = 1 in each animal.

Result: The notified chemical was moderately irritating to the skin of rabbits.

9.1.5 Eye Irritation (Driscoll, 1999d)

Species/strain: Rabbit / New Zealand White

Number/sex of animals: 2 males and 1 female

Observation period: 1, 24, 48 and 72 hours and days 7, 14 and 21.

Method of administration: 0.1 ml placed into conjunctival sac of right eye. Left eye served as control.

Test method: OECD TG 405

Draize scores of unirrigated eyes:

	Time after instillation														
Animal	***1 day***			***2 days***			***3 days***			***7 days***			***14 days***		
Cornea	***o***		***a***	***o***		***a***	***o***		***a***	***o***		***a***	***o***		***a***
1	1[1]		3	1		1	1		1	0		0	-		-
2	1		4	1		4	1		4	2		3	2		2
3	1		4	1		4	1		4	1		1	0		0
Iris															
1	1			1			0			0			-		
2	1			1			1			0			0		
3	1			1			1			0			0		
Conjunctiva	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***
1	2	2	2	2	2	1	1	1	0	0	0	0	-	-	-
2	2	2	2	2	2	2	2	2	2	1	1	1	0	0	0
3	2	2	2	2	2	2	2	2	1	1	1	0	0	0	0

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

Comment: In addition to the above scores, dulling of the normal lustre of the cornea and moderate conjunctival irritation (grade 2) were observed in all treated eyes at 1 hour. Areas of translucent corneal opacity and vascularisation were seen in one treated eye on days 7, 14 and 21. Reversibility of this later effect in one animal was not observed within the time frame of the study.
Mean score for corneal opacity = 1 in each animal.
Mean scores for iris lesions = 0.67, 1 and 1 in the 3 animals respectively.
Mean score for both conjunctival redness and chemosis = 1.67, 2 and 2 in the 3 animals respectively.

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

9.1.6 **Skin Sensitisation** (Driscoll, 1999e)

Species/strain:	Guinea-pig / Dunkin Hartley
Number of animals:	10 test and 5 control animals with an additional group of 5 animals for the rechallenge test.
Induction procedure:	
test group:	
day 0	Three pairs of intradermal injections, of 0.1ml volume, were made in the scapular region: Freunds Complete Adjuvant (FCA) diluted (1:1) in distilled water; 10% test chemical in arachis oil BP; 10% test chemical in a mixture (1:1) of FCA and distilled water;
day 7	75% test material in arachis oil BP applied topically, under occlusive dressing for 48 hours, to same skin area as day 0 treatments.
control group:	
day 0	As for test group with the injections containing: FCA diluted (1:1) in distilled water; Arachis oil BP; 50% arachis oil BP in a mixture (1:1) of FCA and distilled water;
day 7	As for test group, using arachis oil BP alone.
Challenge procedure:	
day 21	50 and 75% test material in arachis oil BP, applied to previously untreated skin sites of test and control animals for 24 hours.
day 34	50 and 75% test material was used to rechallenge test animals at previously untreated skin sites. A new control group of animals was included at rechallenge. These animals had received intradermal injections of FCA but had not been exposed to test material.
Test method:	OECD TG 406 (Maximisation)

Challenge outcome:

	Test animals		***Control animals***	
Challenge concentration	***24 hours****	***48 hours****	***24 hours***	***48 hours***
50%	1/10**	0/10	0/5	0/5
75%	5/10	0/10	0/5	0/5
Rechallenge concentration				
50%	4/10	2/10	0/5	0/5
75%	2/10	2/10	0/5	0/5

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

Comment:

Intradermal Induction: Very slight or well defined erythema was noted at treatment sites in all test animals 24 and 48 hours after injection. Very slight or well defined erythema was also noted in all control animals at 24 hours and was still present in 4 control animals at 48 hours.

Topical Induction: Well defined erythema and incidents of slight oedema were noted at treatment sites in all tests animals 1 hour after treatment. Three animals also showed bleeding at 1 hour. Very slight or well defined erythema was recorded in test animals at 24 hours. Very slight erythema was noted at the treatment site in one control animal at 1 and 24 hours after topical induction. Bleeding was also noted in this control animal at 1 hour.

Challenge: All reactions seen at challenge were grade one erythema and oedema.
Most reactions seen at rechallenge were also grade one erythema and/or oedema, with one animal showing grade 2 erythema with both 50 and 75 % test chemical.

Conclusion: Taking into account the irritant properties of the notified substance, the irritant reactions seen in both test and control animals in this study during induction, the low severity of reactions seen at challenge and the reversibility of the challenge reactions within 48 hours, it is considered that the reactions noted at challenge were irritant reactions and are not an indication of sensitisation.

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

9.2 Repeated Dose Toxicity (Cormack et al, 1999)

Species/strain:	Rat / Sprague-Dawley
Number/sex of animals:	5 males and 5 females per treatment and control group
Method of administration:	Gavage
Dose/Study duration:	0, 15, 150, 500 mg/kg/day (control, low, mid and top dose groups, respectively) in Arachis oil BP, for 28 consecutive days
Test method:	OECD TG 407

Clinical observations:
No deaths occurred. Top and mid-dose males showed a reduced body weight gain during the first three weeks, although this did not always reach statistical significance and was likely to be related to a reduced food consumption also seen in these groups of animals. Top dose animals showed increased salivation from day 3 onwards, with associated red/brown staining and wetting of external body surface. Top dose females also showed increased incidents of diuresis and micturated brown coloured urine from day 10. Brown staining was noted on the cage tray liners of males and females from day 4 onwards.

Males and females treated with 150 mg/kg/day test chemical also showed increased salivation with red/brown staining and wetting of external body surface from day 10, with signs being more evident during the second half of the treatment period.

No signs of toxicity were observed in the low dose animals.

Behavioural assessments, functional performance tests and sensory reactivity assessments did not show any significant findings.

Clinical chemistry/Haematology
There were no changes in haematological parameters in any dose group throughout the study. Top dose males and females showed statistically significant increases in plasma cholesterol (65% increase compared to controls) and plasma bilirubin (87% increase in females and >100% in males). However the bilirubin levels were within historical control ranges and without any associated pathological findings were not considered to be of toxicological significance.

No changes were observed in the low-dose group.

Effects on the organs:
Top dose males and females showed dark kidneys and statistically significant increases in absolute (24% increase in males, 67% in females) and relative (54% increase in males, 71% in females) liver weights. Top dose males also showed statistically significant increases in relative kidney (22%) and heart (19%) weights and statistically significant decreases in absolute spleen (24%) and testes (11%) weights.

Males treated with 150 mg/kg/day showed a statistically significant decrease in absolute

spleen (20%) and increase in relative liver (15%) weights.

Microscopically, top dose males and females showed pigment accumulation in the renal proximal tubular epithelium and an increase in the incidence and/or severity of thyroid follicular cell hypertrophy and/or colloid depletion. The increased colloid depletion was seen in mid-dose males. Top dose males and females also showed centrilobular hepatocyte enlargement. This was not seen in lower dose groups and there was no evidence of degenerative or inflammatory changes in the liver in any of the dose groups.

Result:
A No Observed Effect Level (NOEL) of 15 mg/kg/day was identified with kidney and thyroid toxicity being evident at 150 mg/kg/day.

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* Reverse Mutation Assay (Thompson, 1999)

Strains:	*Salmonella typhimurium :* TA1535, TA1537, TA98, TA 100 *Escherichia coli :* WP2uvrA
Metabolic activation:	Aroclor-induced rat liver S9
Concentration range:	
Experiments 1 & 2	15, 50, 150, 500, 1500, 5000 μg/plate, with and without S9
Experiment 3	500, 1500, 2000, 3000, 4000 μg/plate with TA100, with and without S9 150, 300, 500, 1000, 1500 μg/plate with TA1535, without S9
Test method:	OECD TG 471 (plate incorporation method)
Comment:	The test chemical caused either a decrease in revertant colonies or a reduction in background lawn with 5000 μg/plate, in all tester strains, both with and without exogenous activation. Small but statistically significant increases in the number of revertant colonies were observed with TA 100, with and without exogenous activation, in experiments 1, 2 and 3. A dose-response relationship was evident in experiment 1 and 3 with activation and in experiment 2 without. However all increases were less than two-fold and are not considered positive reactions. Small but statistically significant increases in the number of revertant colonies were observed with TA 1535 in Experiment 1 with and without activation and in experiment 2 and 3 without. Only in experiment 1 without activation did the increases reach 2-fold and show a dose-response relationship. Overall the only result considered positive with TA 1535 was in experiment I, without activation. As this result was not reproducible the findings are questionable but suggest mutagenic activity.

Result: The notified chemical was weakly mutagenic under the conditions of the test.

9.3.2 *In Vitro* Mammalian Cell Gene Mutation Test (Wright and Nolan, 1999)

Cells: Mouse lymphoma L5178 TK +/-

Metabolic activation system: Phenobarbitone/B-naphthoflavone–induced rat liver S9

Dosing schedule:

Metabolic Activation	Experiment Number	Test concentration (μg/mL) and procedure	Controls
-S9	1	test concentrations = 40, 80, 160, 320, 480, 640μg/mL treatment time = 3 hours expression time = 2 days selection time = 10-14 days	Positive: EMS Negative: DMSO
	2	test concentrations = 25, 50, 100, 150, 200, 300μg/mL treatment time = 24 hours expression time = 2 days selection time = 10-14 days	
	3	test concentrations = 50, 100, 150, 200, 250, 300μg/mL treatment time = 24 hours expression time = 2 days selection time = 10-14 days	
+S9	1	test concentrations = 40, 80, 160, 320, 480, 640μg/mL treatment time = 3 hours expression time = 2 days selection time = 10-14 days	Positive: CP Negative: DMSO
	2	test concentrations = 50, 100, 200, 300, 350, 400μg/mL treatment time = 3 hours expression time = 2 days selection time = 10-14 days	

EMS - ethyl methanesulphonate
CP - cyclophosphamide
DMSO – dimethylsulphoxide

Test method: OECD TG 476

Comment: Expt.1 No statistically significant or dose-related increases were seen with or without exogenous activation. Toxicity was observed with 320 μg/mL and above and due to excessive toxicity, the top two dose plates were not included in the statistical analyses.

Expt.2 A statistically significant and dose-related increase in mutation frequency was seen without S9. The response was predominantly due to an increase in small colonies. No statistically significant or dose related increases were seen with S9. Toxicity was observed with 100µg/mL and above without S9 and with 400µg/mL with S9. Due to excessive toxicity without S9 the top dose plates were not included in the statistical analysis.

Expt.3 A statistically significant and dose-related increase in mutation frequency was seen without S9. The response was predominantly due to an increase in small colonies. Toxicity was observed with 100µg/mL and above; due to excessive toxicity, the top two dose plates were not included in the statistical analysis.

Vehicle and positive controls produced results within the expected ranges.

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

9.3.3 Micronucleus Assay in the Bone Marrow Cells of the Mouse (Durwood and Nolan, 1999)

Species/strain: Mouse / Crl:CD-1 (ICR)BR

Number and sex of animals: 7 males per group

Doses: 0, 300, 600 and 1200 mg/kg, animals were sacrificed 24 hours after treatment. Additional control and top-dose groups were sacrificed 48 hours after treatment. A positive control group was sacrificed 24 hours after treatment.

Method of administration: Oral gavage

Test method: OECD TG 474

Comment: There were no statistically significant increases in the frequency of micronuclei with test or control groups. The positive control group showed a marked increase in the frequency of micronuclei.

A statistically significant decrease in polychromatic erythrocyte to normochromatic erythrocyte ratio was observed with 300 and 1200 mg/kg in the 24-hour sacrifice groups. No deaths occurred. General signs of toxicity such as laboured breathing, ataxia, prostration, decreased respiratory rate and lethargy were seen with top dose animals.

Deaths occurred in a sighting study with an intraperitoneal

dose of 2000 mg/kg. General signs of toxicity, increasing in severity with dose, were observed with 800 mg/kg and above in an oral sighting study, with no differences in toxicity between males and females. Therefore males only were used in the main test.

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

9.4 Toxicokinetic Assessment (Blackwell, 1999)

The chemical is a substituted cyclopentylidene alcohol of molecular weight that does not preclude absorption. It is a low melting point, waxy solid of low volatility, therefore the chemical is unlikely to be available for inhalation. The chemical hydrolyses readily in an acidic environment, therefore exposure to degradation products may occur.

Acute and repeated dose oral studies showed clear evidence of systemic effects, indicating that absorption from the gastro-intestinal tract occurs. No signs of toxicity were observed in an acute dermal study, so there is no direct evidence to determine whether or not absorption via this route occurs. However the chemical has a high log P value indicating that absorption is possible via the dermal route. Hydrolysis may occur in the acid conditions of the stomach and it is therefore possible that hydrolysis products will also be available for absorption.

The log P value indicates that wide distribution and bioaccumulation is possible, however there was no evidence from the available information to support this. Following repeated oral dosing, effects were observed in the liver and thyroid, suggesting that metabolism by enzymes from these organs may occur. The most likely biotransformation is by oxidative processes and possibly also by hydrolysis. In view of the thyroid effects seen in the repeated oral study, it is possible that conjugation reactions may occur involving glucuronyl transferase.

Pigment deposits in the renal tubules and coloured urine were observed in the repeated oral study demonstrating excretion via the kidneys. In view of the limited water solubility, it is likely that metabolites are excreted via this route. The chemical is not sufficiently volatile for elimination via the lungs.

9.5 Overall Assessment of Toxicological Data

The notified chemical is of very low acute oral and low acute dermal toxicity. LD_{50} values of >2000 mg/kg were obtained in rodents for both routes of exposure. There was no assessment of the inhalation toxicity of the notified chemical. The notified chemical is a moderate skin irritant and a severe eye irritant. The results of a skin sensitisation study were difficult to interpret and data are insufficient to classify the notified chemical as a skin sensitiser.

A No Observed Effect Level (NOEL) of 15 mg/kg/day was identified in a 28 day repeated toxicity study in rats. Kidney and thyroid toxicity was evident at 150 mg/kg/day.

Genotoxicity was observed in *in vitro* studies. However the results of a mouse *in vivo* study

were negative indicating that genotoxic potential was not expressed *in vivo*. Therefore the notified chemical is not considered to be mutagenic to humans.

The notified chemical can be absorbed across the gastro-intestinal tract and dermal absorption is possible. Following absorption, wide distribution throughout the body is likely and biotransformation will occur. At least some excretion via the kidneys is expected.

The notified chemical should be classified Irritant (Xi) with the risk phrases Irritating to skin - R38 and Risk of Serious Damage to Eyes - R41 according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC 1999).

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

The following ecotoxicity studies have been supplied by the notifier. The tests were carried out according to OECD Test Methods.

Test	*Species*	*Results*
96 h Acute Toxicity	Rainbow Trout *Oncorhynchus mykiss*	LC_{50} = 42 mg/L NOEC = 18 mg/L
48 h Acute Toxicity	*Daphnia magna*	EC_{50} = 13 mg/L NOEC = 5.6 mg/L
72 h Growth Inhibition	Algae *Scenedesmus subspicatus*	E_bC_{50} = 29 mg/L E_rC_{50} = 36 mg/L NOEC = 18 mg/L
3 h Activated Sludge Respiration Inhibition	Activated Sewage Sludge	EC_{50} = 690 mg/L NOEC = 100 mg/L

* NOEC - no observable effect concentration

Rainbow trout (*Oncorhynchus mykiss*) were used in a 96 h semi-static acute toxicity study for the notified chemical (Safepharm Laboratories Ltd 2000e). The study was set up using 10 fish per test vessel. 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, 3 and 6 hours, then at every 24 hour period. The observations included mortality, visible abnormalities (eg appearance and behaviour), oxygen, temperature and pH. No visible abnormalities and no mortalities were observed in the test vessels with concentrations of less than 32 mg/L over the period of the study. At 32 mg/L all the fish were observed swimming at the surface and pigmentation increased over time while at 56 and 100 mg/L all fish died within the first 3 hours. Therefore, the LC_{50} was determined to be 42 mg/L and the no observable effect concentration (NOEC) to be 18 mg/L. This indicates that the notified chemical is slightly toxic to fish.

Daphnia was used in a 48 h static acute toxicity study for the notified chemical (Safepharm Laboratories Ltd 2000f). The study was set up using 20 animals per concentration distributed into 2 groups of 10 animals in glass beakers. The nominal concentrations of notified chemical

were 0, 1.8, 3.2, 5.6, 10, 18, 32, 56 and 100 mg/L. Observations were made at the start of the experiment then at every 24 hour period. The observations included immobility, oxygen, temperature and pH. No immobilisation was observed in concentrations below 5.6 mg/L, immobilisation increased between 10 and 18 mg/L while 100% was observed in concentrations at 32 mg/L and above. The EC_{50} was determined to be 13 mg/L and NOEC was 5.6 mg/L. These results indicate that the chemical is slightly to moderately toxic to daphnia.

Algae (*Scenedesmus subspicatus*) was used in a 72 h growth inhibition study for the notified chemical (Safepharm Laboratories Ltd 2000g). The study was set up using glass flasks with initial algae cell concentration of 10^4 cells/mL, with cell counts every 24 hours. The nominal concentrations of notified chemical were 0, 2.5, 5.0, 10, 20, and 40 mg/L and samples were taken at 0, 24, 48 and 71 hours. The E_bC_{50} was determined to be 29 mg/L, and the ErC_{50} was 36 mg/L. These results indicate that the chemical is slightly toxic to algae.

A mixed population of activated sewage sludge micro-organisms was used in the assessment of the inhibition on respiration of activated sewage sludge (Safepharm Laboratories Ltd 2000h). The test involved nominal concentrations of 10, 32, 100, 320 and 1000 mg/L of Indoclear and aeration for 3 hours at 21°C in the presence of activated sludge plus synthetic sewage as a respiratory substrate. The rate of respiration was measured after 30 minutes and 3 hours. The positive control was 3,5-dichlorophenol. The 3 hour EC_{50} and NOEC for Indoclear are 690 and 100 mg/L, respectively.

The ecotoxicity data indicate the notified chemical is slightly toxic to fish and algae and slightly to moderately toxic to daphnia.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The notified chemical will be used as a fragrance ingredient of domestic cleaning and personal care formulations, and most will eventually be released into domestic sewage systems as a consequence of product use. It is not readily biodegradable (14% over 28 days, CO_2 Evolution test), has a moderate n-octanol/water partition coefficient of 3.09, log K_{oc} of 2.34, moderate water solubility (466 mg/L at 20°C), all indicating that most would remain in the sewage water. Consequently, most of the released chemical is likely to be discharged eventually to receiving waters where some may associate with soils and sediments, and slowly degrade to water, carbon dioxide and methane through biological processes.

The ecotoxicity data indicate that the notified chemical is moderately toxic to daphnia and slightly toxic to fish and algae. However, based on annual imports of 1 tonne/annum, all of which is eventually released to sewer and not removed during sewage treatment processes, the daily release on a nationwide basis to receiving waters is estimated to be 2.74 kg/day. The predicted concentration in sewage effluent on a nationwide basis is estimated as 1 μg/L, as follows:

Amount of Indoclear entering sewer annually	970 kg
Population of Australia	18 million
Amount of water used per person per day	150 L
Number of days in a year	365
Estimated Predicted Concentration in sewer	0.001 mg/L (1.0 ppb)

When released to receiving waters the concentration is generally understood to be reduced by a factor of at least 10, so the Predicted Environmental Concentration (PEC) is around 0.1 μg/L. The notifier also provided a PEC based on 100% use of the compound in a large capital city (Melbourne). This calculation gave a PEC value of 0.6 μg/L after release to receiving waters.

Both PEC estimates indicate that after discharge to receiving waters the environmental concentration of the new compound will be nearly four orders of magnitude less than the demonstrated toxicity to the *Daphnia magna* (EC_{50} = 13 mg/L).

The above considerations indicate minimal hazard to the environment when the notified chemical is used as a component of domestic products in the manner indicated by the notifier.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Assessment of Toxicological Hazard

The notified chemical is of very low acute oral and low acute dermal toxicity. There was no assessment of the inhalation toxicity of the notified chemical. The notified chemical is a moderate skin irritant and a severe eye irritant. Positive reactions were observed in a skin sensitisation study but given the irritant properties of the notified chemical, were insufficient to classify the notified chemical as a skin sensitiser.

A No Observed Effect Level (NOEL) of 15 mg/kg/day was identified in a 28 day repeated toxicity study. Kidney and thyroid toxicity were evident at 150 mg/kg/day.

The notified chemical is not considered to be mutagenic to humans.

The notified chemical can be absorbed across the gastro-intestinal tract and dermal absorption is possible.

The notified chemical should be classified Irritant (Xi) with the risk phrases Irritating to skin - R38 and Risk of Serious Damage to Eyes - R41 according to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC 1999).

Occupational Health and Safety

Exposure to up to 1% Indoclear may occur during warehouse activities, production of products, cleaning of equipment and sampling or analysis tasks. Acute dermal and ocular exposure may occur during accidental splashing. Cleaning operations are likely to involve the highest exposures.

The notified chemical is a skin irritant and a severe eye irritant. Therefore, dermal, ocular and respiratory contact with the notified chemical should be avoided. Despite the low concentration of notified chemical in the imported product, personal protective equipment consisting of impervious clothing, gloves and eye protection should be worn when handling the notified chemical. Such personal protection with the addition of facial protection would be indicated if exposure to hazardous concentrations of the notified chemical is envisaged.

Taking into account the limited exposure and very low concentration of notified chemical likely to be encountered occupationally, the risk of adverse health effects following occupational exposure to Indoclear, as handled in Australia, is expected to be low.

Public Health

The notified chemical will be sold to the public in a variety of personal and household products. Air fresheners may contain up to 1% of the notified chemical, with other products likely to contain around 0.005%.

The notifier has submitted estimations of consumer exposure, based on the daily use of a skin cream, a soap or shower gel, and a household product containing 0.005% of the notified chemical. At an estimated exposure of 0.0088 mg/kg/day, the safety margin (compared with the NOEL from the 28-day study) is 1705. Consequently, based upon the low concentrations in products available in the public domain, the risks to public health associated with the notified chemical are likely to be low.

13. RECOMMENDATIONS

Labelling

Suppliers should label Indoclear with the signal word Irritant (Xi) and the risk and safety phrases:

R38	Irritating to skin
R41	Risk of serious damage to eyes
S24	Avoid contact with skin
S25	Avoid contact with eyes
S26	In case of contact with eyes, rinse immediately with plenty of water and seek medical help
S39	Wear eye/face protection

Hazard classification

The NOHSC Chemicals Standards Sub-committee should consider the above health and physico-chemical hazard classification for Indoclear.

Occupational Health and Safety

To minimise occupational exposure to Indoclear, the following guidelines and precautions should be observed:

- Eye protection, chemical resistant industrial clothing and footwear and impermeable gloves should be used during occupational use of the notified chemical. Where engineering controls and work practices do not preclude the potential for aerosol and vapour exposure, a negative pressure organic vapour respirator should also be used;
- Spillage of the notified chemical should be avoided. Spillages should be cleaned up promptly with absorbents which should then be put into containers for disposal;
- A copy of the MSDS should be easily accessible to employees.

Guidance in selection of protective eyewear may be obtained from Australian Standard (AS) 1336 (Standards Australia, 1994) and Australian/New Zealand Standard (AS/NZS) 1337 (Standards Australia/Standards New Zealand, 1992); for industrial clothing, guidance may be found in AS 3765.2 (Standards Australia, 1990); for impermeable gloves or mittens, in AS 2161.2 (Standards Australia/ Standards New Zealand, 1998); for occupational footwear, in AS/NZS 2210 (Standards Australia/ Standards New Zealand, 1994a); for respirators, in AS/NZS 1715 (Standards Australia/ Standards New Zealand, 1994b) and AS/NZS 1716 (Standards Australia/ Standards New Zealand, 1994c) and other internationally acceptable standards.

If products containing the notified chemical are hazardous to health in accordance with the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999), workplace practices and control procedures consistent with State and Territory hazardous substances regulations must be in operation.

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* (NOHSC, 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, the director must be informed if any of the circumstances stipulated under subsection 64(2) of the Act arise, and secondary notification of the notified chemical may be required. No other specific conditions are prescribed.

16. REFERENCES

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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.
		Swelling with lids half-closed	3 mod.	Discharge with moistening of lids and hairs and considerable area around eye	3 severe
Diffuse beefy red	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

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