File No: NA/711

September 2000

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

AGARBOIS

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Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT

AGARBOIS

1. APPLICANT

Quest International of 6 Britton Street SMITHFIELD NSW 2164 has submitted a standard notification statement in support of their application for an assessment certificate for N-ethyl-N-(3-methylphenyl) propionamide.

2. IDENTITY OF THE CHEMICAL

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

Chemical Name: N-ethyl-N-(3-methylphenyl) propionamide

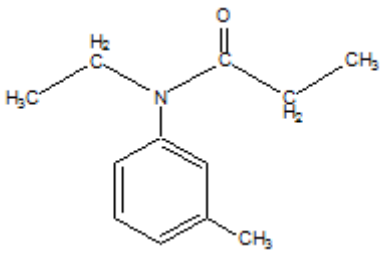
Chemical Abstracts Service (CAS) Registry No.: 179911-08-1

Other Names: none

Trade Name: Agarbois

Molecular Formula: $C_{12}H_{17}NO$

Structural Formula:



Molecular Weight: 191

Method of Detection and Determination: ultraviolet-visible (UV/Vis), nuclear magnetic resonance (NMR) and infrared (IR) spectroscopy and GC/MS spectroscopy

Spectral Data: major characteristic IR peaks were identified at the following wavelengths: 2 930, 2 810, 1 720, 1 630, 1 610, 1500, 1 375, 1 200, 1 100, 1 050 and 760 cm^{-1}.
UV/Vis spectrum: absorbance maxima in ethanol 215 nm

Comments on Chemical Identity

The notified chemical is a simple well defined aromatic amide derived from 3-methylaniline (3-methyltoluidine). The primary N-ethyl-N-(4-methylphenyl) propionamide is present in trace quantities, and is due to the presence of a small quantity of 4-methyltoluidine in the precursor materials used for synthesising the notified chemical. Similarly, the trace of N-ethyl-N(3-methylphenyl) acetamide is due to small amount of acetyl chloride in the precursors.

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa:	colourless to pale yellow non viscous liquid
Freezing Point	10°C at 101 kPa
Boiling Point:	274°C at 101 kPa
Relative Density:	0.99174 at 20°C $\pm$ 0.5°C
Vapour Pressure:	3.35x 10^{-1} kPa at 25°C
Water Solubility:	4.1 g/L at 20°C $\pm$ 0.5 °C (see notes below)
Partition Co-efficient (n-octanol/water):	log P_{ow} = 2.03 at 20°C
Henry's Law Constant	15.6 Pa/m^3/mole (calculated, see comments below)
Hydrolysis as a Function of pH:	$t_{1/2}$ > 1 year at 25°C and at pH 4, 7 and 9 (see comments below)
Adsorption/Desorption:	not determined (see comments below)
Dissociation Constant:	not determined (see comments below)
Surface Tension:	moderately surface active (see comments below)
Flash Point:	136 $\pm$ 2°C
Flammability Limits:	not classified as flammable
Autoignition Temperature:	400 $\pm$ 5°C
Explosive Properties:	not determined; experience with materials with a similar structure indicates that it is not likely to be

	explosive
Particle Size:	not applicable
Reactivity/Stability:	the notified chemical is stable at ambient temperatures; contact with strong acids, alkali and oxidising agents should be avoided

Comments on Physico-Chemical Properties

The water solubility was determined by stirring an excess of the test substance with 100 mL of distilled water at 30ºC, equilibrating for not less than 24 hours at 20ºC, and then separating the aqueous and non aqueous layers by centrifugation and filtration. The content of the notified chemical in the aqueous phase was then determined by High Performance Liquid Chromatography (HPLC). The average of three separate determinations gave the water solubility as 4 100 ± 50 mg/L at 20°C.

The Henry's law constant was calculated from the molecular weight, the measured water solubility and vapour pressure from the equation:

H= MW (g/mole) x Vapour Pressure (Pa)/Water solubility (g/L).

The notified chemical contains a single aromatic amide linkage which may be susceptible to hydrolysis under extreme pH conditions, but is stable (half life > 1 year) in the environmental pH region where 4<pH<9. The rate of hydrolytic degradation of aqueous solutions containing measured concentrations of the test material (1 470-1 610 mg/L) were determined at pH 4, 7 and 9 at 50°C over a five day test period. Samples were analysed for the non degraded Agarbois at three different times after commencement of the tests (approximately 24 h, 48 h and 120 h) using HPLC. The percentage loss was used to derive the half lives listed above assuming pseudo-first order kinetics. This data is interpreted to indicate a half life of greater than one year at 25°C under the usual environmental pH conditions.

The n-octanol/water partition coefficient was determined using the HPLC method, where the retention time of the test compound on C_{18} columns is compared with those of eight reference compounds with known values for K_{ow} ranging from 1.6 (benzonitrile) to 5.7 (triphenylamine). The relatively low value for log K_{ow}, determined as 2.03 indicates the new chemical has a low affinity for hydrocarbon like environments.

The notifier indicated that log K_{oc} was not determined due to the volatility of the compound. However, this parameter can be estimated from that for Log K_{ow} using the relationship:

$\log K_{oc} = 0.33 \times \log K_{ow} + 1.25$

which provides an estimate of 1.92 for this quantity. This relationship is appropriate for compounds normally classified as amides, and is a member of a class of QSARs (quantitative structure activity relationships) recommended by the EEC for calculating K_{oc} for various classes of organic compounds (European Commission, 1996a). The calculated value for Log K_{oc} as 1.92 indicates that the chemical has a small tendency to partition into the organic

component of soils and sediments, and become associated with these materials. However, the small value of log K_{oc} indicates only weak binding, and this, together with the high water solubility indicates that it would be mobile in soils.

The compound contains no functionalities capable of dissociating or otherwise becoming ionised in aqueous media, and the notifier indicates that dissociation constant data are not applicable.

The surface tension of an aqueous solution containing approximately 1 110 mg/L (37% saturation) of the test substance was 54.5 m/Nm at 21±0.5°C (water = 72.6 m/Nm), which indicates the material is moderately surface active.

Calculations based on the molecular structure using the QSARs of the US Environment Protection Agency ASTER database (USEPA, 1998) furnished the following estimates for environmentally relevant physico-chemical parameters. Where comparison with data supplied by the notifier is possible, the agreement is reasonable except for the estimated value of vapour pressure and the derived value of the Henry's Law constant which are significantly lower than the corresponding data listed above.

ASTER DATA (all calculated using QSARs)

PROPERTY	***QSAR ESTIMATE***
Boiling Point	270 °C
Vapour Pressure	1.7 x 10^{-4} K Pa
Water Solubility	624 mg/L
Henry's Law Constant	0.0522 Pa/m^3/mole
log K_{ow}	2.66
log K_{oc}	2.79
Hydrolysis	hydrolytic degradation $t_{1/2}$ = 190 days.

4. PURITY OF THE CHEMICAL

Degree of Purity: > 99%

Hazardous Impurities: none

Non-hazardous Impurities: < 0.2% of 4-methylphenyl isomer due to traces of N-ethyl-p-toluidine in the starting material; < 0.1% of N-ethyl-N-(3-methylphenyl) acetamide due to traces of

acetyl chloride in the starting acid chloride

Additives/Adjuvants: none

5. USE, VOLUME AND FORMULATION

The notified chemical will not be manufactured in Australia. It will be imported as a component of compounded fragrances and added to consumer products such as air-fresheners, personal washing products and household cleaners. The notifier indicated that compounded fragrances may contain up to 25% of the notified chemical, but 20% was more typical.

Typically end use products would contain 0.3% fragrance (containing 20% of the new chemical) giving a final concentration of 0.06% of the notified chemical in the product.

Import volumes for the notified chemical will be up to 5 tonnes per annum for each of the first five years.

6. OCCUPATIONAL EXPOSURE

The notified chemical will be imported in 200 L lacquered or polythene lined steel kegs. The total imported volume will be transported by road to the notifier's warehouse. At the warehouse the steel kegs will be unloaded from the container, stored and shipped to product manufacturers. The notifier has not submitted information on packaging of end use products.

Transport and Storage
Transport workers and storemen are unlikely to be exposed to the notified chemical except when packaging is accidentally breached.

Reformulation
Agarbois will be used to formulate consumer products. The notifier has not provided a detailed description of the processing operations, as final product manufacturers have not been identified. It is anticipated that reformulation would be carried out predominantly in closed systems. If open vessels are used for mixing, adequate ventilation should be provided to remove aerosols that may arise during the process. It is very likely that the product containing the notified chemical will be added to the mixing vessel manually.

Exposure to the notified chemical is possible when opening and closing kegs, weighing and charging it to the blending vessel, mixing in open vessels and cleaning operations. Possible routes of exposure would be inhalation, ocular and dermal. However, inhalation exposure is unlikely to be significant due to the low vapour pressure of the notified chemical.

The notifier states that all workers handling the notified chemical and involved in open mixing operations should wear suitable gloves, eye and face protection and protective clothing. The notifier anticipates that between 5 to 20 workers, including warehouse

workers, production workers and laboratory workers, could be potentially exposed to the notified chemical for not more than 1 hour/day, 50 to 200 days per year.

7. PUBLIC EXPOSURE

The general public will be repeatedly exposed to a low concentration of the notified chemical via a number of different consumer products. The air fresheners (solid or liquid) containing up to 25% Agarbois are designed to release fragrance slowly over 30-90 days. Other products (leave on, rinse off and household products) which contain 0.06% of Agarbois include skin creams, soaps and shower gels, and household cleaners.

The majority of the notified chemical will be released to the environment through the sewage system and mainly from the final use of the products (e.g. being washed from the hair and skin). Used containers containing 1% of residual material will be discarded via the household waste system.

8. ENVIRONMENTAL EXPOSURE

Release

The notifier indicated that production activities involving use of the notified chemical would be performed by a number of different companies (number and locations were not known by the notifier at the time of the submission), and it is expected that production activities will take place in purpose constructed facilities.

The notifier indicates that around 1% of the new chemical (annually 50 kg) may be lost as a consequence of cleaning the blending and filling equipment, and this would be discharged to the sewer system. Assuming production takes place for 200 days each year, this equates to a daily release of 250 grams. No reference to the quantities of chemical likely to be lost and released as results of accidental spillage was made in the submission. However, it is estimates that a further 1% of total import quantity could be lost through accident, which amounts to an annual release of another 50 kg, and this again is expected to be washed into the sewer system.

The empty steel and polythene drums of fragrance will be washed and reused. No estimates of the amount of residual chemical left in the drums was presented in the application, but it is estimated that this could amount to 0.5 - 0.1% of the import quantity, or around 2.5-5.0 kg per annum. It is probable that this would also be washed into the sewer. Consequently it is estimated that annually around 100 - 105 kg of the imported chemical could be discharged directly to the sewerage system as a consequence of formulating and manufacturing activities.

However, the new chemical is a fragrance for use in domestic cleaning and personal care products, and consequently all will be eventually released into the environment as a result of normal product usage. It is expected that a high proportion of the chemical would be released

into the sewerage system, although due to the moderate vapour pressure some would be expected to volatilise and be directly released to the atmosphere.

Empty containers of the consumer products are likely to contain some residual unused product, and these packages would be discarded with domestic garbage and be disposed of into landfill. However, this release could be expected to be uniform across the nation, and consequently very diffuse, and at low levels.

Fate

- **Models**

All new chemical will eventually be released into the environment, and the majority could be expected to be discharged into sewerage systems. However, once released in this manner the moderately high vapour pressure indicates some partitioning to the atmospheric compartment. For that proportion of the chemical which reaches sewage treatment plants (ie is not volatilised or otherwise destroyed during passage to the plant), it is possible to estimate the equilibrium partitioning of the chemical from the SimpleTreat Model (European Commission, 1996b). These estimates are based on the chemical having a calculated Henry's Law Constant of 15.6 $Pa.m^3$/mole, a log K_{ow} = 2.03 and not being biodegradable (see further below). The model indicates that the chemical could be expected to partition into the air, water and sewer sludge compartments as follows:

Air	*Water*	*Sewer Plant Sludge*
5%-40%	53-94%	1-6%

Although the calculated estimates of vapour pressure and Henry's Law Constant from the ASTER database (USEPA, 1998) are lower than the experimental data, Mackay Level 1 calculations based on the calculated data also indicate that at equilibrium the chemical would partition primarily to water. The Mackay model also assumes equilibrium is established between all phases, and the partitioning into the various environmental compartments resulting from this model is:

Atmospheric compartment	1.68%
Soil compartment	3.36%
Sediment compartment	3.13%
Water compartment	91.82%
Aquatic biota compartment	0.00%

However, in the environment an equilibrium state will not be reached as chemical which reaches the atmosphere will be effectively removed from the system by diffusion and degradation through reaction with hydroxyl radicals (see further below). This mechanism will continuously remove the compound from the water compartment.

- **Biodegradation**

The notifier provided a laboratory report on the assessment of the biodegradation of Agarbois conducted in accordance with the OECD Test Guideline TG 301F (Manometric Respirometry Test). The results of this test (performed in triplicate) indicated only 0.2 % loss of initial COD of the test material after 28 days, while the reference substance (sodium benzoate) was 80% degraded after 28 days. Accordingly the compound Agarbois cannot be classed as either readily biodegradable or as inherently biodegradable.

- **Atmosphere**

Once released to the atmosphere, the chemical would be quickly decomposed through photolytically promoted free radical reactions. Hence, over time the sediment/water and water/air partitioning will be driven toward the loss of the chemical to the atmosphere. In the atmosphere it is likely that the substance will be degraded through reaction with hydroxyl radicals (primarily through hydroxyl addition to the aromatic moiety). A calculation based on the methods described in OECD (1992), indicate that in the troposphere the new chemical would react in this manner with a rate constant estimated as 17×10^{-12} cm^3 molecule/sec. Rate constants of this order are indicative of fast degradation in the troposphere (OECD, 1992), and the compound is not expected to persist in the atmosphere. Assuming a typical atmospheric concentration of hydroxy radicals of 5×10^5 radicals/cm, this rate constant gives an atmospheric half life for the compound of approximately 22 hours.

- **Sediment**

The new chemical is not hydrophobic and has a moderate partition coefficient log K_{ow} = 2.03 and estimated log K_{oc} = 1.92. Consequently when released into the sewer system it is unlikely to become strongly bound to soils and sediments and is expected to be mobile.

- **Soil**

Residual chemical disposed of to landfill with empty drums, discarded consumer packaging or with residual solids derived from water treatment at the production facilities would also be expected to volatilise and enter the atmosphere. However, despite the low estimated value for log K_{oc} some chemical may become weakly associated with the organic component of soil particles and would be expected to be eventually destroyed by abiotic and slow biological processes (particularly anaerobic processes). Any waste material containing the notified chemical placed into compost facilities could also be expected to be destroyed through aerobic and anaerobic biological degradation processes. Incineration of material containing the new chemical would decompose the substance with production of water vapour and oxides of carbon and nitrogen.

- **Bioaccumulation**

The ASTER calculations mentioned above also provide an estimate of 51 for the bioaccumulation factor for the compound in fish (fathead minnow), indicating the compound

has little potential for bioaccumulation. The compound is soluble and volatile and is not expected to have prolonged residence times in the aquatic compartment.

9. EVALUATION OF TOXICOLOGICAL DATA

Tests were conducted according to EEC and OECD test guidelines. Testing facilities complied with the OECD principles of Good Laboratory Practice and full study reports were provided. All tests were performed on the notified chemical.

9.1 Acute Toxicity

Summary of the acute toxicity of the notified chemical

Test	*Species*	*Outcome*	*Reference*
acute oral toxicity	rat	LD_{50} > 2 000 mg/kg	(McRae, 1998)
acute dermal toxicity	rat	LD_{50} > 2 000 mg/kg	(McRae, 1996)
skin irritation	rabbit	moderate irritant	(Parcell, 1996)
eye irritation	rabbit	moderate irritant	(Watson, 1996)
skin sensitisation	guinea pig	non sensitiser	(Selbie & Lea, 1996)

9.1.1 Oral Toxicity (McRae, 1998)

Species/strain:	rat/Sprague Dawley
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	oral (gavage), single dose 2 000 mg/kg in water
Clinical observations:	all animals showed piloerection, increased salivation, hunched posture, pallor of the extremities, waddling gait, lethargy, decreased respiration, partially closed eyelids, unsteadiness, cold body surface and prostration (collapsed state); all males recovered by day 4 and all females by day 5
Mortality:	none
Morphological findings:	none

Test method:	EEC Directive 92/69/EEC, Part B, Method B.1
LD_{50}:	> 2 000 mg/kg
Result:	the notified chemical was of very low acute oral toxicity in rats

9.1.2 Dermal Toxicity (McRae, 1996b)

Species/strain:	rat/Sprague Dawley
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	single dose of 2 000 mg/kg body weight applied to the shorn dorsolumbar skin, under semi-occlusive dressing for 24 hours
Clinical observations:	desquamation (dryness/sloughing/scaling or localised spot scab formation) was observed in 4 females during the early part of the study; one animal exhibited slight erythema on day 3; no erythema or oedema was observed in other animals; no clinical signs of systemic toxicity in any animal
Mortality:	none
Morphological findings:	none
Test method:	EEC Directive 92/69/EEC, Part B, Method B.3
LD_{50}	> 2 000 mg/kg
Result:	the notified chemical was of low acute dermal toxicity in rats

9.1.3 Inhalation Toxicity

Study not provided.

9.1.4 Skin Irritation (Parcell, 1996a)

Species/strain:	rabbit/New Zealand white

Number/sex of animals:	3 (sex not specified)
Observation period:	14 days
Method of administration:	a single, 4 hour semi-occlusive application of 0.5 mL of the test substance to shaved dorsal skin
Mortality:	none
Morphological findings:	none
Test method:	EEC Directive 92/69/EEC, Part B, Method B.4

Clinical observations:

Draize scores

Rabbit number		***Day***													
		1*	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***	***13***	***14***
1	E	0	1	2	2	2	2a	1a	1a	1a	0a	0a	0a	0a	0
	O	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	E	0	2	2	2	2	1	1	1	1	1	1	1	1	1
	O	0	1	0	0	0	0	0	0	0	0	0	0	0	0
3	E	0	2	2	2	2	2a	1a	1a	1a	1a	1a	1a	1a	1a
	O	0	1	1	2	1	0	0	0	0	0	0	0	0	0

* approximately 60 minutes after removal of the dressing
a desquamation (characterised by dryness and sloughing)
E erythema; O oedema

Comment:	all test animals demonstrated slight to well defined erythema with or without slight oedema during the first 24 hours post application; erythema persisted throughout the observation period in 2 animals and resolved on day 10 in the other animal; desquamation was observed in 2 animals from day 6 through to day 13 and 14 respectively; no signs of toxicity or ill health was noted
Result:	the notified chemical was a moderate skin irritant in rabbits

9.1.5 Eye Irritation (Watson, 1996)

Species/strain: rabbit/New Zealand White

Number/sex of animals: 3 (sex not specified)

Observation period: 14 days

Method of administration: 0.1 mL of the test substance was applied to the conjunctival sac of one eye of each animal; the contra-lateral eye served as a control

Test method: EEC Directive 92/69/EEC, Part B, Method B.5

Clinical observations:

Draize scores of unirrigated eyes:

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

[1] see Attachment 1 for Draize scales
o = opacity a = area r = redness c = chemosis d = discharge
* = not provided

Comments: corneal opacity developed in one animal (only) within 24 hours of instillation but resolved by day 7; conjunctival redness and chemosis was observed one hour post application in all test animals; conjunctival changes resolved in 2 to 3 animals by day 7 and by day 14 in the third rabbit; none of the animals exhibited signs of toxicity or ill health

during the observation period

Result: the notified chemical was slight to moderate eye irritant in rabbits

9.1.6 Skin Sensitisation (Selbie and Lea, 1996)

Species/strain: guinea pigs/albino Dunkin- Hartley

Number/sex of animals: 10/sex (test group); 5/sex (control group)

Induction procedure: Test Group
day 1: 3 pairs of intradermal injections (0.1 mL) were made on shaved shoulder area of each animal:

- front row: Freund's Complete Adjuvant (FCA) without notified chemical emulsified with 0.9% physiological saline (1:1 w/v)
- middle row: 0.3% (w/v) of the notified chemical in 0.01% dodecylbenzene sulphonate in 0.9% physiological saline
- back row: 0.6% (w/v) of the notified chemical in physiological saline mixed 1:1 with FCA to give a final concentration of 0.3% (w/v) of the notified chemical

control groups were treated similarly without the notified chemical

day 8:-(topical induction)-dermal application of 100% test substance under occlusive dressing for 48 hours; control animals were treated similarly but without test substance

Challenge procedure: day 22: one occluded application to the shaved flank of a 50% solution of the notified chemical in Alembicol D and another occluded solvent application to the opposite flank for 24 hours

Test method: OECD TG 406; Magnusson & Kligman method

Challenge outcome:

	Test animals		Control animals	
Concentration of notified chemical	***24 hours[a]***	***48 hours[a]***	***24 hours[a]***	***48 hours[a]***
50%[c]	[b]0/20	0/20	0/10	0/10

[a] time after patch removal
[b] number of animals exhibiting positive response
[c] 50% notified chemical in distilled water

Comments: test and control animals demonstrated intense skin irritation in both induction periods; cutaneous abnormalities were not observed on challenge; chemical related toxicity was not detected

Result: the notified chemical was not a skin sensitiser in guinea pigs at a challenge concentration of 50%

9.2 Repeated Dose Toxicity (Horner, 1997)

Species/strain: rat/Alpk: Apf SD (Wistar derived)

Number/sex of animals: 5/sex/dose group; 5/sex/recovery group

Method of administration: gavage; corn oil as vehicle

Dose/Study duration:: 0, 50 (low dose), 150 (mid dose), 1 000 (high dose) mg/kg/day for 28 consecutive days; recovery groups at 0 and 1 000 mg/kg/day (recovery period 28 days)

Test method: OECD TG 407

Clinical observations

Salivation and/or staining around the mouth and nose were observed in mid and high dose males and high dose females (salivation only) but not in the recovery group.

No mortality, adverse effects on body weight gain or food consumption were observed in study or recovery group animals throughout the study. Slightly higher water consumption was observed in all high dose animals.

Clinical chemistry

The following observations were made in week 4, in all high and mid dose animals: slight increase in mean total plasma protein, slight decrease in mean albumin/globulin ratio and increased mean plasma cholesterol levels. High dose animals also demonstrated increased plasma triglyceride levels and lower mean total plasma bilirubin (males only). All changes resolved following a 28-day recovery period.

Incidental findings unrelated to treatment included: higher overall motor activity in low dose males, high alkaline phosphatase activity in one high dose male and inter-group differences in plasma albumin and plasma alanine aminotransferase activity in high dose recovery group males only.

Low dose animals did not demonstrate treatment-related changes in blood clinical chemistry parameters.

Haematology

There were no changes in any of the haematology parameters that could be attributed to administration of the notified chemical.

Incidental haematological findings (as determined by the lack of a clear dose response) included: higher mean white blood cell and lymphocyte count in high dose males, higher mean platelet and large unstained cell counts and lower mean activated partial thromboplastin time in mide dose males, lower mean haemoglobin levels and mean cell haemoglobin concentration in low and high dose females and lower mean red cell counts and higher mean cell volumes in low and/or mid dose females.

Organ weights

An increased mean liver:body weight ratio was observed in mid dose males (by 11%) and high dose males and females (by 22% and 29% respectively). High dose males demonstrated a decrease in mean heart:body weight ratio (by 13%). No changes were observed in mean liver and heart weights in high dose animals of either sex following the 28 day recovery period.

No treatment related effects on organ weight parameters were noted in low dose animals.

Gross pathology

No treatment related macroscopic changes were observed at necropsy.

Histopathology

High dose males demonstrated a bilateral slightly increased incidence and severity of renal basophilic tubules and increased severity of renal tubular hyaline droplet formation in the kidneys. The incidence and severity of bilateral renal basophilic tubules persisted to week 8 in high dose males within the recovery group, however hyaline droplet formation did not progress through the recovery phase. No kidney changes were observed in low and mid dose males or females at any dose level.

Minimal reversible centrilobular hypertrophy of the liver was noted in high dose males.

Comment

All indications of liver involvement, as suggested by changes to blood clinical chemistry parameters (eg plasma albumin/globulin ratio, total plasma protein, cholesterol and triglycerides), increased liver weights and centrilobular hypertrophy were reversed by the end of the recovery period. This is suggestive of an adaptive response.

Renal tubular basophilia was irreversible at the high dose. Renal tubular hyaline droplet formation was reversible.

Result

On the basis of renal effects at 1 000 mg/kg/day and as the increased relative liver weight seen at 150 mg/kg/day was not supported by histological changes, the No Observed Adverse Effect Level (NOAEL) is determined at 150 mg/kg/day. Based on clinical signs, alterations in blood clinical chemistry parameters and liver and kidney effects seen at higher doses, the NOEL is 50 mg/kg/day.

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* Reverse Mutation Assay (Grant, 1996)

Strains:	*S. typhimurium* TA 98, TA 100, TA 1535 and TA 1537
Concentration range:	test 1 – preincubation method; 0, 15, 50, 150, 500, 1 500 and 5 000 μg/plate in the presence or absence of metabolic activation provided by Aroclor-1254 induced rat liver S9 fraction test 2 – standard plate method; similar to above dosage and conditions minus 15 μg/plate dose
Positive controls:	without S9: N-ethyl-N`-nitro-N-nitrosoguanidine

	(TA 1535 and TA 100); 9-aminoacridine (TA 1537) and 2-nitroflurene (TA 98) with S9: 2-aminoanthracene (TA 1535,TA 1537, TA 98 and TA 100)
Negative control:	dimethyl sulphoxide (DMSO)
Test method:	OECD TG 471
Comment:	no substantial increases in relevant colonies were observed at any dose level in the presence or absence of S9; toxicity was observed towards all strains at 5 000 and 1 500 μg/plate in both tests positive and negative controls responded appropriately
Result:	the notified chemical did not induce gene mutations in the strains of bacteria tested with or without metabolic activation under the conditions of the experiment

9.3.2 Chromosomal Aberration in Human Lymphocytes *in vitro* (Fox, 1997a)

Cell lines:	duplicate lymphocyte cultures from healthy human male donors, stimulated to divide with phytohaemagglutinin (PHA)
Treatment regime:	donor 1 - phenobarbitol and β naphthoflavone induced rat liver microsomal preparations (S9 fraction) were added to cultures treated for 68 hours with 50*, 250* or 500* μg/mL of the notified chemical; cultures without S9 were also treated for 68 hours with 25*, 150* or 250* μg/mL donor 2 was treated similarly in addition cultures were harvested for 92 hours with or without S9 fraction with 500 μg/mL and 100 μg/mL of the notified chemical respectively 1000 cells were examined per dose level; concentrations selected for analysis are marked '*' above for donor 1; concentrations analysed from donor 2 were 500 μg/mL (with S9) and 100 μg/mL (without S9)

Positive controls:	0.2 μg/mL mitomycin C and 50 μg/mL cyclophosphamide
Negative control:	DMSO
Test method:	OECD guideline TG 473
Comment:	small but statistically significant increase of up to 6% (historical control range 5.5%) in the number of chromosomal aberrations (either including or excluding gaps) occurred in both donor 1 and 2 cultures at 68 hour sampling time in the absence of rat liver S9 fraction similar increase was also noted at 92 hour sampling time in donor 2 cultures in the absence of S9 positive and negative controls responded appropriately and all conditions for a valid study were met
Result:	the notified chemical is weakly clastogenic *in vitro* under the conditions of the experiment

9.3.3 Micronucleus Assay in the Bone Marrow Cells of the Mouse (Fox, 1997b)

Species/strain:	mouse/CD-I
Number and sex of animals:	5/sex/dose
Doses and sampling times:	experiment 1 - treated with 125, 250 and 500 mg/kg of the notified chemical; 65 mg/kg of cyclophosphamide (positive control) and corn oil 10 mL/kg (negative control); 24 hour bone marrow sampling (all doses and controls) and 48 hour sampling (negative control and 500 mg/kg only) experiment 2 - conducted under the same conditions with bone marrow sampling at 24 hours (all doses and controls) only
Test method:	OECD TG 474
Comment:	in experiment 1 a small but statistically significant increase was observed in the incidence of micronucleated polychromatic erythrocytes in

	males dosed at 250 and 500 mg/kg at 24 hour sampling; this effect was not observed at 48 hours at 500 mg/kg
	a similar increase was observed in females dosed at 125 mg/kg
	these results were not reproduced in experiment 2
	positive and negative controls responded appropriately and all conditions for a valid study were met
Result:	under the conditions of the test potential for weak clastogenicity cannot be excluded

9.4 Overall Assessment of Toxicological Data

The notified chemical was of very low acute oral and low acute dermal toxicity (oral and dermal LD_{50} > 2 000 mg/kg, respectively) in rats. It was a moderate skin irritant and a slight to moderate eye irritant in rabbits but not a skin sensitiser in guinea pigs. No data were provided to determine the acute inhalation toxicity potential of the notified chemical.

In a 28-day oral repeated dosing study including a 4 week recovery period, changes in blood chemistry and histopathological changes in the liver were suggestive of reversible liver involvement. These effects were determined to be adaptive in nature. Renal effects observed only in high dose male animals included irreversible tubular basophilia and reversible tubular hyaline droplet formation. Based on renal effects the NOAEL was determined to be 150 mg/kg/day. The NOEL established in this study was 50 mg/kg/day.

The notified chemical was not mutagenic in a reverse mutation assay in bacteria. It was determined to be weakly clastogenic in human lymphocytes *in-vitro*. When the notified chemical was tested *in-vivo* in a mouse bone marrow cell Micronucleus Assay, the possibility of weak clastogenic activity could not be excluded.

The notified chemical would be determined to be hazardous according to NOHSC *Approved Criteria for Classifying Hazardous Substances* (National Occupational Health and Safety Commission, 1999), on the basis of skin irritation and requires the risk phrase R38 irritating to skin.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

The notifier provided the following ecotoxicity data in support of the application. The ecotoxicity tests were performed in accordance with OECD Test Guidelines.

Test	*Species*	*Results (Measured) [mg/L]*
Acute Toxicity [OECD 203]	*Brachydanio rerio* (Zebra fish)	LC_{50} (96 h) = 66 NOEC (96 h) < 5.4
Acute Immobilisation [OECD 202]	*Daphnia magna*	EC_{50} (48 h) > 75 NOEC (48 h) > 25
Algal Growth Inhibition [OECD 201]	*Scenedesmus subspicatus*	EC_{b50} (72 h) = 35 $NOEC_b$ (72 h) = 24 EC_{r50} (72 h) = 55 $NOEC_r$ (72 h) = 24
Inhibition of Bacterial Respiration [OECD 301]	Activated Sludge Bacteria	No significant inhibition – see notes below.

The tests on zebra fish were performed using solutions of the test material made up in carbon filtered tap water at measured concentrations of 0 (control), 5.4, 12, 22, 43, and 101 mg/L. The tests were conducted in a semi-static (renewal) system over a 96 hour period at a controlled temperature of 26ºC, with water removed daily and replaced with fresh water containing the respective concentrations of the test material. Solution analysis was conducted by gas chromatography for determination of the test chemical concentrations. Seven fish were tested at each concentration, and during these tests the pH of the test solutions was always between 7.6 and 8.1, while dissolved oxygen levels were always between 6.9 and 7.2 mg/L and water hardness between 101 and 119 mg/L as $CaCO_3$.

No fish mortality occurred over the duration of the test, although some behavioural aberration, specifically erratic swimming activity was observed at all concentrations. The test results indicate that Agarbois is at least slightly toxic to the zebra fish with a 96 hour NOEC less than 5.4 mg/L.

The acute immobilisation tests on daphnia were performed using solutions of the test material in a static non renewal system over a 48 hour period at a controlled temperature of 19 ± 1 ºC. Five solutions of the chemical with (geometric mean) measured concentrations of 25, 47, 91, 150 and 290 mg/L were tested, together with one control. Solution analysis (gas chromatography) for the test compound was conducted on samples of both old and fresh test media. Five juvenile daphnia were tested at each concentration, with four replicate tests conducted at each concentration. During these tests the pH of the test solutions was always between 7.6 and 8.0, while dissolved oxygen levels were between 7.5 and 8.4 mg/L and hardness was around 245 mg/L as $CaCO_3$.

No reduction in daphnia mobility was observed after 48 hours for the test concentration of 24.5 mg/L, but for the higher test concentrations significant immobility was observed, and all the daphnia were immobile after 48 hours exposure to a concentration of 151 mg/L. These test results indicate that Agarbois is slightly toxic to daphnia with a 48 hour EC50 of 25 mg/L.

A test on the inhibition of algal growth was also conducted on *Scenedesmus subspicatus* over a 72 hour incubation period at 25 ± 2 ºC with (geometric mean) measured concentrations for

the test material of 6.3, 12, 24, 45 and 89 mg/L (nominal 10, 18, 32, 56 and 100 mg/L respectively) together with a control containing no chemical. The solutions were made up in distilled water, and the concentration of the test substance in the media determined was at 0, 24, 48 and 72 hours after commencement of the test. The geometric mean of the measured test concentrations were always > 66% of the nominal concentrations, which indicates minimal adsorption of the test material by the algal mass. The results show the new chemical is at most slightly toxic to this species of green algae, with the 72 hour NOEC = 24mg/L – based on both the increase in algal biomass and the growth rate.

No dedicated test for the inhibition of bacterial respiration was conducted, but a subsidiary test performed as part of the tests for ready biodegradability (OECD 301 F) indicated no significant inhibition of respiration when the new chemical was present at 50 mg/L of the theoretical oxygen demand (ThOD) (ie around 18.3 mg/L of the new chemical).

The QSAR calculations of the ASTER database (USEPA, 1998) also furnished predicted acute toxicity LC_{50} data for several fish species which included Rainbow trout (14.6 mg/L), Fathead minnow (34.6 mg/L), Bluegill (27.2 mg/L), and Channel catfish (14.9 mg/L). These calculations also furnished an acute EC_{50} of 18.8 mg/L for immobilisation of daphnia, and a chronic maximum acceptable toxicant concentration (MATC) of 5.6 mg/L for Fathead minnow. These results are in reasonable accord with the experimental data, and support the conclusion that the new chemical is at most slightly toxic to aquatic species.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

All of the new chemical will be used as an ingredient of domestic cleaning formulations, and most of the material would eventually be released into domestic sewage systems as consequence of product use. However, due to the volatility of the material, a high proportion is likely to enter the atmosphere where it is expected to degrade through reactions with hydroxyl radicals primarily through initial addition of the hydroxyl species to the aromatic centre.

The ecotoxicity data indicates that the new chemical is at most slightly toxic to those aquatic species against which it was tested. Based on annual imports of 5 tonne, all of which is eventually released to sewer, the daily release on a nationwide basis is 13.6 kg/day. Assuming a national population of 18 000 000 and that each person contributes an average 150 L/day to overall sewage flows, the predicted concentration in sewage effluent on a nationwide basis is estimated as 5.0 µg/L. When released to receiving waters the concentration is generally understood to be further reduced by a factor of at least 10, so the Predicted Environmental Concentration (PEC) after final release is around 0.5 µg/L. This PEC is several orders of magnitude less than the concentrations at which the compound is likely to demonstrate toxicity to aquatic species.

The SimpleTreat and Level 1 Mackay calculations mentioned above indicate that due to the moderately high vapour pressure much of the chemical would eventually partition into the atmosphere, to be destroyed by reactions with hydroxyl free radicals. This is expected to be the dominant mechanism for removal of the compound from the environment, and the final degradation products are expected to be to water, and oxides of carbon and nitrogen. These

biological and abiotic mechanisms (particularly photodegradation reactions) would operate to continuously remove the chemical from the environmental compartments, so that overall environmental concentrations would be unlikely to increase with prolonged release of the chemical.

The modest values for Log K_{ow} (2.06) and Log K_{oc} (estimated as 1.96) indicate only low affinity for the organic component of soils and sediments. The relatively high water solubility and low value for Log Koc indicate that if assimilated into soils and sediments, the notified chemical is likely to be mobile in these media. Nevertheless, if it became associated with soils and sediments, it is expected to be degraded through slow biotic and abiotic processes.

Although soluble in water (4,100 mg/L), the notified chemical is expected to have low persistence in the water compartment due to volatilisation to the atmosphere and subsequent degradation through photochemical processes. The compound is not anticipated to bioaccumulate.

The above considerations indicate a low hazard to the environment when the new 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

According to the NOHSC *Approved Criteria for Classifying Hazardous Substances* (National Occupational Health and Safety Commission, 1999), the notified chemical is classified as hazardous based on its skin irritation effects. Risk phrase R38 (Irritating to skin) is assigned to the notified chemical.

The notified chemical had a very low acute oral (LD_{50} > 2 000 mg/kg) and low dermal (LD_{50} > 2 000 mg/kg) toxicity in rats. It was a moderate skin irritant and a slight to moderate eye irritant in rabbits, but was not a skin sensitiser in guinea pigs. No inhalation study was conducted. A 28 day repeat oral study with recovery groups in rats showed a NOAEL of 150 mg/kg/day based on the renal effects of irreversible tubular basophilia and reversible tubular hyaline droplet formation. The NOEL established in this study was 50 mg/kg/day. The notified chemical was not mutagenic in a bacterial reverse mutation assay. An *in vitro* mouse micronucleus assay showed positive in one sex and one test under the experimental conditions. As the result was not dose related and was not reproduced in a second test, the notified chemical was not determined to be clastogenic. However, in an *in vitro* cytogenetic assay in human lymphocytes, there was a dose-related, small but statistically significant increase of aberrant cells in the absence of S9 metabolic activation system. This is consistent with the negative findings *in vivo* and the weak positive cytogenetic effect is considered to have little relevance to human health.

The notified chemical does not contain impurities that present major toxicological hazards.

Occupational Health & Safety

The notified chemical will be imported in 200 L steel kegs with lacquer or polythene lined in compounded fragrances at a concentration up to 25%. Occupational exposure to the notified chemical will only occur through contact with the solutions of the notified chemical.

Waterside, warehouse and transport workers will only be exposed to the notified chemical in the event of an accident or damage to packaging. The occupational health risk to these workers is negligible, given the low concentrations of the notified chemical in compounded fragrances and the packaging type of steel kegs.

At the formulation sites, exposure of workers involved in the mixing of compounded fragrances containing the notified chemical may occur, but is likely to be restricted to the stage of normally pouring and weighing the required amounts of the notified chemical for the mixing batch. Skin exposure and potentially eye contact via splashing, may occur at this point. Workers are to carry out this work on a regular basis (50-200 days per year), however the duration of handling, up to 1 hour, is short. Fragrance products formulation process will involve mainly enclosed and automated equipment. The main occupational health risk to workers involved in formulation during transfer of the compounded fragrances containing the notified chemical is skin irritation. This can be minimised by the use of protective gloves and clothing as outlined below. Eye irritation is a potential health risk but ocular exposure is likely to be rare. Inhalation exposure is considered to be negligible because of the low vapour pressure of the notified chemical and the fact that local exhaust ventilation is in place. Some dermal and (accidental) eye exposure may occur during the packaging of the final fragrance products. The highest concentration of the notified chemical present in the final products is given as 0.06%. Details on worker controls operating during the formulation was not provided, however, according to the information submitted, workers would be wearing gloves, eye and face protections, and protective clothing. Considering all of the above measures, the risk of dermal and ocular exposure to the notified chemical during formation is low.

Public Health

The general public will be repeatedly exposed to a low level of the notified chemical via a number of different consumer products. An air freshener containing 25% Agarbois in 10 g of solid or liquid fragrance, will release approximately 80 mg/day or 3.3 mg/hour of the notified chemical over 30 days. The air concentration of the notified chemical in a normally ventilated room of 12 m^2 will be approximately 0.1 mg/m^3. In the worst case, this will lead to a maximum daily exposure of 0.6 mg/day (0.01 mg/kg bw/day for a woman of 60 kg) for a person with normal alveolar ventilation of 4.2 L/min. Other consumer products which contain 0.06% Agarbois, include skin cream, soaps and shower gels, and household cleaners. In the case of a skin cream ("leave on" products), assuming 10 g cream applied per day and 100% absorption, the daily exposure will be at a level of approximate 6 mg/day (0. 1 mg/kg bw/day for a woman of 60 kg) of the notified chemical. Customers who use "rinse off' or household products will be exposed to a much lower dose. The sum of daily exposure will be less than 7 mg/day if several types of products are used simultaneously. Given the low level of exposure to the notified chemical by using the products in the proposed manner, the risk to the general public is considered to be low.

13. RECOMMENDATIONS

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

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

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

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

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

To minimise public exposure to Agarbois the following guidelines and precautions should be observed:

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

14. MATERIAL SAFETY DATA SHEET

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

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

15. REQUIREMENTS FOR SECONDARY NOTIFICATION

Under the Act, secondary notification of the notified chemical shall be required if any of the circumstances stipulated under subsection 64(2) of the Act arise. No other specific conditions are prescribed.

16. REFERENCES

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Attachment 1

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

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

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

CORNEA

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

CONJUNCTIVAE

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

IRIS

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