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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

Formaldehyde, reaction products with branched 4-nonylphenol and 1-dodecanethiol (WINGSTAY X78678)

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FULL PUBLIC REPORT

Formaldehyde, reaction products with branched 4-nonylphenol and 1-dodecanethiol (WINGSTAY X78678)

1. APPLICANT

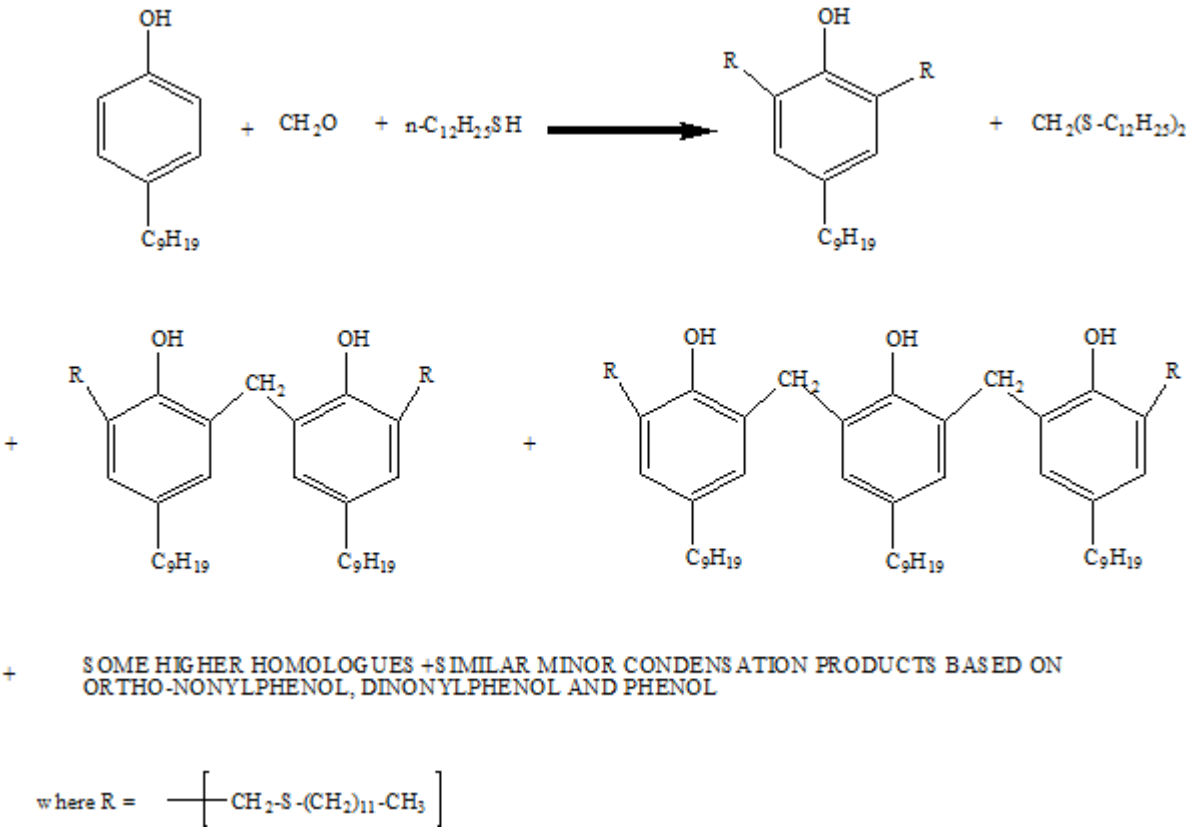
South Pacific Tyres of Hume Highway SOMERTON VIC 3062 has submitted a standard notification statement in support of their application for an assessment certificate for Formaldehyde, reaction products with branched 4-nonylphenol and 1-dodecanethiol.

No claims for exempt information were made by South Pacific Tyres.

2. IDENTITY OF THE CHEMICAL

Chemical Name:	Formaldehyde, reaction products with branched 4-nonylphenol and 1-dodecanethiol
Chemical Abstracts Service (CAS) Registry No.:	203742-97-6
Other Names:	Self-synergised phenolic antioxidant; Alkylated phenol/formaldehyde/mercaptan reaction product.
Marketing Name:	WINGSTAY K; Low VOC WINGSTAY K; WINGSTAY X78678.
Molecular Formula:	UVCB

Structural Formula:



Molecular Weight:

Final product consist of a large number of components with a molecular weight range of 150 to >1250. Minimum average molecular weight was said to be 1099.

Estimated molecular weight distribution is:

Molecular weight	Concentration
150	2
650	7.0±2.0
950	29.0±4.0
1250	21.0±4.0
>1250	41.0±4.0

No supporting information for the above estimates was provided or sought.

Method of Detection and Determination:

WINGSTAY X78678 is a complex reaction product and there are no specific methods relating to its detection and determination. However, diagnostic spectral data are available and the notified chemical may be quantitatively determined via UV/VIS spectrophotometry.

Spectral Data:
The major peaks of the respective spectra were consistent with the accepted structural molecular properties of WINGSTAY X78678.
(Covance 1998e)
IR Spectrum:
3343,3048, 2956-2853, 1602,1483-1466, 1377-1293, 1238-1187, 770, 722 cm^{-1}.

UV/VIS Spectrum:
287.6 nm, absorbance = 0.070 at a concentration of 12.0 µg/mL.
203.2 nm, absorbance = 0.914 at a concentration of 12.0 µg/mL.

NMR Spectrum:
Complex multiplet at 6.6-7.2 ppm.
Complex series of peaks at 5.2-5.4 ppm; and 3.6-4.0 ppm.
Complex series of multiplets at 2.2-2.8 ppm; and 0.4-1.7 ppm.

3. PHYSICAL AND CHEMICAL PROPERTIES

The following investigations were performed according to corresponding OECD test guidelines. The tests were conducted at facilities that comply with the OECD principles of good laboratory practice and full test reports were submitted (Covance 1999c) (Covance 1999d).

All tests were conducted on the notified chemical, WINGSTAY X78678.

Appearance at 20°C & 101.3 kPa:	Clear, viscous liquid
Boiling Point:	Decomposes above 249ºC
Relative Density:	0.960
Vapour Pressure:	4.8×10^{-14} kPa at 25ºC
Water Solubility:	0.027 mg/L at 20ºC
Particle Size:	The material is not in particulate form
Partition Co-efficient (n-octanol/water):	log $P_{ow} > 6.3$
Hydrolysis as a Function of pH:	Unable to perform – see comments below
Adsorption/Desorption:	log $K_{oc} > 4.46$ (estimated)
Dissociation Constant:	Unable to perform – see comments below
Flash Point:	>110°C (closed cup)

Flammability Limits:	Combustible liquid, not flammable
Autoignition Temperature:	295°C
Explosive Properties:	Not explosive
Reactivity/Stability:	Stable to water, stable to air at below 295°C

3.1 Comments on Physico-Chemical Properties

An analysis of the melting temperature was undertaken using OECD TG 102 - Differential Scanning Calorimetry. No clear freezing or melting point was observed between –70 and 20°C, while the chemical appeared to decompose above 249°C.

The water solubility of the largest component of the notified chemical was determined using OECD TG 105 – Shake Flask method by HPLC. The chromatographic profile suggested the water solubility of the other components would be similar. The water solubility was found to be 0.027 mg/L, which indicates that the notified chemical is very slightly soluble (Mensink 1995).

The partition coefficient was determined following OECD TG 107 and 117. Comparing the chromatograms of DDT and the notified chemical indicated that the majority of the notified chemical components would elute after DDT. Therefore, the log P_{ow} would be greater than 6.3. This indicates that the notified chemical would be hydrophobic, immobile in soils and associate with the soil matrix.

The adsorption/desorption was estimated by the draft OECD TG (1997) – Screening method for the determination of adsorption coefficient on soil (K_{oc}) using high performance liquid chromatography (HPLC). Comparison of chromatograms of sulprofos and the notified chemical indicated that the majority of the notified chemical components would elute after sulprofos. Therefore, the log K_{oc} would be greater than 4.46. This result indicates that the notified chemical would be immobile in soil.

Hydrolysis, as a function of pH, and the dissociation constant of the chemical could not be determined because the notified chemical is very slightly soluble in water. The chemical does not contain any groups likely to hydrolyse under environmental conditions. Some dissociation may occur in the environmental pH range of 4 to 9 as the notified chemical contains phenol which is weakly acidic (Morrison RT & Boyd RN 1976).

4. PURITY OF THE CHEMICAL

Degree of Purity: >99%

Hazardous Impurities:

Chemical name:	Toluene
CAS No.:	108-88-3
Weight percentage:	0.1-0.5 w/w
Toxic properties:	R20: harmful by inhalation. (NOHSC 1999b)
Chemical name:	Formaldehyde
CAS No.:	50-00-0
Weight percentage:	<0.0004
Toxic properties:	R23/24/25: toxic by inhalation, in contact with skin and if swallowed. R34: causes burns. R40(3): carcinogen category 3. R43: may cause sensitisation by skin contact. (NOHSC 1999b)
Chemical name:	1-dodecanethiol
CAS No.:	112-55-0
Weight percentage:	0.1-0.3
Toxic properties:	unknown
Chemical name:	4-nonylphenol, branched
CAS No.:	84852-15-3
Weight percentage:	<0.0001
Toxic properties:	corrosive

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

Additives/Adjuvants: None

5. USE, VOLUME AND FORMULATION

The notified chemical, WINGSTAY X78678 is an antioxidant which will be imported as a stabiliser in bulk solid synthetic rubber slabs and bales, for use in the rubber industry. The concentration of the antioxidant (stabiliser) in the polymers is said to vary but would not exceed 0.35% by weight. The stabilised polymers will be polybutadiene and styrene-butadiene rubber, which will be used to manufacture vehicle tyres at the notifier's sites in Victoria.

In the first year, 250 000 to 300 000 kg of bulk rubber containing 900 to 1000 kg of WINGSTAY X78678 is expected to be imported. Over the next four years, a maximum of 300 000 to 500 000 kg per year of such stabilised rubber is expected to be imported. On this basis, the amount of WINGSTAY X78678 imported will be 1000 to 1800 kg per year.

The baled rubber is is sealed in polyethylene film and shipped and stored in sealed, heavy-duty wooden/cardboard or aluminium containers. The containers would be transported by truck or rail within Australia.

The tyre manufacturing process is mainly automated and consists of mixing, blending and moulding at temperatures less than 200ºC. Synthetic rubber slabs containing the notified chemical are blended with a number of additives in a large mixer. Once the required blend is achieved, it is either stored or sent to moulding equipment for immediate use in the production of tyres. Once the 'green' tyre has been built, it is cured and the finished tyre stored in the warehouse.

6. OCCUPATIONAL EXPOSURE

Transport and Storage
Skin contact with the solid rubber would occur where a clean up is required following breakage of the transport containers and rupture of the encasing film. The notified chemical is present at up to 0.35% in solid rubber, is bound and chemically inert and not available for separate contact. Exposure during clean up is considered negligible for transport and storage workers.

Processing into Finished Articles - Tyres
Less than 25 workers at each of the notifier's sites will be involved in processing tasks. The stabilised rubber is used in mixing or blending operations where several people on each shift may handle the baled rubber intermittently. The rubber is wrapped in film and direct contact would be limited.

Processing involves mixing, blending, and moulding of the stabilised rubber into tyres using automated, self-contained machinery. The processing equipment operates at temperatures of less than 200ºC under local exhaust ventilation systems to remove heat and rubber processing fumes. Processing fumes are passed through a scrubber before being emitted to the atmosphere. The antioxidant remains chemically bound within the rubber matrix structure in a stable, inert form and does not diffuse, migrate or vapourise out of the polymer or

contribute to fugitive emissions during processing. Operators handling raw and mixed rubber use gloves and eye protection. Appropriate face masks are used where required.

Under these conditions, inhalation or ingestion of WINGSTAY X78678 is unlikely. Dermal contact to the rubber solid would be limited and direct exposure to WINGSTAY X78678 negligible.

Little solid waste material is expected and would be recycled and reused wherever possible. Where waste disposal is required, there would be little direct contact of workers with the solid waste rubber.

Occupational Health Conditions
The notifier states that industrial experience with the manufacture and use of WINGSTAY X78678 in the USA since 1986 has not indicated any occupational health conditions associated with its use.

7. PUBLIC EXPOSURE

The notified chemical is not available for sale to the public. The potential for public exposure to the notified chemical during transport, reformulation or disposal is assessed as negligible. Members of the public may make occasional dermal contact with tyres manufactured using the notified chemical.

8. ENVIRONMENTAL EXPOSURE

8.1 Release

Little or no imported material will be lost due to spill since the bales are individually wrapped and in heavy-duty containers.

Residual amounts of the blended 'rubber' mixture and trimmings from the 'green' and cured tyres may exist. These are likely to be recycled into the process or used in the manufacture of other products. It is estimated that the overall waste from the reformulation process is <1% (maximum of approximately 18 kg/annum in year 5) of the notified chemical. No information was provided on how this waste will be disposed, but it can be assumed that disposal to landfill is most likely where the chemical is expected to remain bound within the solid rubber matrix.

Since the rubber is a solid inert mass, the wrapping material is unlikely to be contaminated with any residual material.

The notified chemical in treadwear in the environment is expected to remain firmly bound in the inert rubber matrix of the tyre wear particles and not leach out. At the end of their useful life, the tyres containing the notified chemical will typically be disposed of to landfill.

8.2 Fate

The fate of the notified chemical will be tied to that of the rubber tyres in which it is incorporated, strongly bound to the rubber matrix. Used tyres may be shredded and used to make various other articles such as rubber bricks or disposed of directly to landfill. Chemical in rubber articles disposed of to landfill will remain bound to rubber and undergo slow degradation. If the waste tyre or used-rubber article is incinerated, the notified chemical will be destroyed by conversion to oxides of carbon, nitrogen and sulphur and water vapour.

Ready Biodegradation

The ready biodegradation of the notified chemical was tested using the Japanese official test method - Method for Testing the Biodegradability of Chemical Substances by Micro-organisms (Kurume Research Laboratories 1998). The analyst claims that this method is the same as the OECD TG 301C, Ready Biodegradability, Modified MITI Test (I). The study was set-up with 6 test vessels (1 sludge culture control, 1 water/chemical control, 1 reference standard with sludge and 3 chemical and sludge). In the vessels containing the test chemical, the concentration of the chemical was 100 mg/L. In vessels containing sludge, 300 mL of the culture medium was added giving a concentration of the suspended solids of 30 mg/L. The reference standard vessel contained aniline at 100 mg/L. The temperature was maintained at 25ºC for the 28 days of the study. Biodegradation was measured by a BOD meter and chemically via HPLC. Aniline had degraded by 73% by day 14. The test chemical had not undergone any degradation by day 28. The notified chemical was not readily biodegradable.

Bioaccumulation

A bioaccumulation study with carp (*Cyprinus carpio*) was conducted following the Japanese official test method - Method for Testing the Degree of Accumulation of Chemical Substances in Fish Body (Kurume Research Laboratories 2000). The method is similar to OECD TG 305C, Degree of Bioconcentration in Fish. The study consisted of a control, an 8 week level 1 test and a 10 week level 2 test. The level 1 vessel contained the notified chemical at 1 mg/L and dissolved oxygen at 7.0 to 7.7 mg/L, while the level 2 vessel contained the notified chemical at 0.1 mg/L and dissolved oxygen concentration at 7.3 to 8.1 mg/L. Five fish were used in the control while 20 were used in each of the level 1 and 2 tests. The temperature of all test vessels was maintained at 25ºC.

The concentration of notified chemical in water was determined twice a week, while the concentration in the test fish was analysed in weeks 1, 2, 4, 6 and 8 for level 1 and 2, and week 10 for level 2, and at the beginning and end of the study in the control. Mostly, the water concentration in level 1 and 2 did not drop below 75% of the nominal concentrations. Chemical analysis in water and fish was done by HPLC where there were 10 peaks, grouped in 5. The resultant BCF for each peak is given below:

Level 1	Peak 1	103-253
	Peak 2	≤ 8.2-11
	Peak 3	≤ 16
	Peak 4	≤ 28
	Peak 5	≤ 8.9
Level 2	Peak 1	361-1260
	Peak 2	≤ 88-96
	Peak 3	≤ 1170
	Peak 4	≤ 301
	Peak 5	≤ 92

Results indicate that at the higher concentration (1 mg/L, level 1) the chemical is slightly bioconcentrating. At the lower concentration 0.1 mg/L (level 2) there appears to be a high possibility of bioconcentration (Mensink 1995), particularly for the components that form peak 1 and 3.

A depuration study, using peak 1, was conducted also (Naoaki Yakata, 2000b). After the bioaccumulation study the fish were transferred to tanks with clean water (ie no test chemical). Fish were analysed on days 1, 3 and 9 for level 1 and days 1, 3 and 8 for level 2 for residual notified chemical. In level 1 (1 mg/L) the amount of the proportion of the notified chemical in fish accounted for in peak 1 had decreased to 11.4% by day 9, and in level 2 (0.1 mg/L) it had decreased to 41.5%.

Results indicate that, while components of the notified chemical may bioaccumulate in fish, they will be slowly eliminated. Bioaccumulation is possible but unlikely to occur due to the low aquatic exposure.

9. EVALUATION OF TOXICOLOGICAL DATA

The following toxicological investigations were performed according to corresponding EC and OECD test guidelines. The tests were conducted at facilities that comply with the OECD principles of good laboratory practice and full test reports were submitted. All tests were conducted on the notified chemical, WINGSTAY X78678.

9.1 Acute Toxicity

Summary of acute toxicity

Test	*Species*	*Outcome*
acute oral toxicity	rat	LD50>5000 mg/kg
acute dermal toxicity	rat	LD50>2000 mg/kg
skin irritation	rabbit	Slight irritant
eye irritation	rabbit	Slight irritant
skin sensitisation	guinea pig	Weak to mild sensitiser

9.1.1 Oral Toxicity (WIL Research Laboratories Inc 1998c)

Species/strain:	Rat/Crl:CD (SD)BR
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	5000 mg/kg by gavage
Test method:	OECD TG 401; EC Method B1.
Mortality:	Nil
Clinical observations:	Various discoloured areas due to discharge/excretion (described as wet or dried red and yellow around the nose, hindlimb(s), anogenital or urogenital area) were noted in eight animals. All animals appeared normal from day 10 onwards.
Morphological findings:	No abnormalities were noted at necropsy.
Estimated LD_{50}:	> 5000 mg/kg
Result:	WINGSTAY X78678 was of very low acute oral toxicity to the rat.

9.1.2 Dermal Toxicity (WIL Research Laboratories Inc 1998b)

Species/strain:	Rat/Crl:CD(SD)IGS BR
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	A single, 24-hour semi occluded dermal application to intact skin at a dose level of 2000 mg/kg.
Test method:	OECD TG 402; EC Method B3.
Mortality:	Nil
Clinical observations:	One female lost 2 g from day 0 to day 7. By day 14, this animal had gained weight and surpassed its day 0 bodyweight.
Dermal response:	No signs of erythema or oedema were noted. Eight animals had desquamation and three animals had focal eschar followed by exfoliation. Desquamation persisted in one animal to day 14. All other dermal findings had completely subsided by day 11.
Morphological findings:	No abnormalities were noted at necropsy.
LD_{50}:	> 2000 mg/kg
Result:	WINGSTAY X78678 was of low dermal toxicity to the rat.

9.1.3 Inhalation Toxicity

Study not conducted. WINGSTAY X78678 is non volatile and does not form aerosols.

9.1.4 Skin Irritation

Species/strain:	Rabbit/New Zealand White
Number/sex of animals:	2 females, 4 males
Observation period:	3 days
Method of administration:	A single 4 hour, semi occluded application of 0.5 mL to intact skin.
Test method:	OECD TG 404; EC Method B4.
Clinical Observations:	There were no deaths or remarkable body weight changes.

Draize scores:

Time after treatment (days)	*Animal #*					
	1♂	*2*♂	*3*♂	*4*♂	*5*♀	*6*♀
Erythema						
1 hour	[a]1	0	0	1	0	0
1	1	0	0	1	1	1
2	0	0	0	1	0	1
3	0	0	0	0	1	1
4	-	-	-	-	-	0
Oedema	*All individual scores were zero.*					

[a] see Attachment 1 for Draize scales

Dermal response: The test substance induced very slight erythema on four animals. There was no oedema. All erythema was reversible and completely subsided by day 4.

Result: WINGSTAY X78678 was slightly irritating to rabbit skin.

9.1.5 Eye Irritation (WIL Research Laboratories Inc 1999)

Species/strain: Rabbit/New Zealand white

Number/sex of animals: 2 females; 1 male

Observation period: 7 days

Method of administration: A single instillation of 0.1 mL into the conjunctival sac of the right eye. The left eye served as the control.

Test method: OECD TG 405; EC Method B5.

Clinical Observations: There were no deaths or remarkable body weight changes.

Draize[1] scores of unirrigated eyes:

Animal	***1 hour***			***24 hours***			***48 hours***			***72 hours***		
Conjunctiva	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***	***r***	***c***	***d***
1♂	2	1	1	1	0	0	0	0	0	0	0	0
2♀	1	1	1	1	0	0	1	0	0	0	0	0
3♀	1	1	1	1	0	0	0	0	0	0	0	0
Cornea	*All individual scores were zero*											
Iris	*All individual scores were zero*											

r = redness, c = chemosis, d = discharge.

Ocular response: There were no corneal or iridial effects. Conjunctival irritation was noted for all animals and had completely subsided 72 hours post instillation.

Result: WINGSTAY X78678 was slightly irritating to rabbit eye.

[1] See Attachment 1 for Draize scales.

9.1.6 Skin Sensitisation (WIL Research Laboratories Inc 1998d)

Species/strain: Guineapig/Hartley [Crl:HA)BR]

Number of animals: 10/sex test animals; 5/sex control animals.

Test method: OECD TG 406 Magnusson and Kligman Maximisation Method; EC Method B6.

Induction procedure:
Intradermal Induction
Day 1 - three pairs of intradermal injections (0.1 mL) to the dorsal midline:
Test animals: Freund's Complete Adjuvant (FCA) in sterile saline (1:1);
test substance at 5% w/v in olive oil;
test substance at 5% w/v in a (1:1) mixture of FCA:saline.

Negative control animals: FCA in sterile saline (1:1);
olive oil;
50% w/v concentration of olive oil in 1:1 mixture of FCA:saline.

Positive control animals: FCA:sterile saline (1:1);
5% w/v mixture of hexylcinnamaldehyde (HCA) in acetone;
5% w/v solution of HCA in a 1:1 mixture of FCA:saline.

Topical Induction:
Day 7 – A 48-hour semi occluded application to the treated area using filter paper saturated with:
Test animals: test substance at 100%.
Negative control animals: deionised water.
Positive control animals: 50% HCA in acetone.

Challenge procedure:
Day 21 - A 24 hour, occluded application (Hill Top Chamber) of 0.2 mL of:
Test and Negative control animals: test substance at 2% in acetone and 100% acetone to the left and right anterior flank of each animal, respectively.
Positive control animals: 0.5% HCA in acetone and 100% acetone to the left and right anterior flank of each animal, respectively.

Day 28 – Test and Negative control animals were treated as above, but the application site was the posterior flank.

Rechallenge procedure:
As above for Day 28.

Clinical observations:
There were no deaths, clinical findings or remarkable body weight changes.

Challenge Outcome

			Dermal Scores - Incidence					
Group	***Substance***	***Interval hours***	***0***	***0.5***	***1***	***2***	***3***	***No of animals***
1st Challenge								
Test	2% TS in acetone	24	5	10	4	0	0	19
		48	5	13	1	0	0	19
Test	100% acetone	24	18	1	0	0	0	19
		48	17	2	0	0	0	19
Negative control -I	2% TS in acetone	24	3	7	0	0	0	10
		48	2	8	0	0	0	10
Negative control -I	100% acetone	24	10	0	0	0	0	10
		48	10	0	0	0	0	10
Positive control	0.5% HCA in acetone	24	1	6	3	0	0	10
		48	4	4	1	1	0	10
Positive control	100% acetone	24	10	0	0	0	0	10
		48	9	1	0	0	0	10
2nd Challenge								
Test	2% TS in acetone	24	10	8	1	0	0	19
		48	15	4	0	0	0	19
Test	100% acetone	24	14	5	0	0	0	19
		48	19	0	0	0	0	19
Negative control-II	2% TS in acetone	24	9	1	0	0	0	10
		48	10	0	0	0	0	10
Negative control-II	100% acetone	24	10	0	0	0	0	10
		48	10	0	0	0	0	10

TS = test substance. Grade 0.5 very slight dispersed redness (faint residual redness that is not clearly related to the test substance). Grade 1 discrete or patchy erythema. Grade 2 moderate and confluent erythema.

Challenge Outcome – Comment:
One test male was considered an outlier due to the elevated degree of irritation noted for this animal at 2nd challenge.
The incidence of positive reactions at first challenge was 21% (4 of 19 test animals) and at second challenge 5% (1 of 19 test animals). A reaction is considered positive when it is more intense than the responses to the vehicle and the responses to the substance in the negative control group. The incidence of positive reactions with HCA was 30% (3 of 10 animals).
According to the Kligman classification scheme a sensitisation incidence of 9 to 28 is classified as mild.

Result:	WINGSTAY X78678 was weakly to mildly sensitising to guineapig skin.

9.2 Repeated Dose Toxicity (WIL Research Laboratories Inc 1998a)

Species/strain:	Rat/Crl:CD(SD)BR
Number/sex of animals:	10/sex/group, plus 5/sex for recovery group
Method of administration:	Oral, gavage in a dose volume of 10 mL/kg
Dose/Study duration:	Treatment phase: 0, 125, 400, 1000 or 2500 mg/kg/day of test substance in 0.5% carboxymethylcellulose/0.5% Tween 80 for 28 or 29 consecutive days. Recovery phase: a treatment free period of 14 days. Recovery groups were separately provided for animals of the control and 2500 mg/kg/day test groups.
Test method:	OECD TG 407; EC Method B7

Clinical observations
There were no deaths during the treatment or recovery phase. Food consumption, and bodyweight gain were unaffected by treatment. There were no clinical signs attributable to treatment.

Locomotor Activity and Functional Observational Battery (FOB)
Findings from the locomotor activity evaluation were unremarkable. The FOB revealed test substance related effects for neuromuscular observations: significantly decreased hindlimb grip strength and hindlimb footsplay means in females at 2500 mg/kg/day on study days 26 and 27. During the recovery phase, day 41, the effect on grip strength was reversible. Footsplay means remained decreased, however, although not significantly.

Clinical chemistry/Haematology/Urinalysis

There were no changes attributable to treatment with the test substance in clinical chemistry, urinalysis or haematology parameters at the end of the treatment phase.

Findings reaching statistical significance in the recovery phase occurred in males at 2500 mg/kg/day and consisted of: decreased mean red cell count; increased mean cell volume and mean cell haemoglobin concentration; and decreased creatinine and alkaline phosphatase. On the basis of the absence of similar findings in the treatment phase these effects were not considered related to treatment.

Necropsy

No test substance-related gross findings were observed in any of the treated animals. There was no test substance-related effect on organ weights at the end of the treatment phase. Recovery phase males at 2500 mg/kg/day had significantly increased mean kidney weight relative to final body weight. This effect was not attributable to treatment.

Histopathology

Histopathological findings were considered common, spontaneous lesions in laboratory rats and not test substance-related.

Comment

Reversible, test substance-related effects on hindlimb grip strength and hindlimb footsplay means were observed in females at 2500 mg/kg/day. No other findings attributable to treatment were observed in any animals.

Result

The no-observed-adverse-effect level (NOAEL) for systemic and neurologic toxicity determined for WINGSTAY X78678 is 1000 mg/kg/day.

9.3 Genotoxicity

9.3.1 Bacterial Reverse Mutation Assay (Covance 1998d)

Strains:	*Salmonella typhimurium*: TA100, TA1535, TA98, TA1537; *Escherichia coli*: WP2uvrA.
Auxillary Metabolic activation system:	Liver S9 fraction from rats induced with Aroclor 1254 at 500 mg/kg
Study design:	The test substance was tested in triplicate at 0, 33.3, 100, 333, 1000, 3330, 5000 μg/plate, both in the presence and absence of metabolic activation, in two independent experiments. The vehicle control was acetone. Appropriate strain specific positive controls were used.
Test method:	OECD TG 471
Comment:	Cytotoxicity, characterised by growth inhibition, was not observed. Slight precipitate was observed at and above 1000 μg/plate. There was no increase in the number of revertant colonies

above the control, or demonstration of a dose response relationship, either in the presence or absence of metabolic activation at any test concentration for any strain.

Result: WINGSTAY X78678 was non mutagenic under the conditions of the test.

9.3.2 Forward Mutation Assay (Covance 1998a)

Cells: Mouse lymphoma L5178Y

Auxillary Metabolic activation system: Liver S9 fraction from rats induced with Aroclor 1254 at 500 mg/kg

Study design: Duplicate cell cultures received 4 hour exposure to the test substance at 0, 39.3, 78.5, 157, 313, 625, 1250, 2500, 5000 µg/mL, both in the presence and absence of metabolic activation, in two independent experiments.
Vehicle control: 1% acetone.
Positive controls: methyl methanesulfonate (without metabolic activation) and methylcholanthrene (with metabolic activation).

Test method: OECD TG 476

Comment:
In the absence of metabolic activation in both experiments, weak to no cytotoxicity was induced. Treatment with the test substance did not induce a mutation frequency that exceeded the criterion for a positive response.
In the presence of metabolic activation in the first experiment weak to no cytotoxicity was induced. Treatment with the test substance did not induce a mutation frequency that exceeded the criterion for a positive response. In the second experiment, a wide range of cytotoxicity was induced without inducing mutant frequencies that were considered elevated.
Analysis of mutant colony size showed the vehicle control produced the expected bimodal distribution and the positive controls produced the expected large and small colonies. The positive controls induced large increases in mutant frequencies.

Result: WINGSTAY X78678 was non mutagenic under the conditions of the test.

9.3.3 Chromosomal Aberration Assay in Mammalian Cells (Covance 1998c)

Cells:	Chinese hamster ovary (CHO) cells
Auxillary Metabolic activation system:	Liver S9 fraction from rats induced with Aroclor 1254 at 500 mg/kg.
Test method:	OECD TG 473
Study design:	The test substance was tested in duplicate in two independent experiments as follows:
<u>Experiment 1:</u>	
-S9	0, 10, 25, 50*, 100d, 300*p, 1000*, 2500* µg/mL; treatment/harvest time = 3/20 hours; positive control: mitomycin C 1.50 µg/mL;
+S9	0, 10, 25*, 50d, 100, 300*p, 1000*d, 2500* µg/mL; treatment/harvest time = 3/20 hours, positive control: cyclophosphamide 5.0 µg/mL;
<u>Experiment 2:</u>	

-S9	0, 10, 25*, 50d, 100, 300*p, 1000*, 2500* µg/mL.
	treatment/harvest time = 17.6/19.8 hours;
	
	0, 10, 25*, 50*p, 100, 300*, 1000d, 2500* µg/mL.
	treatment/harvest time = 41.6/43.9 hours;
	
	positive control: mitomycin C 0.10 µg/mL;

+S9	0, 10, 25*, 50, 100p, 300*, 1000*d, 2500* µg/mL. treatment/harvest time: 3/20.2 hours, 0, 10, 25*, 50*p, 100, 300*, 1000d, 2500* µg/mL. treatment/harvest time: 3/44.3 hours, positive control: cyclophosphamide, 10.0 µg/mL. In both experiments the vehicle control was acetone.

Metaphase analysis:
Cultures selected for metaphase analysis are annotated with (*);
Lowest concentration where oily droplets were observed is annotated with (d);
Lowest concentration where precipitate was observed is annotated with (p).

Comment:
Cytotoxicity was not observed at any concentration.
The test substance did not cause any significant increases in the incidence of cells with chromosomal aberrations, polyploidy or endoreplication, at the concentrations analysed in the presence or absence of metabolic activation. In Experiment 2, a significant increase in endoreplication was observed at 300 and 1000 μg/mL in the 20.2 hour harvest assay with metabolic activation but not when compared to the negative control, and this response was not observed in the 44.3 hour harvest assay, a harvest time designed for numerical aberration evaluation. The response was considered spurious.
Positive controls used in the test caused marked increases in the incidence of aberrant cells and the activity of the S9 fraction was found to be satisfactory.

Result: WINGSTAY X78678 was non clastogenic under the conditions of the test

9.3.4 Micronucleus Assay in the Bone Marrow Cells of the Mouse (Covance 1998b)

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

Study design: The test substance was emulsified in 0.5% carboxymethylcellulose/0.5% Tween 80 and administered by oral gavage in a dose volume of 10 mg/mL. The positive control, cyclophosphamide (CP) was also administered by gavage.

Substance	*Dose Level, mg/kg*	*No of male animals; Kill time after dosing*
Test substance	2000	7; 24 or 48 hours
Test substance	1000	7; 24 hours
Test substance	500	7; 24 hours
Vehicle control:	0	7; 24 or 48 hours
Positive Control:	80	7; 24 hours

Test method: OECD TG 474; EC Method B12

Clinical observations: There was no mortality or clinical signs of toxicity.

Micronuclei score:
At least 2000 polychromatic erythrocytes (PCE) were counted per slide.
There was no significant change in PCE/normochromatic erythrocytes (NCE) ratio in any of the test substance dose groups when compared to the concurrent vehicle control group.
Bone marrow toxicity was not observed – the ratio of PCE:NCE was comparable to the respective control groups for each kill time and dose tested.
The positive control caused a significant increase in % micronucleated PCE, 2.92.

Result:
WINGSTAY X78678 was not clastogenic under the conditions of the test.

9.4 Overall Assessment of Toxicological Data

Hazard Assessment
WINGSTAY X78678 exhibited very low acute oral and dermal toxicity in rats. Inhalation studies have not been conducted. The substance is non-volatile and does not form aerosols. WINGSTAY X78678 was slightly irritating to rabbit skin, and slightly irritating to rabbit eye.

WINGSTAY X78678 is a weak to mild skin sensitiser when tested in guinea pigs using an adjuvant type method. The incidence of positive reactions at first challenge was 21% (4 of 19 animals) and at second challenge 5% (1 of 19 animals). The incidence of positive reactions for the positive control was only slightly higher at 30%.

In a combined 28 day oral repeat dose reproductive and neurotoxicity study in rats, reversible, test substance-related effects on hindlimb grip strength and hindlimb footsplay means were observed in females at 2500 mg/kg/day. No other findings attributable to treatment were observed in any animals. The NOAEL was determined at 1000 mg/kg/day.

WINGSTAY X78678 was non mutagenic in a bacterial reverse mutation assay or a forward mutation assay in mouse lymphoma cells. WINGSTAY X78678 was non clastogenic to Chinese hamster ovary cells in vitro or to bone marrow cells of the mouse in vivo.

Hazard Classification
On the basis of the data supplied, WINGSTAY X786 is not classified a hazardous substance under the *Approved Criteria for Classifying Hazardous Substances*.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

The following ecotoxicological investigations were performed according to corresponding OECD test guidelines. The tests were conducted at facilities that comply with the OECD principles of good laboratory practice and full test reports were submitted. All tests were conducted on the notified chemical, WINGSTAY X78678.

10.1 Summary of effects on biotic systems

Test	*Species*	*Results (mg/L)*
96 h acute OECD TG 203	Rainbow trout *(Oncorhynchus mykiss)*	LC_{50} > 0.064 NOEC > 0.064
48 h acute OECD TG 202	Water Flea (*Daphnia magna*)	EC_{50} > 0.064 NOEC > 0.064
21 day reproduction OECD TG 211	Water Flea (*Daphnia magna*)	LC_{50} > 0.064 EC_{50} > 0.064 NOEC = 0.064
96 h growth OECD 201	Algae (*Selenastrum capricornutum*)	E_RC_{50} > 0.064 E_BC_{50} > 0.064 NOEC = 0.064

* NOEC - no observable effect concentration

10.2 Acute Toxicity to Fish (Covance 1999b)

The 96 h acute toxicity test using Rainbow trout *(Oncorhynchus mykiss)* consisted of two exposure concentrations (0.032 and 0.064 mg/L), a dilution water control and a dilution water and acetone control. The test concentrations were chosen to approximate the limit and double the limit of water solubility of the chemical. The vessels were set-up with continuous renewal of the test media and 7 fish in each. Throughout the study the test media was clear and colourless and no mortality or abnormal behaviour was observed during the study. It was concluded that the no observed effect concentration (NOEC) and LC_{50} were greater than 0.064 mg/L or greater than the limit of water solubility of the chemical.

10.3.1 Daphnia Acute Immobilisation (Covance 1999a)

The 48 h acute toxicity test using *Daphnia magna* consisted of two exposure concentrations (0.032 and 0.064 mg/L), a dilution water control and a dilution water and acetone control. The test concentrations were chosen to approximate the limit and double the limit of water solubility of the chemical. The test media was renewed after 24 h. Five (5) daphnia were added to each vessel. Throughout the study the test media was clear and colourless and no immobility or abnormal behaviour was observed. It was concluded that the NOEC and LC_{50} were greater than 0.064 mg/L or greater than the limit of water solubility of the chemical.

10.3.2 Daphnia Reproductive Effects (Covance 2000)

The 21 day reproduction test using *Daphnia magna* consisted of ten replicates of five exposure concentrations (0.004, 0.008, 0.016, 0.032 and 0.064 mg/L), a dilution water control and a dilution water and acetone control. The test media was renewed after 24 h. A single daphnia was added to each vessel and fed daily. Throughout the study the test media was clear and colourless. Every 24 h observations were made of mobility, presence of eggs, young and mortality. All young were removed after being counted. No immobility or abnormal behaviour was observed in the solvent control, 0.008 mg/L and 0.064 mg/L vessels. In the dilution water control, 0.016 and 0.032 mg/L vessels one dead adult daphnia was observed, while in the 0.004 mg/L concentration 3 dead daphnia were observed. Surface trapping of the daphnia was also observed. When this occurred the daphnia were resubmerged. It was concluded that the 21 day NOEC was 0.064 mg/L and the 21 day LC_{50} was greater than 0.064 mg/L or greater than the limit of water solubility of the chemical. The majority of the juvenile daphnia survived. The 21 day reproduction EC_{50} was determined to be greater than 0.064 mg/L.

10.4 Algal Growth Inhibition (Covance 1999e)

The algal growth inhibition study with *Selenastrum capricornutum* consisted of three exposure concentrations (0.0032, 0.032 and 0.064 mg/L), a nutrient medium control and an acetone control. The test concentrations were chosen to approximate the limit and double the limit of water solubility of the chemical. The test vessels were inoculated to give a nominal algal cell count concentration of 10^4 cells/mL. At the beginning of the study the test media was clear and colourless. At the end of the study the vessels inoculated with algal contained bright green opaque algal suspension while the controls remainder clear. Results indicated that the no observed effect concentration (NOEC) was 0.064 mg/L and the 72 h E_bC_{50} and E_rC_{50} were greater than 0.064 mg/L or greater than the limit of water solubility of the chemical.

10.5 Conclusion

The ecotoxicity results indicate that the notified chemical is not toxic to fish, daphnia and algae up to the limits of its water solubility.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The environmental hazard from the notified chemical is not expected to be high when it is used for the manufacture of rubber tyres for motor vehicles. Very little of the chemical is expected to be released to water during manufacturing processes.

The notified chemical is not toxic to aquatic species (fish, daphnia and algae), up to the limit of its water solubility. The notified chemical has the potential for bioconcentration, but it is not expected to enter the aquatic environment in sufficient quantities to bioaccumulate.

The notified chemical will ultimately suffer the same fate as the tyres. The majority of the tyres would be disposed of to landfill with some being recycled into other articles. Once in landfill the notified chemical will not leach out. If the rubber articles are incinerated, the

chemical will be destroyed. A proportion of the notified chemical may enter the soil environment through wear and tear of tyres or shredding of used rubber articles for the manufacture of other items. Entry to soil will be in a highly dispersed manner and the notified chemical is likely to associate with the soil matrix.

The environmental hazard from the notified chemical is rated as low if it is used in the manner specified in the submission.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Hazard Assessment
WINGSTAY X78678 exhibited very low acute oral and dermal toxicity in rats. Inhalation studies have not been conducted. The substance is non-volatile and does not form aerosols. WINGSTAY X78678 was slightly irritating to rabbit skin and eyes. WINGSTAY X78678 is a weak to mild skin sensitiser when tested in guinea pigs using an adjuvant type method.

In a combined 28 day oral repeat dose reproductive and neurotoxicity study in rats, reversible, test substance-related effects on hindlimb grip strength and hindlimb footsplay means were observed in females at 2500 mg/kg/day. No other findings attributable to treatment were observed in any animals. The NOAEL was determined at 1000 mg/kg/day.

WINGSTAY X78678 was non mutagenic in vitro or in vivo.

On the basis of the data supplied, WINGSTAY X786 is not classified a hazardous substance under the *Approved Criteria for Classifying Hazardous Substances* (NOHSC 1999a).

Occupational Health and Safety
The notified chemical may be present at up to 0.35% in synthetic rubber and at lower concentrations once processed into automobile tyres. The potential for occupational exposure to the notified chemical is considered negligible because it is chemically bound within the rubber matrix and is not available for separate exposure during either handling of synthetic rubber or the manufacture and disposal of tyres. The notified chemical presents negligible risk to health under the occupational settings described.

Public Health
The notified chemical is not available for sale to the public. Although members of the public may occasionally make dermal contact with tyres manufactured using the notified chemical, the risk to public health from the notified chemical is likely to be low because the notified chemical is present at very low concentrations and unlikely to be bioavailable.

13. RECOMMENDATIONS

No recommendations are required for the handling of synthetic rubber containing the notified chemical. If the notified chemical itself is introduced in the future, control measures to minimise skin and eye contact in workers would be required.

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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Covance (1998b): Mutagenicity test on Wingstay K (Lot Number 9708-04) in the in vivo mouse micronucleus assay, Amended final report. Covance Laboratories Inc, Vienna, VA (unpublished Report No. 19019-0-455OECD).

Covance (1998c): Mutagenicity test on Wingstay K measuring chromosomal aberrations in Chinese hamster ovary (CHO) cells with a confirmatory assay with multiple harvests. Covance Laboratories Inc, Vienna, VA (unpublished Report No. 19019-0-437CO).

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NOHSC (1999b): List of Designated Hazardous Substances [NOHSC:10005(1999)]. National Occupational Health and Safety Commission, Canberra, AusInfo.

WIL Research Laboratories Inc (1998a): A 28-day oral (gavage) toxicity study of Wingstay K in rats (with functional observational battery and motor activity determinations). WIL Research Laboratories Inc, Ashland, OH (Unpublished Report No. WIL-140009).

WIL Research Laboratories Inc (1998b): Acute dermal toxicity study of Wingstay K in albino rats. WIL Research Laboratories Inc, Ashland, OH (Unpublished Report No. WIL-140015).

WIL Research Laboratories Inc (1998c): Acute oral toxicity study of Wingstay K in albino rats. WIL Research Laboratories Inc, Ashland, OH (Unpublished Report No. WIL-140013).

WIL Research Laboratories Inc (1998d): Guinea pig maximisation study of Wingstay K. WIL Research Laboratories Inc, Ashland, OH (Unpublished Report No. WIL-140014).

WIL Research Laboratories Inc (1999): Acute eye irritation study of Wingstay K in albino rabbits. WIL Research Laboratories Inc, Ashland, OH (Unpublished Report No. WIL-140020).

Attachment 1

The Draize Scale (Draize, 1959) for evaluation of skin reactions is as follows:

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

The Draize scale (Draize *et al*., 1944) for evaluation of eye reactions is as follows:

CORNEA

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

CONJUNCTIVAE

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

IRIS

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

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