File No: NA/587

June 1998

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

Z-25

This Assessment has been compiled in accordance with the provisions of the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act), and Regulations. This legislation is an Act of the Commonwealth of Australia. The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is administered by the National Occupational Health and Safety Commission which also conducts the occupational health & safety assessment. The assessment of environmental hazard is conducted by the Department of the Environment and the assessment of public health is conducted by the Department of Health and Family Services.

For the purposes of subsection 78(1) of the Act, copies of this full public report may be inspected by the public at the Library, National Occupational Health and Safety Commission, 92-94 Parramatta Road, Camperdown NSW 2050, between the following hours:

Monday - Wednesday	8.30 am - 5.00 pm
Thursday	8.30 am - 8.00 pm
Friday	8.30 am - 5.00 pm

Copies of this full public report may also be requested, free of charge, by contacting the Administration Coordinator on the fax number below.

For enquiries please contact the Administration Coordinator at:

Street Address: 92 Parramatta Rd Camperdown, NSW 2050, AUSTRALIA
Postal Address: GPO Box 58, Sydney 2001, AUSTRALIA
Telephone: (61) (02) 9577-9514 FAX (61) (02) 9577-9465

Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT

Z-25

1. APPLICANT

Lubrizol International Inc of 28 River Street SILVERWATER NSW 2128 has submitted a standard notification statement in support of their application for an assessment certificate for Z-25.

2. IDENTITY OF THE CHEMICAL

Trade Name:	Z-25
Generic Name:	alkylamine salt of alkyl phosphoric acid
Molecular Weight:	< 1 000
Method of Detection and Determination:	the notifier supplied ultraviolet/visible, infrared, and nuclear magnetic resonance spectral data which serve to characterise the material

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa:	yellow viscous liquid
Melting Point:	< 20°C
Boiling Point:	123-192°C
Specific Gravity:	0.9458 at 20.5°C
Vapour Pressure:	5.0×10^{-6}kPa at 25°C
Water Solubility:	1.72 $g.L^{-1}$ at 25°C (see comments below)
Fat Solubility:	totally soluble (see comments below)

Partition Co-efficient (n-octanol/water):	log P_{ow} = 1.39 at 20.5 ± 0.5°C (see comments below)
Hydrolysis as a Function of pH:	not determined (see comments below)
Adsorption/Desorption:	not determined (see comments below)
Dissociation Constant:	pK_{a1} = 1.79 @ 25°C (see comments below) pK_{a2} = 7.01 @ 25°C (see comments below)
Surface Activity:	32.4 $mN.m^{-1}$ at 352 $mg.L^{-1}$ and 20.5°C (see comments below)
Flash Point:	114°C
Flammability Limits:	not determined
Autoignition Temperature:	not highly flammable
Explosive Properties:	not explosive
Reactivity/Stability:	not an oxidising agent; not reactive toward water

Comments on Physico-Chemical Properties

Tests were performed according to EEC/OECD test guidelines at facilities complying with OECD Principles of Good Laboratory Practice.

The water solubility was determined as 1.72 $g.L^{-1}$ using the flask method. A quantity of the test material considerably in excess of that readily soluble was shaken in distilled water at 30°C for a period of at least 24 hours. The supernatant was centrifuged, filtered (filter pore size not specified) and the phosphorous concentration determined using Inductively Coupled Plasma Emission Spectroscopy (ICPES) against a distilled water blank. The solubility of the test substance was calculated on the basis that this material contains phosphorus. This water solubility is high for a material containing a large proportion of saturated hydrocarbon, but the ionic head groups of both anionic and cationic portions of the material somewhat mitigate the hydrophobic character and are likely to bestow considerable affinity for water on the material. However, although an apparently homogeneous mixture was obtained in these tests it is likely that the material is not truly soluble to the level indicated at the molecular level, but that the solubility is enhanced through the formation of colloidal aggregates like micelles. This is supported by the low surface tension of the aqueous solutions, a very characteristic attribute of micelle forming systems, which is discussed below.

The compound is apparently completely miscible in fat, and this is a consequence of the high hydrocarbon content of the compound. However, the ionic charges carried by the two portions of the molecule will not favour incorporation into a fat matrix. To explain the very high miscibility with fat, it must be assumed that the new compound is assimilated into the fat phase as ion pairs (hence neutral), possibly in association with some bound water. In respect of this point, similar mechanisms must be operative to explain the apparently high solubility in n-octanol which is at least 2 $g.L^{-1}$ as described in the report on the determination of partition coefficient - see below.

No data on hydrolytic degradation was provided, and the notifier indicated that no suitable test methods were available for appropriate tests on the new chemical. The anionic component(s) of the compound contain phosphate ester linkages (in some ways similar to those found in biological phospholipids) which are expected to be stable in the usual environmental pH region between 4 and 9. Similarly the primary amino bond in the cationic portion of the material is expected to be stable in the usual environmental pH region.

The partition coefficient was determined using the shake flask method. A stock solution of the notified material prepared at a concentration of 1.98 $g.L^{-1}$ in water saturated n-octanol was shaken in a flask with various volumes of distilled water for 5 minutes, and the phases allowed to separate. Phosphorus content in each phase was analysed using ICPES and the solubility of test material is calculated as described above for water solubility. The mean partition coefficient (6 samples) was determined as 24.5±4.3, which indicate considerable affinity for water. This is probably due to formation of colloidal aggregates as mentioned above in connection with water solubility.

The screening test for adsorption/desorption was not performed for the new chemical. It is possible to make some predictions in respect of this property on the basis of the partition coefficient data and general chemical constitution of the material. Both the anionic and cationic portions of the molecule carry substantial aliphatic hydrocarbon groups which have a high affinity for natural organic matter. However, the potential for strong association with organic material will be mitigated by the ionic charges which favour association with water, and the "conflict" between these two opposing tendencies is reflected in the modest n-octanol/water partition coefficient. These considerations lead to the conclusion that the new chemical would have some affinity for organic matter, but if released into the environment is unlikely to be completely immobilised through association with the naturally occurring organic component of soils and sediments. In respect of this point, it should also be appreciated that most natural organic matter exists in the form of negatively charged colloidal particles, and consequently the binding of the cationic moieties to organic material is likely to be enhanced over that for the negatively charged phosphate ester ions.

Both the cationic and anionic portions of the new compound are structurally very similar to conventional ionic surfactants, and consequently the new chemical could be expected to exhibit surfactant properties. This is confirmed by the significant lowering of surface tension in aqueous solution from around 72.5 $mN.m^{-1}$ for the blank (distilled water saturated with n-octanol) to less than half this value for a 352 $mg.L.^{-1}$ solution of the test material. As pointed out above in connection with the water solubility, lowering of surface tension to the degree

observed is characteristic of surfactant solutions which can form colloidal aggregates such as micelles.
The dissociation constant data provided by the notifier correspond to the first and second acid dissociation constants of mono and di esters of phosphoric acid, and indicate that the material would normally display acidic behaviour in an aqueous environment. However, pH measurements of the solutions taken during the water solubility and partition coefficient tests were between pH 4.4 and pH 5.8 indicating only modest acidic reactivity.

4. PURITY OF THE CHEMICAL

Degree of Purity: high

Toxic or Hazardous Impurities: none

5. USE, VOLUME AND FORMULATION

The notified chemical will not be manufactured in Australia, but will be imported for use as an antiwear agent for gear oil lubricants. Z-25 will be imported as a component of an additive package, which will contain up to 12% of the notified chemical. The notified chemical will be used as a component of gear oil at concentrations of up to 1%.

Less than one tonne of Z-25 will be imported in the first year following notification. Import volumes will increase to greater than one tonne per year for the following four years.

6. OCCUPATIONAL EXPOSURE

Drums of product containing up to 12% of the notified chemical will be imported, and transported to customers Australia-wide by road or rail. Waterside, warehouse and transport workers would not be exposed to the notified chemical under normal circumstances.

At the customer's site, workers may be dermally exposed while decanting the product containing Z-25 into a trough, from where it would be pumped into the blend tank. Alternatively, the imported product may be pumped directly from drums into the blend tank. Accidental ocular exposure may also occur if there is splashing during this transfer process. Inhalation exposure is not expected, because of the notified chemical's low vapour pressure.

The imported products will be mixed with oil and possibly other additives during blending operations. The notifier states that customer blending and packaging facilities are expected to be well ventilated and fully automated. Some dermal exposure to the imported product within the finished gear oil product (containing up to 1% of the notified chemical) is likely to occur when workers are cleaning and maintaining machinery and equipment.

The gear oil containing the notified chemical is packaged in containers of 1 to 205 litres. Gear oil will be sold to commercial operators (such as fleet operators) for use in trucks. It is anticipated by the notifier that gear oil will be changed in these trucks approximately every 800 000 km, and mechanics will be routinely dermally exposed to the gear oil during oil changes. Ocular exposure is also likely while draining gearbox oil, as mechanics will be working under vehicles when carrying out this operation. Indirect eye exposure is also possible by transfer of grease from hands to eyes.

7. PUBLIC EXPOSURE

Public exposure from transport or reformulation is expected to be negligible except in the event of an accident, where spills will be contained, collected and disposed of to approved industrial facilities or recycled. Used gear oils are expected to be disposed of according to government regulations or recycled, and public exposure from disposal is expected to be low.

Public exposure may occur through the dermal or ocular route when changing gear oils for do-it-yourself services, but exposure will be infrequent.

8. ENVIRONMENTAL EXPOSURE

Release

During blending of the additive concentrate containing Z-25 into lubricant products, there is little likelihood of release, since these processes are conducted in purpose constructed facilities which are expected to be fully automated. The notifier indicates that during the blending operation (typical blend sizes are 1 000 to 2 000 kg), the contents of the 200 L drum of Z-25 are decanted into a storage vessel and then pumped to a blend tank. Alternatively, Z-25 is pumped directly to the blend tank from the drums. Following the blending operations, the product is repacked into containers for distribution to customers. The container sizes used for product distribution are between 1 and 205 L, with little release of the blended product expected during the re-packing operations since fully automated equipment is used. Any spills resulting from either the blending or repackaging operations would be contained within bundling, and would very likely be soaked up in earth or sand and sent to an approved industrial facility for appropriate disposal. This is expected to be either incineration or placement into landfill. Residuals left in the drums are anticipated to be small. In typical operations involving transfer of drum contents to other vessels, approximately 1.0% of the drum contents may remain as residual. In the present case this would account for an annual release of around 50 kg of the notified material. The notifier indicated that these residuals would be removed during drum reconditioning and would probably be incinerated.

Release during transfer of the product during the filling of transmissions would be low. While no information was provided by the notifier, it is estimated (on the basis of experience in assessing lubricant products with similar use patterns) to be a maximum of 50 mL per transfer operation. If it is assumed that each transfer uses 10 L of lubricant these losses amount to

0.5% of lubricant. In the majority of cases the filling of transmissions with the product would take place at sites of vehicle production or maintenance workshops. These releases could be expected to be contained and disposed of with other lubricant and petroleum product waste. In most cases this would be through incineration or oil recycling. When used as a component of automotive transmission oil the material will be contained in an enclosed system, and release is expected to be insignificant. The notifier indicated that gear oils are changed infrequently, and typically would not be changed till the vehicle had "clocked up" around 800 000 km. Few of these major transmission overhauls are likely to be undertaken by independent owners, and in the majority of cases such operations be performed in specialist heavy transport maintenance workshops.

The fate of the majority of transmission fluid would be associated with that of the old transmissions and differentials. In most cases the old transmissions would be drained and the recovered oil sent for recycling. Some old gear oil may be disposed of in an inappropriate manner, and in light of the ecotoxicity data (see below), this could be a concern. However, due to the high aliphatic hydrocarbon content of both the amino and phosphate ester ions, it is expected that the material will have some affinity for organic matter (but see notes on Physico-Chemical Properties above) and is likely to be immobilised through association with the organic component of soils and sediments. The old gear assemblies would be sent for metal recovery where it is likely that the residual oil would be destroyed as a consequence of smelting operations.

Fate

The notified material is not readily biodegradable in aerobic environments, as the modified Sturm test [1] indicated only 31% degradation after 28 days. However, although slow, this test indicated that the new chemical is biodegradable.

The new chemical has a relatively low Log P_{ow} (1.39) which indicates modest affinity for water. Consequently the chemical is not expected to have strong potential for bioaccumulation.

Incineration of the notified material would lead to its complete destruction with production of water vapour and oxides of carbon and nitrogen. The contained phosphorus is likely to be converted to inorganic phosphate salts and to become assimilated into the mineral component of ash. The notifier recommends incineration as the preferred method for destruction of the material.

If placed into landfill, the reasonable water solubility of the compound indicates that it would be leached into the surrounding soils where it would probably become weakly associated with the organic components of soils (see discussion on adsorption/desorption above). It is likely that the positively charged ion will be significantly more strongly immobilised through this mechanism than will be the negatively charged ions, and once bound in this manner would be slowly degraded as a consequence of biological and abiotic processes. The negative ions are likely to be considerably more mobile, but these species have considerable chemical similarity with natural phospholipids, and consequently are expected to eventually be degraded or

assimilated by naturally occurring bacteria or fungi etc. Overall degradation of the new chemical would lead to production of water, methane, carbon dioxide and phosphate.

The notified material contained in oils and lubricants sent for recycling is likely to be destroyed during the re-refining process or to become associated with waste sludge from the recycling plant waste treatment facilities. In the latter case the sludge would be either incinerated or sent to landfill where the fate of the material would be as described above.

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

Summary of the acute toxicity of Z-25

Test	*Species*	*Outcome*	*Reference*
acute oral toxicity	rat	5 000 $mg.kg^{-1}$	[2]
acute dermal toxicity	rabbit	> 2 000 $mg.kg^{-1}$	[3]
skin irritation	rabbit	moderate to severe irritant	[4]
eye irritation	rabbit	moderate to severe irritant	[5]
skin sensitisation	guinea pig	non-sensitiser	[6]

9.1.1 Oral Toxicity [2]

Species/strain: rat/Sprague Dawley

Number/sex of animals: 10/sex

Observation period: 14 days

Method of administration: single administration by gavage of 5 000 $mg.kg^{-1}$ (5 males/5 females) or 2 000 $mg.kg^{-1}$ (5 males/5 females);
vehicle was corn oil for animals dosed at 2 000 $mg.kg^{-1}$; no vehicle was required for animals dosed at 5 000 $mg.kg^{-1}$

Clinical observations: clinical signs observed in animals dosed at 5 000 $mg.kg^{-1}$ included shedding of bloody tears (chromodacyorrhea), decreased activity, abnormal gait and stance; decreased muscle tone; tremors and poor grooming

	clinical signs observed in animals dosed at 2 000 $mg.kg^{-1}$ included shedding of bloody tears (chromodacyorrhea); salivation; diarrhoea; tremors and elevated gait
Mortality:	5 animals (2 males/3 females) dosed at 5 000 $mg.kg^{-1}$ died on the day following dosing; none of the animals in the 2 000 $mg.kg^{-1}$ group died during the study
Morphological findings:	necropsy of the 5 animals that died during the study revealed distended stomachs and fluid-filled intestines; necropsy of the remaining animals at the end of the study revealed mottled kidneys in one animal in the 5 000 $mg.kg^{-1}$ dose group, and three animals in the 2 000 $mg.kg^{-1}$ dose group
Test method:	according to OECD guidelines [1]
LD_{50}:	5 000 $mg.kg^{-1}$
Result:	the notified chemical had a LD_{50} of 5 000 $mg.kg^{-1}$ when administered orally to rats

9.1.2 Dermal Toxicity [3]

Species/strain:	rabbit/New Zealand White
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	single dermal dose of 2 000 $mg.kg^{-1}$ applied to an intact skin site (no vehicle was required); site covered with occlusive dressing; dressing removed after 24 hours and residual test material removed with mineral oil
Clinical observations:	dark material around the mouth and/or nose, faecal stains and/or urine stains were noted in a number of animals at times up to and including day 7; no clinical signs were noted in animals from day 8 through to day 14; there were also signs of local skin irritation at the application site
Mortality:	none

Morphological findings:	none
Test method:	according to OECD guidelines [1]
LD_{50}:	> 2 000 $mg.kg^{-1}$
Result:	the notified chemical was of low dermal toxicity when administered to rabbits in a limit test

9.1.3 Inhalation Toxicity

Not performed

9.1.4 Skin Irritation [4]

Species/strain:	rabbit/New Zealand White
Number/sex of animals:	4 males/2 females
Observation period:	14 days
Method of administration:	0.5 mL of the test material was administered as supplied to a small intact dorsal skin site; site was covered with occlusive dressing for 4 hours; dressing removed and residual test material removed from the test site using gauze moistened with the test material; observations were made at 1 hour, 1, 2, 3 and up to 14 days after removal of dressing and scored according to the method of Draize [7]
Draize scores [7] :	see table on next page
Test method:	according to OECD guidelines [1]
Result:	the notified chemical was a moderate to severe skin irritant when tested in rabbits

Draize scores [7]:

Time after treatment	*Animal #*					
	1 (F)	*2 (F)*	*3 (M)*	*4 (M)*	*5 (M)*	*6 (M)*
Erythema						
1 hour	2[a]	1	1	1	1	2
1 day	3	2	3	3	2	4
2 days	4	2	2	3	3	4
3 days	4	2	2	4	2	4
7 days	2	1	1	2	1	1
10 days	1	0	0	1	0	1
14 days	0	-	-	0	0	0
Oedema						
1 hour	1[a]	1	1	1	0	1
1 day	4	2	4	4	3	4
2 days	4	2	3	4	4	4
3 days	4	1	2	4	4	4
7 days	0	0	0	0	0	0
10 days	0	0	0	0	0	0
14 days	0	-	-	0	0	0

[a] see Attachment 1 for Draize scales

9.1.5 Eye Irritation [5]

Species/strain:	rabbit/New Zealand White
Number/sex of animals:	6/female
Observation period:	21 days
Method of administration:	0.1 mL of the test substance was instilled into the conjunctival sac of the right eye of each animal; untreated eyes served as controls

Draize scores [7]) of unirrigated eyes:

	Time after instillation									
Animal	***1 day***		***2 days***		***3 days***		***4 days***		***7 days***	
Cornea	***o[a]***	***a[b]***	***o[a]***	***a[b]***	***o[a]***	***a[b]***	***o[a]***	***a[b]***	***o[a]***	***a[b]***
1*	2[1]	4	2	4	2	3	1	2	1	1
2[#]	2	3	2	2	2	1	0	0	0	0
3[$]	2	4	2	3	2	2	1	2	1	1
4[$]	2	4	2	2	2	2	2	1	1	1
5[#]	2	4	2	3	2	2	2	1	0	0
6[+]	2	4	2	4	2	4	2	4	2	4

Iris															
1*	1			1			0			0			0		
2[#]	1			0			0			0			0		
3[$]	1			0			0			0			0		
4[$]	1			0			0			0			0		
5[#]	1			0			0			0			0		
6[+]	1			1			1			1			0		
Conjunctiva	***r[c]***	***c[d]***	***d[e]***	***r[c]***	***c[d]***	***d[e]***	***r[c]***	***c[d]***	***d[e]***	***r[c]***	***c[d]***	***d[e]***	***r[c]***	***c[d]***	***d[e]***
1*	3	2	3	2	1	2	2	1	0	2	1	0	1	1	0
2[#]	2	2	2	2	1	0	2	1	0	1	1	0	0	0	0
3[$]	2	2	2	2	1	2	2	1	2	2	1	2	1	1	1
4[$]	2	1	2	2	1	0	2	1	0	1	1	0	1	0	0
5[#]	2	1	1	2	1	0	2	1	0	2	1	0	0	0	0
6[+]	2	2	2	2	1	1	3	1	1	3	1	1	1	1	1

[1] see Attachment 1 for Draize scales
[a] opacity [b] area [c] redness [d] chemosis [e] discharge

*one more reading was taken on Day 10; all values were found to be zero
[#]final reading was taken on Day 7; all values were found to be zero, as shown
[$]further readings were taken on Days 10 and 14; slight effects (scores of 1 or zero for each of the parameters) were found on Day 10; all readings were found to be zero on Day 14
[+]further readings were taken on Days 10, 14 and 21; conjunctival effects had cleared by Day 21, however corneal effects were still present at this time; this animal was euthanised following the Day 21 reading.

Test method:	according to OECD guidelines [1]
Result:	the notified chemical was a moderate to severe eye irritant in rabbits; evidence of permanent damage to the cornea was present in one animal

9.1.6 Skin Sensitisation [6]

Species/strain:	guinea pig/Hartley-derived albino
Number of animals:	18/sex: 10/sex, test; 5/sex, negative control; 3/sex positive control (DNCB)
Induction procedure:	Day 0: topical application of 0.3 mL of 50% solution of test material in mineral oil to clipped site on left side of test animals; application site covered with 25 mm Hilltop chamber; torso of animal wrapped with elastic wrap; wrap and chamber removed after 6 hours and residual test material removed using gauze moistened with mineral oil Day 7: induction procedure repeated as per day 0 Day 14: induction procedure repeated as per day 0
Challenge procedure:	Day 28: topical application of 0.3 mL of 10% solution of test material in mineral oil to clipped site on right side of test animals; application site covered with 25 mm Hilltop chamber; torso of animal wrapped with elastic wrap; wrap and chamber removed after 6 hours and residual test material removed using gauze moistened with mineral oil 10 animals which had not undergone the induction process were treated as described above; these animals served as negative controls

Challenge outcome:

Challenge concentration	*Test animals*		*Control animals*	
	*24 hours**	*48 hours**	*24 hours*	*48 hours*
10%	0**	0	0	0

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

Comments: 'slight patchy erythema' (insufficient redness at test site for a score of 1 on the Draize () scale) was noted at the test sites following challenge as shown below:

time	test	control
24 hours	15/20	7/10
48 hours	9/20	4/10

Test method: according to OECD guidelines [1]

Result: the notified chemical was not a skin sensitiser when tested in a modified Buehler test in guinea pigs

9.2 Repeated Dose Toxicity [8]

Species/strain: rat/Sprague-Dawley

Number/sex of animals: 35/sex: control: 10/sex
low dose: 5/sex
mid dose: 10/sex
high dose: 10/sex

Method of administration: oral gavage; vehicle was arachis oil

Dose/Study duration: dose levels were based on the results of an oral repeat dose 14 day range finding study in rats

the notified chemical was administered daily for a period of 28 days:
control: 0 $mg.kg^{-1}$ per day
low dose: 15 $mg.kg^{-1}$ per day
mid dose: 150 $mg.kg^{-1}$ per day
high dose: 750 $mg.kg^{-1}$ per day

5 animals/sex from the control, mid and high dose group were maintained for an additional 2 week treatment-free period before sacrifice

Mortality:	one female from the high dose group was killed *in extremis* on day 14; there were no further deaths during the study
Clinical observations:	animals in the high dose group exhibited increased salivation, hunched posture, piloerection, noisy respiration, diuresis, fur loss, red/brown staining and wetness of the fur; isolated instances of lethargy, shedding of bloody tears (chromodacyorrhea) and tiptoe gait were also observed; food intake of animals in the high dose group was unaffected by treatment, however males in this group showed a decreased weight gain during the first 2 weeks of treatment when compared with controls animals in the mid dose group showed increased salivation; animals in the low dose group showed no clinical signs during treatment clinical signs found in rats in the mid and high dose groups were no longer evident following the 2 week treatment free period
Clinical chemistry/Haematology:	no toxicologically significant changes were found in haematological parameters in any of the treatment groups increased mean alanine aminotransferase levels were found in female animals in the high dose group – this effect was no longer evident at the end of the two week treatment free period diuresis and reduced urine specific gravity was noted in three males and one female in the high dose group; this was no longer evident at the end of the treatment free period
Gross Pathology:	animals of either sex in the high dose group showed an increased mean liver weight (both absolute and relative to bodyweight) in comparison with controls; these changes are consistent with increased hepatic activity associated with

	detoxification mechanisms which had largely reversed by the end of the treatment free period; males in mid and high dose groups also showed slightly increased mean relative kidney weights when compared with controls three males in the high dose group showed speckled kidneys; one male in this group exhibited pale kidneys; no effects were noted in the high dose group at the end of the treatment free period animals of both sexes in the mid and high dose group showed centrilobular hepatocyte enlargement; males in these groups also showed accumulations of eosinophilic droplets in the renal proximal tubular epithelium; these effects had only partially reversed by the end of the treatment free period; these renal effects are characteristic of hydrocarbon nephropathy, which is unique to male rats following hydrocarbon administration
Histopathology:	animals of both sexes in the mid and high dose group showed centrilobular hepatocyte enlargement; males in these groups also showed accumulations of eosinophilic droplets in the renal proximal tubular epithelium; these effects had only partially reversed by the end of the treatment free period; these renal effects are characteristic of hydrocarbon nephropathy, which is unique to male rats following hydrocarbon administration; this effect is therefore unlikely to occur in humans
Test method:	according to OECD guidelines [1]
Result:	when administered to rats orally over a 28 day period, the notified chemical caused alterations in the liver which were consistent with detoxification mechanisms; kidney effects seen in male animals of mid and high dose groups are likely to be associated with hydrocarbon nephropathy

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay [9]

Strains:	*Salmonella typhimurium* TA 98, TA 100, TA 1535, TA1537 and TA1538; *Escherichia coli* WP2 *uvrA*
Experimental Design:	**experiment 1:** *S. typhimurium*: 0.5 - 167 µg test material per plate; evaluated with or without metabolic activation provided by rat liver S9 mix; *E. coli:* 5-1 000 µg test material per plate; test material was soluble at all doses statistically significant (1.5-2.2 times control levels) increases in revertant colonies were noted in strains TA 1535 and TA 98 without S9; increases were not, however, dose dependent **experiment 2:** test material re-evaluated in all test strains: when S9 was included in the incubation mix, doses ranged from 16.7 - 5 000 µg test material per plate; when S9 was not included, doses ranged from 1.67 - 1 000 µg test material per plate; test material was not completely soluble at 5 000 µg per plate revertant colonies for all strains were the same or less than the control values; there was excessive toxicity in TA 1538 (with S9) and TA 1535 (without S9) in all but three dose levels **experiment 3:** test material evaluated in all test strains with S9 included in the incubation mix, doses ranged from 16.7 - 3 330 µg test material per plate; test material evaluated only in *S. typhimurium* strains without S9 mix, doses ranged from 1.67 – 167 µg test material per plate; test substance was incompletely soluble at 3 300 µg test material per plate revertant colonies for all doses in all tester strains with S9 were less than the control values; significant increases (1.6 – 1.9 times higher than controls) in revertant colonies in strain TA 98 at doses 1.67 and 5 µg test material per plate

(without S9) were observed; however, these were not dose-related

experiment 4: test material re-evaluated in strains TA 1535 and TA 98 without S9; doses ranged from 0.167 to 167 μg test material per plate

for all doses, revertant colonies were the same or less than the control values; increases in revertant colonies in previous experiments were considered to be statistical aberrations due to random fluctuation of the spontaneous revertant colonies

Test method: according to OECD guidelines [1]

Result: the notified chemical was considered not to be mutagenic in this test system, in the presence or absence of metabolic activation

9.3.2 Chromosome Aberration Assay in Chinese Hamster Lung Cells [10]

Dosing schedule: test material was evaluated at doses ranging from 1.25 to 120 $\mu g.mL^{-1}$ according to the following experimental design

without metabolic activation:
12 hour exposure time prior to cell harvest;
24 hour exposure time prior to cell harvest;
6 hour exposure time, cells harvested 18 hours after exposure

with metabolic activation:
4 hour exposure time, cells harvested 8 hours after exposure;6 hour exposure time, cells harvested 18 hours after exposure;

Test method: according to OECD guidelines [1]

Result: the notified chemical did not induce structural chromosomal aberrations in Chinese hamster V79 cells, in either the presence or absence of metabolic activation

9.3.3 Micronucleus Assay in the Bone Marrow Cells of the Mouse [11]

Species/strain:	mouse/CD-1
Number and sex of animals:	30/sex
Doses:	37.5, 75 and 150 $mg.kg^{-1}$; vehicle was arachis oil; animals were sacrificed 24 or 48 hours after treatment
Method of administration:	intraperitoneal injection
Test method:	similar to OECD guidelines [1]
Result:	the notified chemical did not induce micronuclei in mouse bone marrow cells when administered at a dose which induced slight toxic effects

9.4 Overall Assessment of Toxicological Data

The notified chemical exhibited low acute oral toxicity in rats (LD_{50} = 5 000 $mg.kg^{-1}$) and dermal toxicity in rabbits (LD_{50} > 2 000 $mg.kg^{-1}$). Inhalation toxicity testing was not performed by the notifier. Z-25 was a moderate to severe ocular and skin irritant in rabbits. The ocular irritation study showed evidence of permanent damage to the cornea of one out of six rabbits. Scores for the 24, 48 and 72 hour readings did not warrant classification of Z-25 as preventing a risk of serious damage to eyes according to the *Approved Criteria for Classifying Hazardous Substances* [12], but it would be classified as irritating to eyes and skin. The notified chemical was not a skin sensitiser when tested using a modified Buehler test in guinea pigs.

Repeated oral administration of Z-25 to rats over a 28-day period induced liver and kidney effects in animals dosed with 150 or 750 $mg.kg^{-1}$ per day. The alterations in the liver were consistent with detoxification mechanisms and kidney effects seen in male animals of mid and high dose groups are likely to be associated with hydrocarbon nephropathy.

The notified chemical was found on balance to be non-mutagenic in a bacterial reverse mutation assay. No clastogenicity was observed in Chinese hamster lung cells *in vitro*, and it did not cause chromosome damage in mouse bone marrow cells when tested *in vivo*.

Based on the information provided by the notifier, Z-25 would be classified as hazardous according to the Approved Criteria, based on its skin and eye irritant effects.

9. ASSESSMENT OF ENVIRONMENTAL EFFECTS

The following ecotoxicity studies have been supplied by the notifier. The tests were carried out using OECD Test Methods [1], and indicate that the notified chemical is at least moderately toxic to those aquatic species against which it was tested.

Test	*Species*	*Results (WAF Nominal)*
acute toxicity [1]	Rainbow trout	LLR_{50} (96 hour) = 11 $mg.L^{-1}$
	Oncorhynchus mykiss	LOEC (96 hour) = 5.6 $mg.L^{-1}$
acute immobilisation [1]	Water flea	ELR_{50} (48 hour) = 8.3 $mg.L^{-1}$
	Daphnia magna	LOEC (48 hour) = 3.26 $mg.L^{-1}$
chronic toxicity	Water flea	EC 50 (21 day) = 2.0 mg. L^{-1}
(reproduction test) [1]	*Daphnia magna*	LOEC (21 day) = 1.0 $mg.L^{-1}$ (see notes below)
growth inhibition [1]	Algae	E_bLR_{50} (96 hour) = 4.5 $mg.L^{-1}$
	Scenedesmus subspicatus	NOEC = 2.5 $mg.L^{-1}$
respiration inhibition [1]	Aerobic Waste Water Bacteria	EC_{50} (3 hour) = 660 $mg.L^{-1}$

All tests on fish, daphnia and algae were conducted using water accommodation fractions (WAFs) of the notified material since, when preparing the water used in all the ecotoxicity tests, cloudiness - indicative of incomplete solubilisation - was encountered, even at substance loading well below the water solubility in distilled water of 1.7 $mg.L^{-1}$. This can be explained in terms of aggregate formation by the surfactant molecules. The ecotoxicity tests were all conducted in either tap water or in specially prepared culture medium, both of which contain appreciable quantities of divalent calcium and magnesium ions. These ions are well known to induce ionic surfactant molecules to form large colloidal aggregates which impart a cloudy appearance to the water.

No solubility enhancing adjuvants were used. The WAFs were prepared by stirring weighed amounts of the test material into appropriate volumes of water for 24 hours, then filtering through a 0.45 micron membrane. It should be noted that analysis of the phosphorus content of the WAFs so prepared (using the ICPES procedure) indicated that the actual substance loadings in the waters was around 50% of the nominal loadings (range 9-100%).

The tests on rainbow trout were performed in duplicate (with controls) using 10 fish per test vessel. The WAFs of the test material were prepared with the nominal loadings of 3.2, 5.6, 10, 18 and 32 $mg.L^{-1}$ in dechlorinated tap water. A semi-static methodology was employed with daily removal and replacement of 80% of the appropriate WAF containing water from each test vessel. Temperature, pH and dissolved oxygen were maintained at 14ºC, 7.3±0.1, and 7.9±0.1 $mg.L^{-1}$, respectively throughout the 96 hour test period. The results indicate the material to be moderately toxic to this species, with the Lethal Loading Rate (LLR_{50}) of the WAF determined as 11 $mg.L^{-1}$. However, phosphorus analyses of the solutions (ICPES method) over the 96 hour test period indicated that the test material was stable. Based on the phosphorus content of the solutions, an LC_{50} of 4.5 $mg.L^{-1}$ for the notified chemical was

derived, with the corresponding LOEC of 2.1 mg.L^{-1}. During the tests it was noted that abnormal behavioural responses occurred at exposures to 5.6 mg.L^{-1} (WAF) and greater. These included increased pigmentation, swimming at the top or bottom of the test vessels, loss of equilibrium and general lethargy.

The acute immobilisation tests on daphnia were performed in a static test over a 48 hour test period with WAFs of the notified substance prepared in dechlorinated tap water with nominal loadings of 1.0, 1.8, 3.2, 5.6, 10, 18, 32, 56 and 100 mg.L^{-1}. The tests were performed in duplicate using 10 daphnids per test vessel for each substance loading. Temperature, pH and dissolved oxygen were maintained at 21ºC, 8.0±0.1, and 8.0±0.1 mg.L^{-1} respectively, throughout the 48 hour test period. The criteria for establishing immobilisation was if the daphnia failed to begin swimming within 15 seconds after being agitated through swirling of the containment vessel. The data indicate the test material to be moderately toxic to this species, with a 48 hour 50% Lethal Effect Loading (ELR) of 8.3 mg.L^{-1}, with a corresponding LOEC of 3.3 mg.L^{-1}. As with the tests on rainbow trout, the total solution phosphorus was analysed, and these data furnished an EC_{50} (48 h) of 2.9 mg.L^{-1}, and the corresponding LOEC of 1.0 mg.L^{-1}.

Chronic toxicity tests on daphnia were also conducted in accordance with OECD Test Guideline 202. The 21 day EC_{50} (WAF) was determined as approximately 2.0 mg.L^{-1} with the corresponding LOEC as approximately 1.0 mg.L^{-1}. These tests were performed (against controls) in a static test over a 21 day test period with WAFs of the notified substance prepared in dechlorinated tap water with nominal loadings of 0.5, 1.0, 2.0, 4.0 and 8.0 mg.L^{-1}. After 21 days exposure no mortality of young daphnia or unhatched eggs was observed for the WAF with nominal WAF loading of 1 mg.L^{-1} - ie 100% survival. However, at 2 mg.L^{-1}, only 43% survival was noted and for the higher WAF loadings there was zero survival. On the basis of the phosphorus analyses the 21 day EC_{50} was estimated as 0.55 mg.L^{-1}, with the corresponding LOEC of 0.42 mg.L^{-1}.

Inhibition of increase in algal (*Selenastrum capricornutum*) biomass was also tested in a static test over a 96 hour test period at a temperature of 24±0.1ºC. WAFs of the notified substance were prepared in aqueous culture medium (details provided with the reports in the notification) at nominal loadings of 0.625, 0.25, 1.25, 2.5, 5.0 and 10.0 mg.L^{-1}. The tests were performed in triplicate for each substance loading, and the 96 hour E_bLR_{50} based on cell concentration was determined as 4.6 mg.L^{-1}, with the NOEC of 2.5 mg.L^{-1}. As with the tests described above, the total solution phosphorus was analysed, and these data furnished an E_bC_{50} (96 hour) of 2.0 mg.L^{-1} , and the corresponding NOEC of 1.3 mg.L^{-1}. These results indicate that the test material is moderately toxic to this algal species.

A test on the effect of the new material on the respiration of activated sludge bacteria was also conducted over a 3 hour period at 21ºC using nominal loadings of 32, 100, 320, 1 000, and 3 200 mg.L^{-1}. No reduction in the rate of oxygen uptake was observed, and it was concluded that the material does not inhibit bacterial respiration. The control used in this test was 3,5-dichlorophenol, for which an EC_{50} of 7.2 mg.L^{-1} was determined.

It should be noted that the toxicity of the new chemical probably lies primarily with the amino

portion of the molecule, which is well known to be toxic to aquatic life [13].

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The environmental hazard from the notified chemical is low provided it is used in the manner indicated.

Releases of the material to the environment are expected to be low as both product formulation and the majority of transmission filling operations are performed under well-controlled conditions where spills and other losses would be expected to be minimal. Similarly, gear oils are changed very infrequently (around every 800,000 km), and in the majority of cases under well-controlled conditions. The ultimate fate of the majority of the material is expected to be incineration of waste oil resulting in its destruction with production of non-hazardous gases and phosphate minerals. Some used oil containing the notified chemical may be disposed of into landfill, as may some oil lost during transfer operations when filling transmission assemblies. If placed into landfill it is likely the material would become weakly associated with the organic component of soils and sediments, and be slowly degraded as a consequence of slow biological and abiotic processes. These degradation processes would lead to production of water, methane, carbon dioxide and phosphate.

The material is toxic to aquatic organisms, particularly to daphnia and algae, and release into the water compartment should be avoided. However, the potential toxicity, which would mainly reside in the amino ion, is likely to be mitigated in most cases through this portion of the chemical becoming associated with particulate organic matter in the water, and eventually becoming assimilated into sediments where it would be slowly degraded.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Based on the results of animal studies, the notified chemical is a moderate to severe irritant to the eyes and skin. There is a low occupational health risk for waterside, warehouse and transport workers who will be handling unopened drums and smaller packages of products containing the notified chemical.

Workers involved in blending operations at customer sites may experience skin and eye irritation if in contact with the notified chemical. The imported product contains Z-25 at a maximum of 12%, ie below the concentration cut off level for skin and eye irritation. The product overall does not require classification as hazardous on this basis. However, to avoid the potential for irritant effects to occur due to dermal and ocular exposure, appropriate personal protective equipment should be worn by workers handling the imported formulation (see Recommendations section below) to avoid dermal and ocular exposure.

Mechanics changing truck gear oil may experience frequent dermal and occasional ocular

exposure. However, the 1% (maximum) concentration, the notified chemical is not expected to cause adverse health effects. Repeated exposure of the notified chemical and other mineral oil components of the gear oil should, however, be avoided, as repeated exposures can cause skin drying and defatting. Employers need to ensure that the exposure standard for mineral oil mist of 5 $mg.m^3$ (TWA) [14] is not exceeded during either blending or oil changing operations.

Public exposure may occur when changing gear oils for do-it-yourself services, but the exposure will be infrequent. The notified chemical will be present at low levels (less than 1%) in gear oils, which is unlikely to cause serious skin or eye damage. Given the proposed use, Z-25 is not expected to present a significant risk to the public.

13. RECOMMENDATIONS

To minimise occupational exposure to Z-25, the following guidelines and precautions should be observed:

- Safety goggles should be selected and fitted in accordance with Australian Standard (AS) 1336 [15] to comply with Australian/New Zealand Standard (AS/NZS) 1337 [16];
- Industrial clothing should conform to the specifications detailed in AS 2919 [17] and AS 3765.1 [18];
- Impermeable gloves or mittens should conform to AS 2161 [19];
- All occupational footwear should conform to AS/NZS 2210 [20];
- Spillage of the notified chemical should be avoided, spillages should be cleaned up promptly with absorbents which should then be put into containers for disposal;
- Good personal hygiene should be practised to minimise the potential for ingestion;
- A copy of the Material Safety Data Sheet (MSDS) should be easily accessible to employees.

Workers should be aware of the potential for skin drying and defatting following repeated exposure to the mineral oils present in the blended gear oils. In addition, employers should ensure that the exposure standard for mineral oil mists of 5 $mg.m^3$ (TWA) [14] is not exceeded during blending and gear oil change operations

Formulations containing the notified chemical at concentrations greater than or equal to 20%, will be classified as hazardous on the basis of skin and eye irritation according to the *Approved Criteria,* and the MSDS and label would need to be modified accordingly [21,22].

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* [21].

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

1. Organisation for Economic Co-operation and Development, *OECD Guidelines for the Testing of Chemicals on CD-Rom*. 1995-1996, Paris: OECD.
2. Mallory, V., *Acute Exposure Oral Toxicity, Project No., PH 402-LU-011-93*, . 1993: Pharmakon USA, Pennsylvania, USA.
3. Bonnette K.L., *An Acute Dermal Toxicity Study in Rabbits with Z-25, Project No., 3263.85*, . 1995: Springborn Laboratories Inc., Ohio, USA.
4. Bonnette K.L, *A Primary Skin Irritation Study in Rabbits with Z-25, Project No. 3263.87*, . 1995: Springborn Laboratories Inc., Ohio, USA.
5. Bonnette, K.L., *A Primary Eye Irritation Study in Rabbits with Z-25, Project No. 3263.86*, 1995: Springborn Laboratories Inc., Ohio, USA.
6. Douds D. A., *A Dermal Sensitisation Study in Guinea Pigs with Z-25; Modified Buehler Design, Project No. 3263.72*, . 1995: Springborn Laboratories Inc., Ohio, USA.
7. Draize J.H., *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics.* Association of Food and Drug Officials of the US, 1959. **49**: p. 2-56.
8. Wragg M. S., *TwentyEight Day Sub-Acute Oral (Gavage) Toxicity Study in the Rat, project No. 827/001*, 1996: Safepharm Laboratories Limited, UK.
9. Stankowski, L.F., *Ames/Salmonella-E. coli Liquid Pre-Incubation Assay on Z-25, Project No. 301-LU-019-93*, . 1993: Pharmakon USA, Pennsylvania.
10. Wright N. P., *Z-25: Chromosome Aberration Test in CHL Cells In Vitro, Project No. 827/002*, . 1995: Safepharm Laboratories Limited, UK.

11. Dunward R., *Z-25: Micronucleus Test in the Mouse, Project No., 827/003.* Safepharm Laboratories Limited, UK, 1995.
12. National Occupational Health and Safety Commission, *Approved Criteria for Classifying Hazardous Substances [NOHSC:1008(1994)]*. 1994, Canberra: Australian Government Publishing Service.
13. Nabholz, J.V., P. Miller, and M. Zeeman, *Environmental Risk Assessment of New Substances under the Toxic Substances Control Act Section Five*, in *Environmental Toxicology and Risk Assessment, American Society for Testing and Materials*, W.G. Landis, J.S. Hughes, and M.A. Lewis, Editors. 1993, ASTM STP 1179: Philadelphia. p. 40-55.
14. National Occupational Health and Safety Commission, *Adopted National Exposure Standards for Atmospheric Contaminants in the Occupational Environment, [NOHSC:1003(1995)]*, in *Exposure Standards for Atmospheric Contaminants in the Occupational Environment: Guidance Note and National Exposure Standards*. 1995, Australian Government Publishing Service: Canberra.
15. Standards Australia, *Australian Standard 1336-1994, Eye protection in the Industrial Environment*. 1994, Sydney: Standards Association of Australia.
16. Standards Australia/Standards New Zealand, *Australian/New Zealand Standard 1337-1992, Eye Protectors for Industrial Applications*. 1992, Sydney/Wellington: Standards Association of Australia/Standards Association of New Zealand.
17. Standards Australia, *Australian Standard 2919-1987, Industrial Clothing*. 1987, Sydney: Standards Association of Australia.
18. Standards Australia, *Australian Standard 3765.1-1990, Clothing for Protection against Hazardous Chemicals Part 1 Protection against General or Specific Chemicals*. 1990, Sydney: Standards Association of Australia.
19. Standards Australia, *Australian Standard 2161-1978, Industrial Safety Gloves and Mittens (excluding electrical and medical gloves)*. 1978, Sydney: Standards Association of Australia.
20. Standards Australia/Standards New Zealand, *Australian/New Zealand Standard 2210-1994, Occupational Protective Footwear*. 1994, Sydney/Wellington: Standards Association of Australia/Standards Association of New Zealand.
21. National Occupational Health and Safety Commission, *National Code of Practice for the Preparation of Material Safety Data Sheets [NOHSC:2011(1994)]*. 1994, Canberra: Australian Government Publishing Service.
22. National Occupational Health and Safety Commission, *National Code of Practice for the Labeling of Workplace Substances [NOHSC:2012(1994)]*. 1994, Canberra: Australian Government Publishing Service.

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