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October 1999

NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME

FULL PUBLIC REPORT

Component of Additive 3

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Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT

Component of Additive 3

1. APPLICANT

Mobil Oil Australia Ltd. of Millers and Kororoit Creek Road ALTONA VICTORIA 3018 has submitted a standard notification statement in support of their application for an assessment certificate for Component of Additive 3. Additive 3 is also known as MCP 818.

2. IDENTITY OF THE CHEMICAL

The chemical name, CAS number, structural formulae, molecular weight, spectral data, purity and trace amounts of non-hazardous impurities have been exempted from publication in the Full Public Report and the Summary Report.

Marketing Names:	Additive 3; MCP 818
Method of Detection and Determination:	Nuclear Magnetic Resonance (NMR) Spectroscopy

Comments on Chemical Identity

Infra red spectroscopy and NMR Spectra for the chemical have been provided (Stanley, 1997).

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa:	Amber, waxy solid at room temperature
Boiling Point:	Not determined, see comments below
Pour Point:	56°C
Specific Gravity:	1.0191 at 20°C (Pycnometer method)
Vapour Pressure:	< 9.9 x10^{-5} Pa at 25°C (vapour pressure balance)

Water Solubility:	< 7.25 x10 $^{-5}$ g/L at 20°C (Flask method)
Partition Co-efficient (n-octanol/water):	log P_{ow} >5.26 (Flask method)
Hydrolysis as a Function of pH:	Not determined, see comments below
Adsorption/Desorption:	log_{10} K_{oc} >4, see comments below
Dissociation Constant:	Not determined, see comments below
Particle Size:	Not applicable as notified chemical is a waxy solid
Flash Point:	Not determined, as chemical is a solid
Autoignition Temperature:	>56°C, see comments below
Flammability:	Combustible but not highly flammable (Method A10 in EC Directive)
Explosive Properties:	Non explosive (Method A14 in EC Directive)
Reactivity/Stability:	Stable to light and heat

Comments on Physico-Chemical Properties

Physicochemical properties were determined using methods specified in European Commission Directive 92/69/EEC (EC, 1992).

The melting/freezing point could not be determined as the chemical is a multi-component mixture, which undergoes a transition between a very viscous liquid and a glassy solid over a wide temperature range covering approximately 25-35ºC, but with no other indications of melting (Bates, 1997b).

A regression slope was imposed on a chosen data point to provide an estimate of the maximum value for the vapour pressure at 25ºC (Tremain, 1997). No statistical analyses were performed because the balance readings were too low and variable.

The water solubility was determined using the flask method (Hogg, 1997) as the column elution method could not be performed due to the physical nature of the chemical. Flasks containing samples of the notified chemical and distilled water were shaken for 17 hours (at 30ºC) and left to stand for not less than 24 hours (at 20ºC) before being filtered and analysed by HPLC.

Due to the low water solubility of the chemical it was not possible to experimentally determine the dissociation constant or hydrolytic behaviour. However, the substance contains no functionalities likely to hydrolyse in the environmental pH range of 4-9.

An amine group is present in the chemical, which has the potential to become cationic in the

environment. However, taking into consideration the pKa of diphenylamine (0.7), the chemical is not expected to become protonated in the environment.

The partition co-efficient and adsorption/desorption constant are high and the notified chemical can be expected to bind strongly to, or be associated with, soil and sediment (Hogg, 1997, Bates, 1997a).

The autoignition temperature test was only conducted up to the pour point of the notified chemical (56ºC). Method A16 of the EEC Directive was used.

4. PURITY OF THE CHEMICAL

Degree of Purity: 31% in Additive 3

Hazardous Impurities:

Chemical name:	Phenyl-1-naphthylamine
Synonyms:	N-phenyl-1- naphthylamine
CAS No.:	90-30-2
Weight percentage:	1.0% maximum
Toxic properties:	Reported skin sensitiser in laboratory animals and humans; Chemicals of this class induce methaemoglobinaemia. (IPCS, 1998)

Chemical name:	Diphenylamine
Synonyms:	Benzenamine, N-phenyl
CAS No.:	122-39-4
Weight percentage:	0.5%
Toxic properties:	Hazard Classification (NOHSC, 1999b): Toxic (T), R23/24/25 – Toxic by Inhalation, in Contact with Skin and if Swallowed; R33 – Danger of Cumulative Effects. Exposure Standard (NOHSC, 1995): 10 mg/m^3 TWA.

5. USE, VOLUME AND FORMULATION

Use
The notified chemical forms part of an additive package, Additive 3, which will be used in jet engine oils. The jet oils are used in commercial and military aircraft, including turbo-jets, turbo-fans, turbo-props, and turbo-shafts (helicopter).

The additive package was in use in Australia during 1998 under a commercial evaluation permit granted under section 21G of the Act.

Volume
Imported volume will be greater than one tonne for the first two years increasing to more than 5 tonnes by the fifth year.

Formulation
No manufacture of the notified chemical will occur in Australia. Additive 3, containing the notified chemical, will be imported as a component of finished jet oil in one litre stainless steel cans. No blending or reformulation will occur in Australia. The finished oil contains less than 1% of the notified chemical.

6. OCCUPATIONAL EXPOSURE

Exposure
Dockside and Transport
The jet oil containing the notified chemical will be imported in one litre stainless steel containers. Occupational exposure is not likely except in the event of a spill.

Engine Maintenance
Aircrafts undergo a series of maintenance checks, depending upon age of craft, flight hours and maintenance log. Not all service checks require exposure to the jet oil. It is anticipated that less than 50 workers throughout Australia will be exposed to the finished oil containing the notified chemical. For airline and military personnel, exposure would primarily be to the hands and arms during engine maintenance and repair and filling operation tasks; opportunity also exists for eye and other body contact and where aerosols are generated, inhalation exposure. Exposure to the jet oil during engine checks is expected to be incidental[1].

Inflight
Pressurised air is introduced into the aircraft passenger/crew cabin via air compressors and heat exchangers in the engine. Under conditions of engine seal failure, small amounts of oil from engine bleed air could enter the crew and passenger compartments. The notifier indicates there have been anecdotal reports of jet engine oil and hydraulic odours in jet aircraft air, but measurements of cabin air have not detected the oils or their degradation products. A study by Crane et al. 1983, cited by the notifier, showed that the toxicity of experimentally generated thermal degradation products of jet oil was accounted for by

[1] Incidental exposure is assumed to be one event per day and would typically involve splashes or spills which arose from the way in which the process was carried out ([EC, 1996).

production of carbon monoxide.

Disposal
There is potential for skin contact during disposal of used product. The used oil will be collected by waste oil contractors. The used oil will either be burned for fuel or incinerated.

Control measures
To prevent exposure the notifier recommends that workers who come into contact with the jet oil wear impervious gloves and clothing, and safety glasses.

Worker Education and Training.
The pattern of use is considered as non-dispersive[2]. The notifier states that airline workers, maintenance, ground and aircraft crew are trained in order to meet specific job requirements. Training is expected to encompass the *Core Training Elements for the National Standard for the Control of Workplace Hazardous Substances* (NOHSC, 1997).

7. PUBLIC EXPOSURE

The public may be exposed to the notified chemical in certain uncommon situations, such as accidental or engine seal failure, and from disposal or combustion of used oil.

According to the Material Safety Data Sheet (MSDS), spillages are to be contained, absorbed onto a suitable fire-retardant material (treated saw dust, diatomaceous earth etc) and collected for disposal in accordance with local and state EPA regulations. Spills should be prevented from entering drains or waterways.

At the use site, it is estimated that minimal amounts (less than one gram) will be left in the container after each use. Used oil is collected by contractors and either burned for fuel or incinerated. It is estimated that the maximum amount of the notified chemical in used oil disposed of by these methods will be less than 60 kg per annum.

8. ENVIRONMENTAL EXPOSURE

Release

Release of the chemical could occur in the event of accident during transport of the oil product. The MSDS provides adequate clean-up instructions for clean up operations. The small containers used for packaging also mean that any spills should be on a small scale.

The notified chemical will share the fate of the used jet oil. Engine maintenance will only occur at airline maintenance facilities, which should be equipped with adequate spill

[2] Non dispersive use refers to processes in which substances are used in such a way that only certain groups of workers, with the knowledge of processes, come into contact with these chemicals. Procedures are normally worked out to achieve adequate control of exposure commensurate with risk (EC, 1996).

containment facilities. Oil losses during top-ups and oil changes are not estimated by the notifier. These are expected to be low and to be contained by concrete pads from entering the environment.

Supplementary information obtained for the purpose of this assessment indicates that in commercial jet aircrafts, engine oil is topped up only. Complete removal and refilling of oil occurs only during engine rebuilding. When the engine oil is changed the used oil is transferred to drums and passed on to used oil contractors for disposal. The used oil will either be burned for fuel or incinerated.

The notifier estimates the amount of residue left in the original imported cans to be less than one gram and it is assumed to share the same fate as the used oil removed from the aircraft, that is, burnt for fuel or disposed to landfill. Airlines are encouraged to recycle the steel cans. Alternatively, they may go to landfill.

Fate

Any waste chemical disposed to landfill will remain bound to or associated with soil particles. Due to the low solubility and high partition coefficient of the chemical, the potential for aquatic contamination through leaching would be very low. In landfill sites the chemical will undergo slow biodegradation. A modified Sturm test (Bealing, 1997) was performed on the notified chemical, which resulted in less than 2% conversion to carbon dioxide after 28 days. Incineration will destroy the substance converting it to oxides of carbon and nitrogen and water vapour.

Engine oil removed from aircraft engines is collected and disposed of by waste oil collection services. While some of the waste oil collected is recycled, most of it will be burned as fuel. The chemical in the oil will be destroyed by burning.

The potential for bioaccumulation in the environment was not determined, and the chemical is considered to have the potential to bioaccumulate. The notified chemical has a high value for log P_{ow}, which would favour its incorporation into fatty tissues. However, this potential is likely to be mitigated by the moderately high molecular weight and very low water solubility (Connell, 1990). Furthermore, its exposure to the environment should be limited by the specialised use as an aviation oil.

9. EVALUATION OF TOXICOLOGICAL DATA

The following studies were conducted on Additive 3. Additive 3 contains the notified chemical at approximately 30% w/w. It also contains residual starting materials (alkylated arylamines ~50% w/w) and fatty acid esters. Toxicity studies were conducted according to OECD guidelines (OECD, 1995-1996).

9.1 Acute Toxicity

Summary of the acute toxicity of Additive 3:

Test	*Species*	*Outcome*	*Reference*
Acute oral toxicity	Rat	LD_{50} >2 000 mg/kg	(Mallory, 1996)
Acute dermal toxicity	Rat	LD_{50} >2 000 mg/kg	(Denton, 1997a)
Skin irritation	Rabbit	Non irritating	(Denton, 1997a)
Eye irritation	Rabbit	Inconclusive	(Denton, 1997b)
Skin sensitisation	Guineapig	Non sensitising	(Denton, 1997c)

9.1.1 Oral Toxicity (Mallory, 1996)

Species/strain:	Rat/Sprague-Dawley
Number/sex of animals:	5/sex
Observation period:	14 days
Method of administration:	Oral (gavage), 2 000 mg/kg of test substance administered in corn oil (10 mL/kg)
Clinical observations:	Soft and watery faeces, bright yellow urine, discolored hair and poor grooming were observed at 1 hour post-dose through to day 7; for the remainder of the study all animals appeared normal; there was a mean body weight gain on day 7 and 14
Mortality:	nil
Morphological findings:	No visible lesions were observed in any animal at terminal necropsy; no treatment related abnormalities were noted.
Test method:	Limit test OECD TG 401
LD_{50}:	>2 000 mg/kg

Result:	Additive 3 was of very low acute oral toxicity in the rat.

9.1.2 Inhalation Toxicity

An acute inhalation study was not conducted. Claims were made and accepted for variation of schedule requirements for this toxicological endpoint on the basis of the physical nature of the test substance (waxy solid at room temperature).

9.1.3 Dermal Toxicity (Denton, 1997a)

Species/strain:	Rat/Sprague-Dawley
Number/sex of animals:	5/sex
Observation period:	15 days
Study design:	Animals were also observed for dermal reactions for the duration of the study.
Method of administration:	2 000 mg/kg applied to the clipped dorsal skin (3 mL/kg of warmed test substance in Alembicol D) held under semi-occlusive dressing for 24 hours
Clinical observations:	No clinical signs of reaction to treatment were observed
Mortality:	Nil
Dermal reactions:	Yellow staining of the fur persisted to day 4 for the five female rats; dermal reactions were not observed during the observation period.
Morphological findings:	Pelvic dilation was seen in the left kidney of one male and bilateral uterine distension in one female; these are common background findings in rats of this age and strain and were not considered to be toxicologically significant; no other macroscopic changes were observed.
Test method:	OECD TG 402
LD_{50}:	>2 000 mg/kg
Result:	Additive 3 was of low acute dermal toxicity in rats

9.1.4 Skin Irritation

An assessment of the skin irritancy potential for Additive 3 was made in the acute dermal toxicity above. Dermal responses were not noted during the 15 day observation period following a 24 hour exposure to the test substance, other than yellow staining of the fur at the dose site in female animals.

9.1.5 Eye Irritation (Denton, 1997b)

Species/strain:	**Rabbit/New Zealand White**
Number/sex of animals:	1 male
Observation period:	3 days
Method of administration:	0.1 mL of warm undiluted test substance shaped into a pellet placed into the conjunctival sac of one eye. The lower eyelid was gently prised away from the eyeball to create a receptacle for the dose. After instillation the eyelids were held closed for a few seconds to prevent loss of dose.
Test method:	OECD TG 405
Comment:	Although instillation of the test substance caused no initial sting response, on release of the eyelids the pellet was ejected from the conjunctival sac and the material became matted into the hairs adjacent to the eye where it rapidly cooled and solidified. Instillation of the test substance into the conjunctival sac caused no iridial, corneal or conjunctival changes; however, due to the physical nature of the material, it was concluded that administration of the material in compliance with OECD study guidelines was not possible and that no adequate determination could be made of the test substance ocular irritancy.

9.1.6 Skin Sensitisation (Denton, 1997c)

Species/strain:	Guineapigs /Dunkin-Hartley
Number of animals:	20 test, 10 control/females
Induction procedure:	**Test animals** Day 1: three pairs of intradermal injections to the clipped dorsum as follows:

	- Freund's Complete Adjuvant (FCA) with distilled water (1:1) - test substance, 15% m/v in warm Alembicol D - test substance 15% m/v in FCA emulsion (1:1) Day 7: 0.4 mL of 10% m/m sodium lauryl sulphate in petrolatum, rubbed into the same dorsal area Day 8: test substance (70% in alembicol D) applied to filter paper and held to the shaved dorsum under occlusive dressing for 48 hours; Control animals: Control animals were treated similarly, but omitting the test substance
Challenge procedure:	Day 21: 60% and 30% of test substance in Alembicol D applied to the posterior and anterior shorn right flank, respectively, via Finn chambers, for an exposure period of 24 hours
Challenge outcome:	Slight desquamation was observed in some control and test animals; dermal responses were no different to those seen in the controls
Test method:	OECD TG 406, Magnusson and Kligman Maximisation Test
Result:	**Additive 3 was non sensitising to guineapig skin**

9.2 Repeated Dose Toxicity (Hunter, 1998)

Species/strain:	Rat/Sprague-Dawley
Number/sex of animals:	Treatment phase: 5/sex/dose group Recovery phase: 5/sex/control and high dose group
Method of administration:	Oral (gavage)
Dose/Study duration:	0, 15, 150 or 1 000 mg/kg/day for 28 consecutive days, followed by a 14 day treatment free period for animals assigned to the recovery group
Mortality:	Nil

Clinical observations:
Treatment phase: food consumption in high dose animals was generally more than control animals during treatment. There were no clinical sings attributable to treatment.
Recovery phase: comparable to controls.

Clinical chemistry/Haematology:
Treatment phase: animals of the high dose group had higher plasma total bilirubin (6 fold increase above controls) and in males only lower plasma cholesterol concentration and slightly higher aspartate aminotransferase (AST) activity when compared to control values. The study authors suggest that the increased total bilirubin level may be due to the method of detection for bilirubin as it could be also detecting the test substance.
One female of the low dose group had a significantly decreased total leucocyte count, decreased lymphocyte count, and increased neutrophil count. The study authors suggested that this variation could be because the sample was taken at necropsy.
Recovery phase: significantly increased alanine transferase (ALT) activity was observed in high dose males and significantly increased alkaline phosphatase (AP) activity and inorganic phosphatase (Pi) levels were observed in high dose females.

Organ weight:
Treatment phase: significantly increased adrenal and liver weights in high dose animals and low dose males, increased kidney weights in high dose males, increased thymus weights in low dose males, increased brain weights in mid dose males.
Recovery phase: comparable to controls.

Necropsy:
Treatment phase: a large liver was noted in four of the high dose animals (3 males, 1 male), one mid dose female, and one control male.
Recovery phase: comparable to controls.

Histopathology:
Treatment phase: in high dose animals:
hepatic centrilobular hypertrophy – charecterised by a minor increase in cell size and/or cytoplasmic eosinophilia resulting in a slight accentuation of the lobular pattern;
thyroid follicular cell hypertrophy – charecterised by a minor increase in cell height; and
adrenal cortical hypertrophy/vacuolation – charecterised by a minor increase in width of the zona fasciculata with slight compression of the zona reticularis in some cases.
Recovery phase: tissues not examined as there was considered to be no toxic effect of test substance at the end of the main study period.

Comment:
There was no adverse affect on body weight or body weight gain throughout the treatment phase. Changes in clinical chemistry and haematology parameters observed at the end of the treatment phase were not found after the 14 day treatment free period. At the end of recovery, changes in liver enzyme (ALT, AP and Pi) activity were recorded in high dose animals. Macroscopic changes and organ weights were comparable to the control group after the recovery phase. Findings consistent with adaptive physiological responses related to test article administration were found; the adrenal, thyroid and liver of rats of the high dose group showed minor treatment related effects (hypertrophy) in the high dose group following treatment. Morphologic findings were not suggestive of specific organ toxicity related to test substance administration.

Because of increased adrenal and thymus weights observed in low dose animals a no observed effect level (NOEL) cannot be established. Based upon the hypertrophy observed in the adrenals, thymus and liver of high dose animals, the no observed adverse effect level (NOAEL) for this study is determined to be 150 mg/kg/day.

Test method:	OECD TG 407
Result:	**The NOAEL determined for this study is 150 mg/kg/day**

9.3 Genotoxicity

9.3.1 *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay (Wagner, 1997)

A series of investigations were conducted to the mutagenicity of the notified chemical.

In the first investigation (Investigation 1), Additive 3 solubilised in acetone induced a positive response in *Salmonella typhimurium* strains TA 98 and TA 1538, in the presence of an external metabolic activation system.

An Ames study (not provided by notifier in their notification dossier) was performed at Mobil Environmental and Health Sciences Laboratory with Additive 3 at a concentration of 50% w/w in ester. When solubilised in tetrahydrofuran (THF) and tested at doses comparable to those in Investigation 1, the formulation did not produce a positive response.

Therefore, to determine the role of diluent in the observed mutagenicity, Additive 3 (85% w/w) and Additive 3A (50% w/w) were solubilised in either acetone or THF and tested in the presence of metabolic activation (Investigation 2). Positive responses were obtained with Additive 3 and acetone. In a second trial Additive 3 was tested in varying volumes of acetone to determine if the amount of acetone used to solubilise Additive 3 would alter the magnitude of the previously observed mutagenic response.

Investigation 1

Strains:	*Salmonella typhimurium* TA 1537, TA 1535, TA 1538, TA 100 and TA 98 and *Escherichia coli* WP2*uvrA*
Metabolic activation system:	Liver fraction (S9 mix) from rats pretreated with Aroclor 1254
Test method:	Similar to OECD TG 471, 472
Concentration range:	Each concentration was tested in triplicate, in two independent experiments: <u>Experiment 1:</u> With S9: TA 98, TA1538: 100, 333, 1 000, 3 333, 5 000 μg/plate; Without S9: TA 98, TA1538: 10, 33, 100, 333, 1 000 μg/plate; With and without S9: TA 100, TA 1535, TA 1537, WP2*uvrA,* 10, 33, 100, 333, 1 000 μg/plate; <u>Experiment 2:</u> **With S9: TA 98 & TA1538: 333, 1 000, 3 333, 4 000, 5 000 μg/plate;** **Without S9: TA 98 & TA1538: 333, 1 000, 3 333, 4 000, 5 000 μg/plate;** **With and without S9: TA 100, TA 1535, TA 1537, WP2*uvrA* 10, 33, 100, 333, 1 000 μg/plate;** Appropriate strain specific positive control reference substances were used in each experiment.
Solubility and Toxicity:	The test substance was soluble in acetone at a concentration of 500 mg/mL; precipitation was observed at and above 333 μg/plate but no appreciable toxicity was observed up to 5 000 μg/plate.
Comment:	Two fold increases in the frequency of revertant colonies were seen in tester strain TA98 and TA 1538 in the presence of metabolic activation, in both experiments; no significant increases were observed without metabolic activation; There were no significant increases in other tester strains, either in the presence or absence of metabolic activation; Concurrent positive controls used in the test induced marked increases in the frequency of revertant colonies and the activity of the S9 fraction was found to be satisfactory.
Result:	Additive 3 is considered mutagenic in bacterial strains TA 98 and TA 1538 in the presence of metabolic activation.

Investigation 2

Strain:	*Salmonella typhimurium* TA98
Test substances:	Additive 3: 85% of notified chemical in ester vehicle; Additive 3A: 50% of notified chemical in ester vehicle
Test Substance Vehicle:	Acetone, THF
Metabolic activation system:	Liver fraction (S9 mix) from rats pretreated with Aroclor 1254
Test Substance Solubility in Vehicle	Test substances were soluble in both acetone and THF at and above 200 mg/ml
Experiment 1 – Study Design and Test Concentrations:	Each concentration was tested in triplicate, with S9 as follows: Additive 3 or Additive 3A at 0, 3.3, 10, 33, 100, 333, 1 000 or 5 000 μg/plate in acetone or THF; positive control: 2-aminoanthracene 1.0 μg/plate; Vehicle controls: acetone, THF.
Comment:	Precipitation observed at ≥ 333 μg per plate, but no toxicity; Additive 3 with acetone as vehicle: no significant increase in revertant colonies; Additive 3a with acetone as vehicle: non-dose responsive increase (2.2 fold at 5 000 μg) in revertant colonies; Additive 3 and Additive 3a with THF as vehicle: no significant increase in revertant colonies.
Experiment 2 – Study Design and Test Concentrations:	Each concentration was tested in triplicate, with S9 as follows: Additive 3 at 0, 3 333 or 4 000 μg/plate each in plating aliquots of 5, 10, 25, 50 100 μL of acetone; positive control: 2-aminoanthracene 1.0 μg/plate; vehicle control: acetone

Comment:
Precipitation was observed at ≥ 3 333 μg, but no toxicity. Increases in the number of revertant colonies were observed at and above 25μL plating aliquots at concentrations of 3 333 and 4 000 μg/plate. Volumes of acetone did not alter the consistent increase in TA98 revertants.

The notifier has provided commentary by Schreiner CA on the significance of the results from the above experiments. "These findings suggest that acetone either more effectively solubilizes a very small volume component of Additive 3 making it more available for metabolism to a mutagenic form, or reacts with a metabolite producing the low level response which remains constant due to the presence of very little material available to react. It is also possible that THF reacts with and thereby deactivates a mutagenic component that is present at low levels. In either case, it is unlikely that the principal components of Additive 3 (85% w/w) were responsible for this weak mutagenic activity."

Test method:	In-house investigation
Result:	Under the conditions of this study Additive 3 demonstrated mutagenic activity in *Salmonella typhimurium* strain TA98 in the presence of metabolic activation and when acetone was used as the solvent.

9.3.2 *In vitro* Chromosome Aberrations (Gudi, 1997)

Cells:	Human male peripheral lymphocytes
Metabolic activation system:	Liver fraction (S9 mix) from rats pretreated with Aroclor 1254
Study Design:	The initial assay (Experiment 1) was performed by exposing duplicate cultures to the test substance or controls. Cells were exposed continuously for 20 hours in the absence of metabolic activation (S9-) or for 4 hours in the presence of metabolic activation (S9+). Dividing cells were harvested at 20 hours from initiation of treatment. A repeat independent assay was performed (Experiment 2) to confirm the positive responses seen in Experiment 1. In Experiment 2, duplicate cultures were exposed to the test substance or controls for 20 or 44 hours in the absence of S9 and for 4 hours in the presence of S9. Dividing cells were harvested at either 20 or 44 hours from initiation of treatment.

Experiment 1	Without S9: Treatment/Harvest Time: 20/20; 0, 0.25, 0.75, 2.5, 7.5, 25, 75, 250, 750, 2500* µg/ml; positive control: mitomycin C (MMC) 0.25µg/ml; vehicle: acetone. With S9: Treatment/Harvest Time: 4/20; 0, 0.25, 0.75, 2.5, 7.5, 25, 75, 250, 750, 2500*µg/ml; positive control: Cyclophosphamide (CP) 25µg/ml; vehicle: acetone. * highest concentration showing at least 50% reduction in mitotic index.
Comment:	**Without S9,** The percentage of treated cells with structurally aberrant cells, at any concentration level was not significantly different to that of the vehicle control. With S9, A significant dose-related increase in the percentage of cells with numerical and structural aberrations (chromatid type) in cells exposed for 4 hours with a 16 hour recovery period (20 hour harvest) at 75 to 750 µg/ml when compared to the vehicle control.
Experiment 2:	Without S9, Treatment/Harvest Time: 20/20 or 44/44 0, 28.2, 56.3, 112.5, 225, 450, 750, 1250, 2500*µg/ml; positive control: MMC 0.13µg/ml; vehicle: acetone. With S9, Treatment/Harvest Time (hours): 4/20 or 4/44, 0, 28.2, 56.3, 112.5, 225, 450, 750**, 1250, 2500µg/ml. positive control: CP 25 µg/ml vehicle: acetone. * highest concentration showing at least 50% reduction in mitotic index at 20 hour harvest time. ** highest concentration showing at least 50% reduction in mitotic index at 44 hour harvest time.
Comment:	**Without S9,** The percentage of treated cells with structurally

	aberrant cells, at any concentration level or harvest time, was not significantly different to that of the vehicle control. With S9, In the 20 hour harvest group, a significant increase in the percentage of cells with structural aberrations (chromatid type) at 450 µg/ml when compared to the control. No significant increase was observed at the higher concentrations. At the 44 hour harvest**,** a small dose related increase in the percentage of cells with structural aberrations (chromatid type). A small increase in the percentage of numerical aberrations was observed but it was not dose-related. The increases observed in both experiments were within the range of 6% allowed for solvent controls. The study authors regarded the positive responses as being not biologically significant.
Test method:	(Evans, 1976)
Solubility:	The test substance was soluble in acetone at a concentration of 500 mg/ml; in the treatment medium, the test substance was a workable suspension at 2 500 µg/ml, was soluble but cloudy with no visible precipitate at 75, 250 and 750 µg/ml and was soluble at all other concentrations tested.
Metaphase analysis:	The highest dose level demonstrating at least 50% reduction in mitotic index and three lower doses were evaluated microscopically for structural chromosome aberrations.
Result:	Additive 3 was considered to be weakly positive, in the presence of metabolic activation, for the induction of structural or numerical chromosome aberrations in this assay. Additive 3 was negative in the absence of metabolic activation.

9.3.2 Micronucleus Assay in the Bone Marrow Cells of the Mouse (Gudi, 1998)

Species/strain:	Mouse/ICR
Number and sex of animals:	5/sex/vehicle control, low and mid dose groups; 10/sex/high dose group; 5/sex/positive control group
Doses/Method of	Test substance: 500 mg/kg (low), 1 000 mg/kg (mid)

administration:	or 2 000 mg/kg (high); positive control: cyclophosphamide 50 mg/kg; vehicle control: corn oil; all administered via intraperitoneal injection at a constant volume of 20 ml/kg bw.
Sampling schedule:	Vehicle control (5/sex), low, mid and high (5/sex) dose animals were sacrificed 24 hours after dosing; remaining animals of the vehicle control group and high dose animals were sacrificed 48 hours after dosing; positive control group sacrificed 24 hours after dosing.
Clinical observations:	No mortality; all animals appeared normal throughout the observation period
Micronuclei score:	No significant increase in micronucleated polychromatic erythrocytes (PCEs) due to treatment with test substance at either sampling time; the positive control caused a significant increase in micronucleated PCEs
Test method:	(Mavournin, 1990), (Hayashi, 1973), (Heddle, 1973) similar to OECD TG 474
Result:	Additive 3 did not induce a significant increase in micronucleated PCEs in bone marrow cells of the mouse *in vivo*

9.4 Overall Assessment of Toxicological Data

The oral and dermal acute toxicities of Additive 3 were very low and low, respectively, LD_{50}>2 000 mg/kg. An acute inhalation study was not performed because the notifier judged that inhalation exposure would not be substantial given that the material is present at small concentrations in the finished oil and at room temperature it is a waxy solid. Eye irritancy could not be equivocally determined under OECD test guidelines because the test substance immediately solidified and did not remain in contact with the eye. However, initial instillation caused no sting response. A study for dermal irritancy was not conducted. However, there was no evidence to suggest dermal irritancy potential based on the findings of the acute dermal toxicity study. In an adjuvant type test, Additive 3 was non-sensitising to guineapig skin.

In a repeated oral dose study there was no adverse affect on body weight or body weight gain throughout the treatment phase. Changes in clinical chemistry and haematology parameters observed at the end of treatment were reversible after a 14 day treatment free period. At the end of recovery, minor changes in liver enzyme activity were recorded in high dose animals. Macroscopic changes and organ weights were comparable to the control group after the recovery phase. Findings consistent with adaptive physiological responses related to test article administration were found in the adrenal, thyroid and liver of rats of the high dose

group. The NOAEL determined for this study is 150 mg/kg/day.

Additive 3 was assessed for mutagenicity in three standard screening assays. In a series of bacterial reverse mutation assays using 5 strains of *Salmonella typhimurium* and *E. coli* strain WP2 uvrA, the notified chemical induced a mutagenic response in *Salmonella typhimurium* strain TA 98 and TA 1538, in the presence of a rat liver metabolic activation system and using the solvent vehicle acetone. In a supplementary study with TA 98 in the presence of metabolic activation, the mutagenicity in acetone was confirmed, however, the test was negative when tetrahydrofuran was used as the solvent. Additive 3 was found to be weakly clastogenic to human peripheral blood lymphocytes *in vitro* in the presence of metabolic activation and is considered to be a weak mutagen in this system. Additive 3 was not considered clastogenic *in vivo* in the mouse micronucleus test.

Human Health Effects
During 1998 the notified chemical was in use in Australia under a commercial evaluation permit granted under Section 21G of the Act. The notifier reports no adverse health effects were reported from the use of the notified chemical during the evaluation period. The notifier also indicates there have been no reports of adverse effects for the notified chemical, either at US manufacturing or blending facilities or in the European Union or Canada where it has been notified.

Hazard Classification
Additive 3 would not be classified as a hazardous substance under the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999a) in relation to the data provided.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

Ecotoxicity studies were conducted using Additive 3 according to OECD guidelines (OECD, 1995-1996). The method of dispersion of the substance in water for daphnia and algae studies was the water accommodated fraction (WAF). The LC_{50}, LC_{100} and NOEC are based on the lethal loading rates of water. In the acute fish toxicity study, fish were exposed to the test material, maintained as a distribution of small droplets using shielded propeller stirrers to give a mechanical dispersion. Attempts were made to provide qualitative analysis of the WAFs using HPLC. However, it was not possible to detect Additive 3 at the concentrations tested; therefore, no qualitative comparison was possible. Less than 2% of the substance biodegraded after 28 days.

Test	*Species*	*Results*
Acute toxicity Static system [OECD TG 203]	Rainbow trout (*Oncorhynchus mykiss*)	96 hour LC_{50} >1 000 ppm NOEC (96 hour) = 1 000 mg/L (Test performed on dispersion)
Acute Immobilisation Static system [OECD TG 202]	Water flea *Daphnia magna*	48 hour EL_{50} >2 000 ppm NOEC (48 hour) = 2 000 mg/L (Test performed on WAF)
Growth Inhibition [OECD TG 201]	Freshwater Green Algae (*Raphidocelis subcapitata*)	EbL_{50} (72 hour) > 2 000 mg/L $E\mu L_{50}$ (72 hour) > 2 000 mg/L NOEC (72 hour) = 2 000 mg/L (Test performed on WAF)

* NOEC - no observable effect concentration.
EbL_{50} determined using area under the growth curve.
$E\mu L_{50}$ determined using growth rates.

Acute toxicity studies on fish were performed on continuously stirred dispersions of the notified chemical in water and the results are based on nominal concentrations. Due to the low water solubility of the notified substance, the studies for the effects on daphnia and algae were performed on the WAF. A series of WAFs were prepared at the following nominal loading rates: 125, 250, 500, 1 000 and 2 000 mg/L, and stirred for 20 hours. After settling for 4 hours, the WAF was removed and used as the treatment solution. The WAFs were clear and colourless.

The notified substance can be classed as non-toxic to rainbow trout, daphnia and algae, up to its limit of solubility (the WAF), (Mattock, 1998a; Mattock, 1998b; Mattock, 1998c)

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

The notified chemical is unlikely to present a hazard to the environment when it is used as specified. No formulation or repackaging will occur in Australia, any accidental spills would be small because the chemical is held in low volume containers. Losses during transfer and oil exchanges would be held within spill containment facilities. Waste disposed to landfill is unlikely to pose a hazard to the aquatic organisms, due to the low ecotoxicity and low solubility of the notified chemical.

The notified chemical will share the fate of the jet engine oil. This will be removed from aircraft engines, collected and most likely incinerated or used as fuel oil. This will destroy the chemical converting it to oxides of carbon and nitrogen and water vapour.

The environmental hazard from the notified chemical can be rated as low.

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

During 1998 the notified chemical was in use in Australia under a commercial evaluation permit granted under Section 21G of the Act. The notifier reports that no adverse health effects were reported from the use of the notified chemical during the evaluation period. The notifier also indicates there have been no reports of adverse effects for the notified chemical, either at US manufacturing or blending facilities or from use in the European Union or Canada.

The acute oral and dermal toxicity of Additive 3 was very low and low, respectively, in experimental animals. Since Additive 3 is a waxy solid at room temperature, acute inhalation studies were not performed. Eye irritancy studies were inconclusive, however, the substance does not appear to have eye irritancy potential because it immediately solidifies on entering the eye. There was no evidence to suggest Additive 3 is a dermal irritant, nor was it a skin sensitiser in guineapigs.

The notified chemical did not cause specific organ or systemic toxicity in a 28-day repeat oral dose study in rats. The NOAEL determined for the study is 150 mg/kg/day.

Additive 3 was positive in *Salmonella typhimurium* strain TA 98 and TA 1538, in the presence of metabolic activation and using acetone as the solvent vehicle. In a supplementary study with TA 98 in the presence of metabolic activation, the test was negative when tetrahydrofuran was used as the solvent. Additive 3 was found to be weakly clastogenic to human peripheral blood lymphocytes *in vitro* in the presence of metabolic activation. Genotoxic activity was not observed *in vivo* in the mouse micronucleus test.

Based on the data provided, Additive 3 is not considered to be a hazardous substance under the NOHSC *Approved Criteria for Classifying Hazardous Substances* (NOHSC, 1999a).

Occupational Health and Safety

During import and transport of the jet oil containing the notified chemical, worker exposure is unlikely, except in the event of a spill. Exposure after a spill would be controlled by use of the recommended practices for spillage clean up given in the MSDS supplied by the notifier.

Occupational exposure may occur during engine checks, oil change and disposal of spent oil. Additive 3 has low vapour pressure (less than 9.9×10^{-5} Pa) and the jet oil containing the notified chemical is viscous and therefore unlikely to generate aerosol under normal conditions. Consequently, inhalation will be a minor route of exposure and skin contamination is expected to be the main route of exposure. The notified chemical has a molecular weight of greater than 500 and is not expected to be readily absorbed through normal, intact skin. The hydrocarbon content of the jet oil itself may cause skin irritancy upon repeated or prolonged exposure. The process in which the jet oil containing the notified chemical will be handled is non-dispersive with the level of exposure expected to

be incidental. Personal protective equipment is also recommended by the notifier to workers who have contact with the jet engine oil. Given the above, together with the low hazard associated with the notified chemical, and its low concentration in the jet oil (less than 1%), the occupational health risk posed to workers involved in maintenance of aircrafts is considered to be low.

The notifier reports that oil or degradation products have not been detected in cabin air.

Public Health
Due to the low toxicity of Additive 3, the low concentration of the notified chemical (less than 1.0%) in jet oils and the low potential for public exposure, it is considered that the notified chemical will not pose a significant hazard to public health when used in the proposed manner.

13. RECOMMENDATIONS

To minimize occupational exposure to jet oil containing the notified chemical the following guidelines and precautions should be observed:

- Workers should be advised to follow good personal hygiene practices to minimise the possibility for skin contact and promptly wash contaminated skin with soap and water. Contaminated clothing permeable to the oil should be removed without delay;
- Safety goggles should be selected and fitted in accordance with Australian Standard (AS) 1336 (Standards Australia, 1994) to comply with Australian/New Zealand Standard (AS/NZS) 1337 (Standards Australia/Standards New Zealand, 1992);
- Industrial clothing should conform to the specifications detailed in AS 2919 (Standards Australia, 1987) and AS 3765.1 (Standards Australia, 1990);
- Oil impermeable gloves or mittens should conform to AS 2161.2 (Standards Australia, 1998);
- All occupational footwear should conform to AS/NZS 2210 (Standards Australia/Standards New Zealand, 1994);
- Spillage of the notified chemical should be avoided. Spillages should be cleaned up promptly and disposed of as recommended in the MSDS;
- A copy of the MSDS should be easily accessible to employees.

If the conditions of use are varied from the notified use (as a component of jet oils), greater exposure of the public to the product may occur. In such circumstances, further information may be required to assess the hazards to public health.

14. MATERIAL SAFETY DATA SHEET

The MSDS for Additive 3 (also known as MCP 818) was provided in accordance 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, 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.

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Attachment 1

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

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

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

CORNEA

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

CONJUNCTIVAE

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

IRIS

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