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**NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION
AND ASSESSMENT SCHEME**

FULL PUBLIC REPORT

Difluoromethane

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Director
Chemicals Notification and Assessment

FULL PUBLIC REPORT**Difluoromethane****1. APPLICANT**

Elf Atochem (Australia) Pty Ltd of Level 6 65 Berry Street NORTH SYDNEY NSW 2060, has submitted a standard notification statement with their application for an assessment certificate for difluoromethane. The notified chemical will be used as a low temperature refrigerant for commercial use.

2. IDENTITY OF THE CHEMICAL

Chemical name: difluoromethane

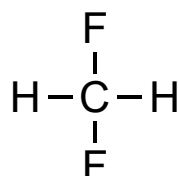
**Chemical Abstracts Service
(CAS) Registry No.:** 75-10-5

Other name: methylene fluoride

Trade name: HFC-32
R-32
Forane FX 220 (blend)
Forane 407C (blend)
Forane FX 230 (blend)
Forane 221 (blend)
Forane FX 40 (blend)

Molecular formula: CF₂H₂

Structural formula:



Molecular weight: 52

Method of detection & determination: Infrared (IR) and Mass Spectrometry (MS), Gas Chromatography (GC) and Nuclear Magnetic Resonance (NMR) data were provided for difluoromethane and are

consistent with the structure of the chemical.

Spectral data: IR; absorption peaks occurred at 3015, 2947, 1451, 1175, 1102 and 1086 cm^{-1}
MS; major peaks observed at 33 and 51 g/mol

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa:	colourless gas
Odour:	ether-like
Melting Point:	-136°C
Boiling Point:	-51.7°C
Vapour Density:	1.8 (air = 1) at 25°C and 101.3 kPa
Vapour Pressure:	1.69×10^3 kPa at 25°C
Water Solubility:	4.4 g/L at 25°C
Fat Solubility:	not provided
Partition Co-efficient (n-octanol/water) log P_{ow}:	0.21 at 25°C
Hydrolysis as a function of pH:	does not hydrolyse
Adsorption/Desorption:	not measured, gas
Dissociation Constant pK_a:	no dissociable groups
Flash Point:	not determined
Flammability Limits:	L.E.L. = 13.6% by volume in air U.E.L. = 32.2% by volume in air
Combustion Products:	hydrofluoric acid and carbonyl fluoride, carbon oxides
Decomposition Temperature:	not provided
Decomposition Products:	hydrofluoric acid and carbonyl fluoride, carbon oxides

Autoignition Temperature:	530°C
Explosive Properties:	explosive with air
Reactivity/Stability:	cylinder damage may result in the explosive release of contents; violent reactions occur with alkali or alkaline earth metals including powdered aluminium, zinc, beryllium, sodium, potassium, barium and calcium.
Particle size distribution: range -	not applicable, gas
Atmospheric Lifetime:	6 years
Ozone Depleting Potential:	0
Global Warming Potential:	500 years - 180 100 years - 580 20 years - 1800

Comments on missing data:

Vapour pressure and water solubility are taken directly from the ECETOC review (1).

Hydrolysis is not expected to be a significant degradation pathway as the notified substance is a stable gas; difluoromethane contains no hydrolysable functionalities. Significant sorption to soil is not expected as the notified substance is a gas.

Testing indicates difluoromethane remains in its undissociated form in water. It contains no dissociable groups.

The "lifetime" is defined as the time necessary for 63% degradation; it is equal to the "half-life" divided by $\ln 2$ ($= 0.69$) (1). These type of gases are slowly destroyed in the stratosphere by sunlight (photodegradation).

Difluoromethane has no effect on stratospheric ozone as it contains neither chlorine nor bromine (1).

The extent to which a greenhouse gas contributes to global warming depends on the volume emitted, the length of time which elapses before it is purged from the atmosphere and the infrared energy absorption properties of the gas. An index termed the Global Warming Potential (GWP) has been developed which provides a simplified means of describing the relative ability of each greenhouse gas emission to affect global climate change. It is the ratio of the warming caused by a substance to the warming caused by CO_2 (on a molecule per molecule basis) to allow a common basis for comparing impacts (2). Thus, with the GWP of CO_2 defined as one, difluoromethane has 180 times the GWP of CO_2 over a 500 year time horizon.

Difluoromethane (HFC-32) has approximately 36% less GWP than HFC-22 which it is replacing.

4. PURITY OF THE CHEMICAL

Degree of purity: $\geq 99\%$

Toxic or hazardous impurities: all impurities are present at less than 250 ppm.

Non-hazardous impurities (> 1% by weight): all impurities are present at less than 250 ppm. Traces of CH_3CF_3 , CH_2ClF and $\text{CH}_2=\text{CHCl} \cdot \text{CH}_3\text{Cl} < 0.01\%$.

Additives/Adjuvants: Difluoromethane will be imported in 5 refrigerant blends that have the following composition:

Forane FX 220 (blend)
21.5% difluoromethane
4.5% trifluoromethane
74% 1,1,1,2-tetrafluoroethane

Forane 407C (blend)
23% difluoromethane
25% pentafluoroethane
52% 1,1,1,2-tetrafluoroethane

Forane FX 230 (blend)
50% difluoromethane
50% pentafluoroethane

Forane 221 (blend)
45% difluoromethane
55% pentafluoroethane

Forane FX 40 (blend)
10% difluoromethane
45% pentafluoromethane
45% 1,1,1 trifluoroethane

None of the other gases used in the mixtures listed above appear in the List of Designated Hazardous Substances (3) or have atmospheric exposure standards (4), however gases in confined spaces, especially those that do not disperse quickly (denser than air, high vapour pressure), can act as asphyxiants by excluding oxygen.

5. INDUSTRIAL USE

Difluoromethane is a low temperature refrigerant that will be used in commercial refrigeration operations such as in supermarkets and in the fishing industry. It has a zero ozone depleting potential and is being introduced to replace ozone depleting chlorofluorocarbons (CFCs), in particular HCFC-22. The notified chemical will initially be imported and used in blends with other refrigerants, including pentafluoroethane, 1,1,1-trifluoroethane and 1,1,1,2-tetrafluoroethane. It will be sold to equipment manufacturers and service contractors as the blends Forane FX220, Forane 407C, Forane FX230, Forane 221 and Forane FX40.

Elf Atochem intends to import 10 - 100 tonnes/annum for the first five years.

It is not anticipated that difluoromethane will be manufactured in Australia, however, reformulation could possibly occur as a result of rebinding recovered blends containing difluoromethane.

6. OCCUPATIONAL EXPOSURE

Difluoromethane (as a component of the imported gas blends) will be shipped into Australia in liquefied form in pressurised tanks and ISO containers. These containers will be taken to warehouses from the dockside for storage. Four to ten dockside workers and transport personnel may be exposed to the notified gas if it is released accidentally. At the warehouse the gas is decanted into smaller cylinders of either 22 or 65 kg for transport to the sites where the gas will be used. The transferral is undertaken using a closed pipe system. However there is the potential for minor losses of gas to occur when connections are made and broken when decanting and during charging and servicing of refrigeration units. During liquefied gas transfer at the warehouse local exhaust ventilation is used to remove gas that escapes. During charging and servicing operations personnel will normally use impervious rubber gloves to avoid cold burns and goggles to prevent eye contact.

Specific operations where exposure to difluoromethane may occur include conversion of refrigeration systems to accommodate the new non CFC gases, installation and servicing and repair of equipment, charging and recharging of equipment (5). Potential exposure may also occur when recycling gases, this involves recovery of refrigerant, filtration, purification via evaporation and transfer to storage cylinders. There is the potential through large scale accidental leakage for fatalities to occur, this has happened overseas with HCFC-22.

Converting equipment (retrofitting) so that it can use difluoromethane involves the following procedure. The existing gases are removed and vented into a recovery system and recycled according to industry regulations. Refrigeration lubricants are removed via draining valves, the system is flushed with new lubricant and recharged with fresh ester lubricant. Difluoromethane or blends containing it are charged from their cylinders into the system via closed hoses. Normally about 0.1g of difluoromethane is released when the hoses are disconnected from the refrigeration system during this procedure.

Exposure to difluoromethane may occur through accidents when the cylinder is badly damaged, or through minor leaks from seals and gaskets in the system. Most accidental exposure will be to the gaseous form of difluoromethane, but liquid contact will also be possible.

7. PUBLIC EXPOSURE

Difluoromethane will not be manufactured in Australia. The notified chemical will be imported in a number of refrigerant blends: Forane FX220, Forane 407C, Forane FX230, Forane 221 and Forane FX40. The concentration of difluoromethane in these blends ranges from 10% to 50%. These blends will be transported by road within Australia. If accidental spillage occurs refrigerant blends will be contained and cleaned up according to practices recommended in the Material Safety Data Sheet (MSDS). No public exposure to the notified chemical is expected to occur during transport.

There will be no reformulation in Australia, only decanting into smaller volumes. At the warehouse, the gas blends will be transferred into small cylinders (22 or 65 kg cylinders) using a closed piping system. The public will not be exposed during the handling of the refrigerant. Public exposure to difluoromethane may occur as a result of a slow leak from a refrigerator, however, this is expected to be minimal.

Difluoromethane will not be disposed of in Australia. Refrigerant gases containing difluoromethane will be recycled and any surplus or unusable refrigerants will be returned to the supplier.

8. ENVIRONMENTAL EXPOSURE

· Release

The notified substance will not enter the environment intentionally when used in refrigeration systems, but any releases during refrigerant transfer, normal operation of cooling systems, following disposal of obsolete equipment or recovery of refrigerants therefrom, will rapidly volatilise to the atmosphere.

Release to the atmosphere may occur through decanting the gas to smaller cylinders. Losses are difficult to estimate but are quoted as approximately 0.1g per charge. If 100 tonnes of the gas was decanted into 22kg cylinders (resulting in ~4545 cylinders), losses of approximately 0.45kg could occur.

Releases can also be expected when hoses are disconnected during charging and servicing of refrigeration units. Estimates also quote losses at approximately 0.1g per charge. If all 4545 22kg cylinders were used in charging and servicing, approximately 0.90kg could be lost (0.1g lost when collecting used refrigerant, then 0.1g lost through charging with new refrigerant).

Total losses through fugitive emissions has been predicted by the industry to be less than 10% of working charge per annum. If 100 tonnes of the notified gas was imported and all put into service, losses through fugitive emissions would be

expected to be approximately 10000kg/year. However, the notifier claims that losses through these emissions is more likely to be 2% per annum, resulting in losses of approximately 2000kg/year. The notifier also claims that it is unlikely that all 100 tonnes of the notified gas would be put into service in any one year.

The Australian Refrigeration and Air Conditioning Code of Good Practice (5) requires that releases of ozone depleting refrigerants to the atmosphere during manufacturing, installation or servicing operations be reduced to the minimum level by re-use of refrigerant recovered. Recovery of refrigerant is required from performance testing during development and production. Refrigerant must be recovered in dedicated cylinders, identified by valving, labelling and colour coding. Where contaminated refrigerants are stored, they must be labelled to indicate the contents.

· Fate

Given its high volatility, any difluoromethane released to the environment will partition almost entirely to the atmosphere.

By analogy with other hydrofluorocarbons and hydrochlorofluorocarbons, it is proposed that degradation of difluoromethane will occur almost exclusively in the troposphere. Here it will react with tropospheric hydroxyl radicals, which abstract hydrogen. Reaction of difluoromethane with hydroxyl radicals leads to carbonyl fluoride (COF_2). This is then removed from the troposphere by uptake into clouds, rain and the oceans, where it is rapidly hydrolysed to CO_2 and HF. This process is expected to occur within a few days to a few weeks (1). The tropospheric degradation mechanism/pathway is outlined below.

The calculated overall atmospheric lifetime is 6 years. This is longer than either the intrahemispheric or interhemispheric mixing times of about 1 month and 1 year, respectively. This would allow difluoromethane to be dispersed over wide areas far from emission sources (1).

Any traces entering water would not be expected to undergo biodegradation at significant rates as degradation by activated sludge in a closed bottle test (OECD Test Guideline 301D) was minimal (28d biological oxygen demand 3 - 5% of theoretical). Any difluoromethane which might be present in aqueous waste streams would be expected to have a half-life with respect to volatilisation of days or a few weeks at the very most (1).

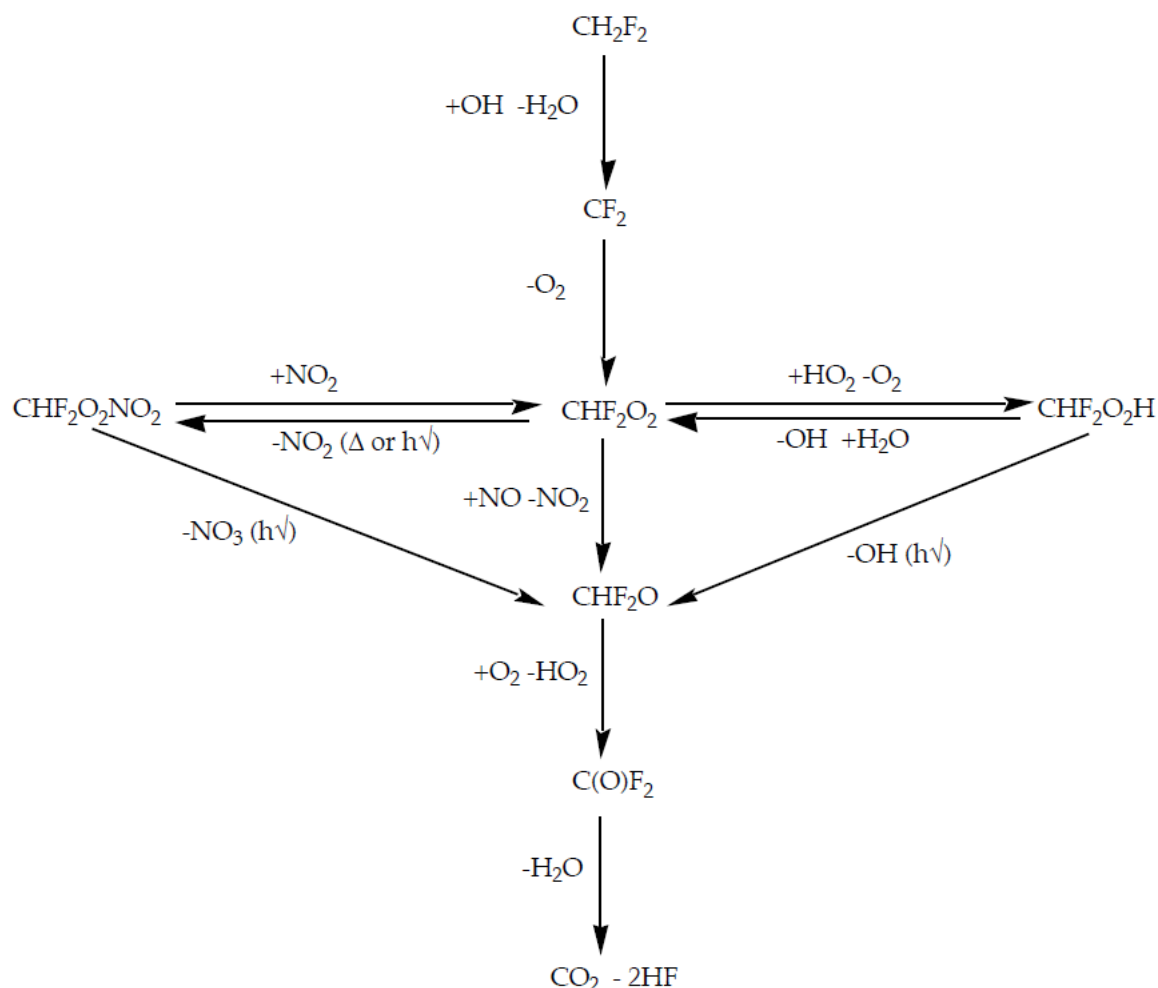


Figure 1. Tropospheric Degradation Mechanism for Difluoromethane (1)

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

9.1.1 Inhalation Toxicity (6)

The study was carried out in accordance with the *OECD Guideline for Testing of Chemicals No: 403*.

Male and female adult rats of a Wistar derived strain were exposed to gaseous difluoromethane for a period of 4 hours. Five animals of each sex were assigned to four treatment groups and placed in restraining tubes with only their noses inside the equilibrated exposure chamber. The chambers were filled with serially diluted difluoromethane and an air exchange system was designed for 20-30 air changes per hour.

The target concentrations of difluoromethane were 0, 5000, 50,000, or 500,000 ppm. The measured concentrations were 0, 7510 ± 4700, 85,900 ± 29,400, or 520,000 ±

19,200 ppm. The latter are used to describe the results of the study. There were problems in controlling volatilisation and flow rates due to the high volatility and low boiling point of the test substance. An artificial atmosphere was created in the cages of those animals exposed to the highest concentration of difluoromethane, due to the substantial reduction in available oxygen. A mixture of nitrogen and oxygen was added to the stream of difluoromethane. Animals were observed for a period of 14 days after exposure during which time daily clinical examinations were carried out and at the end of this period necropsy performed. Lungs were removed and weighed, as were any abnormally appearing body organs.

There were no deaths during the study. Treatment related clinical abnormalities observed during the 4 hour treatment period occurred only in animals treated with middle and high dose difluoromethane. All animals treated with the middle dose showed reduced response to sound. All rats exposed to the high dose showed an absence of response to sound and some showed an increased breathing depth and reduced breathing rate, and tail erections. Following treatment tail erections and an increased period of piloerection were observed in animals treated with middle and high doses of difluoromethane. Reduced activity, salivation and shaking was observed for one day only in the highest treatment group. Animals treated with 520,000 ppm demonstrated reduced splaying reflex but this was thought to be an artefact as it is reported to be found frequently in untreated rats of that strain and age. One male (85,900 ppm) demonstrated reduced stability for the two week observation period, as did 2 females at the highest dose for one day only.

The lungs of all treated animals appeared healthy at necropsy. One or two males showed slight abnormalities in the bladder, kidney, liver, and/or seminal vesicles at the highest dose only. No such abnormalities were noticed in any females.

No toxicologically significant changes in bodyweight, bodyweight gains, absolute or relative lung weights were observed.

Under the conditions of this study the LD₅₀ for difluoromethane is > 520 000 ppm.

9.2 28 Day Repeated Dose Inhalation Toxicity (7)

The study was carried out in accordance with the *OECD Guideline for Testing of Chemicals No: 412*.

Male and female adult rats of a Wistar derived strain were exposed to gaseous difluoromethane for a period of 6 hours per day for 5 days per week for four weeks. Thus the animals were exposed for 20 out of a possible 28 days. Five animals of each sex were assigned to four treatment groups and placed in whole body exposure chambers. The exposure period began when the chambers were sealed and the difluoromethane atmosphere generation begun. Twenty to thirty air changes were made per hour with clean dry air, and a flow rate of 25 L/minute was supplied. The air temperature in the chambers ranged between 19.2 and 20.6°C.

The target concentrations of difluoromethane were 0, 2000, 10,000, or 50,000 ppm. The measured concentrations were 0, 2010 ± 61, 9870 ± 440, or 49,500 ± 1100 ppm. Animals were observed every 30 minutes during exposure and following

exposure. More detailed clinical examinations were made on days 1, 2, 3, 8, 15, 22, and 29. Necropsy was performed on day 29.

There were no deaths during the study. Treatment related clinical abnormalities were observed immediately after exposure but not during exposure. Stains around the nose and piloerection were noticed in some animals exposed to 9870 or 49,500 ppm difluoromethane.

Small but statistically significant variations in body weights compared to controls occurred in several animals at various stages during the study. These were not considered biologically significant and were not accompanied by changes in food consumption.

Several reported changes in the clinical chemistry parameters were noted but are probably not biologically significant as they were mostly not related to exposure levels. An increase in plasma potassium (1.2 fold over control) occurred at the highest dose of difluoromethane in males.

Urinalysis showed small reductions in the urine pH in females (from 6.30 to 5.97) exposed to 9870 ppm and males (from 6.74 to 6.50) exposed to 49500 ppm. This was thought to be toxicologically insignificant, and no other urine parameters were altered.

Haematology tests revealed slight biologically insignificant reductions in the red blood cell count in females (95% of control) exposed to 49,500 ppm difluoromethane. No other haematological parameters were altered.

No changes in organ weights of treated animals compared to controls occurred and no macroscopic findings were noted that suggested a treatment related effect.

Microscopic findings suggested an absence of treatment related effects. However, 3 high dose males had minimal alveolitis as did one female control and one female from the 2010 ppm group. This was of some interest due to the gaseous nature of difluoromethane.

Liver biochemistry as indicated by cyanide-insensitive palmitoyl CoA oxidation activity appeared to be normal. The absence of an effect on β -oxidation enzyme activity indicates that difluoromethane is not a peroxisome proliferator.

In conclusion, treatment of rats with 2010, 9870 or 49,500 ppm difluoromethane for 28 days resulted in a few minor and probably biologically insignificant changes.

9.3 90 Day Repeated Dose Inhalation Toxicity (8)

The study was carried out in accordance with the *OECD Guideline for Testing of Chemicals No: 413*.

Male and female adult rats of a Wistar derived strain were exposed to gaseous difluoromethane for a period of 6 hours per day for 5 days per week for thirteen weeks. The animals were therefore exposed for 64 out of a possible 90 days. Ten

animals of each sex were assigned to four treatment groups and placed in whole body exposure chambers. Ten additional animals from the control and highest dose groups were used as a satellite group and were kept for observation for 28 days after the completion of the 90 day exposure period.

The daily exposure period began when the chambers were sealed and the difluoromethane atmosphere generation begun. Twenty to thirty air changes were made per hour and clean dry air at a flow rate of 70 L/minute was supplied.

The target concentrations of difluoromethane were 0, 5000, 15,000, or 50,000 ppm. The measured concentrations were 0, 4940 ± 160 , $14,600 \pm 470$, or $49,100 \pm 1600$ ppm. Animals were observed every 30 minutes during exposure and following exposure. More detailed clinical examinations were made once weekly. Necropsy was performed in week 14 for the main study group and in week 18 for the satellite group.

There were no deaths and no clinical abnormalities that could be attributed to treatment.

Small but statistically significant variations in body weights and food consumption occurred in several animals at various stages during the study. These were not considered biologically significant and showed no coherent dose response relationship.

Several minor changes in the blood clinical chemistry parameters were observed. In the main study plasma creatinine levels were reduced in males exposed to 15,000 or 50,000 ppm at week 5, and in males exposed to 5,000 or 50,000 ppm, and females exposed to 5,000 or 15,000 ppm both in week 14. Females exposed to 5,000 ppm showed a decrease in triglycerides and a decrease in bilirubin levels at all exposure groups in week 14. All of these differences are considered to be present due to some high individual control values and are therefore not related to difluoromethane exposure.

Additionally, males exposed to 50,000 ppm showed an increase in triglyceride (1.4 fold) at weeks 5 and 15. The serum alanine transferase activity was increased (1.3 fold) in females from all exposure groups at week 5.

Urinalysis revealed an apparent dose related increase in urine volumes and a concomitant reduction in specific gravity in both sexes at week 5. This difference was not evident at week 13 and was therefore thought to be an artefact. Small fluctuations in urine pH occurred in both sexes during the study but did not correlate with the treatment level.

Males in all treatment groups demonstrated an increase in platelet count in week 5 compared to controls. This value appeared to be the result of several low control values, and was not evident in week 14. A slight decrease in mean cell haemoglobin concentration in males from the main study and a slight increase in females from the satellite group treated with 50,000 ppm occurred. In week 18 a decrease in the white blood cell and lymphocyte counts occurred in both sexes from the satellite groups treated with 50,000 ppm. In addition, the females showed a decrease in red

blood cell count. None of these changes followed a coherent dose response or time relationship and are not considered of toxicological significance.

No changes in organ weights of treated animals compared to controls occurred and no macroscopic findings were noted that suggested a treatment related effect. No ophthalmic changes occurred in any animal that were not normal for animals of this strain and age.

Microscopic findings suggested an absence of treatment related effects. In week 14 there was a slight increase over controls of unilateral hydronephrosis in the kidneys of male rats exposed to 50,000 ppm difluoromethane. However, male control rats from the satellite group demonstrated similar levels to the 50,000 ppm exposed group. This finding is thus assumed to be incidental.

In conclusion, it appears that treatment of rats with 4940, 14,600 and 49,100 ppm difluoromethane for 90 days resulted in a few minor and biologically insignificant changes.

9.4 Genotoxicity

9.4.1 *Salmonella typhimurium* and *Escherichia coli* Reverse Mutation Assay (9)

The study was carried out in accordance with the *OECD Guide-lines for testing of Chemicals No: 471*.

Difluoromethane was tested for its ability to cause gene mutations in the *Salmonella typhimurium* and *Escherichia coli* bacterial reverse mutation assay.

The concentrations of difluoromethane selected for the study were 0, 5, 10, 25, 50, 75 and 100% v/v of atmosphere. *S. typhimurium* strains TA98, TA100, TA1535 and TA1537 and *E.coli* strains WP2P and WP2P uvrA were used, in the presence or absence of liver microsomal S9 activation. The positive control used in the absence of activation was N-methyl-N'-nitro- N- nitrosoguanidine. 2-Aminoanthracene was used as the positive control in experiments including the liver S9 mix. An experiment was also performed with vinyl chloride to check that the dosing regime was appropriate for a gaseous compound of known mutagenicity.

Both positive controls produced marked increases in the number of revertant colonies within the anticipated range. At 10 and 20% v/v atmosphere vinyl chloride caused significant increases in revertant colonies over controls in the *S.typhimurium* strain TA 100 tested.

No significant increases in the number of revertant colonies of bacteria were recorded for any of the strains of *S. typhimurium* or *E.coli* used, at any dose level of the test substance, with or without metabolic activation. The test substance caused some toxicity to the *S. typhimurium* bacterial lawn at the 75% and 100% concentration plates.

The results indicate that difluoromethane is not genotoxic toward *Salmonella typhimurium* or *Escherichia coli* under the conditions employed in this study.

9.4.2 Micronucleus Assay in the Bone Marrow Cells of the Mouse (10)

The study was carried out in accordance with the *OECD Guide-lines for testing of Chemicals No: 474*.

Difluoromethane was investigated for its potential to induce micronuclei in bone marrow polychromatic erythrocytes of 6 week old Charles River strain mice.

A single preliminary experiment was performed to determine the protocol of the main experiment. Five animals of each sex were exposed (whole body) to a nominal concentration of 150,000 ppm of difluoromethane for a period of 6 hours. The average measured concentrations was 132,150 ppm. Air was the negative control and cyclophosphamide dissolved in saline administered orally at 65 mg/kg body weight was the positive control. An attempt to use gaseous vinyl chloride as a positive control failed due to "toxicity and technical problems". At 24 hours or 48 hours after treatment the animals were killed and the bone marrow cells were collected for micronuclei analysis.

No cytotoxic effects were observed as indicated by the absence of an increase in the ratio of polychromatic to normochromatic erythrocytes in treated animals compared to controls. No increase in the frequency of micronucleated polychromatic erythrocytes occurred in animals treated with difluoromethane compared to controls. In contrast a distinct increase in the number of micronuclei was noted in animals treated with the positive control cyclophosphamide.

In conclusion, under the conditions of this study difluoromethane was found not to cause chromosomal damage in bone marrow cells of mice *in vivo*.

9.4.3 Cytogenetic Assay in Cultured Mammalian Cells (11)

The study was carried out in accordance with the *OECD Guide-lines for testing of Chemicals No: 473*.

Difluoromethane was investigated for its potential to cause chromosomal aberrations in cultured Chinese hamster lung cells (CHL), with and without exogenous metabolic activation.

Preliminary experiments were performed in order to determine the cytotoxicity of difluoromethane to the cells. Cytotoxicity was not observed both in cell growth inhibition tests and mitotic index even at atmospheric concentrations of difluoromethane of 80%. In the preliminary chromosomal aberration test normal metaphase was seen even at the highest concentration and no induction of chromosomal aberration was observed. In the definitive test CHL cells were exposed to difluoromethane at 10, 20, 40 or 80% difluoromethane or 24 or 48 hours, without metabolic, or for 6 hours without metabolic activation. Ethylchloride was the positive control without activation and vinylchloride was the positive control in the presence of exogenous activation. In addition mitomycin C and benzo(a)pyrene

were used as positive controls. The negative control was purified air. Chromosomal aberrations were very low in the negative controls whereas induction was clearly observed in the positive control groups. No statistically significant chromosomal aberrations were observed in any of the tests using difluoromethane.

In conclusion, under the conditions of this study difluoromethane was found not to cause chromosomal aberrations in cultured Chinese hamster lung cells.

9.4.4 Chernoff-Kavlock Foetotoxicity and Teratogenicity Assay in the Rat (12)

Difluoromethane was investigated for its potential to cause foetotoxicity or teratogenicity in this non-OECD assay (13).

Groups of 10 female Wistar derived rats were exposed to gaseous difluoromethane during days 7-16 of gestation, which includes the period of organogenesis. Animals were exposed (whole body) for a period of 6 hours per day. They then littered and reared their young to 5 days *post partum*. The target atmospheric concentrations of difluoromethane were 0, 10,000 or 50,000 ppm which were measured at 9930 ± 290 , and $49,600 \pm 2620$ ppm respectively. Pups were weighed on days 1 and 5, and the number of dead and live animals was recorded.

There was no effect on maternal body weight gain. One female showed signs of urinary incontinence on days 8-10 of gestation. No other clinical signs were observed in the animals during exposure.

One female (10,000 ppm) was unable to sustain its litter to day 5 *post partum*. One control female and two 50,000 ppm exposed females showed a high incidence of pup mortality to day 5 *post partum* (57%, 64%, and 55% respectively) compared to other females in the study. The percentage of animals surviving to day 5 was 96% for both groups when the 3 litters with increased mortality were removed.

No pups were dead at birth. However the litter size was statistically lower in the two treated groups than controls (9.3 compared to 10.7 for the 10 000 ppm group). The average pup weight at birth was the same in each group but at day 5 pups in the 50,000 ppm group were of low weight compared to controls.

The pups from the 50 000 ppm group showed only 31% weight gain from birth compared to the 40% gain of control animals to day 5. The criteria for this bioassay state that a value of lower than 30% defines a chemical as foetotoxic.

According to the criteria for assessing the Chernoff-Kavlock assay difluoromethane was considered not to be teratogenic due to the relatively small effects of exposure to the chemical on survival, pup weight gain and litter size.

In conclusion, under the conditions of this test difluoromethane is not considered to be a foetotoxin or a teratogen.

9.5 Development Toxicity Study in the Rat (14)

The study was carried out in accordance with the *OECD Guideline for Testing of Chemicals No: 414*.

Difluoromethane was investigated for its potential to cause foetotoxicity or teratogenicity.

Groups of 24 female Wistar derived rats were exposed to gaseous difluoromethane during days 7-16 of gestation, which includes the period of organogenesis. Animals were exposed (whole body) for a period of 6 hours per day. They then littered and reared their young to 5 days *post partum*. The target atmospheric concentrations of difluoromethane were 0, 5000, 15,000 or 50,000 ppm which were measured at 0, 5000 ± 163 , $15,000 \pm 201$ or $49,800 \pm 471$ ppm. On day 22 of gestation the animals were killed and necropsy performed.

None of the animals showed any clinical change during the course of the study. The maternal bodyweights were slightly lower than the control animals at the higher two treatment groups. Animals exposed to 50,000 ppm had a reduced food consumption during days 7-16 compared to controls. No abnormal macroscopic changes were noted at post mortem and maternal performance seemed unaffected by exposure.

Two animals exposed to 50,000 ppm and one each exposed to 5,000 or 15,000 ppm difluoromethane totally resorbed their litters. There was a slight but not statistically significant increase in the number of early intra-uterine deaths amongst the other animals exposed to 50,000 ppm but the number of litters affected was the same as the controls. There was a reduction in the mean number of live foetuses in the 50,000 ppm group, due to the higher incidence of pre-implantation loss (occurring prior to difluoromethane exposure) and slightly but not statistically higher post-implantation loss. A similar reduction in the 15,000 ppm group was considered incidental due to a lower number of corpus lutea compared to controls.

Difluoromethane had no effect on foetus weight. Neither the type nor the incidence of major foetal abnormalities was associated with difluoromethane. A number of foetuses amongst the 50,000 ppm treated group had minor external or visceral defects which consisted of dilated ureters, mottled livers and hepatic cysts. Collectively these defects occurred in statistically significant numbers compared to the control animals, but as individual deformities they did not. There was also an increase in the number of foetuses in the higher treatment group with kinked ureters.

Difluoromethane resulted in an increased incidence of partial ossification of the parietal bones in animals exposed to 5,000 and 50,000 ppm but not 15,000 ppm. These may have occurred as a result of maternal toxicity. Several other skeletal defects were also increased in exposed groups compared to controls. These defects were reduced bone ossification and shortened ribs. No effect on the manus or pes of the mouse was evident.

In conclusion, exposure of pregnant females to difluoromethane at levels at or below 15,000 ppm caused no maternal or foetal toxicity. At higher concentrations maternal and foetal toxicity was seen.

9.6 Pharmacokinetics and Metabolism in Rats and Mice (15)

The study was carried out in accordance with the *OECD Guideline for Testing of Chemicals No: 417*.

Four male rats of a Wistar derived strain and four male mice (Alpk:APfCD-1) were housed individually in glass metabolism cages. Prior to the beginning of the study urine samples were collected and used as control samples for fluoride ion determination. Each animal was then placed in an inhalation chamber and exposed to 10000 ppm [^{14}C]-difluoromethane with a specific activity of 5.27-7.38 $\mu\text{Ci}/\text{mmol}$ for 6 hours.

During the exposure period urine and faeces were collected. Following exposure animals were rapidly transferred back to their metabolism cages where urine, faeces, expired organic material, carbon dioxide and carbon monoxide were collected. Urine and faeces were collected at 6 hourly intervals for rats and 12 hourly intervals for mice for the first 24 hours and at 24 hour intervals thereafter. Gaseous samples were collected in parallel traps and the traps changed at regular intervals. Expired organic material was collected at 15, 30, 45, 60, and 90 minutes and 2 and 6 hours post exposure. Carbon dioxide was collected at 0.5, 1, 1.5, 2, 4, 6, 12, and 18 hours for rats and mice. Additional samples were taken at 24 hours for mice and 30 and 42 hours for rats. Carbon monoxide was collected at the same time points except that the 12 hour point was not included.

The study was terminated after 4 days when the animals were killed. One rat and one mouse were used to measure total carcass radioactivity. The remaining animals were used to collect blood (whole and plasma) and the liver, kidneys, lungs, heart, brain, testes, muscle, renal fat, spleen and bone (femur) removed for radioactivity measurements.

In a separate experiment four male rats and four male mice were exposed to 10,000 ppm unlabelled difluoromethane for 6 hours. Four males of each species were used as controls. Animals were subsequently killed and blood collected for carboxyhaemoglobin assays.

Analysis showed that the target concentration of 10,000 ppm difluoromethane had been reached and maintained. The inhaled dose was estimated to be 78.95 - 84.42 mmol/kg bodyweight for the rats and 122.06-129.39 mmol/kg body weight for the mice. The following results are expressed in terms of this inhaled dose. Most of the absorbed dose was eliminated within one hour of exposure. Only 0.89% and 1.13% of the inhaled dose was actually absorbed in rats and mice respectively. Of the recovered absorbed difluoromethane, 55.7% and 39.8% was eliminated in the faeces, urine and expired air from rats and mice respectively. The levels of organic material collected in traps were too small to identify and was therefore assumed to be difluoromethane.

Urinary and faecal excretion of radiolabel was extremely rapid and began during exposure. Approximately half of the total urinary excretion of radiolabel occurred in the first hour after exposure. Urinary excretion of difluoromethane and its metabolites was the most favoured route for mice (0.34%) and second most

favoured for rats (0.13%). Faecal excretion was minimal and accounted for only 0.03% and 0.07% for rats and mice respectively.

Carbon dioxide exhalation was the major route of excretion of difluoromethane metabolites in rats and the second major route in mice. Of the inhaled dose of difluoromethane, 0.23 % and 0.27% was recovered in the CO₂ from rats and mice respectively. Neither carbon monoxide nor its frequent endpoint carboxyhaemoglobin could be detected as a metabolite of difluoromethane in exhaled air. Therefore, carbon monoxide can be assumed to be an extremely minor component of difluoromethane metabolism if it is produced at all.

At the completion of the study only small amounts of radiolabel remained in the carcass and accounted for 0.11% and 0.12% of the dose in rats and mice respectively. Highest concentrations were present in the lungs, liver and kidneys but a relatively uniform distribution was present for both species.

The metabolism of difluoromethane was expected to result in the excretion of fluoride ion into the urine. Of the inhaled dose 0.51% and 0.80% of the difluoromethane was measured to be metabolised in rats and mice respectively, and carbon dioxide was a major metabolite in both species. It was proposed from the metabolism study that oxidative metabolism was the main route of biotransformation of difluoromethane. The expected major urinary metabolite from this process would be formic acid and a radiolabel arising from the incorporation of formic acid into endogenous material.

From this study it can be concluded that difluoromethane is not readily absorbed by the body but is rapidly excreted unchanged or metabolised via an oxidative pathway. Of the inhaled dose approximately 1% was absorbed, of this excretion via urine was the principle excretory pathway in mice and via exhalation as carbon dioxide in rats.

9.7 Other Toxicological Data

Other studies (16) have found that the lowest inhaled dose cited to cause mortality in rats was 760,000 ppm. The effects of anaesthetisation such as lethargy and lack of co-ordination were observed at concentrations over 100,000 ppm.

Cardiac arrhythmia and possible death are known to result when many halocarbons and hydrocarbons are inhaled during the presence of high circulating levels of endogenous adrenaline present as a result of stress. In an experiment submitted by the notifier (17), dogs were exposed to inhaled concentrations of difluoromethane and then injected with adrenaline. Cardiac sensitisation did not occur at difluoromethane concentrations up to 350,000 ppm. In addition, repeated exposure of guinea pigs (15) to an unstated concentration of difluoromethane was reported to have resulted in bleeding in the lungs and liver discolouration.

9.8 Overall Assessment of Toxicological Data

Difluoromethane was found to have a low acute inhalation toxicity in rats (LD₅₀ > 520,000 ppm). In this study the gas mixture was supplemented with oxygen; it is likely that anoxia would cause mortalities at these concentrations without additional

oxygen. Difluoromethane is essentially non-toxic when administered daily via inhalation at concentrations up to 50,000 ppm for up to 90 days. It was not mutagenic towards *Salmonella typhimurium* or *Escherichia coli*, nor clastogenic towards polychromatic erythrocytes of mouse bone marrow *in vivo*, or human peripheral blood lymphocytes, *in vitro*. Difluoromethane was found to cause foetotoxicity in rats at concentrations higher than 15,000 ppm however only minimal effects were noted at a dose rate of 49,100 ppm in a 90 days inhalation study. It was not teratogenic. It was found to be poorly absorbed by the rat or mouse and to be rapidly excreted mostly through the urine and exhaled CO₂. Metabolic studies showed difluoromethane to be excreted both unchanged and metabolised into formic acid via an oxidative pathway.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

No data were provided. However, effects on organisms are not expected as difluoromethane, like other hydrofluorocarbons, has negligible biological activity. The low partition coefficient suggests a low potential for bioaccumulation and a low risk to the aquatic environment (1). Furthermore, significant exposure of aquatic organisms to this gaseous substance is not expected as it will not remain in solution for any length of time.

Halocarbon refrigerants can affect the atmosphere. Difluoromethane contains neither chlorine nor bromine, and thus will not act as a source of ozone depleting halogen radicals in the stratosphere (1). Scientists from the US National Oceanic and Atmospheric Administration concluded that hydrofluorocarbons have negligible potential to destroy ozone (18).

Like other halocarbons, difluoromethane adds to the global warming potential of the atmosphere. However, its atmospheric lifetime of 6 years is considered short enough that difluoromethane will not contribute significantly to global warming (19).

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

Difluoromethane is not expected to exert a direct effect on living organisms. The high volatility should ensure minimal exposure of aquatic and terrestrial compartments, and therefore minimal hazard to organisms inhabiting them.

Hazard to the atmosphere will be reduced when difluoromethane replaces current chlorofluorocarbon refrigerants, as the replacement refrigerant will not carry chlorine or bromine to the stratosphere. It also possesses a lower global warming potential.

If 100% of the difluoromethane imported into Australia was released to the atmosphere, was converted to COF₂, uniform scavenging of this COF₂ into the global rainfall, followed by hydrolysis to CO₂ and HF, it is concluded that the levels of fluoride and acidity produced would be extremely low compared with those arising from existing sources (1).

12. ASSESSMENT OF PUBLIC AND OCCUPATIONAL HEALTH AND SAFETY EFFECTS

Experiments with rats exposed to difluoromethane at up to 50,000 ppm for 90 days indicate that exposure results in negligible toxicity. Reproductive and teratology studies in rats showed foetotoxicity and maternal toxicity at doses greater than 15,000 ppm but no evidence of teratogenic effects. High concentrations of difluoromethane (100,000 ppm in rats) may lead to an anaesthetic effect. The results suggest that relatively high concentrations of difluoromethane need to be present before toxic effects become apparent. If a large amount of difluoromethane accumulates as a result of a leak, oxygen may be displaced, leading to an asphyxiation hazard. Direct skin or eye contact with the cold liquid or escaping gas will result in frostbite.

Difluoromethane is a flammable liquefied gas that may be reactive at high temperatures. However, it will only be used as a mixture with other HFCs and these mixtures are not flammable (all are classified dangerous goods class 2.2, i.e non-flammable gases). Its vapour is heavier than air which may result in the gas collecting in low areas and displacing oxygen. Difluoromethane when used in confined spaces will not effectively disperse unless positively ventilated. Heated cylinders may cause safety devices that relieve temperature and pressure to operate and thereby cause the release of contents. Cylinders may still rupture in fire situations and hence the maximum recommended temperature for cylinder storage and use is 52°C.

The greatest risk for those employed as refrigeration and air conditioning mechanics apart from cold burns would appear to be asphyxiation arising from the unnoticed accumulation of gas from slow leaks. Frequent leak checks and the installation of permanent leak detectors may be necessary when the chemical is used in confined spaces. Standard Australia's HB40-1992 *The Australian Refrigeration and Air Conditioning Code of Good Practice* (5) provides guidance for the recommended work practices.

Under normal conditions of use, there will be low potential for public exposure to difluoromethane since its application will be restricted to industry. Public exposure to difluoromethane will be limited to contact with refrigeration equipment where it is housed in a closed system. If public contact to difluoromethane does occur during accidental spillages, health hazards arising from acute exposure are anticipated to be low as difluoromethane has low acute inhalation toxicity. Accidental exposure to difluoromethane can also arise as a result of slow leakages of the refrigerant from faulty equipment. Since the overall toxicity of difluoromethane is low as indicated in the toxicology studies, the potential for adverse health effects under such circumstances is anticipated to be low.

13. RECOMMENDATIONS

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

- If engineering controls and work practices are insufficient to significantly reduce exposure to a safe level, then personal protective devices which conform to and are used in accordance with Australian Standards (AS) for eye protection (AS 1336; AS 1337) (20,21), impermeable gloves (AS 2161) (22), protective clothing (AS 2919) (23) and respiratory protection conforming to AS/NZS 1715 (24) and AS/NZS 1716 (25).
- When used in confined spaces positive ventilation is necessary to disperse escaped gases.
- Good work practices should be implemented to avoid spillages.
- Good personal hygiene should be adopted.
- A copy of the MSDS for products containing the notified chemical should be easily accessible to employees working with products containing the chemical.
- Manufacturers, distributors and users must minimise atmospheric emissions of HFC-32 by adhering to the Australian Refrigeration and Air Conditioning Code of Good Practice.
- Training of refrigeration maintenance workers should include relevant information from Australian Refrigeration and Air Conditioning Code of Good Practice and Australian Standard 1677 - refrigeration systems and the MSDS.
- Leak testing of refrigeration equipment should be conducted on a regular basis.
- Storage of cylinders containing difluoromethane should conform to the maximum recommended temperature of 52°C.

14. MATERIAL SAFETY DATA SHEET

The MSDS for a formulation containing difluoromethane was provided in a format similar to Worksafe Australia format (26). This MSDS was provided by Elf Atochem (Australia) Pty Ltd as part of their notification statement. The accuracy of this information remains the responsibility of Elf Atochem (Australia) Pty Ltd.

15. REQUIREMENTS FOR SECONDARY NOTIFICATION

Under the Act, secondary notification of difluoromethane 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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