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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. APPLICANTS

Du Pont (Australia) Ltd, 168 Walker Street, North Sydney NSW 2060; and

ICI Australia Operations Pty Ltd, 1 Nicholson Street, Melbourne VIC 3000.

2. IDENTITY OF THE CHEMICAL

Chemical name:	Difluoromethane
Chemical Abstracts Service (CAS) Registry No.:	75-10-5
Other name:	Methylene fluoride HFC 32
Trade names:	Klea 32 (ICI)
Blends:	SUVA AC 9000
Molecular formula:	CH_2F_2
Molecular weight:	52.024

Method of detection and determination:

Infra Red and Mass Spectrometry, Gas Chromatography and Nuclear Magnetic Resonance data were provided for difluoromethane and are consistent with the structure of the chemical.

Spectral Data:

Infra Red: Absorption peaks occurred at 3015, 1449 and 1059 cm^{-1}.

3. PHYSICAL AND CHEMICAL PROPERTIES

Appearance at 20°C and 101.3 kPa:	Clear colourless gas.
Odour:	Very slight ethereal
Melting Point:	-136°C
Boiling Point:	-51.7°C
Density:	960 kg/m^3 (liquid) at 25°C
Relative Vapour Density:	1.85 (air = 1) at 25°C
Vapour Pressure:	1774 kPa at 25°C
Water Solubility:	4400 ppm at 25°C, kPa.
Fat Solubility:	Not provided.
Partition Co-efficient (n-octanol/water) log $P_{O/W}$:	0.21 at 25°C
Hydrolysis as a function of pH:	Not provided.
Adsorption/Desorption:	Not provided.
Dissociation Constant:	Does not dissociate in water.
Flash Point:	Not provided.
Flammability Limits:	exposure limit (LEL) = 13.6% by volume in air exposure limit (UEL) = 32.2% by volume in air
Combustion Products:	Same as decomposition products.
Decomposition Temperature:	Not provided but cylinder is to be kept below 52°C.
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.

Comments on missing data: The water solubility at atmospheric pressure, soil sorption and hydrolysis are all expected to be very low due to the notified chemical being a gas.

4. PURITY OF THE CHEMICAL

Degree of purity: > 99.99%

Toxicor hazardous impurities: Traces of 1,1,1-trifluoroethane, chlorofluoromethane, chloromethane and chloroethene are present.

Additives/Adjuvants: nil

5. INDUSTRIAL USE

Difluoromethane is a low temperature refrigerant that will be used in commercial refrigeration and air conditioning operations such as in supermarkets and refrigerated transport. 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 HFC-125 and HFC-134a, although other blends may be developed in the future. At a future date it may be imported as a pure substance but will always be sold to customers as a blend. It will be sold to equipment manufacturers and service contractors as the blends Klea 61, Klea 66 and SUVA AC 9000.

ICI and DuPont plan on importing the following quantities:

ICI: 1994-1996: 5-20 tonnes
1997-2000: 20-50 tonnes
Beyond 2000: 50-100 tonnes.

DuPont: 1994-1995: 10-100 tonnes/annum
1996-1998: 100-1000 tonnes/annum

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

6. OCCUPATIONAL EXPOSURE

Difluoromethane will be shipped into Australia in liquefied form in 8 kg and 45 kg steel pressurised cylinders and 650 kg drums. Transport within Australia will be by road freight. Storage shall be in a clean dry area designated for dangerous goods.

Workers likely to be exposed to difluoromethane will include both refrigeration and air conditioning contractors. Contractors will be responsible for the conversion of refrigeration and air conditioning systems to accommodate the new non CFC gases, installation and servicing of equipment, charging and recharging of equipment. It is estimated that the initial number of workers will be 100 and may increase to a maximum of 10,000 by 1997.

Converting equipment 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

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 the gas is housed in a closed system. If public contact with difluoromethane does occur during accidental spillages, health hazards arising from acute exposure are anticipated to be low as difluoromethane has very low acute inhalation toxicity. However, the notifier recommends that in the case of an accidental leak, people should move away from the contaminated site and seek medical advice. 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 toxicity studies, the potential for adverse health effects under such circumstances is anticipated to be low.

8. ENVIRONMENTAL EXPOSURE

- **Release**

The notified substance will not enter the environment intentionally when used in cooling systems, but any releases during filling or use of cooling systems, or following disposal of obsolete equipment or recovery of refrigerants therefrom, will rapidly volatilise to the atmosphere. It is not possible to estimate how much of the refrigerant might be released in this way, but the notifiers indicate that quantities involved will be small (< 10% per annum). Releases during recharge when hoses are disconnected are estimated at about 0.1 g per operation. The new blends are expensive, providing a financial incentive to minimise losses and install area monitors around large installations.

The Australian Refrigeration and Air Conditioning Code of Good Practice (1) 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. The Code is called up in most State legislation. In Tasmania, the Code is practically applied, with legislative backing being developed.

- **Formulation, handling and disposal**

As noted above, new blends may be developed following introduction. Blending will be centralised at Lovelocks in Melbourne.

Recovery, reclamation and recycling of refrigerants is preferred to disposal. For disposal, the Code requires that unusable or surplus refrigerant not be discharged to the atmosphere, but be returned to the supplier or stored in a cool shaded place pending disposal. Reprocessing will not occur in Australia as such activities require a production facility. Local disposal will also not occur as acceptable disposal facilities do not exist currently in Australia.

. **Fate**

Given its high volatility, any difluoromethane released to the environment will partition almost entirely to the atmosphere. 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 (28 d biological oxygen demand 5% of theoretical) (2). The main degradation pathway in the environment is reaction with tropospheric hydroxyl radicals, which abstract hydrogen. The estimated atmospheric lifetime is 7.7 years (3).

Reaction of difluoromethane with hydroxyl radicals leads to carbonyl fluoride. Analogous experiments (4) with chlorine radicals, more readily generated in the laboratory than hydroxyl radicals, indicated that both difluoromethane and chlorodifluoromethane (HCFC-22) degrade to carbonyl fluoride.

Carbonyl fluoride does not react with hydroxyl radicals or undergo photochemical transformation at significant rates in the troposphere. Its fate is somewhat speculative as its Henry's law solubility and reactive sticking coefficient (a measure of the reaction probability on aqueous surfaces) are unknown. However, by using data for phosgene, a tropospheric lifetime of 17 d may be estimated for carbonyl fluoride with respect to incorporation

and hydrolysis in clouds (5). Dry deposition may also be important. Tropospheric loss mechanisms may be inferred from the observation of carbonyl fluoride in the stratosphere, but not in the troposphere (6). However, such conclusions are tentative as stratospheric sources of carbonyl fluoride include the common CFCs, CFC-11 and CFC-12.

9. EVALUATION OF TOXICOLOGICAL DATA

9.1 Acute Toxicity

9.1.1 Inhalation Toxicity (Ref No:7)

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 artifact 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 to the bodyweight, bodyweight gains, absolute or relative lung weights were observed.

Under the conditions of this study the LD_{50} for difluoromethane is > 520 000 ppm.

9.2 28 Day Repeated Dose Inhalation Toxicity (Ref No:8)

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 target concentrations of difluoromethane were 0, 2000, ,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 changes in the clinical chemistry parameters were reported that were probably not biologically significant as there was no relationship to exposure levels. In particular, 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 but would not appear to be toxicological significant.

Liver biochemistry as indicated by cyanide-insensitive palmitoyl CoA oxidation activity appeared to be normal. The absence of an effect on ß-oxidation 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 (9)

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, ,000, or 50,000 ppm. The measured concentrations were 0, 4940 ± 160, 14,600 ± 470, or 49,100 ± 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 plasma 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 artifact. 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 (Ref No:10)

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. . *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 revertent 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 revertent 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 (Ref No:11)

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 Human Peripheral Blood Lymphocytes (12)

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 human peripheral blood lymphocytes.

Preliminary experiments were performed in order to determine the toxicity of difluoromethane to the cells. Duplicate negative controls were air and 100% nitrogen atmosphere, used to determine the effects of anoxia if a positive result was obtained with difluoromethane treated cultures. Mitomycin C (1.0 µg/ml final concentration) and cyclophosphamide (50 µg/ml final concentration) dissolved in nutrient medium were used as positive controls. In addition to these the direct acting clastogen ethylene oxide was used as a positive gaseous control but only in the absence of metabolic activation.

Cells from a male and a female donor were examined after a 72 hour growth period, and cells from the female donor treated at the highest dose were also examined after a 96 hour growth period.

Stimulated cells were exposed to 10%, 50% and 100% v/v of atmosphere of difluoromethane, with and without metabolic S9 activation, approximately 48 hours after culture initiation. Difluoromethane concentrations were nominal and achieved by dilution with air. The cultures were then incubated for approximately 3 hours, after which time they were removed from the presence of difluoromethane and the growth medium replaced. The cells were then allowed to incubate to make up a 72 hour or 96 hour growth period. Lymphocytes were arrested in metaphase by the addition of colcemid to the culture 70 hours or 94 hours after the culture initiation. Cells were harvested and then prepared for microscopic examination at the nominated time.

No significant reductions in the mitotic activity occurred for any of the treated cells compared to the air controls. One hundred metaphases were recorded for each culture with the exception of the ethylene oxide positive control from one donor, from which only 80 were scored. No statistically significant increases in chromosomal aberrations were observed in either

donor, with or without S9 activation at either sampling time. All the positive controls elicited marked increases in chromosomal aberrations compared to controls.

In conclusion, under the conditions of this study difluoromethane was found not to cause chromosomal aberrations in cultured human peripheral blood lymphocytes

9.4 Chernoff-Kavlock Foetotoxicity and Teratogenicity Assay in the Rat (13)

Difluoromethane was investigated for its potential to cause foetotoxicity or teratogenicity in this non-OECD assay (14).

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
,600 ± 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 number of live pups at birth 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 assay 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 (15)

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 ± 201 or 49,800 ± 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 luteum 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 cysts attached to the liver. 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 (16)

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 µCi/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 CO_2 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 metabolized 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 metabolized via an oxidative pathway.

9.7 Other Toxicological Data

A summary of results from other studies was provided. 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 difluoromethane (or many other halocarbons and hydrocarbons) are

inhaled during the presence of high circulating levels of endogenous adrenaline present as a result of stress. In an experiment cited by the notifier, dogs were exposed to inhaled concentrations of difluoromethane and then injected with adrenaline. Cardiac sensitization occurred at difluoromethane concentrations of 250,000 ppm and higher. In addition, repeated exposure of guinea pigs 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 (LD_{50} > 520,000 ppm) and to be 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, but only in the presence of maternal effects. It was not teratogenic. It was found not to be greatly absorbed by the rat or mouse and to be rapidly excreted mostly through the urine and exhaled CO_2. Metabolic studies showed difluoromethane to be excreted both unchanged and metabolized into formic acid via an oxidative pathway.

10. ASSESSMENT OF ENVIRONMENTAL EFFECTS

No ecotoxicity data were provided. However, effects on organisms are not expected as the notified substance, like other hydrofluorocarbons, has negligible biological activity. Furthermore, significant exposure of aquatic organisms to this gaseous substance is not expected.

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. Scientists from the US National Oceanic and Atmospheric Administration concluded recently that hydrofluorocarbons have negligible potential to destroy ozone (17).

Like other halocarbons, difluoromethane makes a positive contribution to the global warming potential of the atmosphere. However, its atmospheric lifetime of 7.7 years is considered short enough that difluoromethane will not contribute significantly to global warming (3). The atmospheric lifetime is less than that of HCFC-22 (15.3 years) (18). According to Du Pont product data sheets, global warming potentials (GWP) for HFC-32 and HCFC-22 over a 100 year time horizon (relative to CO_2 with GWP 1) are 437 and 1600, respectively.

11. ASSESSMENT OF ENVIRONMENTAL HAZARD

Difluoromethane is not expected to exert a direct effect on living organisms as it has negligible biological activity. 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 HFC-32 replaces HCFC-22, as the replacement refrigerant will not carry chlorine or bromine to the stratosphere and has a lower global warming potential.

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 fetotoxicity 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 such as cardiac arrythmia become apparent. If a large amount of difluoromethane accumulates as a result of a leak, oxygen may be displaced. Individuals with pre-existing diseases of the central nervous or cardiovascular systems may be more susceptible to the symptoms of over exposure. Direct skin or eye contact with the cold liquid 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. Its vapour is heavier than air which may result in the gas collecting in low areas and displacing oxygen. Therefore, difluoromethane should not be used in confined spaces. 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 the maximum recommended temperature for cylinder storage and use is 52°C.

The greatest risk for those employed as refrigeration and air conditioning mechanics would appear to arise 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* (1) provides guidance for the recommended work practices.

13. RECOMMENDATIONS

To minimise occupational exposure (and public/environmental if recommendations have been made by these agencies) to difluoromethane the following guide-lines 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) (19,20), impermeable gloves (AS 2161) (21), protective clothing (AS 3765.1 AS 3765.2) (22,23) and respiratory protection (AS 1715) (24) should be worn.

. Good work practices should be implemented to avoid spillages.

. Workers who are taking sympathomimetic medication should be warned about potential cardiovascular sensitization from difluoromethane exposure.

. 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.

14. MATERIAL SAFETY DATA SHEET

The Material Safety Data Sheets (MSDS) for difluoromethane were provided in Worksafe Australia format (Ref No:24). These MSDSs were provided by Du Pont (Australia) Ltd and ICI Australia Operations Pty Ltd as part of their notification statement. They are reproduced here as a matter of public record. The accuracy of this information remains the responsibility of Du Pont (Australia) Ltd and ICI Australia Operations Pty Ltd.

15. REQUIREMENTS FOR SECONDARY NOTIFICATION

Under the *Industrial Chemicals (Notification and Assessment) Act 1989* (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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