Joint Assessment of Commodity Chemicals

No. 9

CHLORODIFLUOROMETHANE

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JACC Report No. 9

THE ECETOC SCHEME FOR THE

"JOINT ASSESSMENT OF COMMODITY CHEMICALS" (JACC)

This report has been produced as part of a programme for making critical reviews of the toxicology, including ecotoxicology, of selected industrial chemicals.

A number of organisations, world-wide, have produced and are continuing to produce such reviews with the aim of ensuring that, based on an up-to-date knowledge of the toxicological and other relevant information regarding existing chemicals they can continue to be produced and used safely. ECETOC is contributing to this activity with its JACC reviews.

In general, commodity chemicals, i.e. those produced in large tonnage by several companies and having widespread and multiple uses, are reviewed jointly by experts from a number of companies concerned. Before it is decided to review a chemical, every effort is made to discover whether an adequate review exists already, in which case no work is necessary.

It should be noted that in a JACC review only the uses of the chemical as such are considered, i.e. its occurrence as an impurity in other products is not normally taken into account.

In this document a critical assessment of the toxicology and ecotoxicology of chlorodifluoromethane is presented. Whenever good scientific reviews on certain toxicological or ecotoxicological aspects exist, their conclusions are summarised and in these cases only the subsequent literature has been assessed.

CONTENTS

CHLORODIFLUOROMETHANE

1. SUMMARY AND CONCLUSIONS

Chlorodifluoromethane is a non-flammable, colourless and nearly odourless gas under normal conditions. A large proportion is used as a chemical intermediate and therefore not emitted into the atmosphere. Other important uses are in refrigeration and in air conditioning systems; with present practice, most of the chlorodifluoromethane so used will eventually be emitted into the atmosphere.

Its moderate water solubility and low octanol/water partition coefficient indicate a negligible bioaccumulation potential. Contamination of water and food would not be expected from its physical and chemical properties and has not been reported.

Most of the chlorodifluoromethane released is destroyed by reaction with naturally occurring $OH^{\cdot}$ radicals in the troposphere. The portion reaching the stratosphere is destroyed by $OH^{\cdot}$ and oxygen atoms in the excited state (O^1D) but not to any major extent by photodecomposition. The calculated Ozone Depletion Potential (ODP) is therefore low, being 0.05 compared to an ODP of 1.0 for the fully halogenated chlorofluorocarbons such as trichlorofluoromethane and dichlorodifluoromethane.

Chlorodifluoromethane is rapidly equilibrated in tissues after inhalation and is eliminated from the blood in expired air with a half life of a few minutes. Over a wide range, the inhalation concentrations and blood concentrations are linearly correlated. Metabolism occurs, if at all, in only minor amounts; the toxicological activities described are unlikely to be due to the formation of reactive intermediates.

Chlorodifluoromethane has an extremely low order of acute toxicity. Atmospheric concentrations over 700,000 mg/m^3 are required to produce a lethal effect.

When liquid chlorodifluoromethane is in contact with skin it causes local freezing. It is not a skin sensitiser.

Chlorodifluoromethane causes cardiac sensitisation when exposure is to concentrations approaching the LC_{50}.

In animal studies, chlorodifluoromethane showed no adverse effects on fertility. It was not teratogenic in rabbits in a study using conventional group sizes at doses up to 175,000 mg/m^3. In initial teratogenicity studies in rats, litters from treated groups contained a low incidence of litters with foetuses with microphthalmia or anophthalmia. In a very large study in which groups of about 400 pregnant rats were exposed to 175,000 mg/m^3, there was a low incidence of anophthalmia and microphthalmia combined (10 foetuses in 383 litters). This increased incidence was statistically significant when compared to concurrent controls; however there was a considerable variation in prospective controls with incidences similar to this high dose group's in studies conducted 6 years later. The concentration used (175,000 mg/m^3) is equivalent to approximately 25% of the 4hr LC_{50}, and there was a slight reduction in maternal body weight gain indicating maternal toxicity. It is concluded that these results are not of significance when considering the health of human beings occupationally exposed at or below recommended permissable exposure levels.

Chlorodifluoromethane is not considered to be genotoxic *in vivo*. Most *in vitro* tests are negative and a positive response in the Ames test is consistent with occurrence of bacteria-specific metabolic pathways.

In studies in which rats and mice were exposed to chlorodifluoromethane for 4 - 131 wks, only minimal effects or no effects were observed. A slight decrease in body weight gain and a small increase in liver and kidney weight occurred with an exposure level of 175,000 mg/m^3. The only exception was a limited study in rats, mice and rabbits which reported changes in the blood, liver, lung and nervous system in animals exposed to 49,000 mg/m^3 over a ten months period; these findings were not confirmed in subsequent studies.

Chlorodifluoromethane did not produce neoplastic changes in female rats and male and female mice exposed to concentrations as high as 175,000 mg/m^3. In one study in male rats, a concentration of 175,000 mg/m^3 was associated with an increase in the number of fibrosarcomas and Zymbal gland tumours; these occurred late in the study and are not considered to be relevant to man. Lower concentrations (35,000 mg/m^3 and below) did not increase tumour rates in this study or in any of the other studies. Overall the data do not indicate that chlorodifluoromethane constitutes a carcinogenic hazard to man.

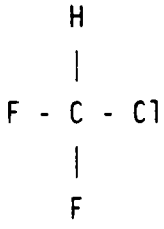
Although the material has been used for several decades, reports on adverse health effects in man are rare and are consistent with findings in experimental animals.

The national permissible occupational exposure limits vary between 1,750 and 3,500 mg/m^3 (8hr TWA). It is concluded that this provides adequate health protection.

2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, ANALYTICAL METHODS

2.1 Identity

Chemical structure:



Chemical formulae: $CHClF_2$

Common name: Chlorodifluoromethane

Common synonyms: Algeon 22; Arcton 22; Chlorofluorocarbon 22; Difluorochloromethane; Difluoromonochloromethane; Electro-CF 22; Eskimon 22; F 22; FC 22; Flugene 22; Fluorocarbon 22; Forane 22; Freon 22; Frigen 22; Genetron 22; HCFC 22; HFA 22; Hydrochlorofluorocarbon 22; Hydrofluoroalkane 22; Isceon 22; Osotron 22; Khladon 22; Methane,chlorodifluoro-; Monochlorodifluoromethane; Propellant 22; R 22; Refrigerant 22; UCON 22.

CAS Registry Number: 75-45-6

Conversion factors: 1 ppm = 3.54 mg/m^3

1 mg/m^3 = 0.282 ppm

2.2 Physical and Chemical Properties

Chlorodifluoromethane is a nonflammable, volatile, colourless gas at room temperature and normal atmospheric pressure. It is nearly odourless. Chlorodifluoromethane is moderately soluble in water and soluble in most organic solvents (Hawley, 1981, Weast, 1985). Some physical and chemical data for chlorodifluoromethane are given in Table 1.

Chlorodifluoromethane is available as a liquified gas either with a minimum purity of 99.9% or in a variety of blends and azeotropes.

2.3 Analytical Methods

A number of methods of analysis have been described for chlorodifluoromethane. These include gas chromatography/mass spectroscopy (Brunner et al, 1981), gas chromatography with electron capture detection (Shiamohara et al, 1979) and photothermal deflection spectrophotometry (Long and Bialkowski, 1985).

3. PRODUCTION, STORAGE, TRANSPORT AND USE

3.1 Natural occurrence

There is no known natural source of chlorodifluoromethane. Although Stoibe et al (1971) reported its presence in volcanic emissions, Rasmussen et al (1980) did not observe any excess of this halocarbon compared with normal atmospheric levels in their studies of volcanic emissions.

3.2 Man-made sources

3.2.1 Production levels

The total annual worldwide production of chlorodifluoromethane in 1987 was estimated to be 246,000 tonnes (Du Pont, 1989).

3.2.2 Producers

Chlorodifluoromethane is produced by 5 companies in the United States of America, 11 companies in Western Europe, 4 companies in Japan, 7 in Latin America, and 14 elsewhere (Du Pont, 1989).

3.2.3 Manufacturing process

Chlorodifluormethane is prepared by reaction of chloroform with anhydrous hydrofluoric acid in the presence of an antimony halide catalyst (Hawley, 1981); various reaction temperatures and pressures can be used (SRI, 1985).

3.2.4 Loss during disposal of waste

When air conditioning and refrigeration equipment reaches the end of its useful life, the metal may be recovered for scrap or disposed of as landfill. This inevitably leads to release of the refrigerant either

immediately or over a period of time. Trace quantities of chlorodifluoromethane have been detected in landfill gas (Dent et al, 1986).

3.2.5 Release from transport, storage, and accidents

3.2.5.1 Transport and storage

All equipment used for the transport and storage of chlorodifluoromethane is designed to withstand high pressures. Containers are filled to contain a maximum of 1.03 Kg/l and are fitted with safety valves, bursting discs, and fusible plugs complying with DOT (Department of Transportation [USA]), EEC directives and other local regulations; there are also requirements for labelling and leak pressure testing. Losses of product during transport and storage are relatively small because of the completely closed system used.

3.2.5.2 Accidents

Little information is available on accidental release. Morita et al (1977) described an accidental release on a fishing vessel (see Sections 7.2.2 and 9.2.1.).

3.3 Use patterns

3.3.1 Major uses

Chlorodifluoromethane has been sold for more than 50 years. It is used primarily as a refrigerant in residential, commercial and mobile air-conditioning units. An azeotropic mixture (HCFC-502) of chlorodifluoromethane/chloropentafluoroethane (CFC-115) (48.8 : 51.2 wt-%) is used as a refrigerant in food display cases, ice makers, home freezers and heat pumps.

Chlorodifluoromethane is used as an intermediate in production of tetrafluoroethylene which then is polymerised. Du Pont (1988) estimates that approximately 34 % of the total amount is currently used in the production of polymers.

In contrast to other halocarbons, chlorodifluoromethane is not currently used to any significant extent as a blowing agent for polyurethane foams although this application is now being investigated. It is used as a blowing agent for polystyrene (see Section 5.1.3). Its potential as a propellant in aerosol formulations is also being examined.

3.3.2 Release from use; controlled or uncontrolled

The major loss of chlorodifluoromethane to the environment results from equipment and system leaks during use and after scrapping. Assuming no significant loss from use as a polymer intermediate, it is estimated that the maximum current worldwide loss of chlorodifluoromethane is approximately 120,000 tonnes per year (Du Pont, 1988). For comparison, the total production of chlorofluorocarbons at the present time is about 1.3 million tonnes.

4. ENVIRONMENTAL TRANSPORT, DISTRIBUTION, AND TRANSFORMATION

4.1 Introduction

Assessment reports are prepared periodically by atmospheric scientists to define current status of atmospheric chemistry and physics. These reports provide the most up-to-date and reliable information on atmospheric transport and transformation of chemicals, including chlorodifluoromethane.

4.2 Sources of Information

The reports have been sponsored by international and national scientific organisations such as the United Nations Environmental Programme (UNEP), the World Meteorological Organisation (WMO), the Commission of the European Communities (CEC), the United States' National Aeronautics and Space Administration (NASA), the Federal Aviation Administration, the National Oceanic and Atmospheric Administration and, in the Federal Republic of Germany, the Bundesministerium für Forschung und Technologie. Recent reports include the WMO Report No. 16: "Atmospheric Ozone 1985, Assessment of Our Understanding of the Process Controlling its Present Distribution and Change" (WMO, 1986) and the Ozone Trends Panel Report cosponsored by NASA, WMO and other agencies (OTP, 1988).

The provisions of the Montreal Protocol on Substances that Deplete the Ozone Layer of 1987 require that international assessments of the state of knowledge of atmospheric science are conducted at least every four years (Article 6). The will be completed in 1989. A brief summary of the key points is provided here.

4.3 Transport, Distribution and Transformation

Once emitted, chlorodifluoromethane is rapidly mixed within the lower region of the atmosphere, the troposphere, by the normal tropospheric mixing processes. Mixing is complete in the hemisphere of the emission (northern or southern hemisphere) within months and in the entire troposphere within about two years. Most of the chlorodifluoromethane is destroyed within the troposphere by reaction with naturally occurring hydroxyl radicals ($OH^{\cdot}$) The atmospheric half life related to this reaction is about 14 years (Makide and Rowland, 1981; WMO, 1986).

Although the detailed mechanism of decomposition of chlorodifluoromethane following the initial reaction with $OH^{\cdot}$ is not fully understood, the most likely product of gas phase reactions in the atmosphere is COF_2 (Atkinson, 1985). The COF_2 would be hydrolysed almost instantly in atmospheric water aerosols (Merck Index 1983) and removed by precipitation.

The small fraction of chlorodifluoromethane not destroyed in the troposphere slowly enters and mixes with the upper layer of the atmosphere, the stratosphere, were the major destruction mechanism is by reaction with $OH^{\cdot}$ and excited oxygen atoms (O^1D). Photodecomposition by solar ultra-violet radiation (a major loss process for the fully halogenated chlorofluorocarbons) does not play a significant role in the destruction of chlorodifluoromethane in this region and leads to the release of a relatively small amount of chlorine: chlorodifluoromethane is responsible for less than 1 % of the amount of chlorine in the stratosphere which can react to deplete ozone. Currently the majority of chlorine in the stratosphere comes particularly from fully halogenated chlorofluorocarbons.

Since the above considerations indicate that most of the chlorodifluoromethane emitted is destroyed before it can reach the stratosphere, chlorodifluoromethane has only a small relative Ozone Depletion Potential (ODP). ODPs are calculated using computer model simulations based on knowledge of the atmospheric chemistry and physics.

The ODP is defined as the model calculated ozone depletion due to the emission of a unit mass of chlorodifluoromethane divided by the ozone depletion calculated to be due to the emission of a unit mass of trichlorofluoromethane; calculations are based on steady-state conditions. The accepted ODP of chlorodifluoromethane is 0.05 (Hammitt et al, 1987; UNEP, 1987; 1988). This means that continuous emissions of chlorodifluoromethane would have to be 20 times as large as continuous emissions of trichlorofluoromethane to have the same effect on ozone.

Therefore, during a UNEP meeting in The Hague in October 1988, delegates from the EEC and US viewed chlorodifluoromethane as part of the solution to ozone depletion because expanding its use could decrease consumption of dichlorodifluoromethane.

Current assessment of "greenhouse warming" of chlorodifluoromethane based on a comparison with the atmospheric lifetime of dichlorodifluoromethane indicates that the potential of chlorodifluoromethane to cause global warming would be lower by a factor of at least 10 than existing chlorofluorocarbons (AFEAS Workshop, 1989).

Krüger and Fabian (1986) also presented work on estimations of ozone depletion, but their models grossly overestimated production levels and are not considered to have produced valid results. Although discussed by atmospheric scientists at the UNEP conference in the Hague in October 1988, the conclusions were not incorporated into the final statements of the conference, where the formerly suggested ODP of 0.05 was confirmed (UNEP 1988).

4.4 Biodegradation

No information is available on the biodegradability in the environment.

4.5 Bioaccumulation

Bioaccumulation can be directly related to the octanol/water partition coefficient (P_{ow}) of a compound. The log P_{ow} of chlorodifluoromethane is

calculated as 1.08 (Hansch and Leo, 1979), indicating that this compound will not have any appreciable potential for bioaccumulation.

5. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

5.1 Environmental levels

5.1.1 Air

Rasmussen et al (1980) measured the atmospheric burden of chlorodifluoromethane. An average global concentration of 45 parts per trillion vol. (pptv, $1/10^{12}$) was found in mid-1979. This corresponds to 0.16 ng/m^3. The concentration in the northern hemisphere was 50 pptv and 42 pptv in the southern hemisphere. These values are considerably higher than the value of 25-30 pptv, calculated from estimates of anthropogenic emissions.

Khalil and Rasmussen (1981) measured concentrations of chlorodifluoromethane in 100 atmosphere samples collected between April 1978 and January 1981 at 45°N latitude in the Northwest Pacific. The chlorodifluoromethane concentration increased exponentially at an average rate of 11.7%/y; their last determinations (January 1981) showed a chlorodifluoromethane concentration of about 65 pptv. The same authors calculated the concentration expected from estimated industrial release of chlorodifluoromethane since 1950. The observed concentrations were, on average, 17 pptv higher than the estimated concentrations. The authors considered that the most probable explanation was underestimation of past industrial releases.

Rasmussen and Khalil (1983) found an average concentration of 73.2 pptv of chlorodifluoromethane in the lower arctic atmosphere (0-4 km) at 70°N in May 1982. This concentration is 12 times greater than that found at 30°-40°S in November 1981 (Rasmussen et al 1982).

In the Arctic (72°N in Alaska) the winter concentrations of chlorodifluoromethane, other halocarbons, CO and soot from combustion are higher than at other times of the year which is attributed to faster

transport of man made emissions in this season. The average winter concentration of chlorodifluoromethane was 61.2 pptv and the average during the summer was 56 pptv. The rate of increase from August 1979 to February 1982 was 11.9 %/year (Khalil and Rasmussen, 1983). The most recent information indicates that the concentration of chlorodifluoromethane was 92 pptv in 1986 (NASA, 1988). The rate of increase of the atmospheric concentration is somewhat uncertain due to the limited number of measurements in the northern hemisphere, but measurements indicate that its concentration is increasing at the rate of about 7% per year in the southern hemisphere.

5.1.2 Water

No data are available on the concentration of chlorodifluoromethane in water.

5.1.3 Food and other edible products

No information was available on the concentration of chlorodifluoromethane in food and other edible products. The physical and chemical properties of the material are such that residues of chlorodifluoromethane in food would be expected to be negligible.

Chlorodifluoromethane is used in the U.S.A., the U.K., and several other countries as a blowing agent for polystyrene foam, an authorised food contact plastic.

5.2 Human exposure

Simulated use studies have been carried out to assess the potential human exposure to chlorodifluoromethane arising from its proposed use as an aerosol propellant.

After a single spray (5 or 10 seconds duration) of an aerosol containing 17% chlorodifluoromethane in a closed 22 m^3-room, the air concentration was determined at various positions relative to the spray

cone. When the spray was directed towards the sampling tube, peak concentrations were 5,075 or 8,050 mg/m^3, depending on spray duration. The concentrations declined after about 10 or 20 seconds respectively and stabilised rapidly at levels of 25 or 45 mg/m^3. In all other spray positions the chlorodifluoromethane concentrations did not exceed the level calculated for homogeneous distribution in the air of the room (Bouraly and Lemoine, 1988).

Hartop and Adams (1989) reported a series of similar studies in which chlorodifluoromethane concentrations were measured in the "breathing zones" of an experimental manikin and an "accompanying child". They examined hairspray containing 20-40%, whole body deodorant containing 20-65%, and antiperspirant containing 20-40 % chlorodifluoromethane. The peak concentrations found in a closed 21 m^3-room ranged from 53 mg/m^3 for a 4 second spray of an antiperspirant containing 18.8%, to 4,950 mg/m^3 for a 20 second spray of a deodorant containing 65% chlorodifluoromethane. This corresponds to time weighted average concentrations over 10 minutes from spraying (TWA10) of about 50 mg/m^3 or 1,440 mg/m^3.

In another part of this study simulated salon use of a hair spray gave TWA10 values of 160 to 225 mg/m^3 for the "customer" and 8 hour TWA values of 90-125 mg/m^3 for the "stylist", based on the assumption that he uses one 10 second spray every 15 minutes with the door of the salon left open. This compares with TLVs of 1,750 (in the Federal Republic of Germany, see Section 10) or 3,500 mg/m^3 (in the USA).

6. EFFECTS ON ORGANISMS IN THE ENVIRONMENT

No data are available on the effects of chlorodifluoromethane on non-human organisms in the environment.

7. KINETICS AND METABOLISM

7.1 Animal studies

7.1.1 Absorption

The relationship between inhaled concentrations of chlorodifluoromethane and blood levels was studied in the anaesthetised rats by Carney (1977). The chlorodifluoromethane concentrations in air were metered through a mixing chamber and pump to a canula inserted into the exposed trachea. After 15 min. a sample of blood was withdrawn from the carotid artery, the supply of chlorodifluoromethane was stopped and further blood samples were taken at timed intervals to estimate the rate of clearance from the blood. Four male and four female rats were used for experiments using nominal air concentrations of either 35,000 or 175,000 mg/m^3. The results showed a direct correlation between the inhaled air and blood concentrations of chlorodifluoromethane. With an inhaled concentration of 35,000 mg/m^3, the mean blood concentration was 31 mg/litre. At 175,000 mg/m^3 the mean blood concentration was 155 mg/litre. After exposure the clearance of chlorodifluoromethane was rapid, with a half-life of approximately 3 minutes.

Comparable results have been reported by Sakata et al (1981) with experiments in rabbits. Animals which had been anaesthetised with phentobarbitone (25 mg/kg ip) received chlorodifluoromethane/air mixture via a plastic mask and blood samples were taken from a catheter in a femoral artery. The concentration of chlorodifluoromethane inhaled ranged from 175,000 mg/m^3 (5 %) to as high as 1,400,000 mg/m^3 (40 %). Blood concentrations of chlorodifluoromethane increased rapidly from the beginning of inhalation at every concentration. Saturation was reached in about 5 min. The blood concentration was directly proportional to the inhaled concentration of chlorodifluoromethane; at 175,000 mg/m^3 (5%) it was calculated to be 43 ul/g (148 mg/l), which is similar to the blood level found in the rat under the same conditions (Carney, 1977), and at 700,000 mg/m^3 (20 %) it was calculated to be 170 ul/g (583 mg/l). When exposure ceased, the blood concentration decreased rapidly, with a maximum

half-life of 1 minute. After 15-30 min., blood chlorodifluoromethane concentrations were 8-9 µl/g (27-31 mg/l) irrespective of the inhaled concentration. It took a further hour for the blood concentration to fall below the limit of detection.

Ding et al (1980) reported the alveolar absorption rate to be 3.15% of the total dose in rabbits exposed to 3,500 mg/m^3 chlorodifluoromethane. The study cannot be evaluated because of the lack of details.

Pregnant rats were exposed to atmospheric concentrations between 350 and 175,000 ppm of chlorodifluoromethane. Blood samples taken at various intervals again showed that chlorodifluoromethane rapidly reached equilibrium with blood and was eliminated quickly following removal from exposure. At the highest dose the blood level reached 118.5 mg/l after 30 min. with no significant increase (121 mg/l) after another 5.5 hours of exposure. Thirty min. following cessation of exposure the blood level had decreased to 3.55 mg/l (Woollen, 1988).

7.1.2 Distribution

Sakata et al (1981) determined the amount of chlorodifluoromethane in the tissues of rabbits receiving up to 1,400,000 mg/m^3 by inhalation. (Conditions are described in Section 7.1.1). The concentration of chlorodifluoromethane in tissues is detailed in Table 2. No major differences were found in the tissues examined except for fat tissue in which there was a difference after prolonged and short inhalation times at high concentrations. The authors postulated the effect was related to the poor vascular blood supply of adipose tissue and that the distribution of chlorodifluoromethane to the tissues depended on partial pressures. Those tissues with a good supply would reach equilibrium quickly, whereas fat would only equilibrate slowly.

7.1.3 Metabolic transformation

In vivo experiments have been carried out by Salmon et al (1979) using ^{14}C and ^{36}Cl labelled chlorodifluoromethane. Rats were exposed individually to atmospheres of chlorodifluoromethane in specially constructed chambers in which all surfaces in contact with gas were either glass or metal. With the ^{14}C-labelled material, exposure levels were 1,750 mg/m^3 in air in three experiments and 35,000 mg/m^3 in three others, the exposure times being 15-24 h. Exhaled CO_2 was collected by absorption in barium hydroxide and the radioactivity was subsequently measured. Separate collection of urine and faeces into containers cooled to 0°C was followed by radiochemical counting, directly in the case of urine and after appropriate oxidation for the faeces. Similar exposure and collection conditions were used for the ^{36}Cl-label experiments, in which the concentration of chlorodifluoromethane was 35,000 mg/m^3 for a 17.5 h exposure.

These experiments showed that metabolism of chlorodifluoromethane in the rat was minimal. The amount of $^{14}CO_2$ released was equivalent to approximately 0.1% of the inhaled chlorodifluoromethane at an air concentration of 1,750 mg/m^3 and 0.06% at 35,000 mg/m^3 chlorodifluoromethane. The amounts of ^{14}C label in the urine were also small, equivalent to approximately 0.03 and 0.01% of the inhaled doses of 1,750 and 35,000 mg/m^3 chlorodifluoromethane respectively. Insignificant quantities were found in the faeces. The results of the experiments with the ^{36}Cl label supported those obtained with the ^{14}C label; only 0.01% of the inhaled dose was detected in the urine. It is questionable whether the minimal metabolism observed was of chlorodifluoromethane or of an impurity present in the test compound.

Salmon et al also conducted in vitro studies utilising a microsomal preparation derived from Aroclor 1254 induced rat liver homogenates. Microsomes, NADPH and ^{36}Cl-labelled chlorodifluoromethane were incubated in a repeat-dosing syringe, samples being removed for analysis at 2 min intervals. Released $^{36}Cl^-$ was isolated as AgCl and estimated by

scintillation counting. Under the test conditions there was no release of chloride ion from chlorodifluoromethane (studied in a range of concentrations from 0.2 to 1.3 mM) further indicating the compound's resistance to breakdown in biological systems and suggesting that any potential biological activity of chlorodifluoromethane was unlikely to be due to formation of reactive intermediates.

Peter et al (1986) found that chlorodifluoromethane was not metabolised by Wistar rats after either ip or inhalation exposure. In their first experiment, rats received a single ip injection of chlorodifluoromethane after which they were placed in a closed desiccator system with a gas sample loop connected to a chromatograph. Injected chlorodifluoromethane was almost completely exhaled, and its further decline in the system could not be distinguished from that of the compound introduced into the atmosphere of the system. When chlorodifluoromethane was added directly to the gas phase of the system, again the clearance values did not differ from those of controls without animals. In addition, pretreatment of the animals with phenobarbital (80 mg/kg ip, followed by 3 days of 0.1% phenobarbital in the drinking water) or DDT (200 mg/kg, 1 week prior to the experiments) did not alter the observations. The authors concluded that there was no detectable elimination of metabolites of chlorodifluoromethane. It is stated that these experiments were confirmed in B6C3F1 mice, although no data are provided.

7.1.4 Elimination

Experiments to detect metabolites of chlorodifluoromethane (Salmon et al, 1979) showed that rats exposed to 35,000 mg/m^3 chlorodifluoromethane only yielded 0.01% of the dose in the urine (Section 7.1.3). The work by Peter et al (1986) demonstrated that after ip injection the compound was exhaled unchanged.

Elimination of chlorodifluoromethane was studied in rabbits after exposure to 175,000 and 1,400,000 mg/m^3 (Sakata et al, 1981, see also Section 7.1.1). After exposure ceased, the blood concentration decreased

rapidly with a maximal half-life of 1 minute. After 15-20 minutes, blood chlorodifluoromethane concentrations were 8-9 ul/g irrespective of the inhaled concentration. It took a further hour for values to fall below the sensitivity of analysis. When the partial pressure of chlorodifluoromethane in alveolar air became zero, chlorodifluoromethane was rapidly cleared from the blood, followed by moderate elimination from poorly perfused tissues.

In conclusion, the studies show that chlorodifluoromethane is rapidly absorbed into the bloodstream by all routes of administration, is not metabolised to any significant extent and is eliminated unchanged in the expired air. Only a very small amount of radiolabeled material (<0.1 % of administered dose) has been detected in urine.

7.2 Human

7.2.1 Absorption and elimination

The uptake and elimination of chlorodifluoromethane has been studied in man. Two groups of 3 male subjects were exposed to average atmospheric concentrations of 320 or 1810 mg/m^3 chlorodifluoromethane for 4 hours. Blood and expired air samples were taken during the exposure period and for up to 26 hours after exposure and analysed for chlorodifluoromethane. Urine samples were collected for up to 22 hours after exposure and analysed for chlorodifluoromethane and fluoride ion. During the exposure period blood concentrations of chlorodifluoromethane approached a plateau and the maximum blood concentrations of 0.25 and 1.36 ug/ml were proportional to dose. The concentrations of chlorodifluoromethane in expired air were similar to the exposure concentrations during the exposure period. The ratio between blood and expired air concentrations towards the end of the exposure period was, on average, 0.77. This is consistent with *in vitro* measurements of the solubility of chlorodifluoromethane in human blood (blood/air partition coefficient 0.79).

In the post-exposure period 3 phases of elimination of chlorodifluoromethane were apparent with half lives of 0.05, 0.23 and 2.8

hours. The first phase, which could be identified only from expired air measurements, is thought to correspond to elimination from blood and rapidly perfused tissues. The second and third phases are believed to correspond to the elimination from slowly perfused tissues and fat respectively. Chlorodifluoromethane was detected in urine samples taken in the post-exposure period at both dose levels and the rate of decline was consistent with the terminal rate of elimination determined from blood and breath measurements. Fluoride ion concentrations in urine did not increase significantly following exposure, indicating that chlorodifluoromethane is not metabolised to any significant extent (Howard et al, 1989).

7.2.2 Distribution

Three days after a fatal accident on board a fishing vessel, samples of major tissues were taken from two of the deceased for estimation of chlorodifluoromethane content by gas chromatography. The findings are given in Table 3; the concentrations were similar to those found in a rabbit examined 3 days after death by asphyxiation with chlorodifluoromethane (Morita et al, 1977).

In a survey of organic compounds found in human milk, chlorodifluoromethane was detected in one of twelve samples. Chlorodifluoromethane was one of 184 compounds detected in the survey. No information on exposure nor quantification of the amount found is given (Pellizzari et al, 1982).

7.2.3 Transformation

There are no data on the transformation of chlorodifluoromethane in man.

8. EFFECTS ON EXPERIMENTAL ANIMALS AND IN VITRO TEST SYSTEMS

8.1 Single exposures

The concentration and duration of exposure at which chlorodifluoromethane proved lethal to a variety of species is given in Table 4. All of the studies showed that chlorodifluoromethane has an extremely low order of acute toxicity. Despite the variety of conditions used and the different laboratories involved, there is a consistency between experiments on each species. Chlorodifluoromethane is less toxic to the dog than to rodents, although in all cases the concentration required to cause death is above 700,000 mg/m^3 (=20% v/v).

The signs of chlorodifluoromethane toxicity in rats were tremors of the limbs and head, convulsions, narcosis, shallow respiration and death from respiratory depression. Death always occurred during exposure, never afterwards. Recovery from non-lethal exposure was rapid and rats appeared normal within 10 minutes with no delayed after-effects. The EC_{50}(10 mins) for the CNS effects described above was determined as 490,000 mg/m^3 for rats, as compared to the LC_{50} value of 700,000 mg/m^3 (Clark and Tinston, 1982).

Signs in rabbits were similar to those in rats, namely incoordination and other signs of CNS depression followed by respiratory depression and asphyxiation. Post-mortem examination of rabbits that had died from exposures up to 1,400,000 mg/m^3 confirmed death by asphyxiation (Sakata et al, 1981).

Thus the primary toxic effect of the acute inhalation was central nervous system depression which only occurred at extremely high exposure levels.

8.2 Repeated exposures

Toxicity studies with repeated exposure up to 13 weeks are detailed here. Studies designed to examine the effects on the cardiac system are

described in Section 8.5.1. and studies in excess of 90 days in Section 8.3.

Few toxic effects were seen on repeated exposure to chlorodifluoromethane. Karpov (1963a) exposed rats to 35,000 mg/m^3 for 6 hours/day for 63 days without observing histopathological effects.

Weigand (1971) exposed rats, guinea-pigs, dogs and cats to 175,000 mg/m^3 chlorodifluoromethane by inhalation, 3.5 hr/d, 5 d/wk for 4 weeks. No effects were seen on body weight, haematology, urine analysis, organ weights or the macroscopic and microscopic appearance of the tissues.

Groups of 16 male Sprague-Dawley rats were exposed to 0 (control) or 175,000 mg/m^3 chlorodifluoromethane for 5 hr/day for 8 weeks (Lee & Suzuki, 1981), after which six rats in each group were killed and blood and tissue samples taken for haematological and biochemical assays and for histopathological examination. The remaining animals were retained for a fertility study (Section 8.6). No signs of toxicity were apparent in the chlorodifluoromethane exposed animals, and body weight was not affected. The weights of a range of organs were not affected although prostate weight was decreased slightly. No histopathological lesion was related to exposure in any of the organs examined. Haematological parameters were unaffected but plasma glucose and triglyceride levels were depressed and plasma cholesterol slightly raised in the chlorodifluoromethane group.

Sprague-Dawley rats and beagle dogs were exposed to 35,000 and 17,500 mg/m^3 chlorodifluoromethane, respectively, for 6 hr/d for 13 weeks by whole-body exposure (Leuschner et al, 1983). Control and chlorodifluoromethane-exposed groups were composed of 20 male and 20 female rats and three male and three female dogs. Clinical behaviour, body weight, haematology, clinical biochemistry, organ weights and histopathology was examined in both species and dogs were also subjected to ECG measurements and to examination of circulatory function. The clinical biochemistry examinations included assay for serum alanine transaminase (ALAT), aspartate transaminase (ASAT) and alkaline phosphatase activities and liver-function tests. Histopathological examinations were undertaken on

a wide variety of tissues. No changes due to chlorodifluoromethane exposure were seen in any of these investigations and it was concluded that the no-effect level for the compound was at least 35,000 mg/m^3 in the rat and 17,500 mg/m^3 in the dog.

In a limited experiment designed to determine whether exposure for 5 hr/d, 5 d/wk for 8-12 weeks to 210,000 mg/m^3 chlorodifluoromethane might induce cardiac arrhythmia, only one of 14 rabbits, which was also receiving sodium phenobarbital in the drinking water, developed an arrhythmia. Since no controls were used, the value of this one observation is questionable. In addition, some rabbits (number unknown) also receiving phenobarbital showed slight histopathologic liver damage and a modest elevation in unspecified serum enzymes. The technical limitations of the experimental design, and the lack of detail in the published abstract (never published as a full paper) mean that no meaningful conclusions can be drawn from this study (Van Stee and McConnell, 1977).

In an inhalation study Knox-Smith and Case (1973) exposed 1 male and 1 female beagle puppy to a 60:40 % mixture of chlorodifluoromethane and dichloromonofluoromethane at a concentration equivalent to 1714 mg/kg bw/d for 5 d/wk for 2 weeks. After 1 to 2 minutes in each exposure period the puppies became sedated and ataxic but recovered fully within a few minutes when removed from the atmosphere. The contribution of chlorodifluoromethane to this observation is unclear. No other effects were noted. Examinations included blood chemistry and urinary analysis. Gross and microscopic examinations of the lungs failed to reveal any changes.

In conclusion, repeated exposure up to 13 weeks to chlorodifluoromethane appears to be associated with a relatively low order of toxicity in several species. The only consistent effect was a weak anaesthetic effect, and no consistent target organ could be identified in any of the studies.

8.3 Long-term exposures

Chlorodifluoromethane shows a low order of toxicity in long-term experiments.

Karpov (1963b) studied the effects of chlorodifluoromethane in rats, mice and rabbits exposed to 49,000 mg/m^3 and in rats and mice exposed to 7,000 mg/m^3 for 6 hr/d on 6 d/wk over a 10 month period. Body weights, oxygen consumption, "nerve function" and biochemical and haematological parameters were recorded and histopathological examination of some tissues was undertaken at termination of the test. Changes noted in the animals at 49,000 mg/m^3 included depressed body-weight gain in mice after 4-6 months, depressed oxygen consumption in the rat, "nerve function" changes in the rat and mouse, decreased haemoglobin concentration in the rabbit and histopathological (dystrophic) changes in the liver, lungs and nervous tissue. No effects due to chlorodifluoromethane were seen at the 7,000 mg/m^3 exposure level. None of these effects seen in rats and mice at 49,000 mg/m^3 have been confirmed in subsequent studies even at much higher exposure levels.

Tinston et al (1981a) conducted a chronic toxicity experiment on mice to assess the carcinogenic potential of chlorodifluoromethane and its effects on haematological and biochemical parameters. Eighty male and eighty female Alderley Park Swiss derived mice per group, were exposed to concentrations of 0, (two groups) 3,500, 35,000 and 175,000 mg/m^3 of chlorodifluoromethane 5 hr/d, 5 d/wk for up to 83 weeks (females) and 94 weeks (males), at which time there was 80% mortality. At week 38, 10 mice per group were killed in order to perform blood and biochemical assays including red and white blood cell measurements, platelet count, prothrombin and kaolin-cephalin times and bone marrow examination. Plasma ALAT and ASAT activity as well as urine analyses were also undertaken. The only consistent finding was hyperactivity in the male mice exposed to 175,000 mg/m^3. No effects were noted on mortality, body weight gain, haematology and biochemistry or in histopathology other than neoplasia described in Chapter 8.8.

A similar study using the same group sizes and exposure levels was performed in rats (Tinston et al, 1981b). The study was continued to 118 weeks in females and 131 weeks in males (80% mortality). Some animals were killed at week 52. The same investigations were done as for the mouse study. No clinical abnormalities, increased mortality or haematological or biochemical changes could be attributed to chlorodifluoromethane at any dose level. At the highest exposure level (175,000 mg/m^3) there was a decrease in body-weight gain in males (up to week 80) and increased liver, kidney, adrenal and pituitary weights in the females. A number of non-neoplastic lesions were observed histologically in all groups but there was no evidence of an increased incidence due to chlorodifluoromethane. There was a clear no-effect level of 35,000 mg/m^3 chlorodifluoromethane for chronic effects in the rat.

8.4 Skin and eye irritation, sensitisation

8.4.1 Skin irritation

Quevauvillier et al (1964) stated that a 10 second spray on the shaved belly of the rat twice a day 5 d/wk for 6 weeks caused reddening of the skin and slight swelling of the surface. There was also a delay in hair regrowth. A more recent report (Atochem, 1986) classified dichlorofluoromethane as a skin irritant but only after application of 0.5 ml in liquified form under a capsule to the intact and abraded skin of rabbits.

These effects were probably caused by freezing of tissues rather than the toxic activity of the substance.

8.4.2 Eye irritation

The ocular irritation of chlorodifluoromethane has been assessed by exposing the cornea of albino rabbits to the gas for 5 or 30 seconds. Chlorodifluoromethane was considered to be slightly irritant (Atochem, 1986).

8.4.3 Allergic Sensitisation

The skin sensitising potential of chlorodifluoromethane has been tested in 10 male and 10 female Hartley albino guinea pigs using a technique derived from the Magnusson-Kligman maximisation test. On day 0, Freund Complete Adjuvant was injected intradermally and 0.25 ml of liquified chlorodifluoromethane was applied topically on the skin under a capsule for 48 h (occlusive). On days 2, 4, 7, 9, 11 and 14, 0.5 ml of liquified chlorodifluoromethane was applied topically in the same place on the skin for 48 h (occlusive). After a rest period of 2 weeks, the challenge exposure was performed on day 28 at the opposite side of the body: 0.25 ml of liquified chlorodifluoromethane, which was the maximum non irritating dose determined in a previous experiment, was applied on the skin under a capsule for 48 h (occlusive). Macroscopic and histological evaluations of skin reaction were scored 1, 6, 24 and 48 h after removal of the occlusive patch. Under these experimental conditions, chlorodifluoromethane did not produce any cutaneous sensitising reaction (Atochem, 1986).

No respiratory sensitisation data are available but it is extremely unlikely that chlorodifluoromethane possesses any immunogenic potential since it is unreactive with nucleophiles in tissues.

8.5 Special studies

Early studies on the toxicity of certain hydrocarbons, especially anaesthetics, showed that they could render the mammalian heart abnormally reactive or sensitive to adrenaline (epinephrine) resulting in cardiac arrhythmias. Halocarbons have been screened for this effect. Section 8.5.1 details the studies on chlorodifluoromethane.

8.5.1 Cardiovascular function

Aviado and Belej (1974) investigated 15 propellants for their ability to induce cardiac arrhythm. Anaesthetised Swiss mice were exposed to

700,000 or 1,400,000 mg/m^3 chlorodifluoromethane for a 6 minute period via a face mask. Electrocardiographic recordings were made throughout and after the exposure period. In the first experiment no adrenaline was given but in a second experiment 6 ug/kg was administered intravenously 2 min after the inhalation phase began to maximise the sensitivity of the assay. Chlorodifluoromethane alone did not cause arrhythms, nor did it do so at the 700,000 mg/m^3 dose level with adrenaline. At the 1,400,000 mg/m^3 level with adrenaline arrhythms were detected.

Fourteen rabbits (7 of each sex) received exposures of 210,000 mg/m^3 chlorodifluoromethane for 5 hr/d, 5 d/wk for 8-12 weeks. One female rabbit, which was also receiving phenobarbital, developed an arrhythmia, probably of supraventricular origin (Van Stee and McConnel, 1977). As this was only a single observation and no controls were used, no meaningful conclusions from this very limited study can be drawn.

Reinhardt et al (1971) assessed the ability of chlorodifluoromethane to induce cardiac sensitisation to adrenaline in male beagle dogs exposed to 87,500 or 175,000 mg/m^3 via a face mask. Twelve dogs were used per dose level. After 5 minutes exposure a challenge injection of adrenaline (0.008 mg/kg) was given. At 87,500 mg/m^3 no effects were noted but at 175,000 mg/m^3 2 animals displayed cardiac sensitisation as demonstrated by serious arrhythmias detected by an electrocardiogram. Compared to a range of other chlorofluorocarbons, chlorodifluoromethane was classed as a weak cardiac sensitising agent.

To investigate the effects of aerosol abuse at high concentrations of chlorodifluoromethane, Pantaleoni and Luzi (1975a,b) exposed rats to high concentrations and measured various cardiac functions. In the first experiment, inhalation of 525,000 to 2,100,000 mg/m^3 (60% v/v) chlorodifluoromethane for 2 minutes produced a decrease in heart rate and changes in an electrocardiogram. In the second study rats exposed to 1,050,000 mg/m^3 (30% v/v) to 2,100,000 mg/m^3 (60% v/v) in air for 2 minutes showed decrease in cardiac contractile strength, followed by a decrease in carotid pressure, electrocardiogram changes and arterial hypotension. Vagotomy partially inhibited the appearance of the electrocardiogram

changes. In both experiments the parameters returned to normal within 2 minutes of breathing normal air.

Experiments in monkeys by Belej et al (1974) demonstrated that chlorodifluoromethane alone did not cause a significant cardiac response at exposure levels of up to 700,000 mg/m^3 (20% v/v). Three monkeys were anaesthetised with sodium pentobarbitone; the trachea of each was cannulated and the chest was opened to enable direct measurement of cardiac function. Following exposure to 350,000 or 700,000 mg/m^3 chlorodifluoromethane for 5 minutes, the following indices of cardiac function were measured: heart rate, myocardial force, aortic blood pressure and left atrial pressure. During these experiments, the only change was a slight yet significant drop in aortic blood pressure in animals exposed to 700,000 mg/m^3.

Clark and Tinston (1982) determined the exposure levels causing acute cardiac sensitisation after adrenaline treatment in dogs. The concentration affecting 50 % of the animals (EC_{50}) was 490,000 mg/m^3 for both species. As this was the same concentration as for the EC_{50} of CNS effects in rats, the authors postulated that in man it would be unlikely that cardiac sensitisation would occur before CNS effects were noted.

Leuschner et al (1983) exposed dogs to chlorodifluoromethane at 17,500 mg/m^3 for 6 hr/d, 7 d/wk for 90 days. During this period no effect upon the ECG and circulary functions were noted.

Aviado (1975) reviewed the available data, which was primarily from his own laboratory, on the toxicity of aerosol propellants and proposed a classification scheme. Chlorodifluoromethane was classed as a high pressure propellant of intermediate toxicity. Many other propellants displayed a higher potential to cause cardiac arrhythmia (with or without adrenaline), although most of these were low pressure rather than high pressure propellants.

In summary, chlorodifluoromethane causes cardiac sensitisation but only at extremely high exposure concentrations (approaching the LC_{50}). Compared

to a variety of other chemicals, chlorodifluoromethane was classified as a "weak" cardiac sensitising agent (Reinhard et al, 1971).

8.5.2 Respiratory function

The effect of chlorodifluoromethane on the respiratory function of a rhesus monkey was measured by Aviado and Smith (1974). The animal was anaesthetised by intravenous injection of 30 mg/kg sodium phenobarbital and the trachea was cannulated. Electrocardiogram measurements and femoral arterial blood pressures were recorded. Pulmonary airway resistance and compliance were estimated from measurements of tracheal air flow and transpulmonary pressure. On exposure to 700,000 mg/m^3 chlorodifluoromethane there was no significant change in pulmonary resistance, pulmonary compliance, heart rate or aortic blood pressure. At 1,400,000 mg/m^3 the only change noted was a slight, yet significant elevation in pulmonary resistance.

8.6 Reproductive Effects, Embryotoxicity and Teratology

8.6.1 Experimental data

Lee and Suzuki (1981) tested for effects on male reproduction in Sprague-Dawley rats. A group of 16 male rats was exposed to 175,000 mg/m^3 chlorodifluoromethane, 5 hr/d, for eight weeks. A control group of the same size was exposed to filtered air under identical conditions. Control and test animals were examined and weighed weekly. At the end of the eight week period, six rats from each group were killed, organ weights determined, and histopathological, clinical chemical and haematological examinations carried out. The prostate glands were assayed for fructose and acid phosphatase activity. Immediately after the final exposure, blood was collected from the remaining 10 rats in each group. Plasma was assayed for follicle stimulating hormone (FSH) and luteinising hormone (LH). These animals were then used for serial mating, each male being housed with a virgin female for 7 days, after which time the female was replaced with another virgin female; this regime was

followed for 10 week period. Nine days after removal each female was killed, examined and the number of corpora lutea, total implants, live implants, resorption sites and dead implants determined. The chlorodifluoromethane exposure regime did not give rise to any overt sign of toxicity. The major organs including testes and epididymides did not differ in weight from those of controls. There was a slight decrease in weight of the prostate and coagulating gland (an accessory sex gland in the rat) but there were no accompanying histological changes even when examined by electron microscopy. Prostatic fructose and acid phosphatase levels were unaltered. Similarly, FSH and LH levels were not different from controls. Overall there was no effect upon the fertility of male rats exposed to chlorodifluoromethane, and no evidence of a dominant lethal effect.

The teratogenicity of chlorodifluoromethane was evaluated in both rats and rabbits. Earlier three inhalation teratology studies were carried out in 1977 and 1978 at the Haskell Laboratory (reported by CFR 1981) in which 20-40 pregnant CD Sprague-Dawley rats were exposed to several concentrations (ranging from 350 to 70,000 mg/m^3) for 6 hr/d either from day 4 to 13 or 6 to 15 of gestation. There was no evidence of maternal or overt foetal toxicity. No teratogenic abnormalities were seen apart from the presence of one or two litters in several treated groups which contained a low incidence (usually a single foetus) of microphthalmia or anophthalmia (Table 11). Although there was no reason to doubt the general foetal evaluation, no quantitative techniques were applied for the assessment of microphthalmia. This issue was specifically addressed in the later study (Palmer et al, 1978a).

The low incidence of microphthalmia or anophthalmia, which was not dose related, led to the conduct of a very large study which was designed to improve the sensitivity in order to investigate this infrequent malformation. Female CD rats were exposed to concentrations of 0, 350, 3500 or 175,000 mg/m^3. Chlorodifluoromethane was administered for 6 hr/d on days 6-15 of gestation. The top concentration equates to approximately 25% of the LC_{50} (4hr) in rats (Palmer et al, 1978a).

19 batches of time-mated females were used, each batch being made up of 34 control rats and 22 rats in each of the three test groups. In total this enabled examination of more than 6000 control foetuses and more than 4000 foetuses from each exposure group. The animals were observed daily, weighed at regular intervals, killed on day 20 of pregnancy and examined macroscopically. The ovaries were examined for numbers of corpora lutea, and uteri for live young and embryonic foetal deaths; litter and mean pup weights were recorded. The live young were examined externally for overt malformations. The heads of all foetuses were sectioned and examined with particular reference to microphthalmia, anophthalmia and associated anomalies.

Maternal body weights in the group exposed to 175,000 mg/m^3 were slightly lower than controls in most batches, but the body weight gain was consistently lower in this high dose group than in the control group during the first days of exposure. No other observable changes in the dams resulted from chlorodifluoromethane exposure. Overall there was no effect on litter size, post-implantation loss or litter weight. Mean foetal weight was slightly but consistently lower in 175,000 mg/m^3 chlorodifluoromethane exposed animals than in controls, but this was not seen at the two lower levels of exposure. The number of foetuses and incidence of litters with anophthalmia or microphthalmia are shown in Table 5.
The foetuses with microphthalmia or anophthalmia were all small and in all but one case were the smallest in the litter, although not necessarily the smallest in the group.

Statistical analysis on a litter basis (Fisher's exact test, one tailed) showed no significant difference from controls at low and intermediate exposures in respect of the incidence of anophthalmia/microphthalmia alone or in combination. At the highest exposure of 175,000 mg/m^3 chlorodifluoromethane there was a significant ($P<0.05$) increase in the incidence of litters containing foetuses with either anophthalmia or microphthalmia combined or anophthalmia alone principally due to a significant increase in the incidence of anophthalmia alone. The incidence of litters with microphthalmia alone was not significant ($P>0.05$). A limited general examination showed no other gross foetal abnormalities (Palmer et al, 1978a).

The results of the large replicate study (Palmer et al, 1978a) have been compared with the data for rats of the same strain and from the same source produced in the years following the large study. When compared with the overall control data from other experiments in the same laboratory conducted in the 10 year period following the chlorodifluoromethane experiment, there is a significantly increased incidence of eye defects in the top dose group. Figure 1 and Table 6 show the total incidence of eye malformations in blocks of 19 studies, each equivalent in size to the chlorodifluoromethane experiment, which indicates the varying incidence of these malformations. One block shows an incidence (10 in 386 litters) in the control groups similar to that found in the high dose group of the chlorodifluoromethane study. Based on his review, Palmer considers that, whilst the prospective control data do not discount the original interpretation that the results of the replicate study may indicate a threshold response at 175,000 mg/m^3, their attribution to random variation may be of equal merit (Palmer, 1989).

A teratology study was also carried out in the rabbit (Palmer et al 1978b). New Zealand white rabbits were exposed to 0, 350, 3,500, 175,000 mg/m^3 chlorodifluoromethane for 6 hr/d on days 6-18 of pregnancy. There were 14-16 pregnant females/group. The animals were killed on day 29 of pregnancy for the assessment of litter data and for the examination of foetuses for major malformations, minor anomalies and variants. There were no significant treatment-related effects in the chlorodifluoromethane exposed females and pregnancy was normal. Maternal body weight gain was slightly but consistently lower in the animals exposed to 175,000 mg/m^3 chlorodifluoromethane during the first 4 days of exposure but, thereafter, weight gains were comparable with those in the controls. Litter size, post-implantation loss and litter and mean foetal weights were unaffected by chlorodifluoromethane exposure. No significant increase in the incidence of major or minor foetal anomalies resulted from chlorodifluoromethane exposure.

8.6.2 Evaluation

The data available for the evaluation of the teratogenic potential of chlorodifluoromethane are unusually extensive and difficult to interpret. Microphthalmia and anophthalmia are rare conditions in rats occurring at an incidence of 1 in 100-200 litters. Hence, in an experiment of normal size with between 20 and 40 litters per group, groups with microphthalmia/ anophthalmia are seen only occasionally. In 3 studies using conventional group sizes there were one or two litters in all but one treated group with a foetus with microphthalmia or anophthalmia. There was no dose-response relationship in spite of the fact that the doses used ranged from 350 to 70,000 mg/m^3 (a 200 fold difference).

The evaluation of these data is difficult. On the one hand the 3 studies produced similar results; on the other there was no dose-response and there is a genetic component (Browman, 1961; Kinney et al, 1982; Khera, 1985) in the incidence of eye lesions of this type. Hence a further study was commissioned.

In the follow-up study group sizes were selected to provide a much greater statistical power than any conventional study and thus maximise the chance of observing a low incidence effect. In the two low dose groups exposed to 350 and 3,500 mg/m^3 there were no signs of maternal or foetal toxicity and no increase in the incidence of eye malformations. This finding is in contrast to the earlier studies where at similar doses one or two litters were observed with microphthalmia or anophthalmia in groups which were one tenth the size. In the highest dose group (175,000 mg/m^3) a statistically significant increase in litters containing foetuses with either microphthalmia or anophthalmia combined or amophthalmia alone was observed although the incidence (10 or 6 foetuses respectively out of 4031 foetuses/383 litters) was still low. In this group there was an indication of maternal toxicity in that there was a consistent decrease in maternal body weight gain particularly between days 6 and 10 of gestation, the critical period for the development of the eye. The small reduction in foetal weight is an indication of foetal toxicity. Analysis of data of litters showing microphthalmia or anophthalmia failed to show an

association between the occurrence of these effects and the severity of depression of maternal body weight gain (Palmer, 1989).

The interpretation of this high dose finding is difficult. The incidence of these malformations in control groups in the testing laboratory subsequent to the chlorodifluoromethane study varies from time to time. A marked increase in the spontaneous incidence of the malformations was seen in subsequent groups (Set 6 to Set 10), and in one (Set 7) the incidence was similar to that of the high dose group (see Figure 1). Thus there is an increase in incidence, which is statistically significantly different from concurrent controls, but the variation in incidence in prospective controls makes this increase appear less clear-cut.

In a rabbit study using a conventional protocol there was no indication of a teratogenic effect. Although the highest dose group was exposed to the same concentration as the high dose group in the large rat study (175,000 mg/m^3) and there was a slight reduction in maternal body weight gain, no increase in incidence of eye defects occurred.

On the basis of the data available on rat teratogenicity, there is a clear no observed effect level of 3,500 mg/m^3. However, it should be noted that, due to the large difference between the no observed effect level and the exposure of the top dose group, it is possible that a higher no observed effect level could be determined in the rat.

The low incidence of this effect in rats, the high exposure level associated with its occurrence and the absence of effects in rabbits, leads to the conclusion that these results are not of significance when considering the health of human beings occupationally exposed by inhalation to levels of chlorodifluoromethane at or below recommended permissable exposure guidelines (i.e. 1,750 to 3,500 mg/m^3 chlorodifluoromethane, 8hr time weighted average; see Section 10).

8.7 Mutagenicity

The data from in vitro and in vivo studies are summarised in Table 7.

In in vitro systems negative responses were recorded in tests using Schizosaccharomyces pombe, Saccharomyces cerevisiae, as well as in assays for the detection of mutations in mammalian cell lines. However, in Salmonella typhimurium, positive results have been reported in some assays under carefully controlled gaseous exposures but only in the base-pair substitution strains. This response was not dependent upon the presence or absence of a metabolising system as would be expected from the lack of metabolism and reactivity of chlorodifluoromethane in animals. This pattern of response would be consistent with the existence of bacteria-specific metabolism. This is confirmed by negative results in a Chinese Hamster Ovary assay (CHO-HGPRT) (McCooey, 1980).

On exposure of rats to chlorodifluoromethane for 6 hr/d for 5 days there was an apparent increase in chromosomal damage at the lowest exposure level of 3,500 mg/m^3. However, this was not observed at exposures of 35,000 and 175,000 mg/m^3 (Anderson et al, 1977). The experiment was repeated at 3,500 mg/m^3 and at the lower dose levels of 35, 350 and 1750 mg/m^3. Although there was an increase in chromosomal damage, it was again not exposure related. In addition there were widely differing findings at the 3,500 mg/m^3 dose level in the two experiments (Anderson and Richardson, 1979).

An experiment in which chlorodifluoromethane was administered by gavage to test for chromosomal changes in the bone marrow of mice also gave negative results (Loprieno and Abbondandolo, 1980).

The dominant lethal assay in rats gave no evidence of mutagenicity (Lee and Suzuki, 1981). In mice, two dominant lethal assays spanning dose levels of 35-350,000 mg/m^3 gave results that were not reproducible at the same dose levels in the two studies, nor was there a dose-response (Anderson et al, 1977; Hodge et al, 1979).

An inhalation micronucleus test has recently been conducted in the mouse (Howard et al, 1989). Chlorodifluoromethane was administered in concentrations up to 525,000 mg/m^3 together with concurrent positive (vinyl chloride) and negative (nitrogen) inhalation controls. No evidence of mutagenic activity was found.

The results of in vivo cytogenic studies in the rat and the dominant lethal studies in the mouse provide no evidence of significant genotoxic activity. Litchfield and Longstaff (1984) and Longstaff (1986, publ. 1988), held the same view with regard to these studies. It is based on the lack of a consistent dose-response relationship and the failure to reproduce effects at the same dose level in different studies. Taken with the negative result of the more recent inhalation micronucleus test of Howard et al (1989), the findings led to the conclusion that chlorodifluoromethane does not possess significant genotoxic activity.

8.8 Carcinogenicity

8.8.1 Experimental data

In an oral study, a group of 36 male and 36 female Alderley Park (Wistar derived) rats received 300 mg/kg body weight chlorodifluoromethane in corn oil by gavage daily on 5 d/wk for 52 weeks. There were three similar sized control groups. One received no treatment and the other two were dosed with corn oil. The study was terminated after 125 weeks. All animals underwent a full post-mortem examination. Chlorodifluoromethane treatment had no effect on body weight gain or mortality. No increase in the incidence of tumours was observed in any of the organs from the treated group when compared with vehicle dosed or undosed control groups (Longstaff et al, 1982).

Maltoni et al (1982, 1988) exposed groups of 60 male and 60 female Sprague-Dawley rats and Swiss mice to chlorodifluoromethane by inhalation of atmospheric concentrations of 0, 3,500 and 17,500 mg/m^3. Exposure was for 4 hr/d, 5 d/wk for 104 weeks (rats) and 78 weeks (mice). The compound failed to show any carcinogenic effect.

In a detailed study (Tinston et al, 1981b; reviewed by Litchfield and Longstaff, 1984) groups of 80 male and 80 female Alderley Park Wistar derived rats were exposed to chlorodifluoromethane by inhalation of concentrations of 0 (two groups), 3,500, 35,000 or 175,000 mg/m^3 for 5 hr/d, 5 d/wk for 118 weeks in females and 131 weeks for males by which time mortality had reached approximately 80% in any one group. Throughout the study regular analyses of samples of vaporised chlorodifluoromethane taken from the supply line in the exposure laboratory revealed the presence of a number of impurities.

No clinical abnormalities, increased mortality or haematological or biochemical changes due to chlorodifluoromethane were observed at any dose level. The only abnormalities were a body weight reduction in males exposed to 175,000 mg/m^3 (up to week 80) and increased liver, kidney, adrenal and

pituitary weights in females. In males there was no increase in the number of benign tumours but there was a slight increase in the number of animals bearing malignant tumours (Table 8). This increase was primarily due to an increased incidence of animals bearing fibrosarcoma (Table 9). The only organ that was consistently associated with this increase was the salivary gland, but the authors state that it was difficult to identify the origin of the tumours, which may have been generalised subcutaneous fibrosarcomas developing at a submandiblar site and involving the salivary gland only by chance. The increase in fibrosarcomas was observed only in the late stages of the study (between 105 and 130 weeks). No significant increase was found in lower dose animals (Tinston et al, 1981b), which is in agreement with the results of Maltoni et al (1982, 1988). Four male rats in the 175,000 mg/m^3 group were found to have Zymbal gland tumours. The relevance of this tumour to human risk assessment is unclear, since man does not have this organ. Females did not exhibit a significant increase in any tumour type in any of the exposure groups.

In an analogous mouse study (Tinston et al, 1981a) groups of 80 male and 80 female Alderley Park Swiss derived mice were exposed to chlorodifluoromethane by inhalation at levels of 0, (two groups), 3,500, 35,000 or 175,000 mg/m^3. Exposures were given 5 hr/d, 5 d/wk for up to 83 weeks (males) and 94 weeks (females). At these times the mortality rate was approximately 80%. The impurities present in the sample of chlorodifluoromethane were the same as those in the rat study. The only finding which could consistently be related to exposure was hyperactivity in the mice of the 175,000 mg/m^3 groups. There were no significant increases in the incidences of benign or malignant tumours in treated male or female mice, compared to controls (Table 10). There was a small increase in the incidence of liver nodules in males receiving 175,000 mg/m^3 but this was within the range of historical control values for the Alderley Park Swiss derived mouse.

8.8.2 Evaluation

The animal studies provide limited evidence for the carcinogenicity of chlorodifluoromethane to experimental animals. However, only ageing male rats exposed to the top concentration of 175,000 mg/m^3 displayed an increase in tumour incidence. These tumour types occur commonly in the strain of rats used for the study. A clear no effect level of 35,000 mg/m^3 could be demonstrated in the male rats (Tinston et al, 1981b; Maltoni et al, 1982; 1988). No increased tumour incidences attributable to chlorodifluoromethane exposure were diagnosed in female rats or in mice of either sex at 175,000 mg/m^3.

Chlorodifluoromethane undergoes no detectable metabolism so that formation of biologically significant amounts of reactive intermediates is an unlikely explanation for the tumours seen in male rats (Peter et al, 1986). Chlorodifluoromethane has proved to be non-mutagenic in vivo; the positive findings in the Ames test is consistent with bacteria specific activation (Litchfield and Longstaff, 1984). Further, the test material was contaminated by chlorofluoromethane (HCFC-31), a known mutagen and carcinogen; this may have contributed to the positive response.

It is considered that chlorodifluoromethane presents no carcinogenic hazard to man at exposure levels anticipated to occur in industry or from use in consumer products.

9 EFFECTS ON MAN

9.1 General Population Exposure

No adverse health effects from exposure to chlorodifluoromethane have been reported in the general population. One case of acute poisoning was reported: a young boy was found dead in a small room with his mouth around the nozzle of a tank of chlorodifluoromethane (Garriot and Petty, 1980).

9.2 Occupational Exposure

9.2.1 Acute Poisoning

Morita et al (1977) reported six deaths from refrigerant gas exposure in a deepsea fishing boat. Because a valve from the refrigerant plant had been mishandled, the gas completely filled the engine room and overcame the person working there and the chief engineer who went to his assistance. Four other fishermen who could not escape from their quarters also succumbed. The chief engineer and the engine room worker were autopsied on return to port. Gas analysis of their tissues showed high concentrations of chlorodifluoromethane. The autopsy revealed no outstanding macroscopic features. Histopathology of all the major organs showed that the lungs were oedematous and fine lipid droplets were seen in the cytoplasm of hepatocytes, mainly in the peripheral zone. No other findings which could be regarded as associated with death were discovered. The authors considered that the cause of death could be ascribed to suffocation from oxygen deficiency.

9.2.2 Long-term Exposure

In a hospital pathology laboratory aerosols of chlorodifluoromethane were used in the preparation of frozen sections. Following the death of one worker from myocardial infarction, it was noted that certain others were experiencing repeated episodes of palpitations. To determine the extent of palpitation, a highly subjective symptom, Speizer et al (1975)

carried out a questionnaire survey and concluded that there was an association between excess palpitation and exposure to chlorodifluoromethane. However, the test population (pathology department employees) was a biased group and the reliability of reporting episodes of such a highly subjective symptom is in doubt; these and other factors may have influenced significantly the findings of this survey. For example, exposure to substances other than fluorocarbon may have played a part in any increased incidence of palpitation, and the validity of making estimates of exposure from the numbers of frozen sections produced is highly questionable. No comparable control group was equally examined. In addition, the association is not supported by the extensive toxicological information in animals and man developed on chlorodifluoromethane and analogous fluorocarbons over the last few decades. Therefore the study based on the limited data has little merit and the results cannot be taken seriously.

A case of peripheral neuropathy in a commercial refrigeration repair man prompted a survey of the health of refrigeration repair workers (Gunter et al, 1982; Campbell et al, 1986). A group of 27 refrigeration workers were studied. They were likely to have been exposed to the three commonly used refrigerants, chlorodifluoromethane, dichlorodifluoromethane and chloropentafluoroethane during the repair of leaks and to their thermal degradation products produced by welding and soldering, namely, hydrogen chloride, hydrogen fluoride, phosgene, carbon dioxide and chlorine. A control group of 14 workers from allied trades had no exposure to refrigerants. A number of potential reference subjects were excluded because they had histories of neurological problems. All subjects underwent tests for neurological, cardiovascular and respiratory fitness. Blood and urine were screened for biochemical changes. No cases of peripheral neuropathy were identified and chest radiographs, pulmonary function tests, electrocardiograms and blood and urine results were all within normal limits. A questionnaire completed by all subjects indicated that lightheadedness and palpitations were more common in the refrigeration workers than the unexposed control. Again, the study design was inadequate and no conclusions can be drawn from it.

It is concluded that association between chlorodifluoromethane exposure and the occurrence of palpitation has not been established by these studies.

9.2.3 Epidemiology

Although chlorodifluoromethane has been used for more than 50 years, information from retrospective epidemiological studies is limited. A study of 539 refrigeration workers exposed to a combination of chlorofluorocarbons, revealed five deaths due to heart cardiovascular disorders compared to 9.63 expected. There were six deaths due to cancer versus 5.7 expected and two deaths from lung cancer versus 1.0 expected (Szmidt et al, 1981). The authors concluded that the study failed to show association between exposure to chlorofluorocarbons and adverse health effects.

9.3 Conclusion

In over 50 years of commercial use of chlorodifluoromethane, few reports on adverse health effects have been forthcoming. Exposure to extremely high inhaled levels can be lethal due to oxygen replacement. The lack of toxic effects reported in man and the results from extensive toxicity testing in animals demonstrate the low toxic hazard to man at chlorodifluoromethane exposure levels found in industry or in the community.

10. RECENT EVALUATIONS BY OTHER BODIES

IARC (1986) concluded that the animal studies provided limited evidence for the carcinogenicity of chlorodifluoromethane to experimental animals.

The Dutch Expert Committee for Occupational Standards (1987) evaluated a group of 10 chlorofluorohydrocarbons and allocated a Health-based Exposure Limit of 1,000 ppm (3,500 mg/m^3) (8 h-TWA) to chlorodifluoromethane.

On the basis of the more recent investigations and considering the fact that chlorofluoromethane was identified as a contaminant in the long-term inhalation studies (see Chapter 8.8), the German "Senatskommission zur Prüfung gesundheitsschädlicher Arbeitsstoffe" ("MAK Commission") decided to remove chlorodifluoromethane from the list of compounds "which are justifiably suspected of having cancerogenic potential" and allocated to chlorodifluoromethane a MAK value of 500 ppm (1,750 mg/m^3) (8 h-TWA) (Henschler, 1987).

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Table 1. Physical and Chemical Properties of Chlorodifluoromethane.

Molecular weight	:	86.47
Physical form	:	gas
Colour	:	colourless
Boiling point, °C at 1030 HPa	:	-40.8 (a)
Freezing point, °C	:	-146 (a)
Liquid density at -68 °C, g/ml	:	1.4909 (b)
Vapour density in air at 0 °C and 1030 HPa, g/l	:	3.87 (c)
Vapour density at normal boiling point, g/l	:	4.82 (d)
Vapour pressure, HPa at 24 °C	:	10.300 (a)
Solubility in water, g/l at 20 °C and 1030 HPa	:	35 (e)
Solubility in organic solvents	:	soluble
Flammability	:	nonflammable
Reactivity	:	incompatible with aluminium containers (c)
Octanol/water partition coefficient (log P_{ow})	:	1.08 (f)
Spectral Properties	:	Stadtler Reference number 3701, IR Prism (a)

a - Weast (1985), b - Grasselli and Richey (1975), c - Sax (1984), d - Hawley (1981), e - Horrath (1982), f - Hansch and Leo (1979).

Table 2. Amount of chlorodifluoromethane in rabbit tissues after death [a]

	Rabbit No.							
	1	2	3	4	5	6	7	8
Chlorodifluoromethane concentration (%,v/v) [c]	0-32	0-29	0-33	0-42	30	30	40	40
O_2 concentration (%,v/v)	20-14	20-14	20-13	20-12	20	20	20	20
Time of death after inhalation (min)	67	31	25	19	15	92	7	10
Amount of Chlorofluoromethane [b]								
Brain	145	138	76	156	137	148	140	159
Heart	145	150	100	158	135	140	125	129
Lung	167	128	187	231	139	136	121	186
Liver	143	95	72	78	101	153	44	60
Kidney	160	90	81	89	102	142	82	56
Visceral fat	327	93	48	33	38	196	23	27
Blood	219	193	140	161	131	219	147	199

[a] From Sakata *et al* (1981)
[b] Values are ul/g at 20-25°C, 1 atm.
[c] Inhaled concentration. Latter concentration in rabbits 1-4 indicates that at the time of death.

Table 3. Amount of gas in the major tissues after asphyxiation with chlorodifluoromethane (ul/g)

	Brain	Lung	Liver	Kidney	Blood
Subject A	68	18	71	18	69
Subject B	100	20	92	8	130

Table 4. Lethality of single exposure to chlorodifluoromethane

Concentration mg/m^3	Duration (min)	Species	Effect	Reference
2,450,000	90	Dog	Lethal	Poznak & Artusio (1960)
2,100,000	2	Rat	Not lethal	Pantaleoni & Luzi (1975)
1,400,000	120	Rat/guinea-pig	Lethal	Weigand (1971)
1,400,000	90	Dog	Not lethal	Poznak & Artusio (1960)
1,295,000	120	Mouse	MLC	Karpov (1963a)
1,225,000	15	Rat	LC_{50}	Clark & Tinston (1982)
1,120,000	120	Mouse	Not lethal	Karpov (1963a)
1,050,000	120	Rat	MLC	Weigand (1971)
1,050,000	30	Rabbit	MLC	Sakata et al (1981)
1,050,000	120	Guinea-pig	Not lethal	Weigand (1971)
970,000	30	Mice	LC_{50}	Sakata et al (1981)
875,000	240	Rat	MLC	NIOSH (1986)
775,000	240	Rat	LC_{50}	Litchfield & Longstaff (1984)
700,000	120	Rat	Not lethal	Weigand (1971)
700,000	2	Guinea-pig	Approx. lethal concentration	Nickolls (1940) cited in Waritz (1971)
700,000	5	Monkey	Not lethal	Aviado & Smith (1974)

MLC = Minimum lethal concentration
(After : Litchfield & Longstaff 1984)

Table 5. The number and incidence of foetuses with eye malformations (anophthalmia or microphthalmia) in rats exposed on days 6-15 of pregnancy to atmospheres containing chlorodifluoromethane (Palmer et al, 1978a)

Concentration Chlorodifluoromethane (mg/m^3)	Eye malformations		Anophthalmia		Microphthalmia	
	No. of foetuses affected	Incidence per 1000 litters	No. of foetuses affected	Incidence per 1000 litters	No. of foetuses affected	Incidence per 1000 litters
0	3	4.94	1	1.65	2	3.29
350	5	12.66	1	2.53	4	10.13
3,500	3	7.69	1	2.56	2	5.13
175,000	10	26.11*	6	15.67*	4	10.44

The values marked with an asterisk were statistically significant (*P<0.05) by a one-sided stratified contingency chi-squared test.

Table 6. The number and incidence of foetuses with eye malformations in rats occurring in prenatal toxicity studies conducted between 1976 and 1986. More than 15,000 litters were arranged into sets slightly less than the dimensions of the chlorodifluoromethane study (Set 1) and in a chronological progression (see also Figure 1).

	No of Groups	Litters examined	Foetuses examined	Foetuses with a	m	a + m
SET 1						
Contr	19	607	6338	1	2	3
Low	19	395	4204	1	4	5
Medium	19	389	4113	1	2	3
High	19	383	4031	6	4	10
Total	76	1774	18686	9	12	21
SET 2						
Contr	24	421	4298	2	0	2
Low	18	338	3467	0	2	2
Medium	19	350	3587	0	2	2
High	21	351	3512	1	1	2
Total	82	1460	14868	3	5	8
SET 3						
Contr	20	373	3759	3	0	3
Low	19	342	3517	1	2	2
Medium	19	347	3583	0	0	0
High	20	363	3496	2	1	2
Total	78	1425	14355	6	3	7
SET 4						
Contr	19	350	3682	0	0	0
Low	19	345	3586	1	0	1
Medium	20	357	3618	2	1	2
High	18	333	3478	1	1	2
Total	76	1385	14364	4	2	5
SET 5						
Contr	19	346	3743	1	1	2
Low	17	291	3121	0	1	1
Medium	19	343	3711	0	0	0
High	19	338	3620	0	0	0
Total	74	1318	14195	1	2	3

Table 6 (continued)

	No of Groups	Litters examined	Foetuses examined	Foetuses with a	m	a + m
SET 6						
Contr	19	430	4741	0	4	4
Low	18	410	4664	1	5	6
Medium	17	381	4506	2	4	5
High	18	393	4374	2	6	7
Total	72	1614	18119	5	19	22
SET 7						
Contr	17	386	4123	3	8	10
Low	13	293	3164	1	0	1
Medium	13	299	3189	2	2	4
High	13	294	3182	2	6	7
Total	56	1272	13658	8	16	22
SET 8						
Contr	20	421	4727	1	1	2
Low	17	347	3880	2	5	6
Medium	19	386	4459	2	5	7
High	21	448	5067	3	3	5
Total	77	1602	18133	8	14	20
SET 9						
Contr	20	433	5091	2	4	6
Low	18	386	4441	2	8	10
Medium	18	388	4510	1	4	5
High	20	433	5041	1	4	5
Total	76	1640	19083	6	20	26
SET 10						
Contr	19	414	4810	0	5	5
Low	19	415	4787	6	1	6
Medium	17	372	4327	1	3	4
High	17	369	4347	7	5	10
Total	72	1570	18271	14	14	25
Grand Total	739	15060	163728	65	106	159

a = anophthalmia
m = microphthalmia

Table 7. The genetic toxicology of chlorodifluoromethane in *in vitro* and *in vivo* studies

ASSAY	STRAIN/ TYPE	METABOLIC ACTIVATION	RESULT	COMMENT	REFERENCE
Schizosaccharomyces pombe	forward mutation	+/- S-9	-ve	Tested as a gas	Loprieno & Abbondandolo (1980)
Saccharomyces cerevisiae	mitotic gene conversion	+/- S-9	-ve	Tested as a gas	Loprieno & Abbondandolo (1980)
Salmonella typhimurium	TA1535, TA1538, TA98, TA100	+/- Aroclor induced rat liver S-9	S-9 independent +ve for strains TA1535, TA100	Incubated with 50% atmosphere of chlorodifluoromethane for 24 h.	Longstaff & McGregor (1978)
Salmonella typhimurium	TA100	+/- Phenobarbitone or Aroclor induced rat liver S-9	-ve	Tested as a gas 50% for 24 h	Bartsch *et al* (1980)
Salmonella typhimurium	TA100 TA1535	+/- Auxilary metabolising systems	unrepeatable +ve in strain TA1535	6 h exposure upto 40% chlorodifluoromethane. 32 h to air. Result not considered biologically significant	Butterworth (1976)

- - as reported by authors

Table 7 (continued)

ASSAY	STRAIN/ TYPE	METABOLIC ACTIVATION	RESULT	COMMENT	REFERENCE
Salmonella typhimurium	TA100 TA1535	+/- Auxilary metabolising systems	S-9 independent +ve for strains TA1535, TA100	48 h exposure to upto 40% chlorodi-fluoromethane	Russel et al (1980) Krahn (1977)
Salmonella typhimurium	Not stated	Not stated	-ve	Liquid suspension protocol. Flasks gassed and maintained for 2 h.	Russel et al (1980)
Host mediated assay - mouse	Sc. pombe or S. cerevisiae	-	-ve		Loprieno and Abbondandolo (1980)
Chinese Hamster Cell (CHO) - mutation	HGPRT locus	+/- metabolic activation	-ve	Tested as a gas at 0, 33, 67 and 100% atmospheres	McCooey (1980)
Chinese Hamster V-79 - mutation	HGPRT locus	+/- S-9	-ve		Loprieno and Abbondandolo (1980)
Unscheduled DNA synthesis	Human heterploid EUE cell line	-	-ve		Loprieno and Abbondandolo (1980)

Table 7 (continued)

SPECIES	ASSAY	EXPOSURE MG/M^3	RESULT	COMMENT	REFERENCE
Rat	Dominant lethal	175,000 5 hr/day for 8 weeks	-ve		Lee & Suzuki (1981)
Mouse	cytogenetics bone marrow	816 mg/kg in corn oil; gavage	-ve		Loprieno & Abbondandolo (1980)

Table 8. Tumour incidence in rats exposed to chlorodifluoromethane in a lifetime inhalation study

Concentration of chlorodifluoromethane (mg/m^3)	Numbers of rats			
	With tumours			
	Examined	Total	Benign	Malignant
		Males		
0	80	43	38	16
0	80	54	43	18
3.500	80	57	44	27
35.000	80	56	47	22
175.000	80	53	42	33
		Females		
0	80	67	66	18
0	80	69	63	18
3,500	80	69	66	14
35,000	79	68	66	19
175,000	80	70	67	25

(From Litchfield and Longstaff, 1984)

Table 9. Incidence of fibrosarcoma in male rats exposed to chlorodifluoromethane in a lifetime inhalation study

Concentration of chlorodifluoromethane (mg/m^3)	Numbers of male rats		
	With fibrosarcomas		
	Examined	Total	Involving salivary gland
0	80	5	1
0	80	7	0
3,500	80	8	1
35,000	80	5	0
175,000	80	18	7

(From Litchfield and Longstaff, 1984)

Table 10. Tumour incidence in mice exposed to chlorodifluoromethane in a lifetime inhalation study

Concentration of chlorodifluoromethane mg/m^3	Numbers of mice			
		With tumours		
	Examined	Total	Benign	Malignant
		Males		
0	77	20	12	8
0	77	30	21	9
3.500	78	23	15	9
35.000	80	31	20	11
175.000	80	27	19	8
		Females		
0	80	46	22	24
0	78	45	14	31
3.500	79	45	20	25
35.000	79	43	13	30
175.000	80	45	12	33

(From Litchfield and Longstaff, 1984)

Table 11. Incidence (foetuses/litters) of microphthalmia/anophthalmia in 3 preliminary studies in Charles River CD rats carried out at the Haskell Laboratory. The dams were exposed to chlorodifluoromethane for 6hr/d either on days 4-13 or 6-15 of gestation.

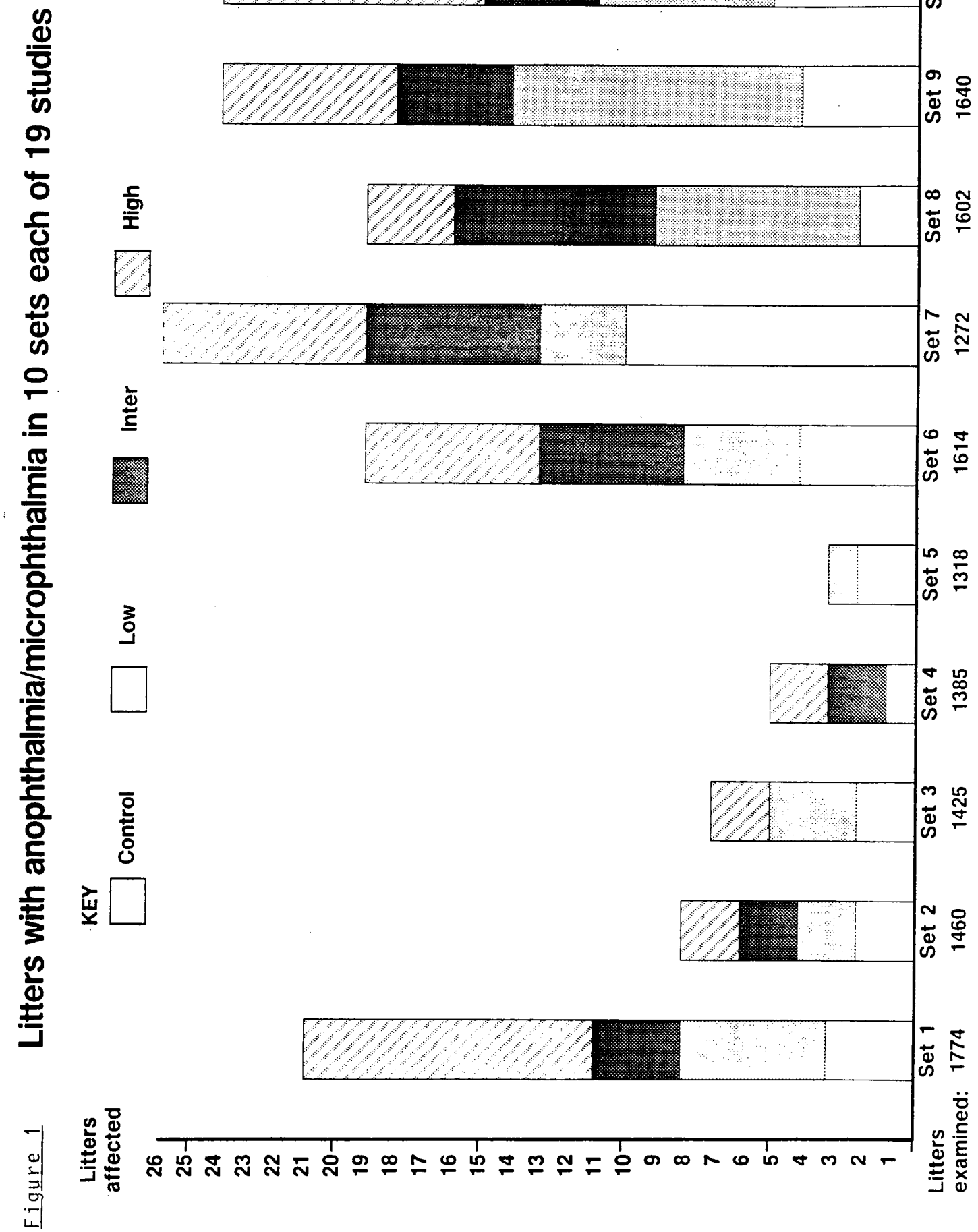
Exposure levels	0	350	1,050	1,750	3,500	35,000	70,000 mg/m^3
Study 1	0/21				1A/22	2A/21	
Study 2	0/34			1M/33	{1A/+ } {1A/33}		1A/35
Study 3	0/38	1M/40	0/35			2M/34	

A = anophthalmia

M = microphthalmia (no quantitative procedures were applied to the assessment of microphthalmia)

+ = two foetuses were affected in the same litter

Figure 1


Litters with anophthalmia/microphthalmia in 10 sets each of 19 studies
KEY
Control
Low
Inter
High
Litters affected
26
25
24
23
22
21
20
19
18
17
16
15
14
13
12
11
10
9
8
7
6
5
4
3
2
1
Litters examined:
Set 1 1774
Set 2 1460
Set 3 1425
Set 4 1385
Set 5 1318
Set 6 1614
Set 7 1272
Set 8 1602
Set 9 1640
Set 10 1570

APPENDICES

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