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1,1-Dichloro-2,2,2-Trifluoroethane (HCFC-123) CAS No. 306-83-2

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Joint Assessment of Commodity Chemicals No. 33

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ECETOC JACC Report No. 33

THE ECETOC SCHEME FOR THE JOINT ASSESSMENT OF COMMODITY CHEMICALS

This report has been produced as part of the ECETOC programme for preparing critical reviews of the toxicology and ecotoxicology of selected existing industrial chemicals.

In the programme commodity chemicals, that is those produced in large tonnage by several companies and having widespread and multiple uses, are jointly reviewed by experts from a number of companies with knowledge of the chemical. It should be noted that in a JACC review only the chemical itself is considered; products in which it appears as an impurity are not normally taken into account.

ECETOC is not alone in producing such reviews. There are a number of organisations that have produced and are continuing to write reviews with the aim of ensuring that toxicological knowledge and other information are evaluated. Thus a Producer, Government Official or Consumer can be informed on the up-to-date position with regard to health, safety and environment, information and standards. Within ECETOC we do not aim to duplicate the activities of others. When it is considered that a review is needed every effort is made to discover whether an adequate review exists already; if this is the case the review is checked, its conclusions summarised and the literature published subsequent to the review assessed. To assist ourselves and others working in this field we published a summary of international activities incorporating work planned, in hand, or completed on the review of safety data for commodity chemicals. Interested readers should refer to our Technical Report No. 30(5) entitled "Existing Chemicals: Literature Reviews and Evaluations".

1,1-Dichloro-2,2,2,-Trifluoroethane (HCFC-123) No.306-83-2

CONTENTS

Table of Abbreviations

ALD	Aldolase
ALT	Alanine amino transferase (SGPT)
AST	Aspartate amino transferase (SGOT)
AD_{50}	50% of animals anaesthesized
CCK	Chlolecystokinin
FSH	Follicle stimulating hormone
GSH	Glutathione
GWP	Global warming potential
HCG	Human chorionic gonadotropin
HRP	Horse-radish peroxidase
LH	Luteinizing hormone
LHRH	Luteinizing hormone releasing hormone
ODP	Ozone depleting potential
PBPK	Physiologically based pharmacokinetic
PPAR	Peroxisome proliferator activated receptor
TFA	Trifluoroacetic acid
UDS	Unscheduled DNA synthesis

1. SUMMARY AND CONCLUSIONS

1,1-Dichloro-2,2,2-trifluoroethane is a non-flammable, volatile, colourless liquid under ambient conditions and has a faint odour of ether. It is produced and used as a substitute for the fully halogenated chlorofluorocarbons with comparable physical properties since it has more favourable environmental properties.

Any dichlorotrifluoroethane released to the environment will partition mainly to the atmosphere where it will be degraded essentially in the troposphere by reaction with naturally occurring hydroxyl radicals, leading to the formation of HCl, HF, CO, CO_2 and CF_3COOH. The overall atmospheric lifetime of dichlorotrifluoroethane is estimated to be 1.4 y. Relative to reference values of 1.0 for CFC-11, dichlorotrifluoroethane has a calculated ozone depleting potential (ODP) of 0.014-0.021 and a global warming potential (GWP) of 0.02.

Dichlorotrifluoroethane would not be considered to be readily biodegradable. Studies conducted in algae, Daphnia, and rainbow trout indicated a moderate level of toxicity to aquatic organisms. The octanol/water partition coefficient (log P_{ow} = 2.0-2.8) is indicative of a low potential to bioaccumulate.

Studies in rodents indicate that dichlorotrifluoroethane is easily absorbed via the respiratory route and undergoes a biphasic uptake. It is distributed in all organs and is more concentrated in the liver. About 90% of unchanged dichlorotrifluoroethane was eliminated via the lungs. The rest was metabolised to trifluoroacetic acid which was excreted in the urine. Small amounts of trifluoroacetylated proteins were detected in exposed rats.

Dichlorotrifluoroethane has a low order of acute toxicity. Its oral approximate lethal dose in rats was 9,000 mg/kg; its 4-hour-inhalation LC_{50} was 200,000 mg/m^3 in rats and 178,000 mg/m^3 in hamsters. Its dermal LD_{50} in rats and rabbits was greater than 2,000 mg/kg. The primary effect was a rapidly reversible central nervous depression. After a few minute's exposure it induced pre-narcotic effects at 31,300 mg/m^3 and anaesthesia at 129,000 mg/m^3 in rats.

Liquid dichlorotrifluoroethane did not induce skin irritation in rabbit or skin sensitization in guinea pigs but it caused moderate eye irritation in rabbits.

Repeated inhalation exposure studies in rats exposed 6 h/d, 5 d/wk, for 4 to 13 weeks, to vapour levels of up to 125,000 mg/m^3, have resulted in bodyweight decreases and liver weight increases but with minimal adverse histological findings. Dichlorotrifluoroethane was found to cause a marked drop in serum cholesterol, triglyceride and glucose levels at 1,880 mg/m^3 and above. Signs of CNS

depressing effects were seen during exposure periods above 6,250 mg/m^3. However, there was no evidence of neurotoxicity when specific testing for neurobehavioural and nervous tissue histology were performed in a 13-week neurotoxicity study in rats.

Dichlorotrifluoroethane elicited cardiac sensitization to adrenaline in dogs acutely exposed by inhalation at a concentration of 119,000 mg/m^3. The no-effect-level was 62,500 mg/m^3.

No clear effects were seen in serum steroid hormone and CCK levels in rats and guinea pigs exposed to dichlorotrifluoroethane. In rats dichlorotrifluoroethane produced minimal decreases in testicular luteinizing hormone and testosterone levels under stimulation conditions only. Dichlorotrifluoroethane was found to induce liver peroxisome proliferation in rats. Its proliferating potential is weak and may be due to its metabolism to trifluoroacetic acid.

Dichlorotrifluoroethane was not teratogenic or embryotoxic to rats and rabbits exposed by inhalation at concentrations of 31,300 and 62,500 mg/m^3 which induced slight maternal toxicity.

In a two-generation inhalation study conducted with dichlorotrifluoroethane in rats exposed 6 h/d, 7 d/wk at concentrations ranging from 188 to 6,250 mg/m^3, there were no effects on mating performance and offspring survival. Growth retardation was observed in pups during nursing at all test concentrations, but normal growth was restored in the post-weaning phase.

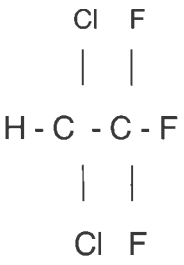
Dichlorotrifluoethane was inactive in several *in vitro* studies including a series of *Salmonella typhimurium* assays, a *Saccharomyces cerevisiae* assay and a cell transformation assay. It was active only in the human lymphocyte chromosome abberation assay. Dichlorotrifluoroethane was clearly inactive in a series of *in vivo* studies, including mouse micronucleus, rat chromosome abberation and rat unscheduled DNA synthesis. In conclusion, dichlorotrifluoroethane is not genotoxic *in vivo* and therefore does not have toxicologically significant genotoxicity.

Rats were exposed 6 h/d, 5 d/wk for 2 years by inhalation in a lifetime study to concentrations of 1,875; 6,250 and 31,300 mg/m^3. Compared to controls, survival was significantly improved in an exposure related pattern in all exposed groups. Significantly depressed bodyweights and levels of serum triglycerides, glucose and cholesterol were observed. Compound-related increased incidences of benign tumours were observed in the liver, testis and pancreas. These tumours were a continuum from hyperplasia and occurred late in life. It is thought that they are related with non-genotoxic mechanisms associated with the peroxisome proliferating potential of dichlorotrifluoroethane or with hormonal disturbances occurring in senescent rats. Considering they occurred late in life and were benign, the relevance of these findings is questionable in terms of a carcinogenic risk to man.

2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, ANALYTICAL METHODS

2.1 IDENTITY

Chemical structure:



Chemical formula: $CHCl_2CF_3$

Common name: 1,1-Dichloro-2,2,2-trifluoroethane

Common synonyms: 2,2-Dichloro-1,1,1-trifluoroethane; Fluorocarbon 123; FC-123; HFA-123; HCFC-123; F-123; Ethane, dichlorotrifluoro-; Propellant 123; Refrigerant 123; R-123

CAS Registry Number: 306-83-2

Einecs Number: 206 190 3

Conversion factors: 1 ppm = 6.25 mg/m^3 at 25º C
1 mg/m^3 = 0.160 ppm at 25º C

2.2 PHYSICAL AND CHEMICAL PROPERTIES

Dichlorotrifluoroethane is a nonflammable, volatile, colourless liquid at room temperature and normal atmospheric pressure. It has a faint ethereal odour. Dichlorotrifluoroethane is slightly soluble in water. Physical and chemical data for dichlorotrifluoroethane are given in Table 1.

Table 1 : Physical and Chemical Properties of Dichlorotrifluoroethane

Molecular weight	152.93
Physical form	liquid
Colour	colourless
Boiling point,°C at 1013 hPa	27.9
Freezing point,°C	-107
Liquid density at 25°C, g/ml	1.46
Vapour density: (air=1.0)	5.3
Vapour pressure at 25°C, hPa	913
Solubility in water at 25°C, (g/l)	3.9
Solubility in organic solvents	miscible with acetone, ethanol, vegetable oil and petroleum solvents[1]
Henry's Law constant at 25°C, g/l.bar	4.3
Log P_{ow}	2.0 - 2.8
Flammability	non-flammable

From Allied-Signal Inc. Product Safety Data Sheet - Genetron ® 123, October 1987 or Genetron ® Products
Bulletin, 1987,1989.

[1] From: E.I. du Pont de Nemours and Company Material Safety Data Sheet - Freon ® 123, E-97116 September 1987.

2.3 ANALYTICAL METHODS

A method for analysis has been described for dichlorotrifluoroethane which involves gas chromatography with dual flame ionization detection (Rusch *et al,* 1994).

3. PRODUCTION AND USE

Possible routes for the production of dichlorotrifluoroethane are hydrofluorination of tetrachloroethylene and hydrodechlorination of 1,1,1-trichloro-2,2,2-trifluoroethane (CFC-113a).

The current production of dichlorotrifluoroethane is estimated to be several kt/y. Like other hydrochlorofluorocarbons, dichlorotrifluoroethane is regulated under the Montreal Protocol and is scheduled for virtual phase-out by 2020.

The main application for dichlorotrifluoroethane is likely to be as a substitute for trichlorofluoromethane (CFC-11) in centrifugal chillers used for air conditioning of large buildings. Other potential applications include foam blowing, solvent cleaning, fire fighting, and as a chemical intermediate.

4. ENVIRONMENTAL TRANSPORT, DISTRIBUTION, TRANSFORMATION AND IMPACT

There is no known natural source of dichlorotrifluoroethane. The figure of 25 kt/y is estimated to represent an upper limit to future annual man-made emissions, in order to assess certain aspects of the potential environmental impact of dichlorotrifluoroethane.

4.1 ENVIRONMENTAL DISTRIBUTION

On the basis of its physical properties, dichlorotrifluoroethane may be expected, when released to the environment, to partition almost exclusively into the atmosphere:

- it is a volatile liquid at room temperature and atmospheric pressure, boiling at 27.9°C;
- its Henry's Law constant for dissolution in water is only 4.3 g/l·bar at 25°C. For an atmospheric concentration of 1.4 pptv* (i.e. the calculated global-average steady-state concentration resulting from emissions of 25 kt/y), the equilibrium concentration in cloud and surface waters would thus be only 0.006 pptw**.

Any dichlorotrifluoroethane which might be present in aqueous waste streams discharged directly into rivers or lakes would be expected, by analogy with similar compounds, to have a half-life with respect to volatilisation of days or a few weeks at the very most. Moreover, any dichlorotrifluoroethane present in surface or ground waters would have only a moderate tendency to partition into biota or soil:

- log P_{ow} is 2.0 - 2.8, indicating the absence of a significant potential for passive bioaccumulation;
- from various correlations, log K_{oc} may be estimated to lie in the range 1.8 - 2.6, which means that dichlorotrifluoroethane is not likely to bind tightly to soils.

The atmospheric lifetime of dichlorotrifluoroethane (about 1.4 y, see below) being longer than either the intrahemispheric or interhemispheric mixing times (about 1 month and 1 y, respectively), this compound will be transported to regions far from its emission source.

* pptv = parts per trillion (volume/volume)

** pptw = parts per trillion (weight/weight)

4.2 ATMOSPHERIC LIFETIME***

The atmospheric degradation of dichlorotrifluoroethane will occur mainly in the troposphere, being initiated by attack by naturally occurring hydroxyl radicals. A few percent of ground-level emissions will reach the stratosphere and be degraded there by photolysis and reaction with hydroxyl radicals. The overall atmospheric lifetime of dichlorotrifluoroethane is calculated to be 1.4 y (WMO, 1994a).

4.3 OZONE DEPLETING POTENTIAL

Ozone Depleting Potentials (ODPs) express the stratospheric ozone loss due to emission of a unit mass of a given compound, divided by the ozone loss due to emission of the same mass of a reference compound.

The latest estimates (WMO, 1994a) of the ODP of dichlorotrifluoroethane, are 0.014 from model calculations and 0.021 by a semi-empirical method, relative to a reference value of 1.0 for CFC-11. The 0.02 value was adopted as the best estimate of the ODP for regulatory purposes in the 1992 Montreal Protocol revision.

4.4 GLOBAL WARMING POTENTIAL

Global Warming Potentials (GWPs) express the radiative forcing (increase in earthward infra-red radiation flux) due to emission of a unit mass of a given compound, divided by the radiative forcing due to emission of the same mass of a reference compound.

Based on the lifetime quoted above, the halocarbon global warming potential (GWP) of dichlorotrifluoroethane is 0.02 (WMO, 1994a) relative to a reference value of 1.0 for CFC-11. This assessment assumes a pulse emission and an infinite integration time horizon (ITH), which is mathematically equivalent to a steady-state calculation.

GWPs may also be expressed relative to CO_2 as the reference substance, and assessed over a finite ITH. For dichlorotrifluoroethane the calculated values are 300, 93 and 29 respectively relative to reference values of 1.0 for CO_2 at each ITH (WMO, 1994a), for ITHs of 20, 100 and 500 y.

*** The "lifetime" is the time necessary for 63 % degradation; it is equal to the "half-life" divided by ln2 (= 0.69)

4.5 TROPOSPHERIC OZONE FORMATION

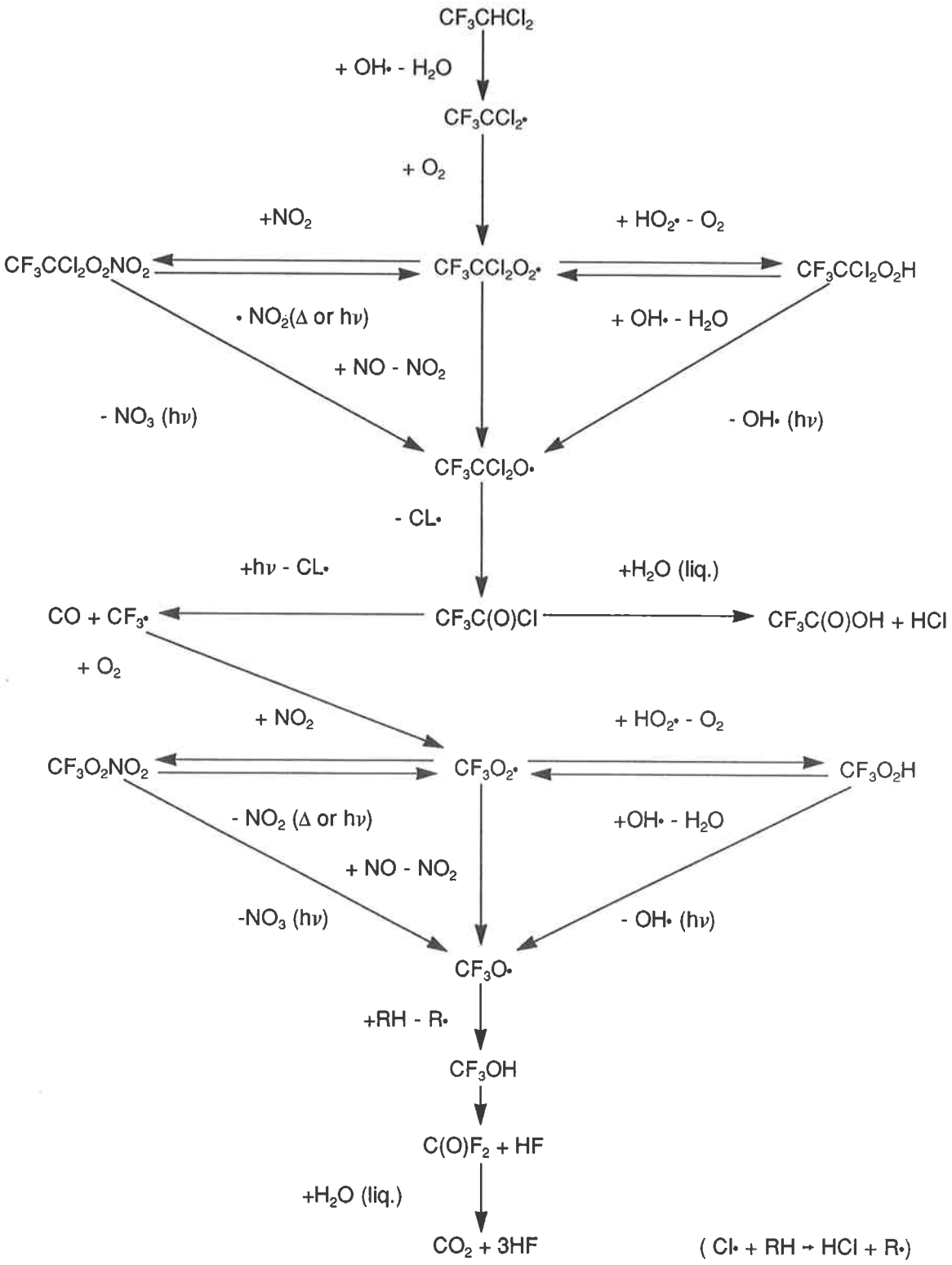
It has been shown that dichlorotrifluoroethane is too unreactive in the atmosphere to make any significant contribution to local urban tropospheric ozone formation, and the related "photochemical smog" near the emission sources (WMO, 1989a).

4.6 DEGRADATION MECHANISM AND PRODUCTS

Support for the basic tropospheric degradation mechanism for dichlorotrifluoroethane proposed by WMO (1989b) has been provided by recent laboratory studies (see, for example: Edney *et al,* 1991; WMO, 1991; WMO 1994b; Tuazon and Atkinson, 1993; Hayman *et al,* 1994).

Breakdown of dichlorotrifluoroethane in the troposphere (see Figure 1. on page 9) will be initiated by OH radicals and will proceed via various free-radical and short-lived molecular intermediates to give HCl and CF_3COCl. The latter molecules are expected to be removed from the atmosphere within a few days to a few months by uptake into clouds, rain and the oceans, CF_3COCl then being rapidly hydrolysed to CF_3COOH (trifluoroacetic acid, TFA) and HCl (AFEAS, 1992).

Figure 1: Tropospheric Degradation Mechanism for HCFC-123



Photolysis of CF_3COCl will compete to some extent with its hydrolysis and will ultimately lead to CO, CO_2, HCl and HF. It has been estimated that the fraction of CF_3COCl photolysed is about 25 - 40% (Rodriguez *et al,* 1993; Hayman *et al,* 1994).

Although peroxynitrates ($CF_3CCl_2O_2NO_2$, $CF_3O_2NO_2$) and hydroperoxides ($CF_3CCl_2O_2H$, CF_3O_2H) may be formed during the degradation of dichlorotrifluoroethane, they are not thought to play a significant role in its atmospheric chemistry, probably being rather short-lived intermediates.

4.7 CONTRIBUTION OF DEGRADATION PRODUCTS TO ENVIRONMENTAL CHLORIDE, FLUORIDE AND TRIFLUOROACETATE AND TO THE ACIDITY OF RAINWATER

Assuming an atmospheric release and degradation rate of 25 kt dichlorotrifluoroethane (conservative upper limit), conversion of two-thirds of the latter into CF_3COOH + 2HCl and one-third into CO_2 + 2HCl + 3HF, followed by uniform scavenging of the acids produced into the global average rainfall of 5×10^{11} kt/y, it follows that the levels of chloride, fluoride and the acidity thus produced are low compared with those arising from existing sources:

- Cl^- production from 25 kt/y dichlorotrifluoroethane would be 12 kt/y, i.e. insignificant compared with the natural atmospheric chloride flux of roughly 1×10^7 kt/y, mainly arising from sea-salt aerosols (WMO, 1989c);

- F^- production would be 3 kt/y, i.e. very small compared with the estimated atmospheric fluoride flux of 1,000 - 8,000 kt/y (WMO, 1989c); the contribution of dichlorotrifluoroethane to the fluoride concentration in rainwater would be 6 pptw. This should be compared with typical fluoride concentrations in "background" rainwater of around 10 ppbw, i.e. 1,700 times greater, and with levels of about 1 ppmw used for the fluoridation of drinking water, i.e. 170,000 times greater (WMO, 1989c);

- the contribution of dichlorotrifluoroethane to trifluoroacetate in rainwater would be 25 pptw;

- the hydrochloric, hydrofluoric and trifluoroacetic acids formed from dichlorotrifluoroethane and scavenged in rainwater would represent an acidity of 6×10^8 mol H^+/y, i.e. 17,000 times less than the acidity arising from natural and anthropogenic emissions of SO_2 and NO_x (UKRGAR, 1990). Thus, the contribution of 25 kt/y dichlorotrifluoroethane to acid rain would be negligible.

4.8 BIODEGRADABILITY

To assess its biodegradability potential, a sample of dichlorotrifluoroethane was incubated with activated sludge in a sealed bottle for 28 days. It was estimated that 24% of the compound had degraded by that time which indicates that dichlorotrifluoroethane is not readily biodegradable (Jenkins, 1992). Aerobic degradation by the methanotrophic bacterium, *Methylosis trichosporium*, was not detected (De Flaun *et al,* 1992). However, when using a mixed culture (MM1) a slow methanotrophic biotransformation occurred (Chang and Criddle, 1995).

5. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

No observations of dichlorotrifluoroethane in the background atmosphere or other environmental compartments have yet been reported.

6. EFFECTS ON ORGANISMS IN THE ENVIRONMENT

6.1 AQUATIC TOXICITY

The effects of dichlorotrifluoroethane on the growth of the unicellular green alga, *Selenastrum capricornutum*, were determined over a period of 96 hours. The measured concentration causing a 50% reduction in growth rate was 96.6 mg/l (nominal concentration 736 mg/l). The wide difference between measured and nominal concentration is probably reflective of the low solubility and high volatility of the compound (Jenkins, 1992a).

With the water flea, *Daphnia magna*, the measured mean concentration causing a 50% reduction in activity during a 48-hour exposure was 17.3 mg/l with no significant effects on immobilization at 2.24 mg/l (Jenkins, 1992b). In the rainbow trout, *Salmo gairdneri*, the 96-hour LC_{50} was 55.5mg/l (Jenkins, 1992c).

The above values are typical for the HCFCs and indicate a moderate level of toxicity to environmental organisms.

7. KINETICS AND METABOLISM

7.1 ANIMAL STUDIES

7.1.1 Absorption

Dose-dependent increases in urinary fluoride levels were observed in 90-day inhalation studies with rats and dogs and in the 2-year inhalation study with rats indicating that absorption and some metabolic transformation of dichlorotrifluoroethane occurred in these species (see Section 8.2 and 8.6).

The partition coefficients of dichlorotrifluoroethane in various tissues have been determined by a number of investigators (Dekant, 1994, Loizou *et al,* 1994; Vinegar *et al,* 1994) utilizing the vial-equilibration method of Gargas *et al,* (1989). Based on the results of these studies, dichlorotrifluoroethane was less lipophilic than halothane (2-bromo-2-chloro-1,1,1-trifluoroethane, HCFC 123B1), the bromine analog. Because of its lipophilic characteristics, however, absorption would be expected to occur readily. Indeed, studies by several investigators (see below) indicate that dichlorotrifluoroethane absorption occurs rapidly. The partition coefficients for dichlorotrifluoroethane are shown in Table 2.

Table 2: Partition Coefficients of HCFC-123 in Rats[a]

Partition between:	Coefficient
Blood/Air	4.06
Liver/Air	1.15
Lean Tissue/Air	1.20
Fat/Air	15.4

[a] From Dekant (1994), Loizou *et al* (1994) and Vinegar *et al* (1994)

Male and female Sprague-Dawley rats were exposed for six hours to dichlorotrifluoroethane starting concentrations of 3,130 mg/m^3, 6,250 mg/m^3, 12,520 mg/m^3, 18,800 mg/m^3, 25,040 mg/m ^{3} or 31,300 mg/m^3. Uptake was measured as the loss of dichlorotrifluoroethane from a recirculating inhalation chamber by gas chromatography. A PBPK model to describe the uptake, distribution and metabolism was constructed with the Advanced Continuous Simulation Language program (Loizou *et al,* 1994; Dekant, 1994). The data were fitted to the model. The kinetic constants obtained from this modelling are shown in Table 3. The uptake of dichlorotrifluoroethane was best described by a single saturable component model, although the model did not accurately predict the uptake in female rats at dichlorotrifluoroethane concentrations of greater than 12,520 mg/m^3. Indeed, a transition from first order to zero order uptake appears to occur at about 12,520 mg/m, with first order kinetics describing

the uptake at lower concentrations. This concentration limiting process may be due to a perfusion limiting process in tissues, e.g., liver, or by suppression of metabolism (Vinegar *et al*, 1994).

Table 3: Metabolic Constants of dichlorotrifluoethane ([a])

Metabolic Constant	Male Rats	Female Rats
Vmax (mg/h)	2.34	2.34
Vmax (mg/kg/h)	7.20 ± 0.28	7.97 ± 0.30
Km (mg/l)	1.2	1.2

[a] From Loizou *et al* (1994)

In another study by Dekant (1994), male Sprague-Dawley rats and male Hartley guinea pigs were exposed by inhalation to ^{14}C-dichlorotrifluoroethane for six hours at a concentration of 12,520 mg/m^3. This investigator measured the disappearance of dichlorotrifluoroethane from the inhalation chamber and found that approximately 50-60% of the radiolabelled material was lost from the chamber of exposed rats, whereas about 95% of the applied dose was lost from the chamber of exposed guinea pigs. Only 20-30% of the applied radiolabel was recovered in these studies, and in the absence of a mass balance the loss of the remaining radiolabel is unknown but assumed to be absorbed.

In separate studies, Dodd *et al* (1993) and Vinegar *et al* (1994) exposed Fischer 344 rats to dichlorotrifluoroethane concentrations of 625 to 62,600 mg/m^3 for 2-6 hours. Vinegar *et al* (1994) reported that the uptake of dichlorotrifluoroethane was biphasic with a rapid initial uptake over 30-45 minutes followed by a slower absorption phase. Both groups of investigators also indicated that uptake was saturable at concentrations greater than 12,520 mg/m^3, a finding consistent with Dekant, (1994). As the concentration approached 62,600 mg/m^3 there appeared to be a suppression of the production of trifluoroacetic acid (TFA). Concurrent with the uptake of dichlorotrifluoroethane, TFA blood concentrations also rose during exposure to the compound (Vinegar *et al,* 1994). Following a four-hour exposure to 6,260 mg/m^3, the dichlorotrifluoroethane and TFA blood concentrations were about 15 and 93 mg/l, respectively. However by the end of the four-hour exposure to a dichlorotrifluoroethane concentration of 62,600 mg/m^3, the dichlorotrifluoroethane and TFA blood concentrations were about 94 and 38 mg/l, respectively. These data suggest that at 62,600 mg/m^3 suppression of the oxidative metabolic pathway occurs, apparently due to substrate inhibition and not to killing of the metabolic enzyme. Indeed < 24 h later, the TFA blood concentrations rebounded and approached 100 mg/l at the exposure concentration of 62,600 mg/m^3.

7.1.2 Distribution

Dodd *et al* (1993) reported that following a two hour exposure to dichlorotrifluoroethane at a concentration of 62,600 mg/m^3 to Fischer 344 or Sprague-Dawley rats, the material was detected in all tissues sampled (tissues were not identified). At 24 h post-exposure, small amounts of metabolites (not identified) were detected in the liver, kidney, muscle and skin.

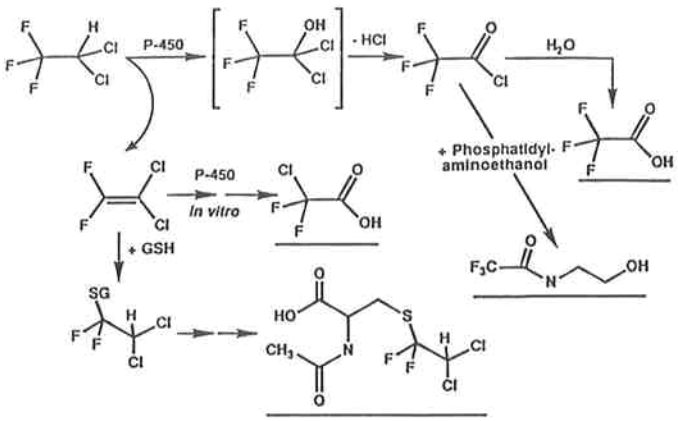
Dekant (1994) examined the tissue and organ distribution of ^{14}C-dichlorotrifluoroethane in the rat and guinea pig. In these studies, two male Sprague-Dawley rats or two male Hartley guinea pigs were exposed by inhalation to ^{14}C-dichlorotrifluoroethane concentration of 12,520 mg/m^3. After the six hour exposure, the animals were placed in glass metabolism cages and excreta collected for 48 hours. The animals were subsequently killed and radioactivity determined in selected tissues and organs.

After 48 hours, only low levels of radioactivity remained in the organs and tissues. Low amounts of radioactivity (pmol/mg protein) were bound to plasma proteins and erythrocytic proteins. Guinea pigs showed a greater variation in this binding compared to the rat. The liver contained the greatest concentration of radioactivity in both species, and represented about 5-10 fold higher level than other tissues and organs. The kidney and lung contained lower concentrations of radioactivity, but still somewhat higher concentrations than the other organs examined (testes, brain, pancreas and spleen).

7.1.3 Metabolism

Routes of metabolism for dichlorotrifluoroethane are shown in Figure 2.

Figure 2: Oxidative Metabolic Pathways of Dichlorotrifluoroethane



Metabolic pathways of dichlorotrifluoroethane in rats *in vivo* and in rat liver microsomes
Metabolites identified are underlined
* After Dekant, 1993

In vitro metabolism

Dekant (1994) and Urban *et al* (1994) examined the metabolism of dichlorotrifluoroethane in rat and human liver microsomal fractions. The final concentration of dichlorotrifluoroethane in the microsomal fractions was 3.3 mM (4.32 μmoles in a final volume of 1.3 ml); the actual exposure concentration is unknown since the air/microsomal partition coefficient was unknown. In these studies, TFA was the primary metabolite that was oxidatively formed. In the absence of NADPH or with the use of heat inactivated microsomes, only a small amount of TFA was observed, indicating the involvement of cytochrome P-450. Chlorodifluoroacetic acid (a minor metabolite), inorganic fluoride and a minor unidentified metabolite were also identified in both rat and human liver microsomal fractions. In human cells, the microsomal oxidation of dichlorotrifluoroethane was significantly greater than in rats (about 10 times).

These investigators also examined the effects of inducing and inhibiting cytochrome P-450 on the metabolism of dichlorotrifluoroethane. In these studies, rats were pretreated with either ethanol (10%, w/v) in drinking water for 10 days or pyridine (100 mg/kg, *i.p.*) once daily for four consecutive days. With pretreatment, the rate of TFA formation was increased approximately 10-fold compared to control values. However, inclusion of diethyldithiocarbamate (300 μM), a cytochrome P-450 inhibitor, into the microsomal reaction inhibited induction of TFA formation by approximately 60%. A similar inhibition of TFA formation was observed with human microsomes. These results suggested that dichlorotrifluoroethane was metabolized by a specific isoenzyme of cytochrome P-450 known as cytochrome P-450 2E1. In humans, there was considerable heterogeneity with the liver. In the rat, greater concentrations of P-450 2E1 and P-450 2B1/2 were identified in the liver with low levels also detected in the lung and kidney. No cross-reactivity was observed in the testes or pancreas.

Godin *et al* (1993) examined the *in vitro* metabolism of dichlorotrifluoroethane in rat liver microsomal preparations by varying the concentration of oxygen to simulate anaerobic metabolism. At 0% oxygen concentrations, HCFC-133a (2-chloro-1,1,1-trifluoroethane) was detected. When microsomes from rats treated with either phenobarbital (0.1% solution in drinking water for 7 days) or pyridine (200 mg/kg *i.p.* for 4 days) were utilized, an increase in HCFC-133a formation, compared to controls, occurred at oxygen concentrations of 2-5%. However, no HCFC-133a formation was evident in either control or induced microsomes at oxygen concentrations greater than 5%. Another microsomal metabolite, 2-chloro-1,1-difluoroethylene was also detected, but only in induced microsomes at 0% oxygen concentrations. Under low oxygen anaerobic conditions (5% O_2, 95% argon) *in vitro*, Dekant, (1994) and Urban *et al* (1994) found no volatile metabolites.

In separate studies presented in an abstract, dichlorotrifluoroethane (1-20 mM, final concentrations) was incubated with rat liver microsomal preparations from either phenobarbital or pyridine induced microsomes (Ferrara *et al,* 1995). The formation of reactive intermediates was studied by measuring the depletion of added glutathione (GSH). After 30 min. of incubation, a 32-47% loss of added GSH was observed with phenobarbital induced microsomes. The loss was dose-dependent. With pyridine induced microsomes, a 57-65% loss was observed, but no dose dependency was observed. Saturating concentrations of carbon monoxide or N-tert-butyl-α-phenylnitrone (a free radical scavenger) afforded nearly complete protection from GSH loss in both phenobarbital and pyridine induced microsomes at 20 mM dichlorotrifluoroethane. These data suggested that dichlorotrifluoroethane under anaerobic conditions may be reductively metabolized to reactive intermediates that inactivate cytochrome P-450, either P-450 2E1 or P-450 2B.

The formation of the oxidative metabolite, TFA, has been observed in all metabolic studies conducted with dichlorotrifluoroethane. In the studies of Godin *et al* (1993), TFA formation was detected in microsomal preparations from control and induced animals, and at oxygen concentrations of 0%, 2%, 5% and 21%. The amount of TFA formation at 0% oxygen levels was low (0.07 ± 0.05 nmol/3 min/mg protein). At the higher oxygen concentrations TFA was formed in much higher quantity. However the TFA amounts were not significantly different one from another between 2,5 and 21 % oxygen. Both pyridine and phenobarbital induced microsomes increased the levels of TFA production, although there was not a significant increase in TFA with increasing levels of oxygen concentrations. These data indicate that oxidative metabolism of dichlorotrifluoroethane to TFA is a saturable process at low oxygen concentrations. The same authors showed that small changes in oxygen concentrations may have a greater influence on the formation of reductive metabolites than on oxidative metabolites (see above description of reductive metabolism).

In another study, the metabolism of dichlorotrifluoroethane in microsomes prepared from rat kidney and liver was examined (Huwyler *et al,* 1992). In this study, the release of Cl^- was measured as reflecting the metabolism of dichlorotrifluoroethane. Compared to liver metabolism, the kidney was a poor metabolic tissue with the metabolic capability being approximately 10-fold less than the liver. The authors did not attempt to identify any metabolites.

In vivo metabolism

Loizou *et al* (1994) examined the *in vivo* metabolism of dichlorotrifluoroethane in rats. In this study, male and female Sprague-Dawley rats were exposed once whole body for six hours to starting concentrations of 3,130 mg/m^3, 6,250 mg/m^3 , 12,250 mg/m^3, 18,800 mg/m^3 , 25,040 mg/m^3 or 31,300 mg/m^3. After exposure, the animals were transferred to metabolism cages and urine was

collected for determination of TFA. There were no sex related differences in formation of TFA, but there was a dose-dependent increase in the amount of TFA formed. This increase in TFA formation was also reported by Dodd *et al* (1993). In the latter study, Fischer 344 or Sprague-Dawley rats were exposed nose-only for two hours to a dichlorotrifluoroethane concentration of 62,600 mg/m^3. No strain differences in TFA formation were observed, and TFA represented the most abundant metabolite. Blood level saturation of TFA appeared by the end of the four hour exposure at all concentrations. In addition, the reductive metabolites HCFC-133a and 2-chloro-1,1-difluoroethylene were identified in the liver and HCFC 133a was also identified in the kidney of the exposed rats (Vinegar *et al,* 1994). Metabolism appears to reach saturation with exposures in the range of 6,250 to 12,500 mg/m^3 (Dodd *et al,* 1993), although Loizou *et al* (1994) reported that metabolic saturation occurs at about 18,800 mg/m^3.

Dekant (1994) also examined the *in vivo* metabolism of dichlorotrifluoroethane. In this study, two male Sprague-Dawley rats and two male Hartley guinea pigs were exposed whole body for three hours to 12,520 mg/m^3 of ^{14}C-dichlorotrifluoroethane. After three hours, a second injection into the chamber of ^{14}C-dichlorotrifluoroethane was made; the total exposure duration was six hours. TFA was the major metabolite for both species. In rats, approximately 25% of the administered radiolabel was recovered in the urine as TFA. In the guinea pig about 20-30% of the administered label appeared in the urine as TFA. Other minor metabolites were also detected. One metabolite identified was N-acetyl-S-(2,2-dichloro-1,1-difluoroethyl)-L-cysteine which can be formed through reductive metabolism of dichlorotrifluoroethane, possibly to 1,1-dichloro-2,2-difluoroethene, and conjugation with glutathione, a metabolic process observed *in vitro* (Ferrara, 1995). In addition, N-trifluoroacetyl-2-aminoethanol was also identified in the urine of rats and guinea pigs. Two other minor metabolites were only identified in urine collected in the first 24 hours. TFA was identified in the urine collected throughout the 48h collection period.

Dekant (1994) and Loizou *et al* (1994) pretreated rats, *i.p.*, with diallyl sulphide, a cytochrome P-450 2E1 inhibitor (Brady *et al,* 1988), at doses of 10, 20 or 50 mg/kg 14 hours prior to a six-hour exposure to dichlorotrifluoroethane at a concentration of 6,250 mg/m^3 . A dose-dependent reduction in dichlorotrifluoroethane uptake was observed, with a significant reduction occurring at 20 and 50 mg/kg diallyl sulphide. Further, a dose-dependent reduction in urinary TFA excretion (22% at 50 mg/kg) was evident providing further evidence of the involvement of cytochrome P-450 2E1 in the metabolism of dichlorotrifluoroethane.

7.1.4 Elimination

Data from pharmacokinetic and biotransformation studies with dichlorotrifluoroethane and the urinary excretion of TFA indicate that hepatic elimination is the rate limiting step in the biotransformation and elimination of dichlorotrifluoroethane.

The major route of elimination was rapid exhalation of unchanged dichlorotrifluoroethane. About 90% or more was eliminated by this route . Within a period of 8 hours, 98% of inhaled dichlorotrifluoroethane was cleared from the blood. This is in agreement with the disappearance of clinical symptoms after about 15 minutes of cessation of exposure. The majority of metabolised dichlorotrifluoroethane was excreted as TFA in the urine (Vinegar *et al,* 1994).

In a study by Dekant *et al* (1994), male and female Sprague-Dawley rats were exposed whole body to ^{14}C-dichlorotrifluoroethane concentrations of 6,250 mg/m^3, 9,375 mg/m^3 , 12,520 mg/m^3, 18,800 mg/m^3 or 31,300 mg/m^3 for six hours, and male Hartley guinea pigs were exposed to 12,520 mg/m^3 for six hours. Urine was collected for 48 hours after exposure, and TFA levels determined. An increase in urinary TFA excretion was observed. The kinetic constants for urinary TFA excretion for male rats was 75.2 µmol/l (K_m), and the V_{max} was 292 µmol/kg/24 h. For female rats, the K_m was 24.4 µmol/l and the V_{max} was 232 µmol/kg/24 h.

Similar results have been reported by other investigators. Dodd *et al* (1993) and Vinegar *et al* (1994) reported separately on exposures of Fisher 344 rats to dichlorotrifluoroethane concentrations of 625 mg/m^3, 6,250 mg/m^3 or 62,600 mg/m^3 for four hours. Vinegar showed that the major route of elimination was through exhalation of unchanged dichlorotrifluoroethane. Indeed, about 90% or more is eliminated by this route. These investigators also followed the time course of TFA elimination for 24 hours. They showed that blood TFA concentrations initially increased for about five hours post-exposure, followed by a slow elimination. They further reported that the venous blood concentration of dichlorotrifluoroethane after a four hours exposure to 6,250 mg/m^3 decreased from 4.5 mg/l to 1.5 mg/l in about one hour, and the concentration decreased further (to 98% after 8 h post-exposure) during the remainder of the study. The blood elimination half-life ($t_{1/2}$) was estimated to be 2-4 h (from Dodd *et al*, 1993). Fat concentrations of dichlorotrifluoroethane decreased in a manner similar to the blood levels. However this source of dichlorotrifluoroethane influenced the post-exposure, elimination kinetics by serving as a source of additional material.

7.1.5 Covalent Binding

Metabolic studies reveal the potential for formation of a reactive intermediate, trifluoroacetyl chloride, in *in vitro* studies (see above). Since halothane forms the same reactive intermediate which has previously been shown to bind to liver macromolecules, several investigators have focused on the covalent binding of dichlorotrifluoroethane metabolites to liver macromolecules. Furthermore, with halothane, this binding has been shown to result in an immunoreactive product responsible for halothane induced hepatitis (Pohl *et al,* 1988). This has never been reported with any other chemical.

Harris *et al* (1991) exposed male Fischer 344 rats to approximately 43,800 or 68,800 mg/m^3 dichlorotrifluoroethane for two hours. After exposure, microsomes were prepared, and the level of binding determined by immunoblotting. These authors identified a trifluoroacetylated lysine cross link that was identical to the TFA-protein cross link found with halothane. In an extension of these studies, Harris *et al* (1992) exposed male Fischer 344 rats for six hours to dichlorotrifluoroethane concentrations of 625 mg/m^3, 6,250 mg/m^3 or 62,600 mg/m^3 and to 80,800 mg^3 halothane. Twelve hours after exposure, liver subcellular fractions were prepared for immunoblotting. TFA-proteins were detected in the liver of animals exposed to both compounds. A similar degree of cross linking was seen in rats exposed to 80,800 mg/m^3 of halothane and 62,600 and 6,250 mg/m^3 of dichlorotrifluoroethane. Less TFA-protein cross link was detected at 625 mg/m^3.

Huwyler and Gut (1992) and Huwyler *et al* (1992) examined the binding of dichlorotrifluoroethane or halothane to macromolecules in the liver, kidney and heart. In each of these studies, a 10 mmol/kg dose dichlorotrifluoroethane (1529 mg/kg) or halothane (1974 mg/kg) was administered *i.p.* to male Sprague-Dawley rats in sesame oil. Kidney, heart and liver subcellular fractions were prepared six hours after treatment. TFA-protein cross-links were determined by immunoblotting sequentially using anti-TFA antibody, goat anti-rabbit HRP conjugated second antibody and enhanced chemiluminescence. TFA-protein cross-links were detected in each of these tissues for both compounds, although the intensity of the kidney and heart cross-linking was much less than that in the liver; about 5% and < 0.5% of the amount formed in the liver was found in kidney and heart, respectively. Prior treatment of the homogenate with piperidine (1 M) or glutathione (5 and 10 mM) abolished the reactivity with protein indicating that cytochrome dependent metabolism of dichlorotrifluoroethane was occurring in the kidney. The half-life of the cross-links in the kidney was estimated to be between 18 and 90 h, whereas in the heart, the half-life of the cross-links was estimated to be about 12 h. This is in contrast to the apparent half-life in the liver which was higher than 90 h but less than 10 days. Cryosections of the kidney tissue revealed that the tubular proteins of the renal cortex were the prime targets of acylation. In the liver, there was a gradient of staining that occurred from the central vein to the portal triad, with the central vein containing the greater staining

intensity. These data confirm the ability of the liver and kidney to transform dichlorotrifluoroethane into a potentially reactive metabolite that can bind to tissue macromolecules.

7.1.6 Summary

Overall, dichlorotrifluoroethane appears to be easily and quickly absorbed via the respiratory route. It undergoes a biphasic uptake which is saturable in the rat at concentrations greater than 12,520 mg/m^3. It is distributed in all tissues/organs and found in greatest quantity in the liver. Under aerobic conditions, it undergoes cytochrome P450 2E1 mediated oxidative metabolism resulting in the formation of trifluoroacetylchloride and trifluoroacetic acid. Reductive metabolism to 2-chloro-1,1,1-trifluoroethane (HCFC 133a) could be detected only under anaerobic conditions and this pathway seems of little relevance for human hazard assessment. The major route of elimination is rapid exhalation of unchanged dichlorotrifluoroethane. About 90% was eliminated by this route in the rat. Within a period of 8 hours, 98% of inhaled material was cleared from the blood. The majority of the metabolised dichlorotrifluoroethane was excreted in the urine as trifluoroacetic acid. Similar to halothane, trifluoroacetylated proteins can be detected *in vitro* and *in vivo*.

7.2 HUMAN STUDIES

No data are available on human absorption, distribution or elimination of dichlorotrifluoroethane. *In vitro* metabolism has been reported, and is described above under animal studies.

8. EFFECTS ON EXPERIMENTAL ANIMALS AND *IN VITRO* TEST SYSTEMS

8.1 SINGLE EXPOSURES

8.1.1 Oral Toxicity

The approximate lethal dose was reported to be 9,000 mg/kg of body weight for male rats administered a corn oil solution of dichlorotrifluoroethane by intra-gastric intubation (Henry, 1975).

8.1.2 Inhalation Toxicity

The results of inhalation toxicity studies are summarized in Table 4.

From an acute inhalation toxicity study of dichlorotrifluoroethane using Chinese hamsters, the 4-hour LC_{50} was determined to be approximately 178,000 mg/m^3 (equals 2.84 % dichlorotrifluoroethane in air) indicating a low order of acute inhalation toxicity (Darr, 1981). Surviving animals were anaesthetized, and recovered upon cessation of exposure. All animals died at 194,000 mg/m^3 (3.1%) and no mortality was observed at approximately 163,000 mg/m^3 (2.6%) indicating a steep dose-response curve.

Another acute inhalation study (Hall, 1975) found the LC_{50} to be 200,000 mg/m^3 in Sprague-Dawley rats exposed for 4 hours. The animals showed signs of anaesthesia: loss of mobility, lethargy, prostration, unresponsiveness to sound, and dyspnea within 5 minutes after initiation of exposure to concentrations of dichlorotrifluoroethane ranging from approximately 129,000 to 344,000 mg/m^3. Surviving rats showed no clinical signs 30 minutes after the cessation of exposure.

A 6-hour LC_{50} was determined to be approximately 329,090 mg/m^3 for rats. One group of rats exposed to a concentration of about 145,000 mg/m^3 experienced mild convulsions after 3 hours of exposure (Coate, 1976). In comparison to other studies described above, the 6-hour LC_{50} would seem to be rather high.

Behavioural effects were evaluated in rats before, during and after 15-minute exposures to dichlorotrifluoroethane at concentrations of 0; 6,250; 15,600; 31,300 and 62,500 mg/m^3 (v/v).
The exposures were carried out in a "glovebox" thereby allowing the measurement of unconditioned reflexes, locomotor activity and coordination. At concentrations of 31,300 and 62,500 mg/m^3 these behavioural parameters were affected. At 31,300 mg/m^3 the animals recovered fully within 5 to 15

minutes and at the high dose the animals recovered fully within 30 minutes post-exposure. A no-observable-effect level (NOEL) was determined to be 15,600 mg/m^3 (Trochimowicz, 1989).

An LC_{50} of approximately 463,000 mg/m^3 was reported for mice exposed to dichlorotrifluoroethane vapours for 30 minutes; the AD_{50} for anaesthesia in mice was found to be 148,800 mg/m^3 also for a 30-minute exposure (Raventos and Lemon, 1965).

Dekant (1993) exposed guinea pigs to a level of 12,500 mg/m^3 of dichlorotrifluoroethane for 6 hours in a closed recirculating exposure system. No effects were reported.

The acute toxicity of dichlorotrifluoroethane to guinea pigs was evaluated in a series of 4-hour exposures at levels of 6,250 to 187,500 mg/m^3. At 48 h post-exposure, animals from all exposure groups showed significant signs of hepatotoxicity, centrolobular vacuola (fatty) change, multifocal random degeneration and necrosis. No effects were seen in the heart and kidney (Marit *et al*, 1994).

The above results demonstrate that dichlorotrifluoroethane has a rather low order of acute inhalation toxicity with the primary toxic effect being central nervous system depression.

Table 4: The Inhalation Toxicology of Dichlorotrifluoroethane

Concentration mg/m^3	Species	Exposure Regimen	Comments	Reference
62,500 to ~ 194,000	Hamster	1 x 4 h	LC_{50} of 178,000 mg/m^3; survivors anaesthetised reversibly; 100% mortality at ~ 194,000 mg/m^3 0 % mortality at ~ 163,000 mg/m^3	Darr, 1981
129,000 to 344,000	Rat	1 x 4 h	LC_{50} of 200,000 mg/m^3; loss of mobility, lethargy, unresponsiveness to sound, dyspnea after 5 min exposure; no clinical signs 30 min post exposure	Hall, 1975
48,600 to 767,000	Rat	1 x 6 h	LC_{50} of 329,900 mg/m^3; mild convulsions after 3 h exposure to ~ 145,000 mg/m^3	Coate, 1976
	Mouse	1 x 30 min	LC_{50} of ~ 463,000 mg/m^3	Raventos and Lemon, 1965
0; 6,250; 15,600; 31,500 and 62,500	Rat	1 x 15 min	Behavioural parameters adversely affected at 31,300 mg/m^3 and above, but full recovery within 5 to 15 min post exposure; NOEL of 15,600 mg/m^3	Trochimowicz, 1989
12,500	Guinea Pig	1 x 6 h	Rapid metabolism, no clinical signs	Dekant, 1993
0; 6,250; 62,600; 125,300; 187,800	Guinea Pig	1 x 4 h	Liver changes at all concentrations (centrilobular vacuolisation; degeneration, necrosis)	Marit *et al*, 1994

8.1.3 Dermal Toxicity

Two acute dermal toxicity studies were performed. In the first study (Trochimowicz, 1989), a single dose of 2,000 mg/kg of body weight of dichlorotrifluoroethane was applied to the clipped, intact skin of 5 male and 5 female New Zealand white rabbits. The application sites were occluded for a period of 24 hours and the rabbits were observed for 14 days post treatment. One day following treatment, 6 of 10 rabbits had slight to moderate erythema which resolved by day 5. No deaths occurred in this study and no gross pathological abnormalities were found in any of the rabbits. It was concluded that the dermal LD_{50} for dichlorotrifluoroethane was greater than 2,000 mg/kg body weight for rabbits.

In the second acute dermal toxicity study (Trochimowicz, 1989), the 2,000 mg/kg of body weight of dichlorotrifluoroethane was applied undiluted to the backs of 5 male and 5 female Crl:CD®BR rats. The application sites were also occluded for a period of 24 hours. No dermal irritation was observed and no deaths occurred during 14 days post dosing. The only clinical signs in this study consisted of red nasal or ocular discharges noted in only one male and one female rat and slight to moderate

body weight losses (described as up to 12% of initial body weights). As with the rabbits, no gross pathological abnormalities were observed in the treated rats. The dermal LD_{50} was determined to be greater than 2,000 mg/kg body weight for rats.

Based on these two studies, dichlorotrifluoroethane is considered as a substance with a low order of acute dermal toxicity.

Dichlorotrifluoroethane was tested for acute skin irritation potential using 4 male and 2 female New Zealand rabbits. Dichlorotrifluoroethane produced no skin irritation when 0.5 ml/6 cm^2/4 h were applied to clipped, intact skin in any of the treated animals (Goodman, 1975; Trochimowicz, 1989).

Dichlorotrifluoroethane, when applied topically to the backs of male guinea pigs as 10% and 50% solutions in propylene glycol, produced no skin irritation or sensitization at challenge (Brock, 1988; Trochimowicz, 1989).

8.1.4 Eye Irritation

Undiluted (0.1 ml) dichlorotrifluoroethane instilled into the conjunctival sac of the rabbit eye without subsequent washing produced mild-to-moderate conjunctival irritation. Washing the eye immediately after dosing still resulted in mild-to-moderate conjunctival irritation, as well as a slight corneal opacity. In either case, complete recovery occurred within 3 to 7 days (Brock, 1988; Trochimowicz, 1989).

8.2 SUBACUTE/SUBCHRONIC TOXICITY

Groups of 10 male rats were exposed to either air (controls) or to 62,600 mg/m^3 dichlorotrifluoroethane by inhalation 6 hours per day, five days per week for 2 weeks. Except for weak anaesthetic effects during exposure, there were no adverse compound-related effects relative to haematological, blood, chemical, urine analytical or histopathological indices (Kelly, 1976).

In a 4-week inhalation study (Rusch *et al,* 1994), four groups of 20 Crl: CD® BR rats (10/sex/group) were exposed for 6 h/d, 5 d/wk to concentration of 6,250, 31,300, 62,500 or 125,000 mg/m^3 dichlorotrifluoroethane. At 31,300 mg/m^3 and above, rats exhibited dose-related anaesthetic effects during exposure. A decreased rate of weight gain was seen in all female exposure groups (not dose-related) and in the two highest male exposure groups (dose-related). No compound-related mortality occurred during the 4-week study. A dose-related increase in liver-to-body weight ratios was observed for all females (up to a maximum of 27% for high dose females) and for high dosed males (18%), without any accompanying histopathologic change. Elevations in transaminases were seen in

male rats only at the 125,000 mg/m^3 level. In addition, a dose-related decrease in cytochrome p-450 occurred in all female exposure groups and in males at the highest two exposure levels. Increases in urinary fluoride at the highest exposure concentrations were also observed. Finally, there were no histopathologic effects seen in this study.

Sprague-Dawley rats (27/sex/group) and four male Beagle dogs were exposed to dichlorotrifluoroethane at concentrations of 0, 6,250 and 62,500 mg/m^3 for 6 hours /day, 5 days a week for 90 days. At the high dose level, both species exhibited lack of motor coordination soon after the start of exposure. This was followed by reduced motor activity and a reduction in responsiveness to noise. After removal from exposure, coordination and activity returned to normal within 20 minutes. Other than final body weight reductions, increased relative liver weights and elevated urinary fluoride concentrations at the two test levels were not observed in rats. At the high dose level, dogs exhibited histopathologic changes in the liver characterized by hypertrophy, clear cytoplasm, and necrosis of liver cells with inflammatory infiltration and clinical chemistry changes including increased levels of serum and liver alkaline phosphatase, ALT and AST, indicative of slight liver damage. No compound-related effects were noted at the lower (6,250 mg/m^3) exposure level (Rusch *et al,* 1994).

In another 90-day study, also described by Rusch, groups of 35 males and 25 female Sprague-Dawley rats were exposed to vapours of dichlorotrifluoroethane. Twenty-five male and 20 female rats in each group were sacrificed following the final exposure. The remaining 10 males and 5 females were held an additional 30 days and then sacrificed. The target levels for this study were 0 (control), 3,130, 6,250 and 31,300 mg/m^3. No treatment-related deaths occurred in this study and mean body weight reductions observed in high dosed males and the two highest dosed groups of females were significant only at the week 13 interval. Slight depressions were observed in heart weight in both male and female rats exposed to 31,300 mg/m^3 dichlorotrifluoroethane. While depressions in kidney weights and kidney/brain weight ratios, but not kidney/body weight ratios, were observed in male rats in all three exposure groups, these effects appeared to be biologically significant only in the 31,300 mg/m^3 exposure group. A depression in kidney weight and kidney/body weight ratio, but not in kidney/brain weight ratio, occurred in the 31,300 mg/m^3 exposed females. Increased liver/body weight ratios, but not liver weight or liver/brain weight ratios, were observed in the 31,300 mg/m^3 exposed males and in all three female exposure groups. However, no significant differences were found for absolute or relative organ weights in animals sacrificed at the end of the 30-day recovery period. The absence of histopathological findings coupled with the absence of effects at the end of the recovery period suggest these effects to be of marginal significance, if at all, in relation to the dichlorotrifluoroethane exposure (Rusch *et al,* 1994).

In a further study, groups of 10 Sprague-Dawley rats/sex/group were exposed to dichlorotrifluoroethane at concentrations of 0, 1,880, 6,250 and 31,300 mg/m^3 for 6 hours/day, 5

days/week for a period of 90 days. Rats exposed at 31,300 mg/m^3 showed slight anaesthetic effects. At this same concentration, as well as at 6,250 mg/m^3, decreased body weight gain was observed. Except for slightly elevated urinary fluoride levels in males at 31,300 mg/m^3, and in all female groups, no other effects relating to haematology or urinary analysis were noted. Serum triglyceride and glucose levels were reduced in both sexes at all levels of exposure. Serum cholesterol was also reduced in female rats at 6,250 and 31,300 mg/m^3. At the 90-day terminal sacrifice, slightly increased liver weights were observed at the two highest exposure concentrations. However, no histopathological effects were noted at any exposure level. Hepatic peroxisome β-oxidation activity to palmitoyl CoA oxidase was increased in rats in all exposure groups. Comparison of liver sections from the control and high-exposure group by electron microscopy revealed a 2- to 3-fold increase in peroxisome number in the high dose animals (Rusch *et al,* 1994).

Two other subchronic studies were conducted with dichlorotrifluoroethane in rats and/or guinea pigs (Lewis, 1990; Warheit, 1993). These studies were undertaken to clarify the findings of reduced serum triglycerides and cholesterol and the increase in benign adenomas of the liver, pancreas and testes observed in a 2-year inhalation study (see Section 8.6). The study by Lewis is described in Section 8.3.4. In the Warheit study, two groups of male Crl:CD®BR rats (n=17/group) were exposed to 0 or 125,200 mg/m^3 for 6 h/d, 5 d/wk for 4 weeks. After 6 exposures and because of severe body weight losses, the dichlorotrifluoroethane exposure concentration was reduced to 112,700 mg/m^3. Similarly, two groups of 15 male Hartley (HABR VAP/PLUS) guinea pigs were exposed to either 0 or 125,200 mg/m^3. After 6 exposures and because of severe body weight losses observed in the guinea pigs, the dichlorotrifluoroethane exposure concentration was reduced ultimately to 31,300 mg/m^3. The total exposure for these groups of animals was 23 days. One group each of 17 male rats and 15 male guinea pigs were fed diets containing 7,500 ppm TFA. Also, one group each of 17 male rats and 15 male guinea pigs were fed diets containing 100 ppm of Wyeth (WY)-14643, a compound known to induce peroxisomes in the liver (see 8.3.4). These animals were treated with compound for 25 consecutive days. A decrease in body weight was observed in the dichlorotrifluoroethane, TFA and WY-14643 exposed animals without concomitant changes in food consumption for rats, although there was a decrease in food consumption of guinea pigs exposed to dichlorotrifluoroethane or TFA. Three guinea pigs exposed to dichlorotrifluoroethane died during the study. These deaths were considered to be compound-related in particular to the initial high exposure concentration. Decreases in serum cholesterol, triglycerides, glucose and insulin levels were observed in rats exposed to either dichlorotrifluoroethane or TFA (Table 5). An increase in serum glucose and cholesterol was observed with WY-14643, but a decrease was observed for triglycerides. In guinea pigs, decreases in triglycerides and glucose were observed with each treatment, although the changes were not as dramatic as those observed in rats. Furthermore, the decrease in these parameters in guinea pigs exposed to dichlorotrifluoroethane may be related to the

severe body weight effects of the compound. Alternatively, dichlorotrifluoroethane may lower serum triglycerides levels via a mechanism unrelated to peroxisome induction.

Increases in mean absolute and relative liver weights were observed in rats exposed to dichlorotrifluoroethane, TFA and WY-14643. The increased liver weights correlated with diffuse hypertrophy observed microscopically in the treated rats. The weight changes and hypertrophy were most severe in the WY-14643 treated rats. In contrast, the liver weights of guinea pigs exposed to dichlorotrifluoroethane were significantly reduced. Minimal to mild centrilobular fatty change was observed microscopically. In guinea pigs exposed to TFA or WY-14643 the liver weights were also reduced, although there were no significant microscopic observations.

In conclusion there are several differences in the degree and type of changes observed within and between the two species. Guinea pigs were more sensitive to the systemic effects of dichlorotrifluoroethane than rats based on body weight losses and mortality. Liver weights were decreased in guinea pigs, whereas liver weights were increased in the rat. These changes correlated with microscopic observations, e.g., the increased liver weights in rats correlated with hypertrophy whereas the decreased liver weights in guinea pigs correlated with cytotoxic fatty changes. In rats repeated subchronic inhalation exposure to dichlorotrifluoroethane at exposure levels of up to 31,300 mg/m^3 appears to be associated with decreased body weights, increased liver weights and effects on lipid metabolism, and reversible anaesthetic effects, without significant evidence of histopathologic damage. The NOEL for anaesthetic effects was 6,250 mg/m^3. The NOEL for hypolipidemic effect was less than 1,880 mg/m^3.

Table 5: Clinical Chemical Results for Rats and Guinea Pigs[1]

Group	Cholesterol (mg/100ml)	Triglycerides (mg/100 ml)	Glucose (mg/100ml)	Insulin (ng/ml)
Rat				
Control	51 ± 17[2]	79 ± 30	105 ± 14	3.9 ± 1.9
Dichlorotrifluoroethane (113,750 mg/m^3)	33 ± 10	31 ± 2.0	77 ± 12	1.9 ± 0.6
TFA (7500 ppm)	43 ± 9	68 ± 18	87 ± 8	3.6 ± 1.4
WY-14643 (100 ppm)	70 ± 15	51 ± 9	121 ± 10	3.7 ± 1.4
Guinea Pig				
Control (0 ppm)	58 ± 13	94 ± 15	155 ± 13	ND[3]
Dichlorotrifluoroethane (58.750 mg/m^3)	36 ± 24	60 ± 24	134 ± 13	ND
TFA (7500 ppm)	63 ± 31	92 ± 2	136 ± 10	ND
WY-14643 (100 ppm)	49 ± 13	87 ± 28	139 ± 12	ND

[1] Groups of rats and guinea pigs were exposed 6 h/d, 5d/wk for 28 days to either dichlorotrifluoroethane or air (controls).
For TFA and WY-14643, rats or guinea pigs were provided diets containing compound for 25 consecutive days.
From Warheit (1993)
[2] Values are the mean ± standard deviation
[3] ND = Not determined

8.3 SPECIAL STUDIES

8.3.1 Cardiac Sensitization to Adrenalin

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. In one study described by Foll, (1976), a concentration of 62,500 mg/m^3 (v/v) of dichlorotrifluoroethane was listed as the concentration which will induce cardiac sensitization in the dog. No details of the experimental conditions were provided.

In another study in Beagle dogs, the cardiac sensitization potential of dichlorotrifluoroethane was evaluated using exposure levels of 62,500, 125,000 and 250,000 mg/m^3. After approximately 5 min. of exposure the dogs were given an intravenous injection of epinephrine (8 µg/kg) and monitored for cardiac arrhythmias. All three dogs developed cardiac arrhythmias at an exposure level of 250,000 mg/m^3 and 4 out of 6 dogs after 125,000 mg/m^3. A concentration of 62,500 mg/m^3 was tolerated without any signs for cardiac arrhythmias. The EC_{50} was estimated to be 119,000 mg/m^3 and the NOEL was 62,500 mg/m^3 for dichlorotrifluoroethane (Trochimowicz and Mullin, 1993).

8.3.2 Neurotoxicity

The potential for neurotoxic effects of dichlorotrifluoroethane was investigated in a 13-week inhalation neurotoxicity study in rats (Coombs, 1994). Groups of 10 male and 10 female Sprague Dawley rats were exposed (whole body) 6 h/d, 5 d/w, to vapour of dichlorotrifluoroethane at concentrations of 0, 1,875, 6,250 and 31,300 mg/m^3 in air.

The behaviour was assessed on several occasions: on 2 consecutive days before starting exposures; then at the end of week 4, 8 and 13 (on the day following the last 6-h exposure of the concerned week); then at the end of a 4-week recovery period. The Irwin behavioural screen methodology (Irwin, 1968) as indicated by WHO (1986) was used. It comprised a series of standardized observations in home cage, on the bench, in the hand and in a few selected situations including testing for grip strength, landing foot splay, startle response, tail pinch and righting reflex. In addition, whole-body perfusion/fixation was performed on 5 males and 5 females of each group at the end of the exposure and recovery periods. The brain was weighed and the following nerve tissues were examined histologically by light microscopy after haematoxylin-eosin staining: brain, medulla/pons, cerebellar cortex, spinal cord, ganglia and dorsal and ventral root fibres (C3-C6 and L1-L4), peripheral nerves (sciatic, sural, tibial).

There were no treatment-related effects at any time or exposure level on the brain weight or on any of the nerve tissues. There was no apparent specific clinical sign although the body weight gain was slightly reduced in the high concentration group. The Irwin behavioural screen showed no treatment-related change, except for an apparent tendency to a reduction in arousal in male rats which attained statistical significance at 6,250 and 31,300 mg/m^3 on week 13.

It should be noted that the behavioural assessment was performed on the day after the last 6-h exposure. By that time the rats should have normally recovered from the anaesthesia-like effects which occurred during the inhalation exposure of dichlorotrifluoroethane at concentrations of 31,250 mg/m^3 and above (see chapters 8.1.2 and 8.2). However, the arousal reduction might be interpreted as a residual effect in animals which may have not fully recovered from the anaesthesia-like effects of the previous day.

The lack of neurotoxicity of dichlorotrifluoroethane in this study is consistent with the lack of any adverse clinical signs in medium and long-term exposed animals (see sections 8.2 and 8.6) at similar concentrations.

8.3.3 Endocrine Evaluations

An evaluation performed by Sandow (1994) designed to investigate the mechanism of testicular changes (Leydig cell hyperplasia) observed in aging Sprague-Dawley rats after long-term treatment with dichlorotrifluoroethane, was conducted as an addendum to a two-generation reproduction study (Hughes, 1994b; and see Section 8.4.2.). Near the end of the study, the pituitary-gonadal function was evaluated by a dynamic stimulation test using luteinizing hormone-releasing hormone (LHRH) injection. At final necropsy, the capacity for androgen biosynthesis was evaluated by incubation of the testes *in vitro* with human chorionic gonadotropin (HCG).

Serum levels of several steroids (testosterone, progesterone, estradiol, 17a-OH progesterone, and d4-androstenedione) were measured using male rats that were exposed for 22 weeks to dichlorotrifluoroethane at levels up to 6,250 mg/m^3, for 6 hours/day, 7 days/week. In addition, testicular levels of luteinizing hormone (LH) and testosterone were determined under both normal and stimulated conditions, with LHRH or HCG, respectively.

No effects were observed on serum steroid levels. Basal levels of LH and testosterone were unaffected by the dichlorotrifluoroethane exposures. With stimulation, the levels of LH in the 1,875 mg/m^3 and 6,250 mg/m^3 exposure level groups were significantly lower than in the control and 625 mg/m^3 exposure level group. Moreover, a slight reduction in testosterone level was seen in all treatment groups compared to the control. These two findings indicate that exposure to dichlorotrifluoroethane may have a minimal effect on the rat endocrine system.

Warheit (1993) also evaluated serum hormone levels in male rats and guinea pigs exposed to levels of 113,750 mg/m^3 or 58,750 mg/m^3, respectively. Levels of testosterone, estradiol and LH were measured in rats following 3 and 21 exposures, in addition, levels of cholecystokinin (CCK) were measured after 21 exposures. In the guinea pigs, levels of testosterone were measured after 3 and 21 exposures and CCK levels were determined after 21 exposures. No significant differences were seen in the dichlorotrifluoroethane exposed rats compared to controls. In guinea pigs, testosterone levels may have appeared lower in dichlorotrifluoroethane exposed animals compared to controls. CCK levels were unaffected by treatment.

These observations may be associated with the testicular findings in the chronic inhalation study with rats.

8.3.4 Peroxisome Proliferation and Hypolipidemia

Dichlorotrifluoroethane produced hypolipidemia and hepatic proliferation of peroxisomes in rats in several studies. These effects suggest that dichlorotrifluoroethane belongs to a particular class of chemicals, commonly referred to as peroxisome proliferators or hypolipidemic agents, whose effects are mediated through a disruption of lipid metabolism (Reddy and Lalwani, 1983). A thorough review of hepatic peroxisome proliferation has been published (ECETOC, 1992). As part of some of the studies conducted with dichlorotrifluoroethane, measurement of certain clinical chemical parameters was included, e.g., triglycerides and cholesterol. In addition, peroxisome proliferation was measured through measurement of β-oxidation of ^{14}C-palmitoyl-CoA, and actual changes in the density and numbers of peroxisomes were measured with electron microscopy.

In 90-day inhalation studies (see Section 8.2), decreases in serum cholesterol, triglycerides or glucose were observed in rats at dichlorotrifluoroethane concentrations of 1,880 mg/m^3 and greater. Likewise, in the chronic/oncogenicity study, decreases in these parameters were observed throughout the 2-year study at concentrations of 1,880 mg/m^3 and greater (see Section 8.6).

In a 28-day inhalation study to assess the effects of dichlorotrifluoroethane on rat liver, microsomal cytochrome P-450 plasma and associated enzymes, male CD rats (n=6/group) were exposed to dichlorotrifluoroethane concentrations of 0, 6,250, 31,300 and 125,200 mg/m^3 resulting in elevated liver and testes weights (Lewis, 1990). A dose-related reduction in plasma cholesterol levels was noted in the dichlorotrifluoroethane exposed rats, with about a 50% reduction observed at the high concentration. Likewise, a reduction of about 60-70% in triglyceride levels was observed in the exposed rats (Table 6). An increase in alkaline phosphatase activity was also observed.

In this study, an increase in β-oxidation activity measured by palmitoyl CoA oxidase activity was observed at 125,200 mg/m^3 (229%), with increases also occurring at 6,250 and 31,300 mg/m^3. Similar results were observed in the 90-day and 2-year inhalation studies at dichlorotrifluoroethane concentrations of 1,880, 6,250 and 31,300 mg/m^3. Cytochrome P-450 or cytochrome b5 activities were unchanged.

Consistent with the increased β-oxidation levels was an increase in peroxisome numbers detected by electron microscopy (Table 6). Peroxisome numbers were 126% and 242% higher in the 31,300 and 125,200 mg/m^3 groups, respectively. Mitochondria levels were also increased. Taken together, these data indicate that dichlorotrifluoroethane is a mild peroxisome proliferating compound. Liver hypertrophy was observed by light microscopy at all concentrations, although the severity of the response was much less at 6,250 mg/m^3.

Table 6: Clinical Chemical and Liver Biochemical Measurements[1]

Parameter	Dichlorotrifluoroethane Inhalation Concentrations (mg/m^3)			
	0	6250	31,300	125,000
Plasma Cholesterol (mg/100 mL)	71 ± 14[2]	55 ± 10	40 ± 13	35 ± 5
Plasma Triglycerides (mg/100 mL)	82 ± 26	30 ± 14	21 ± 8	25 ± 8
Cytochrome P-450 (nmol/mg protein)	0.73 ± 0.17	0.76 ± 0.13	0.81 ± 0.14	0.78 ± 0.11
β-Oxidation (nmol/min/mg protein)[3]	2.8 ± 0.56	5.3 ± 0.58	5.7 ± 1.6	9.2 ± 1.8
Peroxisome Density[4]	1.9 ± 0.7	2.7 ± 0.7	4.3 ± 1.2	6.5 ± 1.1

[1] Groups of rats were exposed 6 hr/day, 5 days/week for 28 days. From Lewis (1990)
[2] Values are the mean ± standard deviation
[3] β-Oxidation activity was measured as ^{14}C-palmitoyl CoA oxidase activity
[4] Morphometric analysis of liver samples with electron microscopy

A "mechanistic study" was conducted by Warheit (1993) using rats and guinea pigs in order to identify a potential mechanism for dichlorotrifluoroethane induced tumours in the liver, pancreas and testes (see 8.6). The study also included the use of Wyeth (WY)-14643, a compound known to induce peroxisomes, and trifluoroacetic acid (TFA) the primary metabolite of dichlorotrifluoroethane (for details see 8.2).

At the end of the exposure phase, the animals were subjected to cell turnover and histopathology evaluations, serum hormonal determinations, β-oxidation and clinical chemistry studies. The rate of hepatic peroxisomal β-oxidation was increased in rats exposed to dichlorotrifluoroethane, TFA or WY-14643 (Table 7). These data correlate with the observed increase in liver weights seen in this study, as well as with the microscopic findings of hypertrophy and the increased peroxisome number. In contrast, no increases in peroxisomal proliferation was observed in the pancreas. In guinea pigs exposed to any of these compounds, the rate of hepatic peroxisomal β-oxidation was unchanged.

The species differences in the response to dichlorotrifluoroethane exposure is associated with the sensitivity of the rat for the induction of peroxisome proliferation, and WY-14643 was clearly the more potent agent causing a greater degree of peroxisome induction and liver hypertrophy.

An evaluation of the human relevance of the rodent liver tumours to dichlorotrifluoroethane is best considered within the context of peroxisome proliferator activated receptor (PPAR) or the pharmacological activity associated with this class of compounds. TFA, the principal metabolite of dichlorotrifluoroethane, has also been shown to act as a peroxisomal proliferator in the liver (Just, 1989 and Warheit, 1993). The nature of the chronic tumorigenic response to dichlorotrifluoroethane in rats and the short-term peroxisomal responses to both dichlorotrifluoroethane and TFA are consistent with expectations based on other peroxisomal proliferators. For all three tissues in which tumours occurred, the cell type associated with the lesion is a cell type which contains peroxisomes, and/or has been a site of tumorigenic activity for other peroxisomal proliferators (Fitzgerald *et al,* 1981; Mennear, 1988; Sibinski, 1987; Svoboda and Azarnoff, 1979; Reddy and Rao, 1977; Cook *et al,* 1994), although peroxisome proliferation with dichlorotrifluoroethane has only been demonstrated in the liver.

Table 7: Hepatic Peroxisomal β-Oxidation Activity in Rat and Guinea Pig

Group	β- Oxidation[1,3]
Rat	
Control (0 mg/m^3)[2]	10.9 ± 1.4 (n = 5)
HCFC-123 (113,750 mg/m^3)[2]	51.2 ± 1.9 (n = 5)
TFA (7500 ppm)[4]	45.8 ± 13.3 (n = 5)
WY-14643[4]	81.2 ± 8.5 (n = 5)
Guinea Pig	
Control (0 mg/m^3)[2]	9.3 ± 1.4 (n = 3)
HCFC-123 (58,750 mg/m^3)[2]	7.1 ± 1.1 (n = 4)
TFA (7500 ppm)[4]	8.8 ± 1.4 (n = 3)
WY-14643[4]	7.1 ± 1.7 (n = 3)

[1] β-Oxidation activity was measured as 14 C-palmitoyl CoA oxidase activity
[2] Exposures were 6 h/d, 5 d/wk
[3] Values are mean ± standard deviation
[4] Administered in the diet

In conclusion, these results provide good evidence that dichlorotrifluoroethane can be classed as a rat liver peroxisome proliferator, although the proliferator activity is weak. The peroxisome proliferating activity of dichlorotrifluoroethane is very likely due to its metabolism to trifluoroacetic acid. This property of dichlorotrifluoroethane is further discussed in association with the tumour findings in rat (see section 8.6).

8.4 REPRODUCTIVE PERFORMANCE, EMBRYOTOXICITY AND TERATOLOGY

8.4.1 Embryotoxicity and Teratology

An inhalation teratology study was conducted with vapours of dichlorotrifluoroethane (Rusch *et al,* 1994). For this study, two groups of 20 pregnant female rats were exposed to 0 and 31,300 mg/m^3 of dichlorotrifluoroethane for 6 h/d from days 6 through 15 of gestation. The animals were sacrificed on day 20 and all dams and foetuses examined. Maternal mean body weights in the exposed groups were significantly depressed on days 12 and 15 of the gestation period (20 and 40 grams, respectively). At termination, maternal mean body weights were still depressed (21 grams), but not to a statistically significant degree. The numbers of corpora lutea, implantation sites, resorption sites and foetuses were similar among control dams and those exposed to dichlorotrifluoroethane. In summary, exposure of pregnant rats to a concentration of 31,300 mg/m^3 of dichlorotrifluoroethane, a level which produced a response in the dams (depressed weight gain), did not result in a teratogenic response.

One other inhalation teratology study, was reported by Kelly *et al* (1978) in which 25 pregnant rats were exposed to dichlorotrifluoroethane at a concentration of 1% (v/v) or 62,500 mg/m^3 for 6 h/d on days 6 through 15 of gestation. Dams and foetuses were sacrificed on day 21 and examined for gross changes. It was concluded that dichlorotrifluoroethane did not cause either embryotoxicity or teratogenic effects under the conditions of this study.

A study was conducted to evaluate the teratogenic potential of dichlorotrifluoroethane when administered to rabbits by inhalation. Initially, in the range-finding study, groups of six pregnant rabbits were exposed to concentrations of 6,250; 31,300, 62,500 and 125,000 mg/m^3 for 6 h/d during days 6 through 18 of gestation. An air exposed control group was included. All test compound exposed rabbits lost weight during the study and food consumption was markedly reduced, especially at 62,500 and 125,000 mg/m^3. Based on marked decreased food consumption, decreased body weights, and an increased number of resorptions seen at 62,500 and 125,000 mg/m^3, all indicating maternal toxicity and possibly embryotoxicity, exposure levels of 3,125, 9,380 and 31,300 mg/m^3 were selected for the main study. This study consisted of 6 h/d exposures of 24 mated females per exposed group during days 6-18 of gestation. No compound-related mortality was observed in any of the exposure groups. Evidence of maternal toxicity occurred during day 6-18 of gestation at all exposure levels and included body weight loss as well as reduced food consumption. No other signs of maternal toxicity were observed. In conclusion, there was no evidence of embryotoxic, fetotoxic or teratogenic effects (Trochimowicz, 1989).

8.4.2 Reproductive Performance

A one-generation reproduction study (Hughes, 1994a) was performed to aid in establishing experimental parameters for the conduct of an inhalation two-generation reproduction study on dichlorotrifluoroethane. Male and female rats and their offspring were exposed by whole-body inhalation to concentrations of 0 (control), 1,880, 6,250 and 31,300 mg/m^3 for 6 h/d, 7d/wk. Parental animals (12 males and 12 females per group) were exposed for 4 weeks prior to pairing, through mating, gestation, lactation and weaning of their offspring and were sacrificed after about 17 weeks of exposure. Exposure to dichlorotrifluoroethane caused a dose-related increase in water consumption and decrease in food consumption. Also, a slight dose-related retardation in body weight gain was seen in the adults of all treatment groups as well as a reduced rate of growth in the pups. As in other repeated-exposure studies in the rat (Section 8.2) reduction in triglycerides was seen at all exposure levels. The administration of dichlorotrifluoroethane did not effect the reproductive ability of the animals. There were no effects on implantations, litter size, or pup survival at any exposure level. Sexual maturation was delayed slightly in males in the 6,250 and 31,250 mg/m^3 groups. This was probably due to lower body weight gain seen in these groups compared to controls.

The effect of dichlorotrifluoroethane on the reproductive performance and growth was evaluated in a two-generation reproduction study conducted by whole body inhalation in the rat (Hughes, 1994b). Male and female rats were exposed to the test material at concentrations of 0 (control), 188, 625, 1,880 and 6,250 mg/m^3. The animals were exposed to the test material for 6 hours per day, 7 days a week.

There were no clinical signs attributable to the exposure. Retarded body weight was observed among F_0 and F_1 males and females of the 6,250 mg/m^3 exposure group. At 1,880 and 625 mg/m^3, a slight retardation of body weight gain was observed only in F_0 males, while there were no effects at 188 mg/m^3. For both generations, weight gain at 6,250 mg/m^3 was lower than controls throughout pregnancy. For both generations during lactation, the overall pattern of weight change was considered comparable among the groups and not obviously affected by exposure. The food consumption was increased only in both F_0 male and female animals at 6,250 and 1,875 mg/m^3. During lactation, food consumption was lower at 6,250 and 1,875 mg/m^3 in both generations. The food utilization was impaired in F_0 animals at 6,250 mg/m^3 and 1,875 mg/m^3 (only females) and F_1 animals only at 6,250 mg/m^3. Increased water consumption was observed at 6,250, 1,875 and 625 mg/m^3 (only F_0 animals). Decrease of cholesterol and triglycerides, and VLDL values were observed in the animals of both generations at 6,250, 1,875 and 625 mg/m^3. As discussed in Section 8.3.3, basal levels of estradiol, testosterone, LH, 4-androstenedione, progesterone and FSH were unaffected by treatment. Increased liver weights of F_0 and F_1 animals, macroscopic changes in the liver with associated microscopic changes of centrilobular hepatocyte enlargement and vacuolation

were observed at 6,250, 1,875 and 625 mg/m^3. At 188 mg/m^3 only a slight increase of liver weight was observed in the F_0 animals. Decreased litter and mean pup weight in F_0 offspring from day 14 post partum to weaning and F_1 offspring from day 7 post partum to weaning was observed at 625 mg/m^3 and above. At 6,250 mg/m^3 a reduction of implantation counts in F_1 females was observed. Male sexual maturation was slightly delayed at 1,875 and 6,250 mg/m^3. The difference in growth rate may have been a factor in the delay of sexual maturation. No effects were seen at 188 and 625 mg/m^3 and in the female sexual maturation. At 188 mg/m^3 a decreased mean pup weight was observed only in the F_1 generation. The fat content in the milk of both F_0 and F_1 lactating dams was highly variable and did not yield useful data.

In conclusion, exposure to dichlorodifloroethane was principally associated with effects on growth, lipid metabolism and on the liver as has been seen in the subchronic studies. For both generations there were no treatment-related effects on mating performance. Retarded weight gains were observed among directly exposed adults as well as among offspring during the pre-weaning period when exposure was confined to the lactating dam. Effects on weight gains were observed at 625 mg/m^3 in adult animals and at the lowest exposure level employed (188 mg/m^3) in offspring during the pre-weaning phase. During the post weaning phase the body weight gain was comparable to the controls at all dose levels except at 6,250 mg/m^3. Liver weights were increased at 625 mg/m^3 but only among F_1 pups. Histopathological changes appeared confined to exposure of 625 mg/m^3 and above. In terms of reproductive performance, the only adverse finding was a decrease in implantation counts among F_1 females at 6250 mg/m^3. As this effect was not seen in the one-generation study, even with exposures up to 31,250 mg/m^3, it is considered to be of marginal significance at most. Regarding development, all exposure levels of dichlorotrifluoroethane were associated with impaired pup growth in the offspring of F1 generation. This was seen only during the preweaning phase. Further studies are envisaged to clarify the mechanism.
Although effects on fertility were not observed, it is not possible to identify a clear NOEL as effects were seen in some parameters at the lowest exposure level (188 mg/m^3).

8.5 MUTAGENICITY

The data from *in vitro* and *in vivo* genotoxicity studies are summarised in Table 8.

Table 8: The Genetic Toxicology of Dichlorotrifluoromethane. in *In vitro and in vivo Studies*

Assay	Strain/Type	Metabolic activation	Result	Comment	Reference
Salmonella typhimurium	TA1535, TA1538, TA98, TA100	± S-9	-ve	Tested as a gas up to 625,000 mg/m^3	Longstaff *et al* (1984)
Salmonella typhimurium	TA1535, TA1537, TA1538, TA98, TA100	± S-9	-ve	Tested as a gas at a nominal conc. of 100% for up to 30 min	Brusick (1976)
Salmonella typhimurium	TA1535, TA1537 TA1538,TA98 TA100	± S 9	-ve	Tested as a liquid up to 0.5 ml per expsoure	Callander (1989)
Saccharomyces cerevisiae	D4 Forward Mutation	± S-9	-ve	Tested as a gas at a nonimal conc. of 100% for up to 72 minutes	Brusick (1976)
Cell Transformation	BHK21	+ S-9	-ve	Tested as a liquid up to 250 µg	Longstaff *et al*, 1984 : Styles (1977)
Clastogenicity	Human lymphocytes	- S-9 + S-9 -S-9 +S-9	+ve +ve +ve -ve	Tested as a vapour at 2.5 and 10% Vapour at 30% Liquid at 0.005%	Edwards (1991) Edwards (1991) Dance (1991)
Clastogenicity	Human lymphocytes	-S-9 +S-9	+ve -ve	Liquid at 50, 250, and 500 µg/ml 100, 500, 1,000 µg/ml	Mackay (1992)
Chromosome Aberration	Rat lymphocytes	*in vivo*	-ve	Tested at 31,250 mg/m^3 (0.5 %)	Marshall (1992)
Unscheduled DNA Synthesis (UDS)	Rat hepatocytes	*in vivo*	-ve	Rats exposed to 1.25% and 2% (78, 125 and 125,000 mg/m^3)	Kennelly (1993)
Micronucleus	Mice Polychromatic Erythrocytes	*in vivo*	-ve	Tested as a gas at 12,500, 37,500 and ~113,000 mg/m^3 (v/v) for 6 hours	Müller and Hoffmann (1988)

Longstaff *et al* (1984) reported that dichlorotrifluoroethane gave negative results when tested in several *Salmonella typhimurium* strains (Ames), both in the presence and absence of rat liver S-9. In addition, these investigators found that dichlorotrifluoroethane also gave negative results in a cell transformation (Styles, 1977)) assay using a permanent cell line of baby hamster kidney fibroblasts (BHK21) in the presence of S-9 mix.

In an earlier study (Brusick, 1976), dichlorotrifluoroethane was tested for mutagenicity in a series of suspension and plate assays using *Salmonella typhimurium* tester strains (Ames), as well as *Saccharomyces cerevisiae* strain D4 (forward mutation assay), in the presence and absence of S-9. Dichlorotrifluoroethane was found to be negative in both of these assays.

Dichlorotrifluoroethane was evaluated for clastogenic potential in an *in vitro* cytogenetic assay using human lymphocytes both in the presence and absence of rat liver-derived S-9 mix (Mackay, 1992). Cultures of these cells were exposed to dichlorotrifluoroethane at dose levels of 50, 250 and 500μg/ml in the absence of S-9 mix and 100, 500 and 1,000 μg/ml in the presence of S-9 mix. In the presence of S-9 mix, no increases in the frequency of chromosomal aberrations were observed. In the absence of S-9 mix, dose-related increases in the chromosomal aberration frequency were observed which were statistically and biologically significant at 500 μg/ml. These results suggest that mammalian liver enzymes are capable of inactivating the mutagenic potential of dichlorotrifluoroethane.

The latter study was repeated at another laboratory, using both vapour phase and liquid exposure designs (Hodson-Walker, 1993). In the vapour phase study, cultured human lymphocytes were exposed to 2.5% and 10% dichlorotrifluoroethane in the absence of S-9, and to 30% in the presence of S-9. In both cases, biologically and statistically significant positive responses were produced (Edwards, 1991). When tested in the liquid phase at 0.005% (v/v), dichlorotrifluoroethane gave a positive response in the absence of S-9, but no significant evidence for a positive response in the presence of S-9 (Dance, 1991).

A micronucleus assay was conducted to further evaluate the genotoxic potential of dichlorotrifluoroethane (Müller and Hoffmann, 1988). NMRI mice were exposed to levels of 113,000 mg/m^3 (1.8%), 37,500 mg/m^3 (0.6%) and 12,500 mg/m^3 (0.2%) in air for six hours. Under the conditions of this assay, exposure to dichlorotrifluoroethane did not result in an increased number of micronuclei and, therefore, was shown to be non-mutagenic.

Dichlorotrifluoroethane was further evaluated for genetic activity in an *in vivo* chromosome aberration study with peripheral blood lymphocytes from rats (Marshall, 1992). The blood samples were taken from male Sprague-Dawley rats which had been exposed for 6h/d, 7 d/w over a period of 14 weeks to dichlorotrifluoroethane concentrations of 1,875 mg/m^3, 6,250 mg/m^3 and 31,250 mg/m^3.

Chromosome aberrations were determined only in the high dose group. The compound was not active under the conditions of this test.

Dichlorotrifluoroethane was also tested for the ability to induce unscheduled DNA synthesis (UDS) in an *in vivo* rat hepatocyte assay incorporating an autoradiographic technique (Kennelly, 1993). Male Alderley Park (Alpk:APfSD) rats were exposed for six hours by inhalation at 50,000 mg/m^3, 78,125 mg/m^3, and 125,000 mg/m^3 in air. Hepatocytes from exposed rats were assessed for the induction of UDS at the two highest dose levels. It was concluded that, when tested in rat liver, *in vivo* at exposure levels up to 125,000 mg/m^3, dichlorotrifluoroethane did not induce DNA repair.

In conclusion, dichlorotrifluoroethane was inactive in several *in vitro* studies including a series of *Salmonella typhimurium* assays, a *Saccharomyces cerevisiae* assay and a cell transformation assay. It was positive only in the human lymphocyte chromosome abberation assay. Dichlorotrifluoroethane was clearly negative in a series of *in vivo* studies, including mouse micronucleus, rat chromosome aberration and rat unscheduled DNA synthesis. In conclusion, dichlorotrifluoroethane is not genotoxic *in vivo* and therefore does not have toxicologically significant genotoxicity.

8.6 CARCINOGENICITY AND CHRONIC TOXICITY

A two-year inhalation toxicity (combined chronic toxicity/ oncogenicity) study was conducted with dichlorotrifluoroethane (Malley *et al* (1995). In this study 80 male and 80 female CD rats per group were exposed to vapours of dichlorotrifluoroethane for 6 h/d, 5 d/w for approximately two years at concentrations of 0 (control), 1,880, 6,250 and 31,300 mg/m^3. Clinical pathology was evaluated at 6, 12, 18, and 24 months and an interim sacrifice of 10 animals per sex per group was conducted at 12 months. Serum triglyceride and glucose concentrations were significantly decreased in both sexes, at all exposure concentrations (Table 9). Serum cholesterol was significantly lower in females exposed to 1,880, 6,250 and 31,300 mg/m^3 and in males exposed to 31,300 mg/m^3. In addition, male and female rats exposed to 31,300 mg/m^3 and females exposed to 6,250 and 1880 mg/m^3 had lower body weight and body weight gain. Males and females exposed to 6,250 mg/m^3 or 31,300 mg/m^3 had significantly greater survival (Table 10). At 24 months, increased liver and kidney weight occurred in 31,300 mg/m^3 in males and females. At the 24-month sacrifice, there were compound-related increases in the incidences of benign hepatocellular adenomas which were statistically significant in males exposed to 31,300 mg/m^3 and in females exposed to 1,880 mg/m^3 and 31,300 mg/m^3. of benign hepatic cholangiofibromas in 31,300 mg/m^3 exposed females; and of benign pancreatic acinar cell adenomas in all test groups of males. Acinar cell hyperplasia of the pancreas was increased in the 6,250 mg/m^3 and 31,300 mg/m^3 in males and females. Moreover, the incidence of cellular alteration (basophilic) in the liver was increased in all test groups of males and females.

Benign interstitial cell adenomas and focal interstitial cell hyperplasia were increased in testes of all test groups of males. Data for tumour incidences are summarized in Table 11. A diffuse retinal atrophy, increased in all test groups of both males and females, was considered an indirect compound-related effect.

8.6.1 Discussion and Assessment

The study described above shows some notable effects of dichlorotrifluoroethane in the rat, being: an increase in survival rate, particularly at 31,300 mg/m^3 dichlorotrifluoroethane (Table 10); a decrease in body weight, particularly at 31,300 mg/m^3 dichlorotrifluoroethane (although this group of rats had the same rate of food consumption); and a dose-related, strong and persistent decrease in serum triglyceride concentration present at all exposure levels. When considered together these data suggest that improved rat survival at the high exposure level was possibly due to lower body weights which itself was probably associated with the reduction of plasma triglycerides.

Also, the study identified some statistically significant increases in tumour incidence in the liver, testis and pancreas (Table 10). These tumours were all benign, were all a continuum from hyperplasia, and all occurred late in life. Age adjustment of tumour rates to correct for the higher mortality in controls compared to dichlorotrifluoroethane exposed rats tended to reduce the actual observed tumour rates. However, these changes did not affect the overall conclusion of the significance of the tumours.

Table 9: Combined Chronic Toxicity/Oncogenicity Study with Dichlorotrifluoethane (Malley et al, 1995)

Summary of Clinical Chemical Findings For Male Rats					
Tests	Concentration (mg/m^3)	Sampling Time (Concentrations in mg/dl blood [a])			
		6 Month	12 Month	18 Month	24 Month
Triglyceride	0	96 (55)	201 (133)	183 (117)	176 (70)
	1,880	45 (23)#	49 (22)#	87 (70)#	95 (30)#
	6,250	20 (10)#	35 (34)#	66 (29)#	85 (24)#
	31,300	8 (9)#	4 (8)#	34 (10)#	44 (12)#
Glucose	0	117 (11)	109 (9)	93 (13)	74 (22)
	1,880	94 (12)*	93 (12)*	84 (15)	73 (10)
	6,250	85 (7)*	92 (11)*	71 (10)*	69 (9)
	31,300	78 (9)*	81 (5)*	73 (9)*	76 (14)
Cholesterol	0	63 (21)	120 (61)	121 (49)	131 (48)
	1,880	69 (22)	101 (24)	85 (39)	100 (48)
	6,250	63 (9)	94 (19)	84 (25)	115 (26)
	31,300	54 (16)	68 (22)#	76 (24)*	78 (32)*
Summary of Clinical Chemical Findings For Female Rats					
Triglyceride	0	104 (70)	207 (173)	148 (46)	122 (87)
	1,880	42 (18)#	72 (54)#	68 (11)#	70 (35)
	6,250	25 (7)#	5 (14)#	66 (30)#	59 (18)#
	31,300	15 (2)#	0 (1)#	37 (5)#	40 (15)#
Glucose	0	110 (12)[a]	97 (9)	85 (10)	77 (17)
	1,880	93 (8)*	78 (11)*	81 (9)	63 (18)
	6,250	77 (11)*	66 (12)*	75 (10)	69 (19)
	31,300	78 (7)*	67 (5)*	69 (9)*	79 (18)
Cholesterol	0	73 (18)	76 (12)	100 (31)	110 (27)
	1,880	57 (9)*	56 (7)	78 (11)	76 (16)*
	6,250	52 (12)*	52 (8)*	79 (25)	94 (36)
	31,300	54 (11)*	52 (10)*	58 (12)*	57 (21)*

[a] Group means and standards deviations (SD)
* Significantly different from control at 5 % level by Dunnett criteria
# Significantly different from control at 5 % level by Mann-Whitney U criteria

Table 10: Combined Chronic Toxicity /Oncogenicity Study with Dichlorotrifluoethane in the rat : Survival at 2 years

Exposure Levels	Survival at 2 Years	
	Males	Females
0 mg/m^3 (control)	26 %	23 %
1,880 mg/m^3	31 %	34 %
6,250 mg/m^3	40 %	46 %
31,300 mg/m^3	43 %	59 %

Table 11: Combined Chronic Toxicity / Oncogenicity Study with Dichlorotrifluoroethane in the Rat : Tumour Findings

Exposure Levels	0 mg/m^3	1 880 mg/m^3	6 250 mg/m^3	31 300 mg/m^3
Males (80 per group)				
Survival at 18 months+	53	54	51	56
Testes, interstitial cell adenoma	4	12*	9	14*
Pancreas, acinar cell adenoma	1	4	12*	14*
Liver, hepatocellular adenoma	3	2	2	8*
Total animals with primary tumours	58	55	56	60
Total animals with benign tumours	55	48	49	53
Total animals with malignant tumours	12	13	21	18
Females (80 per group)				
Survival at 18 months+	50	54	56	65
Liver, hepatocellular adenoma	0	5*	2	6*
Liver, cholangiofibroma	0	0	0	6*
Pancreas, acinar cell adenoma	0	2	0	2
Total animals with primary tumours	60	65	61	62
Total animals with benign tumours	57	63	56	61
Total animals with malignant tumours	19	14	17	16

+ Out of 70 animals

* Statistically different from control $p \leq 0.05$

Assessment of Benign Liver Adenomas:

In the liver there were increased numbers of hepatocellular adenoma in male rats exposed to 31,300 mg/m^3 dichlorotrifluoroethane, although after application of age adjustment statistics the trend was not statistically significant. Incidence was also increased in female rats, and although the dose response relationship was not good, the increase at 1,880 mg/m^3 and 31,300 mg/m^3 and the overall trend were statistically significant.

Dichlorotrifluoroethane is established as a peroxisome proliferator (see Chapter 8.3.4.), probably due to its metabolism to trifluoroacetic acid (see Chapter 7.1.3), a known rat hepatic peroxisome proliferator (Just, 1989; Lock *et al* 1989; Warheit, 1993). Peroxisome proliferation has been identified as a rodent- specific phenomenon, and is causally linked to hepatic tumours in rats and mice (ECETOC, 1992). The finding of a small increase in the incidence of benign hepatic adenoma in rats, following lifetime exposure to dichlorotrifluoroethane, is considered to be associated with its moderate peroxisome proliferating activity, and should not represent a hazard to man.

Female rats exposed to 31,300 mg/m^3 dichlorotrifluoroethane had a small increase in incidence of benign hepatocholangiofibroma. This was not seen at lower exposure concentrations or in males. Also seen in this group of females was a marked increase in the incidence of biliary hyperplasia, and to a lesser degree, cholangiofibrosis. Although hepatocholangiofibromas were also increased, together with hepatocellular tumours, by peroxisome proliferators such as thioacetamide (Becker, 1983) and ethionine (Hiruma, 1983), most peroxisome proliferations do not give rise to this tumour type. Peroxisome proliferation does not appear as an obvious hypothesis for the increase of cholangiofibroma.

Assessment of Benign Testicular Adenomas:

The incidence of benign testicular interstitial cell adenomas was increased in dichlorotrifluoroethane treated groups compared to controls, and the difference was statistically significant for the 1,875 mg/m^3 and the 31,300 mg/m^3 groups. Although adjusting for the increased survival of treated animals showed equivocal significance for a dose-related trend, if only animals surviving to the study end at 2 years are considered, there is clearly a dose-related increase in testicular Leydig cell adenoma in all treated groups.

The benign tumours of the testicular interstitial cells are common in the aging rat. The spontaneous incidence of this tumour type is variable from one strain to another, ranging from a few percent in Sprague-Dawley rats up to 100% in some Wistar derived strains and in Fisher 344 rats (Bär, 1992). These tumours do not usually progress to malignancy in the rat, as is shown by the absence of malignant Leydig cell tumours (*e.g.* none found in several thousands of control Fisher 344 rats: Boorman, *et. al,* 1990; Iawata, *et. al,* 1991). Benign Leydig cell tumours typically appear late in life and are not life-threatening to rats. They are associated with the senescence process. Leydig cells secrete sex hormones (*e.g.* testosterone, dihydroandrosterone, estradiol). The high incidence of hyperplasia and tumours of these testicular cells in old rats is thought to be related to senile endocrine disturbance (Mostofi and Price 1973).

Biegel *et al* (1992) have hypothesized that peroxisome proliferators may increase Leydig cell tumour incidence in rats. This can be seen only if a strain with low spontaneous Leydig cell tumours is used in the bioassay. Most recent carcinogenicity studies were performed on Fisher 344 rats and this may have precluded detecting substances that would have increased Leydig cell adenoma incidences. Substances with rodent hepatic peroxisome proliferation potential that increased Leydig adenoma incidences in non Fisher 344 rat strains were: Gemfibrozil (Fitzgerald *et al,*1981), trichloroethylene (Maltoni *et al,* 1986) and ammonium perfluorooctanoate (Sibinski, 1987). Biegel *et al* (1992) were able to demonstrate an increased formation of Leydig cell adenomas in Sprague-Dawley CRL:CD rats but not in Fisher 344 rats, when treated with the potent hepatic peroxisome proliferator Wyeth 14643. Peroxisome induction was not seen in the testes. At present, therefore, it is not possible to clearly establish causal links between peroxisome proliferation and Leydig cell adenoma.

A more direct hypothesis for the increased incidence of Leydig cell tumours is related with hormonal imbalance. As discussed above, the spontaneous occurrence of Leydig cell adenoma in rats is thought to be related to senile endocrine disturbance (Mostofi and Price, 1973). Some measurements of plasma hormones have been made on rats exposed to dichlorotrifluoroethane for up to 40 weeks (see section 8.3.3). In a study with lansoprazole (Fort *et al,* 1995) decreased testosterone reponsiveness was reported to be associated with the induction of Leydig cell tumours. This effect was not seen in mice and appears to reflect a species specific sensitivity. However these findings were not conclusive although minimal reduction of LH and testosterone were found under HCG stimulation. In contrast to the rat, the Leydig cell tumour occurrence in human is extremely low, representing less than 3% of all testicular neoplasms. The rarity of Leydig cell tumours in human as compared to the high spontaneous incidence in the rat make the relevance of the rat findings to man highly questionable. Consequently, the increased frequency of benign Leydig cell tumours in rats exposed to dichlorotrifluoroethane is not considered to indicate a tumorigenic hazard to humans.

Assessment of Benign Acinor Adenomas:

The incidence of benign pancreatic acinar cell adenoma was increased in a dose-dependent pattern in all male groups exposed to dichlorotrifluoroethane. Although not statistically significant, increased incidence of this tumour was also seen in females exposed at 1,880 mg/m^3 and 31,300 mg/m^3, but not at 6,250 mg/m^3. The incidence was lower in females compared to males. Also increased was the incidence of acinar cell hyperplasia, which is considered the precursor to the adenoma. Interestingly, the incidence of acinar cell atrophy was decreased compared to controls.

The acinar cell's function is to synthesize and secrete digestive enzymes which are released into the duodenum. Release of these enzymes is affected by an increase in circulating levels of the intestinal hormone, cholecystokinin (CCK). Measurement of CCK levels in rats exposed for 26 days with dichlorotrifluoroethane may have shown a slight, non-statistically significant, increase in the level of this hormone (see section 8.3.3). If stimulation of CCK levels is maintained over the duration of the 2-year carcinogenicity study, the increased incidence of acinar cell hyperplasia and adenoma may be a consequence. However, after one year of exposure to dichlorotrifluoroethane there was still no increase in the incidence of hyperplasia, suggesting the changes can be seen only in senescent animals.

It has been noted that treatment with a number of hypolipidemic agents has resulted in pancreatic acinar cell tumours in experimental studies. These have included hepatic peroxisome proliferators such as clofibrate and nafenopin (Svoboda and Azarnof, 1979; Reddy and Rao, 1977; Longnecker, 1983) and gemfibrozil (Fitzgerald *et al,* 1981), which also induced rat hepatocellular and Leydig cell adenomas. However, direct links between peroxisome proliferation and the occurrence of pancreatic tumours are not established at present. Indeed, peroxisome proliferation was not seen in the pancreas of dichlorotrifluoroethane-treated rats (see section 8.3.4).

In view of the marked perturbation of lipid metabolism by dichlorotrifluoroethane, as evidenced by the depression of triglycerides (and cholesterol), it is possible that an endocrine mechanism may lead to stimulation of the exocrine pancreas, and this, over lifetime, may lead to the increase in pancreatic hyperplasia and adenoma.

In conclusion, the above 2-year combined chronic toxicity/carcinogenicity study with dichlorotrifluoroethane in rats has shown increased incidences of liver, testis and pancreatic tumours. These tumours were all benign, appeared late in life and were not life threatening to the animals. In fact animals in the high-level exposure groups had a statistically-significant increase in survival at 2 years compared to controls. Because dichlorotrifluoroethane was assessed as non-genotoxic (see chapter 8.6), the tumours appear to be epigenetic in origin. The hepatic tumours can be linked with the rodent-specific peroxisome proliferation potential of dichlorotrifluoroethane and the testicular and pancreatic tumours may have resulted from enhanced hormonal disturbances in senescent rats. Considering that they occurred late in the life of the rat and were benign, the relevance of these findings is questionable in terms of a carcinogenic risk to man.

9. EFFECT ON MAN

9.1 GENERAL POPULATION EXPOSURE

There are no reported adverse health effects which can be ascribed to dichlorotrifluoroethane exposure.

9.2 OCCUPATIONAL EXPOSURE

There have been no reports of adverse health effects attributable to occupational exposure to dichlorotrifluoroethane.

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