Evaluation of the Genotoxicity Potential and Chronic Inhalation Toxicity of 1,1-Dichloro-1-Fluoroethane (HCFC-141b)*†

R.-J. MILLISCHER‡[1], C. G. de ROOIJ[2], G. M. RUSH[3], C. H. FARR[4],
R. BEN-DYKE[4], C. J. HARDY[5], D. J. LEWIS[5] and G. HODSON-WALKER[6]

[1]Elf Atochem S.A., 4 cours Michelet, Cedex 42, 92091 Paris La Défense, France, [2]Solvay S.A., Brussels, Belgium, [3]AlliedSignal Inc., Morristown, NJ 07962, USA, [4]Elf Atochem N.A., King of Prussia, PA 19406, USA, [4]Ben-Dyke Associates Inc., Whitehouse Station, NJ 08889, USA, [5]Huntingdon Research Centre Ltd, Huntingdon, Cambridgeshire, UK and [6]Pharmaco:LSR, Eye, Suffolk, UK

Abstract—A battery of *in vitro* and *in vivo* tests were conducted on HCFC-141b as a vapour. Bacterial gene mutation assays with *Escherichia coli* and *Salmonella typhimurium* were negative in all tester strains. *In vitro* chromosomal aberration assays were positive on CHO cells but negative on human lymphocytes. Moreover, HCFC-141b was negative *in vivo* in a mouse micronucleus inhalation assay. On the basis of these data and previously reported genotoxicity testing, HCFC-141b is considered non-genotoxic. Groups of 80 male and 80 female Sprague–Dawley rats were exposed, by inhalation (6 hr/day, 5 days/wk) to vapours of HCFC-141b for 104 wk at target concentrations of 0 (control), 1500, 5000 and 20,000 ppm (increased from 15,000 ppm after 17 wk of exposure). No exposure-related effects of toxicological significance were noted with respect to survival, clinical signs, ophthalmoscopy, haematology, clinical chemistry, urinalysis or organ weight analysis. Reduced food intake and body weight gain were noted in both sexes of the 15,000 ppm group during the first 16 wk; thereafter, body weight gains in all groups were similar although the intergroup differences in body weight remained evident. Reduced food intake persisted in both sexes through wk 52 and in females during the second year of exposure. Treatment-related effects on macroscopic pathology were confined to increased incidences of testicular masses and altered appearance. Microscopic pathology examinations confirmed the testes as the target organ with findings of increased incidences of benign interstitial cell tumours and hyperplasia at 5000 and 20,000 ppm. The no-observable-adverse-effect level (NOAEL) was 1500 ppm. The testicular changes at high exposure levels were considered to be due to a change of the senile hormonal imbalance in geriatric rats and of little significance for the assessment of human health effects.

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‡Author for correspondence.

Abbreviations: AIHA = American Industrial Hygiene Association; CHO = Chinese hamster ovary; ECETOC = European Chemical Industry Ecology and Toxicology Centre; HPRT = hypoxanthine phosphoribosyltransferase; IPCS = International Programme on Chemical Safety; LH = luteinizing hormone; NOAEL = no-observable-adverse-effect level; PAFT = Program for Alternative Fluorocarbon Toxicity Testing; PCE = polychromatic erythrocytes; PNE = normochromatic erythrocytes.

INTRODUCTION

1,1-Dichloro 1-fluoro-ethane (CAS No. 1717-00-6, HCFC-141b) is a colourless, volatile liquid (boiling point 32°C, vapour pressure 525 mmHg at 21°C) with a faint ethereal odour. It has been developed primarily as a replacement for CFC 11 in producing rigid polyurethane, polyisocyanurate or phenolic insulating foams. Major uses of these foams include insulation of roofs of commercial buildings (either as foam boards or sprayed directly onto the roof), insulation foam boards for residences, residential cavity insulation or foam-in-place foam fill of surrounding walk-in or domestic refrigerators. A smaller potential application is as a solvent replacement for CFC 113 to remove soldering flux from printed circuit boards, precision cleaning of intricate parts and, when combined with a surfactant, for removing trace water from intricate parts.

The Program for Alternative Fluorocarbon Toxicity Testing (PAFT) is a worldwide

multi-national consortium of chemical manufacturers which undertook an extensive program of toxicity testing for HCFC-141b together with program for other potential CFC replacement materials. Because of the potential exposure during manufacture and use, the testing program included assessment of toxicity and ecotoxicity, as well as oncogenicity, genotoxicity and developmental/reproductive toxicity endpoints. The results presented here are confined to the genotoxicity and oncogenicity testing studies; other aspects of the program will be reported separately. The European Chemical Industry Ecology and Toxicology Centre (ECETOC, 1990) has reviewed much of the available information on HCFC-141b. In previous acute toxicity studies, HCFC-141b was practically non-toxic by the oral, dermal and inhalation routes. The acute LD_{50}/LC_{50} values were greater than 5000 mg/kg (rat), greater than 2000 mg/kg (rabbit) and 61,647 ppm (rat, 4 hr exposure), respectively (ECETOC, 1990). Subchronic inhalation toxicity studies in rats (exposure for 6 hr/day, 5 days/wk) for up to 13 wk indicated a no-observable-adverse-effect level (NOAEL) of 8000 ppm. A minor reduction in body weight gain in this study at 8000 ppm was related to slightly higher exposure chamber temperatures compared with the control chamber, owing to the use of heated volatilization generators (ECETOC, 1990). This NOAEL was reinforced by a similar NOAEL in a subsequent 4-wk study (Hino *et al.*, 1992). Metabolism studies indicated that absorption does occur, but there was no indication of *in vivo* metabolism (ECETOC, 1990). Harris and Anders (1991) confirmed that HCFC-141b is poorly metabolized in the rat; the major metabolite was 2,2-dichloro-2-fluoroethanol, which was eliminated in the urine as a glucuronide conjugate.

Most of the results available on the genetic toxicity of HCFC-141b have been summarized in previous review papers [ECETOC, 1990; International Programme on Chemical Safety (IPCS), 1992]. However, in view of the importance of the genotoxicity data in the assessment of the carcinogenicity hazard, we are reporting more recent information and additional details on the genotoxicity studies conducted on HCFC-141b.

MATERIALS AND METHODS

Test material

HCFC-141b (CAS No. 1717-00-6), a volatile liquid, was supplied for the inhalation study in 1 t pressurized containers by Elf Atochem N.A. and Elf Atochem S.A. with a minimum purity of more than 99.5% (v/v). The purity of each batch of test material was determined by duplicate gas chromatography–mass spectroscopy (GC–MS) analysis in two different laboratories. Smaller samples, all meeting purity criteria as above, were supplied by Elf Atochem N.A., Elf Atochem S.A. and E.I. DuPont de Nemours and Co. Inc. for the genotoxicity studies.

Genotoxicity testing

Test systems/test conditions. Internationally accepted protocols and Good Laboratory Practice rules were followed based on Organisation for Economic Cooperation and Development (OECD), United States Environmental Protection Agency (US EPA), and Japanese Ministry of International Trade and Industry (MITI) guidelines. All studies were conducted using the vapour phase of HCFC-141b. The apparatus used to expose mammalian cell cultures was described in a recent presentation (Hodson-Walker *et al.*, 1993). The bacterial assays were performed in closed stainless-steel vapour exposure vessels. All *in vitro* studies were conducted with and without metabolic activation. Liver S-9 homogenates (S-9 mix) were prepared from Aroclor 1254-treated Sprague–Dawley rats.

Ames test. HCFC-141b was examined for mutagenic activity in five histidine-dependent auxotrophs of *Salmonella typhimurium*, strains TA98, TA1538, TA100, TA1535 and TA1537, and a tryptophan-dependent auxotroph of *Escherichia coli*, strain WP2 uvrA, using a procedure in which agar plates, seeded with the tester strains, were exposed to the test material in the vapour phase. Each test was conducted on two separate occasions. HCFC-141b test concentrations were 0.3, 1, 3, 10 and 30% (v/v) in the first study and 3, 6, 20 and 30% (v/v) in the second study. These exposure levels were selected following a preliminary toxicity test. Following a 48-hr exposure incubation, the plates were removed from the chambers and incubated for a further 24 hr. The actual exposure concentrations were analysed on samples removed from the chambers after 24 hr.

Chromosome aberration assay in Chinese hamster ovary (CHO) K1 cells. The effect of HCFC-141b on chromosome structure was assessed in cultured cells maintained at 37 C in Ham's nutrient F-10 medium supplemented with 10% foetal calf serum and antibiotics. The cells were grown as a monolayer on the internal surface of sterile screw-capped glass bottles, placed on a roller, with a full rotation every 8 min. HCFC-141b was added to the culture vessel to achieve the desired test atmosphere concentrations of 2.5, 5.0 and 10.0% (v/v), based on preliminary assessment of toxicity. The cells were exposed to the test material for 4 hr (with S-9) and for 24 and 48 hr (without S-9). Mitomycin C or cyclophosphamide were used as positive controls. Mitotic indices (calculated as observed metaphases per 1000 cells) and chromosome aberrations (expressed as cells with one or more aberrations from 100 cells) were scored.

Chromosome aberration assay in human lymphocytes. The effects of HCFC-141b on chromosomal structure were investigated in phytohaemagglutinin-stimulated human lymphocytes obtained from a single healthy, non-smoking human male not currently taking any medication. The cultured

lymphocytes were maintained at 37°C for 48 hr, then transferred in sterile screw-capped glass bottles and incubated with HCFC-141b vapours at concentrations of 1.25, 2.5 and 5% (v/v) (without S-9) and 10, 20 and 35% (with S-9). The bottles were placed on a roller with a full rotation every 8 min. Cultures with and without S-9 were incubated for 3 and 24 hr, respectively. Cyclophosphamide or chlorambucil were used as positive control. 1000 metaphase cells spread from two slides of each culture were scored for chromosome aberration.

Mouse bone marrow micronucleus assay. Groups of 15 CD-1 mice per sex were exposed (whole body) for 6 hr to HCFC-141b vapours at concentrations of 0, 3600, 10,000 and 34,000 ppm (v/v) in 20-litre glass cylinder chambers. The test concentrations were selected after an initial range-finding study (10,000, 20,000, 30,000, 40,000 and 80,000 ppm). The exposure to 80,000 ppm induced 60% mortality within 30 min, and 40,000 ppm induced overt narcotic effects. Consequently, 35,000 ppm was selected for the main study as the highest concentration that would not cause excessive toxicity. Bone marrow smears were prepared from the femur of five mice per sex per level, killed 24, 48 and 72 hr after exposure. A group of mice received cyclophosphamide (20 mg, ip) to serve as a positive control. 1000 polychromatic erythrocytes (PCE) were scored for the presence of micronuclei. Micronuclei in normochromatic erythrocytes (PNE) were scored and the PCE:PNE ratio was calculated.

Test atmosphere analyses. Atmospheric samples of each test concentration were removed from the vessels or the chambers, and were analysed by gas chromatography. The actual concentrations were typically very close to the intended concentrations.

Chronic toxicity study

Albino rats of the Sprague–Dawley strain [Crl: CD (SD) BR] were obtained from Charles River Breeding Laboratories (Portage, Michigan, USA). The animals were approximately 4 wk of age at receipt and were housed in groups of four rats by sex in stainless-steel mesh cages. Plastic trays with absorbent liners were placed below each cage to collect excreta. Blood samples were obtained from a sentinel group of 10 males and 10 females prior to study initiation to determine the health status of the colony. Haematological and virological (ELISA; *Mycoplasma pulmonis*, rat corona virus/sialodacryoadenitis virus, pneumonia virus of mice, Sendai virus, Kilham rat virus and Toolan H-1 virus) examinations were undertaken, and all animals were subjected to a detailed autopsy examination.

Animals had *ad lib*. access to food (SDS certified rat and mouse maintenance diet) and tap water

during all non-exposure periods. Analysis of the diet and water indicated that no contaminants were present that could have affected the outcome or integrity of the study. Room temperature and humidity were controlled to target ranges of 21 ± 3°C and 55 ± 15%, respectively. A 12-hr photoperiod was also maintained automatically.

The rats were allocated to four groups containing 80 rats of each sex by a stratified pseudorandomization process based on body weight. The group mean body weights of each group at randomization were 101 g (males) and 81 g (females). An additional 24 rats of each sex were allocated to a sentinel group designated for health screening (ELISA, including pretest and at 8-wk intervals throughout the study duration). Three cages of each sex were allocated to each of the two holding rooms used for the study and were not exposed to the test material.

The treated rats were exposed, using whole-body systems, to vapours of HCFC-141b, 6 hr day, 5 days/wk for 104 wk. The rats were individually housed during exposure. Rotation of the animals within the chamber over a 5-wk period eliminated positional bias. The exposure levels were 0 (control), 1500, 5000 or 15,000 ppm. The high exposure level was increased to 20,000 ppm after 17 wk of exposure in the light of the minimal toxicity noted at that point in the study. Exposures continued during wk 105 and 106 until all surviving rats had been randomly killed. The 5.43 m^3 exposure chambers were constructed of stainless-steel with stainless-steel framed glass panelled doors and were operated under slight negative pressure (15 mmHg below ambient atmospheric pressure) with airflows of approximately 1000 litres/min. Inlet air and the HCFC-141b vapour-containing air stream entered the top pyramidal section of the chamber through a dispersion device. The HCFC-141b vapour was produced by metering the liquid to an all-glass concentric jet atomizer surrounded by an integral water-jacket. The heating jacket was continuously supplied with hot water (80°C) by a thermostirrer pump. The vapour produced by evaporation of the liquid was swept into the chamber with clean, dried, compressed air. At the high exposure level this air supply was heated by passage through a copper coil immersed in the 80°C water-bath. The constant flow of liquid to the atomizers was achieved by the use of nitrogen top pressure to the three reservoirs at 20, 50 and 70 psig* for the 1500, 5000 and 15,000 (20,000) ppm levels. Each liquid reservoir was placed on a load cell to register weight loss during the exposure. In-line filtration systems ensured a clean, particle-free supply from the bulk drums.

Chamber airflow, reservoir weight, temperature, humidity and HCFC-141b chamber concentrations (GC/FID)† were monitored every 7.5 min using a central computerized monitoring and recording system. At the end of each exposure period the data were summarized on a group basis. Chamber atmosphere

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*psig = pounds per sq. in. gauge pressure (1 psig = 6894.76 pascals).
†GC/FID = gas chromatography (flame ionization detector.

samples were withdrawn from four ports in the chamber walls to determine chamber concentration and atmosphere homogeneity.

All cages were checked at least twice daily for dead or moribund animals, and a thorough external examination of all animals was performed once a week. Individual body weights were recorded before the initial exposure, weekly through wk 13 and every 2 wk thereafter. Food intake on a cage basis was recorded weekly throughout the study.

The eyes of all rats used for the study were examined with an indirect ophthalmoscope before exposure initiation and during wk 52 and 104 of exposure. Five rats with ocular abnormalities at the pre-exposure examination were replaced before exposure commenced.

Blood and urine samples were collected during wk 13, 26, 52, 78 and 104. 20 rats of each sex (haematology) or 10 (biochemistry and urinalysis) in each group were sampled, after an overnight period of food deprivation; animals were allowed an approximately 1 hr access to water following exposure and before initiation of urine collection. Blood samples were preserved with sodium citrate for clotting assays, EDTA for all other haematological assays and lithium heparin for clinical chemistry assays. The haematology assays were performed using an Ortho ELT-1500 Hematology Analyzer and included haematocrit, haemoglobin, leucocyte, erythrocyte and platelet counts, cell morphology and thrombotest. Blood smears stained with modified Wright's stain were used for leucocyte differential counts based on counting 100 leucocytes. Mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and mean corpuscular volume were calculated from the measured erythrocytic indices. Following centrifugation at 3200 g for 3 min, plasma samples were analysed using a Roche Cobas Centrifugal Analyzer for glucose, alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, γ-glutamyl transpeptidase, creatine phosphokinase, triglycerides and total phospholipid. Plasma samples were also analysed using a Hitachi 737 Clinical Chemistry Analyzer for total protein, albumin (globulin and albumin/globulin ratio by calculation), urea nitrogen, alkaline phosphatase, total bilirubin, creatinine, sodium, potassium, calcium, inorganic phosphate, chloride and cholesterol. Samples of blood from all rats killed after 52 and 104 wk of treatment were removed prior to autopsy, allowed to clot and analysed for serum fluoride concentration. Urinalysis measurements comprised volume, colour and appearance, pH, specific gravity, osmolality, protein, fluoride and qualitative tests for glucose, ketones, bile pigments, urobilinogen and haem pigments. Following centrifugation at 1500 g for 10 min, the urinary sediment was examined microscopically for epithelial cells, polymorphonuclear leucocytes, mononuclear leucocytes, erythrocytes, organisms, renal tubule cast and other abnormal constituents.

After 52 wk of exposure, 10 predesignated animals of each sex from each group were used for interim autopsy examination (one male in the control, low and high exposure level groups and one female in the control and low exposure groups did not survive to the interim killing interval; these animals were not replaced).

All rats surviving to termination, scheduled for interim killing or either found dead or killed *in extremis*, were subjected to a detailed autopsy examination following exsanguination under pentobarbitone anaesthesia (unless found dead). Organ weights were recorded for brain, pituitary, thyroids, heart, lungs, liver, spleen, kidneys, adrenals and gonads. Approximately 50 tissue samples were preserved in 10% neutral buffered formalin, with the exception of the eyes, which were preserved in Davidson's fluid. The lungs were infused through the trachea prior to immersion in fixative. Light-microscopic examination followed staining with haematoxylin and eosin. Examination of tissues was confined to all animals in the control and high exposure groups, and nasal turbinates, lungs, liver, kidneys, testes and macroscopic abnormalities from the low and intermediate exposure level groups. All tissues from decedents in all groups were also examined. Testicular pathology findings were submitted to an independent peer review. A size criterion was used for distinguishing Leydig cell adenoma from focal hyperplasia: foci with a size larger than the cross-section of one seminiferous tubule were classified as adenoma.

Statistical analysis. Statistical techniques were applied to all measured variables. Bartlett's test was used to test for homogeneity of variance: if the variances were significantly different at the 1% level a logarithmic transformation was applied; if no significant heterogeneity was detected, a one-way analysis of variance followed by a Student's t-test and a Williams' test for dose-related response were performed. For non-parametric evaluations, the Kruskal–Wallis analysis of ranks and the non-parametric equivalent tests for the t-test and Williams' test were used.

RESULTS

Genotoxicity

The two tests conducted to assess the induction of point mutations in prokaryotic organisms in *S. typhimurium* and *E. coli* (May, 1989) and in *S. typhimurium* (May, 1991), respectively, gave negative results in all tester strains with and without S-9 microsome metabolic activation at all test concentrations (up to 35%). Tests to evaluate the potential to cause chromosome damage gave conflicting results. When tested on CHO K1 cells, HCFC-141b increased chromosomal aberration incidences with

and without S-9 metabolic activation (Hodson-Walker, 1990a).

The increases in aberrant cell frequencies over negative control values in non-activated cultures exposed to HCFC-141b at 10% for 4 hr were found to be statistically significant after both excluding and including gaps ($P < 0.001$ and $0.01 > P > 0.001$, respectively). In the presence of S-9 mix, statistically significant increases in aberrant cell frequencies were seen in all treated cultures when gaps were included, and in cultures exposed to 5 and 10% excluding gaps ($P < 0.01$ in all cases). Following 24 hr of exposure to HCFC-141b, no statistically significant increases in the frequencies of aberrant cells were seen at any concentration. However, following 48 hr of exposure, statistically significant increases in aberrant cell frequencies were observed at all HCFC-141b levels, whether gaps were included or excluded.

In the human lymphocyte assay, HCFC-141b was inactive (Hodson-Walker, 1990b). It did not induce statistically significant increases in the frequency of aberrant cells over negative control values at any of the concentrations tested ($P > 0.05$), either in the absence of S-9 mix (HCFC-141b concentrations up to 5%) or in the presence of S-9 mix (HCFC-141b concentrations up to 35%), whether gap-type aberrations were included in or excluded from the analysis.

HCFC-141b was also inactive *in vivo* in the mouse micronucleus assay (Vlachos, 1989). No statistically significant increases in the frequency of micronucleated cells were seen in HCFC-141b-exposed mice at any time of sampling and at any of the test concentrations examined up to and including 34,000 ppm. The PCE:PNE ratios were not significantly changed. Lethargy, tremors and body weight losses were observed in the animals exposed to 34,000 ppm.

Chronic toxicity study

Mean nominal chamber concentrations (standard deviations in parentheses) of HCFC-141b (calculated on weight loss of the reservoir during exposure divided by the total chamber air flow during the 6-hr exposure periods) were 0, 1779 (108), 5932 (258), 14,809 (953) and 21,492 (666) ppm for the control, 1500, 5000, 15,000 and 20,000 ppm groups, respectively. The actual chamber concentrations were 0, 1500 (48), 4998 (132), 15,051 (364) and 19,959 (285) ppm, respectively. Chamber airflows varied between 957 and 1045 litres/minute with temperatures in the range 17.4 to 25.7°C, with the higher temperatures generally recorded in the high exposure level chamber owing to the use of preheated compressed air to facilitate vaporization of the high concentrations needed for this group. The intergroup chamber temperature differences were not considered to have affected the validity of the study. Relative humidity was in the range 31 to 87%.

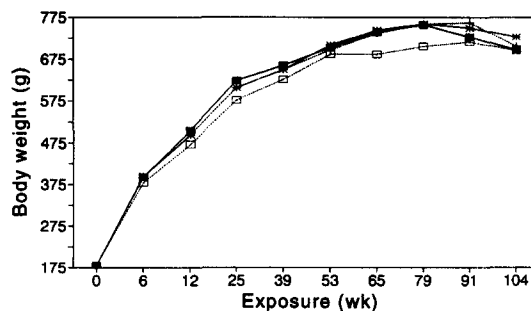No clinical signs were noted in any group associated with exposure to HCFC-141b and overall

Fig. 1. Effect of exposure to HCFC 141b vapour [■, 0 ppm (control); †, 1500 ppm; *, 5000 ppm; □, 20,000 ppm] on the body weight of male Sprague–Dawley rats.

survival in treated groups was similar to, or better than in control groups (approx. 64–69% in males and 46–57% in females). Survival in all male treated groups was higher than in the control group. There were no indications that the presumptive causes of death were related to exposure.

Exposure to HCFC-141b at 15,000 ppm caused a slight, but statistically significant ($P < 0.01$) reduction in food intake and body weight gain (Figs 1 and 2) during the first 15 wk. Food intake was also reduced at 20,00 ppm from wk 17 to 52. The effect on body weight gain was particularly noticeable in males. Subsequently, the rate of body weight gain was similar in treated and control groups despite the increase of the high exposure level to 20,000 ppm. During wk 53–104, food intake in females in the high exposure group was significantly reduced ($P < 0.01$) compared with the controls.

There were no intergroup differences in the types or incidence of ocular abnormalities as detected by ophthalmoscopic examination. Haematological indices and clinical chemistry analyses did not indicate any exposure-related effects, although there were occasional increases in serum triglyceride concentrations. The changes in triglycerides were most obvious during the first months of the exposure period in the high exposure group where levels of increase above control values were +81% ($P < 0.01$), +55% ($P < 0.05$) and +29% (not significant) in females at weeks 13, 26 and 52, respectively, and
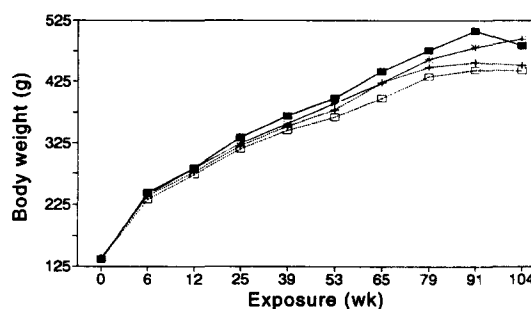

Fig. 2. Effect of exposure to HCFC-141b vapour [■, 0 ppm (control); +, 1500 ppm; *, 5000 ppm; □, 20,000 ppm] on the body weight of female Sprague–Dawley rats.

Table 1. Effect of inhalation of HCFC-141b on the incidence of testicular effects in male Sprague–Dawley rats

	Exposure level (ppm)							
	O (Control)		1500		5000		20,000	
Finding	D†	T‡	D	T	D	T	D	T
No. of animals	32	38	22	48	24	46	25	45
Macroscopic masses	1	1	0	2	0	5*	0	8*
Macroscopic changes at autopsy§	8	21	11	22	12	21	25	29
Tubular atrophy	13	15	5	17	7	19	12	19
Interstitial cell hyperplasia	12	25	10	31	7	35	11	33
Interstitial cell adenoma	1	2	0	4	2	12*	2	10*

†Decedents.
‡Survivors to termination.
§See text for type of changes.
Asterisks indicate significant difference from control (*$P < 0.05$; IARC time-to-tumour test).

were +29% ($P < 0.05$), + 16% (not significant) and + 1% (not significant) in males at wk 13, 26 and 52, respectively. Although relatively small and not always statistically significant, these changes may have been related to exposure to HCFC-141b. Qualitative and quantitative urinalysis data did not indicate any effects of treatment. Occasional statistically significant differences in urinary fluoride concentrations were noted in exposed groups, although these were neither consistent nor of sufficient magnitude to be indicative of an association with treatment.

Organ weight analysis at 52 and 104 wk did not reveal any intergroup differences apart from small increases in kidney weights in females at wk 53 and slightly decreased thyroid weights in females at 20,000 ppm, and slightly increased adrenal weights in females at 5000 and 20,000 ppm after 104 wk. In the absence of any dose response, similar effects in males, and any corroborative microscopic pathology findings, these differences were not considered to be of toxicological significance.

There were no intergroup differences with respect to macroscopic pathology findings after 52 wk. At termination there were no treatment-related macroscopic changes except for testicular masses and testicular abnormalities (small, blue, flaccid, and white subtunical striae), which were slightly in excess over the control group in males at 20,000 ppm (Table 1). Microscopic pathology examinations did not show any treatment-related lesions in females. In males, with the exception of the testes, there were

no effects apart from a small increase in the number of vacuolated sinusoidal histiocytes in the cervical lymph nodes at 20,000 and 5000 ppm. The microscopic pathology examinations confirmed the testes as a target organ with the finding of increased testicular hyperplasia, benign testicular interstitial cell tumour incidence, and marginally increased incidence of seminiferous tubule atrophy. Other differences regarding incidence and occurrence of macroscopic findings were related to geriatric or early neoplastic change and were considered to be unrelated to treatment.

The incidence of benign testicular interstitial cell (Leydig cell) adenomas was increased at 5000 and 20,000 ppm and the increased incidences were outside the historical control range for this strain of rat in the testing laboratory. The tumours were found predominantly in rats killed at termination. Statistical analysis using the International Agency for Research on Cancer (IARC) time-to-tumour method (IARC, 1980), showed that the pairwise comparisons between the control group and the 5000 and 20,000 ppm groups were statistically significant ($P = 0.006$ and $P = 0.043$, respectively). Trend analysis was also statistically significant ($P = 0.037$).

There was no evidence of any treatment-induced neoplasia of the respiratory tract. A polypoid adenoma of the nasal turbinates was found in a male rat at 20,000 ppm, pulmonary adenomas in single male rats at 5000 and 20,000 ppm and a pulmonary adenocarcinoma in a male rat at 20,000 ppm. The

Table 2. Comparison of tumour incidence in male Sprague–Dawley rats after inhalation of HCFC-141b

	Exposure level (ppm)							
	O (Control)		1500		5000		20,000	
Finding	D†	T‡	D	T	D	T	D	T
No. of animals	32	38	22	48	24	46	25	45
Animals with tumours	29	33	20	35	19	35	22	42
Maligant tumours	17	17	12	9	8	7	12	11
Benign tumours	23	28	13	31	15	33	18	39
Multiple tumours	21	22	8	13	10	16	15	21
Single tumours	8	11	12	22	9	19	7	21
Multiple malignant	7	5	3	2	4	1	3	0
Multiple benign	9	15	2	11	6	14	11	18
Metastatic tumours	0	4	1	0	0	0	1	0

†‡As in Table 1.

Table 3. Comparison of tumour incidence in female Sprague–Dawley rats after inhalation
of HCFC-141b

| Finding | O (Control) | | | 1500 | | 5000 | | 20,000 |
	Dt	Dt	D	T	D	T	D	T
No. of animals	35	35	33	37	38	32	30	40
Animals with tumours	32	33	31	28	37	23	27	32
Malignant tumours	13	7	7	11	13	5	11	13
Benign tumours	30	33	30	24	34	22	23	28
Multiple tumours	18	23	14	14	16	12	14	16
Single tumours	14	10	17	14	21	11	13	16
Multiple malignant	2	1	2	3	1	1	4	2
Multiple benign	11	21	10	9	11	10	7	11
Metastatic tumours	2	1	1	0	2	0	1	3

The header also shows "Exposure level (ppm)" spanning the data columns.

t,tAs in Table 1.

types and incidences of these and other neoplasms were within the range of normal expectation and did not reflect any effect of treatment (Tables 2 and 3).

An increased incidence of moderate interstitial hyperplasia (Table 1) was noted in the testes of rats at 20,000 ppm, although this difference from control did not attain statistical significance. A similar increased incidence at 5000 ppm was more marked and did attain statistical significance ($P < 0.05$). No effect was seen at 1500 ppm. The incidence of testicular seminiferous tubules with moderate to marked degrees of atrophy were marginally increased in the 20,000 ppm group compared with the controls but the difference was not statistically significant (Table 1). This finding correlated with the increased incidence of small/blue/flaccid/white striae noted at autopsy. Testicular atrophy was not seen at either 1500 or 5000 ppm.

DISCUSSION

The genetic toxicity of HCFC-141b, with respect to both mutation and clastogenicity endpoints, was evaluated in the testing program. HCFC-141b appeared to be clearly non-mutagenic in the Ames bacterial test on two occasions. ECETOC (1990) and IPCS, 1992 reported results from assays conducted on HCFC-141b prior to standardization of sample purity and impurity content by the testing consortium. One negative Ames test finding was reported, but two positive results with and without S-9 mix with the TA1535 *S. typhimurium* strain were also noted. It is not known whether the HCFC-141b samples used for these ECETOC-reported studies met the analytical criteria established for the majority of the testing program. The potential for abnormal impurities or high levels of expected contaminants may explain the isolated positive results noted in some of the earlier test results.

ECETOC (1990) and IPCS (1992) also reported results of a hypoxanthine phosphoribosyltransferase (HPRT) assay with V79 Chinese Hamster cells exposed to HCFC-141b vapours up to 35%. There was no increase in the mutation frequency at the HPRT gene locus in the presence or in the absence of S-9. Furthermore, negative results were also reported

in a DNA repair assay with *E. coli* (WP2, WP67 and WB71) exposed to HCFC-141b liquid phase up to 10 mg/ml with and without S-9. These negative results in the HPRT V79 cell and DNA repair assays reinforce the overall conclusion that HCFC-141b is not mutagenic.

Tests for chromosome aberration yielded somewhat conflicting results. In the PAFT *in vitro* chromosome aberration assay, HCFC-141b was found positive on CHO cells but negative with human lymphocytes. Furthermore, the material was found negative in an *in vivo* mouse bone marrow micronucleus assay. ECETOC (1990) and IPCS (1992) reported two additional chromosome aberration assays using CHO cells: one study gave positive results with and without S-9 with HCFC-141b vapours, whereas the other was, at most, equivocal after liquid HCFC-141b was incorporated directly in the culture medium. A second *in vivo* mouse micronucleus test was also reported to be negative (ECETOC, 1990). The micronucleus test reported by ECETOC as well as the above-reported micronucleus test were conducted at sublethal concentrations high enough to induce overt toxicity signs in the mice, including nervous system depressant effects. There was no indication of toxicity to bone marrow as evidenced by alteration of the PCE:PCN ratios. However, HCFC-141b is likely to be freely distributed in blood and tissues, including the bone marrow, because it is a small two-carbon molecule that can easily diffuse from the blood to tissue compartments, as demonstrated by the rapid-onset anaesthetic properties that indicate that HCFC-141b crosses the blood–brain barrier.

On the basis of the weight of evidence, ECETOC (1990) concluded that HCFC-141b does not demonstrate toxicologically significant mutagenic activity and the American Industrial Hygiene Association Workplace Environmental Exposure Level (WEEL) Committee (AIHA, 1992) stated that it is not mutagenic.

The statistically significant increases in late-onset benign testicular interstitial tumours at 5000 and 20,000 ppm must be analysed in the context of the absence of genotoxic potential defined in the *in vitro* and *in vivo* mutagenicity assays. A non-genotoxic

(epigenetic) mechanism needs to be considered to explain the increased incidence of these tumours.

Non-germinal cell testicular tumours in humans are extremely rare (Chomette *et al.*, 1985; Damjanov *et al.*, 1979; Mostofi, 1977). The incidence of Leydig cell tumours in these studies was maximally 3% of the total number of testicular tumours. Other epidemiology studies in the US (Damjanov *et al.*, 1979; Devesa and Silverman, 1978), France (Haillot *et al.*, 1989) and Ireland (Thornhill *et al.*, 1988) yield a combined incidence of 0.1–3 Leydig cell tumours per million men. The predominant testicular tumour types in humans are germinal cell tumours such as seminomas, teratomas and teratocarcinomas.

In rats, Leydig cell adenomas are age-associated benign tumours and are not as uncommon as in humans. The natural incidence varies according to strain and is most pronounced in the Fischer 344 strain where it often reaches 90–100% in 18–24-month-old animals (Bär, 1992). There is extensive evidence that the induction of Leydig cell tumours in rodents occurs by an epigenetic mechanism that involves excessive gonadotrophic stimulation, as reviewed by Bär (1992). That author concluded that Leydig cell tumours most commonly occur in rats as a result of increased pituitary luteinizing hormone (LH) secretion, increased testicular LH/human chorionic gonadotrophin (HCG) receptors or impaired androgen biosynthesis or action.

A wide variety of materials (including lactose and lactitol), and procedures have been shown to induce the formation of Leydig cell tumours (Bär, 1992). It has been reported by Bär (1992) and Roberts *et al.* (1989) that an increased incidence of interstitial cell tumours followed ligation of testicular blood vessels, intrasplenic grafting of the testis, gonadotrophin injection and radiation. Administration of a wide variety of unrelated drugs, such as cimetidine, hydralazine, gemfibrizol, carbamazepine, vidarabine, mesulargine, isradipine and metronidazole, has also resulted in elevated interstitial cell tumour masses (Griffith, 1988). Senility *per se* is also a significant contributor to the appearance of Leydig cell tumours in rats: for example, Fischer 344 rats have a 100% incidence of interstitial cell tumours by the end of their life. In the cases of mesulargine (Prentice *et al.*, 1992) and isradipine (Roberts *et al.*, 1989) the association of increased LH and reduced prolactin with the induction of Leydig cell tumours was demonstrated.

There is no evidence in humans that there is any association between the occurrence of Leydig cell tumours and hormonal imbalance. Case–control studies in humans have shown no relationship to prenatal oestrogen treatment (Henderson *et al.*, 1970; Leary *et al.*, 1984; Loughlin *et al.*, 1980; Schottenfeld *et al.*, 1980), cadmium exposure (Armstrong and Kazantzis, 1983; Elinder *et al.*, 1985) or chronically administered drugs (Bär, 1992) that induce Leydig cell tumours in rats. Thus the development of Leydig cell tumours in rats appears to be a species-specific phenomenon that has little, if any, relevance to humans.

The onset time of the hormonal perturbation in rats for mesulargine was approximately 4 wk and for isradipine was 52–66 wk, although the time of appearance of tumours (approx. 2 yr) was the same. In control rats, Leydig cell tumours usually occur very late in life (20–24 months) and old age appears to be a prerequisite for the development of this tumour in rodents. This late onset is also a characteristic of chemically induced Leydig cell tumours. In all cases the tumours were benign and there was no evidence of progression to malignancy. In fact, these tumours in rodents are not considered to progress to malignancy (J. F. Hardisty, personal communication, 1993).

HCFC-141b is chemically unreactive, is poorly metabolized *in vivo* and does not accumulate in the tissues. Metabolism of HCFC-141b is minimal. Approximately 1% of the material was dechlorinated *in vitro* when rat hepatic microsomes were incubated with HCFC-141b (Van Dyke, 1977) and no increases in urinary fluoride were detected in a 90-day rat inhalation study using Fischer 344 rats, or in the present study in Sprague–Dawley rats.

In vivo inhalation exposure of Sprague–Dawley rats in a screening test failed to demonstrate any metabolism using a closed-loop exposure system with continuous monitoring of the exposure atmosphere by infra-red analysis (ECETOC, 1990). The method was estimated to be sensitive enough to detect metabolism of the material above 0.15%. Exposure of male Fischer 344 rats for 2 hr to $55,200 \, mg/m^3$ and subsequent collection of urine and expired air demonstrated the presence of a single fluorinated metabolite (2,2-dichloro-fluoroethanol glucuronide) at a concentration of $1 \, \mu mol$. Analysis of the cytosolic and microsomal fractions of the livers of the exposed rats using ^{19}F nuclear magnetic resonance technology did not detect any covalent binding of fluorinated species (Harris and Anders, 1991). The limited metabolism and retention of HCFC-141b would predict a minimal potential for biological activity through reactive metabolic pathways.

The basic characteristics of the rat spontaneous interstitial cell tumours in the present study were not altered by exposure to HCFC-141b. The tumours (a) occurred predominantly on ageing animals, (b) were all of benign nature and (c) were not life-threatening. Consequently, a pituitary–testicular axis disruption that has been shown to be linked with all known exogenous causes of increased incidence of Leydig cell tumours in rats (Bär, 1992; Cook *et al.*, 1992; Rao and Reddy, 1987) can be considered as a likely explanation for the effect of HCFC-141b.

Estimates of exposure concentrations during the manufacture of insulating foam indicate the levels are significantly below the current generally accepted exposure guideline of 500 ppm (AIHA, 1992). Modelling of residential exposure resulting from the

slow release of HCFC-141b from insulating foam used in home construction indicates that time-weighted average concentrations in the first year of occupancy are likely to be maximally 10–15 ppb, and over the first 9 yr of occupancy approximately 5 ppb (*Environ*, 1992). These potential exposures were estimated using worst-case scenarios of exposure time and concentration.

The strong suggestion of an underlying hormonal mechanism and the absence of genotoxicity potential, in conjunction with the late onset of the Leydig cell tumours in the rat, and the extreme rarity of such tumours in humans, indicate that a threshold exists for HCFC-141b and, thus the risk characterization should be based on the NOAEL for tumours of 1500 ppm, although the NOAEL for systemic toxicity was 5000 ppm. The two-stage linear carcinogenesis model is not appropriate for the epigenetic mechanism of tumorigenesis.

The overall assessment of the toxicological and genotoxicity data indicates a low order of toxicity, a lack of genotoxic potential and a chronic NOAEL several orders of magnitude higher than probable human exposures. On the basis of the minimal human exposure potential compared with the very high exposures received by laboratory animals and the lack of relevance to human risk assessment of the interstitial cell tumours in exposed rats, it is concluded that HCFC-141b is not likely to pose a risk to human health.

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