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RESEARCH ARTICLE

The acute, genetic, developmental and inhalation toxicology of trans-1-chloro,3,3,3-trifluoropropene (HCFO-1233zd(E))

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Abstract

Trans-1-chloro,3,3,3-trifluoropropene (HCFO-1233zd(E)) is being developed as a foam blowing agent, refrigerant and solvent because it has a very low global warming potential (<10), as contrasted to the hydrofluorocarbons (>500). The toxicology profile is described. The acute 4-hour 50% lethal concentration value in rats receiving HCFO-1233zd(E) was 120 000 ppm. The no observed effect level (NOEL) in cardiac sensitization studies in dogs was 25 000 ppm. In a 2-week range-finding study, rats were exposed to HCFO-1233zd(E) at levels of 0, 2000, 7500 and 20 000 ppm 6 hours/day for 5 days/week. Histopathological changes in the heart described as multifocal mononuclear infiltrates were observed in males (mid- and high-exposure group) and females (high-exposure group), suggesting this organ was the target for HCFO-1233zd(E) toxicity. In a 4-week study, rats were exposed to 0, 2000, 4500, 7500 and 10 000 ppm. The only finding was an increase in potassium (mid- and high-exposure males). No increase was observed after a 2-week recovery period, nor in a subsequent 13-week toxicity study. In a 13-week study, rats were exposed to 4000, 10 000 and 15 000 ppm 6 hours/day for 5 days/week. Findings consisted of multifocal mononuclear cell infiltrates in the heart with a NOEL/lowest observed adverse effect level of 4000 ppm. No genetic toxicity was observed in a battery of genetic toxicity studies. In a rat prenatal developmental toxicity study, dilated bladders were observed in the high-exposure group fetuses (15 000 ppm), a finding of unclear significance. HCFO-1233zd(E) was not a developmental toxin in rabbits, even at exposure levels up to 15 000 ppm.

Keywords

Foam blowing agent refrigerant, low global warming

History

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Introduction

Chlorofluorocarbons (CFCs) were introduced in the 1930s as ''safe'' replacements for refrigerants, such as sulfur dioxide, ammonia and carbon tetrachloride. During the next 40–50 years, the number and type of applications expanded to include foam blowing, precision cleaning and propellants for medicinal, cosmetic and general-purpose aerosols, air conditioning and refrigeration. These uses resulted in emission of CFCs into the atmosphere. Because of their low chemical reactivity, they typically have long atmospheric residence times, and, as a consequence, they are distributed globally (AFEAS, 1991; ECETOC, 1994; IPCC, 1994; Lashof & Ahuja, 1990; WMO, 1991a, b, 1999).

In 1974, Molina and Rowland hypothesized that, once CFCs reach the stratosphere, they will undergo breakdown to release chlorine atoms. The chlorine atoms could then react with the stratospheric ozon,e breaking it down into oxygen. Because the stratospheric ozone absorbed much of the sun's ultraviolet β radiation (UVB), decreased ozone levels would lead to increases in ground-level UVB (Molina and Rowland, 1974). This could affect crop growth and lead to increases in cataracts and nonmelanoma skin cancers. Subsequent to reports of a marked drop in column ozone over Antarctica (the ''ozone hole'') during the Antarctic winter, in 1987, most of the nations of the world drafted and signed an agreement calling for the phase-out of CFCs (WMO, 1991a). This agreement is known as the Montreal Protocol.

Initial development focused on two types of ''in-kind'' replacements (ECETOC, 1994; Kawano et al., 1995). The first were hydrochlorofluorocarbons (HCFCs) and the second were hydrofluorocarbons (HFCs). Both contain hydrogen and are susceptible to attack by hydroxyl radicals present in the atmosphere. Therefore, they have a shorter atmospheric lifetime and either are not transported to the stratosphere or are transported there only in small amounts. HCFCs contain chlorine and are still capable of causing ozone depletion, although, because their atmospheric lifetimes are short, their ozone-depleting potential is lower than those associated with CFCs. HFCs do not contain chlorine (or bromine, also associated with ozone depletion). Therefore, they do not cause ozone depletion.

A second concern for CFCs, as well as their replacement, HCFCs and HFCs, is that they are global warming gases.

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They, along with other substances such as carbon dioxide, trap the sun's infrared radiation and convert it to heat. However, they are also good insulating materials, and frequently their use as foam blowing agents in refrigeration equipment can lead to considerable energy savings, reducing carbon-dioxide emissions. Due to their global warming potential, HFCs and HCFC are under environmental and regulatory pressure worldwide. Recent studies have focused on a new class of molecules, fluorinated olefins, which have low global warming and little or no ozone depletion potential. As some members of this class of compounds can be toxic, these substances require careful screening. This article describes the results of several of the studies conducted with trans-1-chloro,3,3,3-trifluoropropene (HCFO-1233zd(E)) as it was being developed as a replacement for the HFCs and HCFCs in foam blowing, solvent, and refrigerant applications.

Methods

All studies were conducted according to good laboratory practices. When available, studies followed Organization for Economic Cooperation and Development (OECD) guidelines. Protocols for all studies involving animals were reviewed by the respective laboratory's institutional review board and approved.

Test substance

HCFO-1233zd(E) (CAS no. 102687-65-0) is a colorless, nonflammable liquefied gas with a boiling point of 19 °C, water solubility of 1.90 g/mL, log octanol:water partition coefficient of 2.2 (measured) and Henry's law constant of 4.1×10^3 bars at 20 °C. It was supplied as a liquefied gas in cylinders from Honeywell (Buffalo, NY) at a minimum purity of 99.8%. All batches were analyzed by gas chromatography (GC)/mass spectroscopy before shipment.

Acute inhalation toxicity

Rat acute toxicity

Four groups of 5 male and 5 female Sprague-Dawley (SD) CD rats (Charles River Deutschland, Sulzfeld, Germany), approximately 10 weeks old, were exposed for 4 hours to vapors of HCFO-1233zd(E) in a nose-only exposure unit. One group served as an air control, and the other three groups were exposed to approximate levels of 96 000 (9.6%), 120 000 (12%) and 156 000 ppm (15.6%) of HCFO-1233zd(E). The test atmosphere was generated either directly as a gas controlled by a calibrated rotameter (96 000 and 156 000 ppm) or by evaporation of a liquid flow controlled by a peristaltic pump (Minipulse; Gilson, Villiers le Bel, France). The actual concentration was measured with a total carbon analyzer (Bernath Atomic, Wennigsen, Germany). Oxygen was added to the test atmosphere, as necessary, to maintain oxygen content at ambient levels. Before exposure, animals were acclimated for 1–2 weeks. During nonexposure periods, rats were group-housed (5 males or 5 females per cage) in Macrolon cages with wood shavings (Lignocel, type ¾; Rettenmaier, Rosenberg, Germany). Food (Rat & Mouse No. 3 Breeding Diet, RM3; SDS Special Diets Services, Witham, UK) and drinking water (tap) were provided *ad libitum*, and the animal room was maintained at 22 ± 2 °C with relative humidity between 30 and 70%. Rats were held for 14 days after exposure and sacrificed by exsanguination under pentobarbital anesthesia. Body weights and detailed clinical observations were recorded on day 0 (before exposure) and days 7 and 14.

Cardiac sensitization

The potential for HCFO-1233zd(E) to induce cardiac sensitization to adrenalin was evaluated following the procedure described by Rusch et al. (1999) and ECETOC (2009). A group of 6 Beagle dogs (Ridglan Farms, Mt. Horeb, WI) was trained to accept the procedure of being exposed to a vapor with a nose exposure device while standing in a sling. The maximum dose of adrenalin was determined for each dog as described in Example II, ECETOC (2009). On the day of exposure, dogs were given an initial injection of adrenalin, then after a 5-minute washout period, exposure to HCFO-1233zd(E) at one of the preset levels (0, 25 000, 35 000 or 50 000 ppm) was initiated. After 5 minutes of exposure, dogs were given a second injection of adrenalin at the same dose as the first. Exposure was continued for 5 minutes, and dogs were monitored for the development of arrhythmias by means of a continuous electrocardiography (EKG) tracing. Test sample atmospheres were prepared in Tedlar® bags and analyzed for HCFO-1233zd(E) by GC before exposure. At the initiation of the exposure, the 3-way valve was turned to the bag position, whereas during nonexposure periods it was in the filtered air position. Each dog served as its own control. The same dogs were used for all exposures. After each exposure, dogs were given at least 2 days of rest before being given the next exposure.

2-Week inhalation toxicity study

Four groups of 5 male and 5 female SD rats (Charles River Deutschland), approximately 7 weeks of age, were whole-body exposed to vapors of HCFO-1233zd(E) (6 hours/day, 5 days/week) for 2 weeks at concentrations of 0, 2000, 7500 and 20 000 ppm. Target values for concentrations were 0, 2000, 7500 and 20 000 ppm. Animals were assigned to groups using a computer randomization program, taking body weights into account. During nonexposure periods, rats were housed 5 per cage, separated by sex, in Macrolan cages with wood shavings (Lignocel, type ¾; Rettenmaier, Rosenberg, Germany) in an animal room maintained at 22 ± 2 °C with relative humidity between 30 and 70%. A 12-hour light/dark cycle was provided. Food (Rat & Mouse No. 3 Breeding Diet, RM3; SDS Special Diets Services) and drinking water (tap) were provided *ad libitum* from arrival until the end of the study, except during inhalation exposures. Animals were observed group-wise. Each animal was observed daily before exposure, and group-wise observations were made during exposure. Body weights were recorded on days −3 and −1, before the first exposure, then on days 0, 7, 13 and 14 before sacrifice. Food consumption was measured weekly.

Animals were exposed to the test atmosphere in 200-L whole-body exposure chambers. Each chamber accommodated 10 wire cages. Animals were rotated each day with respect to position in the chamber.

The test atmosphere was generated by evaporation of a liquid flow of test substance controlled by a peristaltic pump (Minipulse; Gilson). The actual concentration was measured with a total carbon analyzer (RS55 and RS55T; Ratfisch, Munich, Germany). Body weights were measured on days −3, −1 and 0 before exposure and days 1, 4, 7 and 13 (at sacrifice). Food consumption/cage was measured for the first 7 and final 6 days on test. Blood samples were collected at sacrifice on fasted rats using K_2-EDTA (ethylenediaminetetraacetic acid) as an anticoagulant. Blood was analyzed for hemoglobin, packed cell volume, red blood cells (RBCs), reticulocytes, white blood cells (WBCs), differential count, prothrombin time, and thrombocyte count. Mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated. Clinical chemistry parameters that were measured or calculated include alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), total protein, albumin (ALB), albumin/globulin (ALB-GLOB) ratio, urea, creatinine, fasting glucose, bilirubin total, cholesterol, triglycerides (TGs), phospholipids, calcium, sodium, potassium, chloride and inorganic phosphate.

At sacrifice, organ weights were collected on the brain, adrenals, heart, kidneys, liver, spleen, testes and lungs with trachea and larynx. These tissues and the remainder of the respiratory tract, including nasal passages and any gross lesions, were collected in 10% neutral buffered formalin; lungs were fixed under 15 cm of water pressure. All tissues from the high-level group and controls were embedded in paraffin wax, sectioned at 5 µm, stained with hematoxylin and eosin (H&E) and examined under a light microscope. Where microscopic lesions were noted, the same tissues were examined from the low- and mid-level exposure groups.

4-Week inhalation toxicity study

Five groups of 5 male and 5 female SD rats (Charles River Deutschland), approximately 7 weeks of age, were exposed nose only (6 hours/day, 5 days/week) for 4 weeks to vapors of HCFO-1233zd(E) at concentrations of 0, 2000, 4500, 7500 and 10 000 ppm. Animals were assigned to groups using a computer randomization program, taking mean body weights into account. During nonexposure periods, rats were housed 5 per cage, separated by sex, in Macrolon cages with wood shavings (Lignocel, type ¾; Rettenmaier, Rosenberg, Germany) in an animal room maintained at 22 ± 2 °C with relative humidity between 30 and 70%. A 12-hour light/dark cycle was provided. Food (Rat & Mouse No. 3 Breeding Diet, RM3; SDS Special Diets Services, Witham, England) and drinking water (tap) were provided *ad libitum* from arrival until the end of the study, except during inhalation exposures. Each animal was observed daily before exposure, and during exposure animals were observed group-wise approximately mid-way during each exposure. Body weight was recorded on days −3 and −1, before the first exposure, then on days 0, 7, 14, 21 and prior to sacrifice. Food consumption was measured for 7-day increments while on test. Male animals from all exposure-level groups were used for micronucleus evaluation, whereas male rats from the control, 7500 - and 10 000-ppm groups were used for the unscheduled DNA synthesis (UDS) evaluation. These procedures are described below.

Animals were exposed to the test atmosphere in nose-only exposure units (modification of the chamber manufactured by ADG Developments Ltd., Codicote, UK). Each unit consisted of a cylindrical polypropylene or stainless steel column surrounded by a transparent cylinder with a volume of approximately 50 L. Each column consisted of two rodent tube sections with 20 ports for exposures. Empty ports were used for test atmosphere sampling, temperature and humidity measurements and oxygen-level determinations. Animals were secured in nose-only exposure units (Battelle Pacific Northwest Laboratories, Richland, WA) positioned around the central column. All unused ports were closed. The central column was maintained at a slightly positive pressure relative to the laboratory, whereas the outside hood enclosure was kept at a slightly negative pressure relative to the laboratory.

The exposure concentration was generated by mixing a controlled amount of liquid test substance using a peristaltic pump (Minipulse 3; Gilson) with a mass flow-controlled stream (Bronkhorst; Hi Tec, Ruurlo, The Netherlands) of humidified compressed air and oxygen for the two higher exposure levels. The test substance was allowed to evaporate in a U-tube at 30 °C and was led to the entrance at the top of the exposure chamber. The concentrations in the exposure atmospheres were monitored continuously by total carbon analysis (RatfischRS55 or RatfischRS55T; Ratfisch). Readings were recorded every minute on a PC using a CAN transmitter (G. Lufft Mess- und Regeltchnik Gmbh, Fellbach, Germany). Temperature and humidity levels were recorded in the same way.

Blood samples were collected at sacrifice on fasted rats under pentobarbital anesthesia, using K_2-EDTA as an anticoagulant. Because male rats were being used for UDS synthesis on day 28, blood was taken from these animals on day 24. Blood was analyzed for hemoglobin, packed cell volume, RBCs, reticulocytes, WBCs, differential count, prothrombin time and thrombocyte count. MCH, MCV and MCHC were calculated. Blood for clinical chemistry evaluations was collected in heparinized vials. Clinical chemistry parameters that were measured or calculated included ALP, AST, ALT, GGT, total protein, ALB, ALB-GLOB ratio, urea, creatinine, fasting glucose, bilirubin total, cholesterol, TGs, phospholipids, calcium, sodium, potassium, chloride and inorganic phosphate.

At sacrifice, organ weights were collected on the brain, adrenals, heart, kidneys, liver (all females, 2000 ppm; males, 4500 ppm), spleen, testes, thymus, epididymides and lungs with trachea and larynx. These tissues and the remainder of the respiratory tract, including nasal passages, pancreas, skin, testes, thymus, brain, pituitary gland, spinal cord, eyes, thyroid gland, parathyroid gland, heart, stomach, small intestines, colon, Peyer's patch, ovaries, uterus, epidymis, urinary bladder, lymph nodes, sciatic nerve, femur and any gross lesions, were collected in 10% neutral buffered formalin. Lungs were fixed under 15 cm of water pressure. All tissues from the high-level group and controls were embedded in paraffin wax, sectioned at 5 µm, stained with H&E and examined under a light microscope. Where microscopic

lesions were noted, the same tissues were examined from the low- and mid-level exposure groups.

UDS was conducted according to the procedure described by Butterworth et al. (1987) and OECD guideline 486 (OECD, 1997a). As noted above, sections of liver from male rats in the control, 7500 and 10 000 ppm exposure-level groups were analyzed for UDS. In addition, a positive control of 5 male rats, dosed with 2-acetylaminofluorene at 10 mL/kg of a solution of 5 mg/mL in corn oil, was included. At sacrifice (for the positive controls, 12–16 hours after treatment), hepatocytes were isolated from liver using the perfusion technique described by Williams (1977). After isolation, dissociated cells were incubated for 5–10 minutes in a shaking water bath at 37 °C. Thereafter, they were filtered over a 200-mesh nylon filter, centrifuged and resuspended in Williams medium E complete. Cell counts were made with a hemocytometer. Cell viability was determined by trypan blue exclusion. In negative controls, acceptable viability was >50%. Cells were plated on Thermanox® 25-mm round plastic coverslips, washed and incubated in Williams E medium incomplete at 37 °C for 18 hours, washed and immersed in 2 mL of a 1% sodium citrate solution for 10 minutes and then fixed in absolute ethanol/acetic acid (3:1). The resulting slides were processed for autoradiography using Ilford K5D emulsion, developed with Kodak D19, fixed in Kodak Fixer, stained with H&E and embedded in Pertex. Grain counting was conducted with an Artek electronic counter with a microscopic attachment.

The micronucleus assay was conducted according to the procedure described by Aardema (1995), Lovell et al. (1989) and OECD Guideline 474 (OECD, 1997b) using rats from the air control, 2000, 4500, 7500 and 10 000 ppm exposure-level groups. Additionally, a positive control group injected with mitomycin C (10 mL/kg of a solution of 0.15 mg/mL in saline) was included. Briefly, immediately after sacrifice, bone marrow cells were collected in fetal calf serum from one femur and processed into two glass-drawn smears. One smear was fixed with a May-Grunwald Giemsa solution, whereas the other was kept as reserve. The number of polychromatic (PE) and normochromatic (NE) erythrocytes were recorded in a total of 200 cells per animal. The number of micronucleated PE and NE erythrocytes were recorded and an additional 2000 PE cells were scored for the presence of micronuclei (MPE). The total number of MPE was compared to negative and positive control values.

13-Week inhalation toxicity study

Four groups of 10 male and 10 female SD rats (Charles River Deutschland), approximately 7–8 weeks of age, were exposed (6 hours/day, 5 days/week) for 4 weeks to vapors of 0, 4000, 10 000 and 15 000 ppm of HCFO-1233zd(E). Animals were assigned to groups using a computer randomization program, taking body weights into account. During nonexposure periods, rats were housed 5 per cage, separated by sex, in Macrolon cages with wood shavings (Lignocel, type ¾; Rettenmaier, Rosenberg, Germany) in an animal room maintained at 22 ± 2 °C with relative humidity between 30 and 70%. A 12-hour light/dark cycle was provided. Food (Rat & Mouse No. 3 Breeding Diet, RM3; SDS Special Diets Services) and drinking water (tap) were provided *ad libitum* from arrival until the end of the study, except during inhalation exposures. Each animal was observed daily before exposure, groups were observed during exposure, body weights were recorded on days −2 (females only) and −1 (males only), before the first exposure, then on days 0 and 2, weekly thereafter and before sacrifice. Food consumption was measured for combined groups of 5 rats weekly as the rats were group-housed.

Animals were exposed to the test atmosphere in nose-only exposure units. Operation of these exposure units, measurements of exposure levels, environmental conditions and measurements of these conditions were the same as those described above for the 4-week study.

Blood samples were collected at sacrifice on fasted rats under pentobarbital anesthesia, using K_2-EDTA as an anticoagulant. Blood was analyzed for hemoglobin, packed cell volume, RBCs, reticulocytes, WBCs, differential count, prothrombin time and thrombocyte count. MCH, MCV and MCHC were calculated. Blood for clinical chemistry evaluations was collected in heparinized vials. Clinical chemistry parameters that were measured or calculated included ALP, AST, ALT, GGT, total protein, ALB, ALB-GLOB ratio, urea, creatinine, fasting glucose, total bilirubin, cholesterol, TGs, phospholipids, calcium, sodium, potassium, chloride and inorganic phosphate.

At sacrifice, organ weights were collected on the adrenals, brain, heart, kidneys, liver, spleen, testes, thymus, thyroid (with parathyroids) and lungs with trachea and larynx, ovaries, uterus and epididymides. In addition, the remainder of the respiratory tract (including nasal passages), aorta, axillary lymph nodes, cecum, colon, eyes with optic nerve, femur, mammary glands, mandibular (cervical) lymph nodes, nasal passages with teeth, peripheral nerve, esophagus, parotid salivary glands, pituitary, prostate, rectum, seminal vesicles with coagulating glands, skeletal muscle (thigh), skin (flank), small intestines (duodenum, ileum and jejunum), spinal cord, sternum with bone marrow, stomach (glandular and nonglandular), sublingual salivary glands, testes, thymus, tracheobronchial lymph nodes, ovaries, pancreas, parathyroids, pharynx, urinary bladder, uterus (with cervix) and any gross lesions, were collected in 10% neutral buffered formalin; lungs were fixed under 15 cm of water pressure. All tissues from the high-level group and controls were embedded in paraffin wax, sectioned at 5 µm, stained with H&E and examined under a light microscope. Where microscopic lesions were noted, the same tissues were examined from the low- and mid-level exposure groups.

Genetic assays

Reverse mutation

The reverse mutation assay was conducted according to the procedure previously described (Kawano et al., 1995; Maron and Ames, 1983). Four strains of *Salmonella typhimurium* (TA98, TA100, TA 1537 and TA1535) as well as *Escherichia coli* WP2 uvrA were employed. Plates were exposed in desiccators to levels of 1–37 mmol/L (24 500–906 000 ppm) of the test substance in air. A confirmatory assay was also conducted.

Chromosome aberration

Effects of HCFO-1233zd(E) on chromosomal structure were investigated in human lymphocytes obtained from healthy nonsmoking male volunteers not currently taking any medication. This procedure has previously been described (Kawano et al., 1995). For studies without and with metabolic activation (S-9), cells were treated with 469, 783 and 1305 ug/mL of HFO-1233zd(E). In the absence of S-9 exposure was for 21 hours. In the presence of S-9 treatment was for 3 hours, followed by an 18-hour recovery. In the study without metabolic activation, mitomycin C was the positive control, and in the presence of S-9 metabolic activation, cyclophosphamide (CPA) was the positive control.

Mouse micronucleus

Two groups of 10 male CD-1 mice, approximately 8 weeks old, were exposed for a single period of 4 hours to vapors of HCFO-1233zd(E) in a nose-only exposure unit. One group served as an air control, whereas the other was exposed to an approximate level of 50 000 ppm (5%) of HCFO-1233zd(E). Additionally a third (positive control) group was included for the micronucleus test. These mice received a single intraperitoneal (i.p.) injection of 40 mg/kg of CPA. Bone marrow samples were collected from the CO_2-euthanized animals at approximately 24 and 48 hours after exposure or dosing (positive control). Three sets of fixed, unstained slides were prepared from each animal, two were stained for 10 minutes in 10% Giemsa, rinsed in purified water, buffered in purified water, air-dried, mounted with coverslips using DPX (distrene, plasticizer and xylene) and evaluated under light microscopy for the presence and incidence of micronucleated polychromatic erythrocytes per 2000 polychromatic cells per animal.

Prenatal developmental toxicity

Rat

One hundred and twelve female and 56 male Wistar outbred (Crl:WI(WU)BR) rats (Charles River Deutschland) approximately 9 (female) to 11 (male) weeks of age, were obtained. Before mating, males were individually housed and females were housed 4 to a cage. During mating, 1 male was placed with 2 females in type 3 Macrolon cages, until positive signs of copulation, females being sperm positive, were noted. Inseminated animals with similar days of insemination were equally distributed to all groups. Females inseminated by the same male were assigned to different groups. Females were housed in Macrolon cages with wood shavings (Lignocel, type ¾; Rettenmaier, Rosenberg, Germany) in an animal room maintained at $22 \pm 2\,^{\circ}C$ with relative humidity between 40 and 70%. A 12-hour light/dark cycle was provided. Food (Rat & Mouse No. 3 Breeding Diet, RM3; SDS Special Diets Services) and drinking water (tap) were provided *ad libitum* from arrival until the end of the study, except during inhalation exposures.

Groups of 24 presumed pregnant rats were exposed to vapors of HCFO-1233zd(E) (6 hours/day) daily from gestation day (GD) 6 up to and including GD19 at levels of 0 (control), 4000, 10 000 and 15 000 ppm. Animals were exposed to the test atmosphere in nose-only exposure units. Exposures were conducted in the same exposure units and concurrently with the 90-day inhalation study described above. Hence, measurements of exposure levels, environmental conditions and measurements of these conditions were the same as those described above for the 90-day study. Each animal was observed daily before exposure; during exposure, animals were observed group-wise approximately mid-way during each exposure. Body weights were recorded on GD 0, 3, 6, 9, 12, 15, 19 and 21 before sacrifice. Food consumption was measured for each mated female on GD 0–3, 3–6, 6–9, 9–12, 12–15, 15–19 and 19–21 by weighing the feeders. Females were killed by decapitation after CO_2/O_2 anesthesia on GD 21.

After sacrifice, a gross examination was conducted on each dam. The intact uterus was removed from the abdominal cavity and weighed. The number of viable fetuses, dead fetuses, early embryonic deaths, late fetal deaths, sex of the fetuses, number of corpora lutea and number of implantation sites were determined. All live fetuses were individually weighed, examined for external defects and sacrificed by hypothermia. Half of the fetuses of each litter were fixed in Bouin's fixative and evaluated for soft tissue abnormalities using a modification of the procedure described by Barrow and Taylor (1969). The remaining half of the fetuses were processed for staining of ossified skeletal structures using a version of the Alizarin Red S staining procedure modified after Dawson (1926) and examined under a dissecting microscope for skeletal variations and malformations and ossification status.

Rabbit

Subsequent to a pilot inhalation developmental toxicity study in which groups of 6 pregnant New Zealand white rabbits (data not presented) were exposed to levels of 0 (control), 1500, 5000 and 15 000 ppm, a full inhalation developmental toxicity study was performed. Exposure levels were based on the results in the pilot study where exposure at levels up to 15 000 ppm did not result in any significant adverse effects. In the full study, groups of 22 pregnant New Zealand white rabbits, approximately 5–6 months old (Covance Research Products, Denver, PA), were exposed (6 hours/day) daily to levels of 0 (control), 4000, 10 000 and 15 000 ppm of HCFO-1233zd(E) from GD 6–28. Rabbits were handled, and the study was conducted under a program of animal care accredited by the Association for Assessment and Accreditation of Laboratory Animals (Guide for the Care and Use of Laboratory Animals, National Research Council, National Academy Press, 1996). The exact number of animals required for the study (88) were purchased and assigned to the four groups using a computer randomization program based on body weights. Rabbits were individually housed in suspended wire-mesh cages and given food (up to 200 g daily; Global High Fiber Diet #2031C; Harlan Teklad, Madison, WI) and tap water *ad libitum*. The animal housing room was maintained with a 12-hour light/dark cycle, humidity at 30–70% and temperature range of 18–20 °C. During the exposure periods, rabbits were placed in single-housing exposure cages and did not have access to food or water. Animals were removed from their cages and given detailed clinical examinations daily. Body weights were measured on

GD 4, 6, 9, 12, 15, 18, 20, 23, 26 and 29 (before sacrifice). Sacrifice was by injection of sodium pentobarbital.

Exposures were conducted in 1.5-m^3 stainless steel and glass exposure chambers operated at an air flow of 300 liters/min. The exposure atmosphere was generated through a series of metering the liquid test substance into a countercurrent volatilization chamber and then diluting the vapor with filtered air to the final targeted exposure levels. Exposure levels were measured using a MIRAN® infrared ambient air analyzer at least four times during each exposure. Particle-size determinations were performed using a TSI Aerodynamic Particle Sizer (TSI Incorporated, Shoreview, MN) to confirm the absence of any aerosol.

After sacrifice, a gross examination was conducted on each doe. The intact uterus was removed from the abdominal cavity and weighed. The number of viable fetuses, dead fetuses, early embryonic deaths, late fetal deaths and number of implantation sites were determined. All live fetuses were individually weighed, examined for external defects and sacrificed. All fetuses were evaluated for soft tissue abnormalities using a modification of the microdissection procedure described by Staples (1974). After evaluation for soft tissue abnormalities, each fetus was processed for staining of ossified skeletal structures using a modified version of the Alizarin Red S staining procedure Dawson (1926) and examined under a dissecting microscope for skeletal variations and malformations and ossification status. Recently deceased fetuses were examined following the same procedure as used for live fetuses.

Results

Acute inhalation

Groups of 5 male and 5 female SD rats were exposed to analytically determined levels of 0, 95 971 (9.6%), 120 256 ppm (12%) or 155 579 ppm (15.5%) of HCFO-1233zd(E) for 4 hours. All animals exposed to 156 000 ppm died during exposure. At 120 000 ppm 3 males and 1 female died during exposure. There were no deaths at 96 000 ppm. Among the survivors, tremors were observed during exposure. During the 14-day observation period, ataxia, lethargy, hunched posture, restlessness, blepharospasm, exophthalmus, red eyes and piloerection were observed in surviving animals, but only for a few days. No abnormalities were noted after 2–3 days. The 4-hour 50% lethal concentration (LC_{50}) value for both sexes combined was calculated as 120 000 ppm.

Cardiac sensitization

Cardiac sensitization was evaluated in 6 Beagle dogs. There were no test-substance–related deaths. No arrhythmias or other apparent exposure-related effects were induced after exposure to 25 000 ppm of HCFO-1233zd(E). However, at 35 000 ppm, dogs exhibited tremors that made it impossible to conduct EKG monitoring. These types of effects are sometimes observed in dogs exposed to high concentrations of test substance in cardiac sensitization assays. At 50 000 ppm, only 2 dogs were exposed and no EKG measurements were obtained because of severe clinical findings (e.g. tremors or convulsions). Based on these findings, the threshold for effects (not cardiac sensitization) was 35 000 ppm and the no observed effect level (NOEL) was 25 000 ppm. No cardiac sensitization was observed at 25 000 ppm, the highest concentration that could be evaluated.

2-Week inhalation toxicity study

Groups of 5 male and 5 female SD rats were exposed to levels of 0 (control), 1994, 7496 or 19 955 ppm of HCFO-1233zd(E) for 6 hours/day, 5 days/week. There were no treatment-related differences in body weight, body-weight gain or food consumption. Daily clinical observations were generally unremarkable, and all rats survived until the scheduled sacrifice. A few differences in hematology were noted, such as an increase in prothrombin time (high-exposure females) and the number of neutrophils and monocytes (high-exposure males). These were not considered to be a direct effect of the test substance. A significant increase in plasma levels of ALT and AST (high-exposure males) with a slight increase in AST (high-exposure group females), increase in glucose (females mid- and high exposure) and urea (high-exposure males and females) were observed.

The only effect on organ weights was a decrease in absolute, but not relative, spleen weight in the 20 000-ppm males. Gross necropsy revealed pale livers in 3 females at 20 000 ppm, but no other effects. Microscopic examination revealed multifocal mononuclear cell infiltrates in heart of female rats at 20 000 ppm and male rats exposed to 7500 and 20 000 ppm. Several animals, including control rats, had minimal focal mononuclear cell infiltrates in the heart, but these are common findings in rats and were not considered to be adverse (Table 1).

4-Week inhalation toxicity study

Mean exposure concentrations for this study were 0 (control), 1994, 4485, 7492 and 9966 ppm. Test substance usage differed less than 2% from the amount expected from the test atmosphere flow and the concentration. There was no mortality during the study. There were no treatment-related clinical abnormalities, nor effects on body weight, body-weight gain, food consumption or food conversion efficiency. The only hematology finding was an increase in the percentage of reticulocytes in the recovery group. However, this was not considered to be treatment related because it was not noted at the end of the main study, and other parameters, especially RBCs and hemoglobin, were not affected. Clinical chemistry parameters affected were a decrease in creatinine in high-exposure males and increase in potassium in mid- and high-exposure males as well as increase in cholesterol in low- and mid-dose females. Cholesterol levels in females did not show a dose-response relationship. These effects were not statistically significant after a 2-week recovery. There were no significant differences in organ weights, organ/body-weight ratios or urinalysis parameters. Macroscopic and microscopic examination of all rats was unremarkable. The NOEL for this study was reported to be 4500 ppm based on increased potassium levels observed in males at the two higher exposure levels.

13-Week inhalation toxicity study

Mean exposure concentrations for this study were 0 (control), 3987, 9974 and 14 903 ppm. Test substance usage differed

Table 1. Summary of effects observed in the heart of rats exposed to HCFO-1233zd(E)[a].

Study/effects	Males						Females					
2-Week exposure levels in 1000 ppm	0		2	7.5		20	0	2		7.5		20
Very slight to slight focal mononuclear cell infiltrate	2		1	2		0	2	1		0		0
Multifocal mononuclear cell infiltrate												
Very slight	0		0	3		0	0	0		0		3
Slight	0		0	0		4	1	0		0		1
Moderate/multifocal	0		0	0		1	0	0		0		0
Totals	0		0	3		5**	1	0		0		4
4-Week exposure levels in 1000 ppm	0	2	4.5	7.5	10		0	2	4.5	7.5	10	
Slight subepicaridal mononuclear cell infiltrate	1	0	0	0	0		0	0	0	0	0	
Myocardial mononuclear cell infiltrate												
Very slight	2	1	0	1	2		2	0	0	1	4	
Slight	0	1	0	0	0		0	0	0	0	0	
Totals	2	2	0	1	2		2	0	0	1	4	
13-Week exposure levels in 1000 ppm	0		4		10	15	0		4		10	15
Vacuolation	0		1		3	5*	0		0		1	0
Very slight to slight focal mononuclear cell infiltrate	4		5		3	0	1		2		3	0
Multifocal mononuclear cell infiltrate												
Very slight	0		0		5	3	0		0		0	3
Slight	0		0		2	4	0		0		0	2
Moderate	0		1		0	2	0		0		0	0
Totals	0		0		7**	9***	0		0		0	5*

[a]$N = 5$ animals/sex/group in the 2- and 4-week exposure studies; $N = 10$ animals/sex/group in the 13-week exposure study.
*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

less than 1% from the amount expected from the test atmosphere flow and the concentration. No lethality was observed. There were no treatment-related clinical or ophthalmoscopic abnormalities, nor effects on body weight, body-weight gain, food consumption or food conversion efficiency. Hematologic examination showed increases in thrombocytes and monocytes in the male rats exposed to 15 000 ppm and increases in hemoglobin concentration, packed cell volume and, to a slight extent (i.e. in a few animals), monocytes in females exposed to 15 000 ppm. Increases were noted in AST and ALT in serum of males exposed to 15 000 ppm as well as glucose and urea in serum of females exposed to 10 000 and 15 000 ppm and potassium at 15 000 ppm in females. An increase in occult blood was observed in urine of high-exposure-group males. Relative and absolute heart weights were significantly decreased and liver weights were slightly, but significantly, increased in 15 000-ppm-group males and relative heart weight increased in 15 000-ppm-group females. Gross necropsy observations were generally unremarkable. Microscopic examination revealed that HCFO-1233zd(E) induced multifocal mononuclear cell infiltrates in hearts of both males and females in the mid- and high-exposure group. Multifocal inflammatory infiltrates were observed in 9 of 10 males in the 15 000-ppm group, 5 of 10 females in the 15 000-ppm group, 7 of 10 males in the 7500-ppm group and 1 of 10 males exposed to 4000 ppm (Table 1). No changes were noted in the liver or nasal passages or any other organ. According to the study director, the lowest observed adverse effect level for this study was 4000 ppm based on the multifocal infiltrate observed in the heart of 1 male at 4000 ppm. However, a peer review was conducted by an outside expert who utilized a single set of diagnostic criteria for cardiac alterations, as described by Ruben et al. (2000). Alterations that included mononuclear cell infiltrate of the myocardium (focal or multifocal distribution) most commonly in the apex were considered to be spontaneous (Chanut et al., 2013; Lewis, 1992; Ruben et al., 2000). Many of the findings were consistent between the pathologists, but the peer-review expert considered the effects observed at 4000 ppm not to be adverse and determined the NOEL to be 4000 ppm based on the location of the lesion noted in the one male rat at this exposure level.

As can be seen from the data in Table 1, the consistent finding in the three inhalation studies was (multifocal) mononuclear cell infiltrations in the heart, whereas myocardial vacuolation was primarily observed only in the 13-week study and at high concentrations ($\geq$10 000 ppm).

Reverse mutation

In this Ames assay, four strains of *S. typhmurium* (TA98, TA100, TA1537 and TA1535) as well as *E. coli* WP2 uvrA were exposed to levels from 73 000 to 900 000 ppm (7.3–90%) of HCFO-1233zd(E) in air. Exposures were conducted both without and in the presence of metabolic activation. Positive controls were included. HCFO-1233zd(E) was not active in any of these assays.

Mouse micronucleus

CD-1 mice were exposed to 50 000 ppm of HCFO-1233zd(E) for 4 hours. At 24 and 48 hours after exposure, mice were sacrificed and bone marrow from both femurs were extracted and evaluated for the presence of an increased number of micronuclei. Both negative (air-exposed) and positive (mice given i.p. injections of CPA) controls were included in the study. Under the conditions of this study, there was no evidence for an increase in micronuclei formation.

UDS

Cultured hepatocytes from male rats exposed to 0, 7500 and 10 000 ppm for 4 weeks, discussed above, were evaluated for UDS. HCFO-1233zd(E) did not induce UDS under the conditions of this study.

Rat micronucleus

Bone marrow collected from the femur of male rats exposed to levels of 2000, 4500, 7500 and 10 000 ppm, from the 4-week inhalation toxicity study described above, was used for a micronucleus test. No damage to the chromosomes and/or mitotic spindle apparatus (micronuclei) was observed at 10 000 ppm, so the other concentration groups were not evaluated. Cytotoxicity and damage to the chromosomes and/or mitotic spindle apparatus were observed in the positive control rats, indicating the study was valid.

Chromosome aberration in human lymphocytes

Effects of HCFO-1233zd(E) on chromosomal structure were investigated in human lymphocytes obtained from healthy nonsmoking male volunteers not currently taking any medication. Evaluations were conducted both without and with S-9 metabolic activation. Concentrations used in the absence of S-9 were 469, 783 and 1305 ug/mL of HCFO-1233zd(E). In the absence of S-9, exposure was for 21 hours. In the presence of S-9, treatment was for 3 hours, followed by 18 hours of recovery. HCFO-1233zd(E) did not increase the proportion of metaphase figures containing chromosome aberrations or increase the proportion of polyploidy cells, when compared to the solvent control. Positive controls (mytomycin C and CPA) gave the expected responses. Under the conditions of this test, both without and with metabolic activation, HCFO-1233zd(E) was not clastogenic in human lymphocytes.

Prenatal developmental toxicity rats

Average exposure levels achieved during the study were 0, 3988, 9980 and 14 906 ppm. Test substance usage differed less than 1% from the amount expected from the test atmosphere flow and the concentration. There was no mortality and no reports of abnormal test-article–related clinical observations. There were no effects on body weights or food consumption or gross observations of exposure-related abnormalities. There were no statistically significant differences in female fecundity index, gestation index, number of corpora lutea, implantation sites, pre- or postimplantation loss, number of live or dead fetuses, resorptions or sex ratio.

There were no effects noted during gross necropsy or on reproductive organ weights. There were no statistically significant differences in fetal external observations, placental observations, placental or fetal weights, visceral malformations, skeletal malformations or anomalies or variations or ossifications. There were various external, visceral and skeletal findings, but these were scattered among the control and treatment groups. One female in the low-concentration group had one fetus with a soft skull, malformed ears and subcutaneous edema, along with incomplete ossification. Because no similar effects were observed in the mid- or high-exposure groups, these effects were considered to be incidental and not treatment related. Likewise, retardations were noted in skeletal development at various sites in all control and treatment groups. A statistically significant decrease in fetal incidence of three or more incompletely ossified caudal arches in the low- and mid-concentration groups and a decreased incidence of one to two incompletely ossified metacarpals in the low-concentration group were noted. None were considered adverse and all were reported in a low incidence. Visceral variations occurred in all groups and included hemorrhagic areas in the oral and nasal cavity, folded retinas, not well-defined soft lenses, pericard and stomachs filled with hemorrhagic fluid as well as kinked and bent ureters. Incidence of folded retina and stomach containing hemorrhagic fluid were statistically decreased in the high- and low-concentration group, respectively. Overall, the incidence of fetal visceral variations was decreased in the low- and high-exposure groups, compared to controls. A statistically significant increased incidence in dilated urinary bladders, a visceral anomaly, was observed in fetuses of dams exposed to 15 000 ppm. This was considered to be test substance related, but the significance of this finding is not clear, because other possibly related effects, such as kidney abnormalities or increased amniotic fluid, were not observed. In conclusion, the NOEL for this inhalation, prenatal developmental toxicity study in rats was 10 000 ppm.

Prenatal developmental toxicity rabbits

Average exposure levels achieved during the study were 0, 2519, 9945 and 14 952 ppm. These were within 3% of the target concentration. The nominal concentration was determined based on the total test substance usage and differed by less than 0.05% of the amount expected from the test atmosphere flow and concentration. There was no mortality and no reports of abnormal test-article–related clinical observations. There were no effects on body weight or food consumption and no gross observations of exposure-related abnormalities. There were no test-substance–related effects on pregnancy status or pregnancy rates. There was no difference between the groups regarding the number of corpora lutea, number of implantations, sex ratio or postimplantation loss. An increase in preimplantation loss observed at $\geq$10 000 ppm was not considered to be treatment related because these parameters were established before initiation of exposure.

There were no test-substance–related effects on gravid uterine, placental or fetal weights. There were no test-substance–related effects on fetal external, visceral or skeletal abnormalities. Thus, the NOEL for this study was 15 000 ppm, the highest level tested.

Discussion and conclusion

HCFO-1233zd(E) was not acutely toxic. The acute inhalation LC_{50} value in rats after 4-hour exposure is 120 000 ppm (12%). Animals died during exposure to 120 000 and 156 000 ppm. Clinical signs of toxicity were observed in surviving animals, but only for 3–4 days after exposure. In an evaluation of the potential for this material to sensitize the heart to the action of adrenalin, no signs of cardiac sensitization were observed in dogs after exposure to 25 000 ppm. Hence, the NOEL for cardiac sensitization is at least 25 000 ppm. At higher concentrations (35 000 and 50 000 ppm), artifacts in the ECG resulting from toxicity prevented analysis of the ECG signal. The NOEL is similar to that for 1,1,1,3,3-pentafluoropropane (HFC-245fa), where

sensitization was noted at 44 000 ppm, but not at 34 000 ppm (Rusch et al., 1999).

In the 2-week inhalation toxicity study, a few differences in hematology were noted. Clinical chemistry changes, such as elevations in AST, ALT and urea, were observed after exposure to 20 000 ppm of 1233zd(E). Though this may indicate that the liver and kidney could be potential target organs, no changes in organ weight and no histological lesions were present in the 2-week or subsequent 4- and 13-week studies. In the 2-week study, histological changes in the heart included multifocal mononuclear cell infiltrates, which were slight to moderate at 20 000 ppm and very slight to slight in males at 7500 ppm and females at 20 000 ppm. Based on these histological lesions, the NOEL for this study was 2000 ppm.

In the 4-week study, again, some variations were noted in a few serum chemistry parameters, particularly increases in potassium in males exposed to 7500 ppm or higher. However, this effect was not present after a 2-week recovery period. In addition, no exposure-related changes in organ weights, gross necropsy or histopathology were observed. Interestingly, very slight to slight focal mononuclear infiltrates commonly noted in hearts of rats were observed in both control and treated animals, but not multifocal mononuclear infiltrates that were observed in the 2-week study. In the 13-week study, some changes were noted in both hematology and serum chemistry parameters in the high-exposure-level group. Again, there were no exposure-related changes in organ weights or gross necropsy observations. Multifocal mononuclear infiltrates in hearts were observed in males in this study. Focal mononuclear infiltrates in hearts, a common finding in rats, were observed in both control and treated rats and were not considered to be treatment related.

In comparing these three inhalation toxicity studies, there were no consistent observations of hematological differences. In serum chemistry parameters, there were some consistent results (e.g. increase in AST, ALT and urea) in the 2- and 13-week study, but not the 4-week study. However, there were no microscopic changes in the liver or kidneys. Though an increase in potassium was noted in males in the 4-week study, this was not observed after 13 weeks of exposure. Most of these differences were small or were not replicated or associated with organ damage and therefore do not appear to represent adverse effects. The most prominent microscopic finding was multifocal mononuclear cell infiltrates in the heart, which was observed after 2 and 13 weeks of exposure. Focal very slight to slight mononuclear cell inflammation in the heart is part of the background pathology and was observed in all studies. Cardiotoxicity is considered generally to be a result of either a direct myocardial effect of the compound or an indirect effect, namely, by vasoactive properties of a compound (Jokinen et al., 2005). Direct cardiotoxicity generally leads to diffuse vacuolation (degeneration) of the myocardial fibers, which can be accompanied by a minimal inflammatory cell infiltrate. Indirect cardiotoxicity typically leads to multifocal inflammatory cell infiltrates, with hypereosinophilic or infrequent vacuolated myocytes. Here, both types of mechanisms apparently played a role: The direct cardiotoxicity was prominent at high concentration in males after 13 weeks of exposure, whereas indirect cardiotoxicity was observed at lower concentrations and also after 2 weeks of exposure. In the inhalation studies with 1,1,3,3-pentafluoropropane (HFC-245fa) (Rusch et al., 1999), similar effects were reported in hearts of rats exposed to levels of 10 000 and 50 000 ppm for 13 weeks. However, exposure of rats for 13 weeks to levels of up to 50 000 ppm of 1,1,1,2,2-pentafluoroethane (Kawano et al., 1995) did not produce similar effects. Additionally, exposure of rats to levels up to 50 000 ppm of 2,3,3,3-tetrafluoropropene did not result in cardiac effects (Tveit et al., 2013), but exposure of 1,3,3,3-tetrafluoropropene at levels of 10 000 ppm or higher did show similar cardiac effects (Rusch et al., 2013). Taken together, these observations suggest that the cardiac effects are related to a propane or propene with one or more halogens (fluorines or chlorines) on carbon 1 and a trifluoromethyl group in the 3 position.

HCFO-1233zd(E) was not active in a reverse mutation assay (Ames assay), including *E. coli*, a mouse micronucleus assay, a rat micronucleus assay after 4 weeks of exposure at 10 000 ppm, a chromosome aberration assay with human lymphocytes and a UDS assay in rats also after 4 weeks of exposure up to 10 000 ppm. Taken together, these findings imply that HCFO-1233zd(E) has a low potential to be mutagenic or carcinogenic.

Prenatal developmental toxicity studies were conducted in both rats and rabbits. Both species were exposed to levels of up to 15 000 ppm from day 6 of gestation until day 19 (rats) or 28 (rabbits). An increase in dilated bladders, a visceral anomaly, was observed in rat pups from dams exposed to 15 000 ppm. The effect is considered to be treatment related, but the significance of this finding is not clear because no possibly related changes, such as an increase in amniotic fluid or changes in the kidney, were observed. There was no evidence of developmental toxicity or teratogenicity in the rabbit study.

The overall low level of toxicity is supported by the results of studies on the biotransformation of HCFO-1233zd(E). In these studies, male SD rats and female New Zealand rabbits were exposed to levels of 2000, 5000 and 10 000 ppm of HCFO-1233zd(E) for 6 hours. Urinary metabolites were identified by ^{19}F-NMR. In rats, the major urinary metabolites were 3,3,3-trifluorolactic acid (32%) and *N*-acetyl-(3,3,3-trifluoro-trans-propenyl)-L-cysteine (46% rabbits). In rabbit urine, the major metabolite was *N*-acetyl-(3,3,3-trifluoro-trans-propenyl)-L-cysteine. Rabbit urine did not contain 3,3,3-trifluorolactic acid. The urinary half-life was less than 6 hours in both species. Taken together, these metabolites represented approximately 0.002 and 0.02% of the total dose received by rats and rabbits, respectively (Schmidt et al., 2013).

Declaration of interest

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