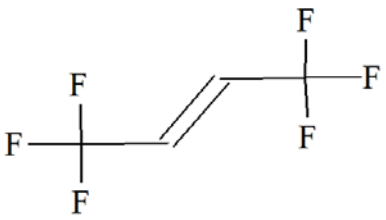
WORKPLACE ENVIRONMENTAL EXPOSURE LEVEL®

OARS WEEL

trans-1,1,1,4,4,4-Hexafluoro-2-butene (HFO-1336mzz-E)(2018)

I. IDENTIFICATION

Chemical Name: trans-1,1,1,4,4,4-Hexafluoro-2-butene
Synonyms: HFO-1336mzz-E; HFO-1336mzz-(E)
CAS Number: 66711-86-2
Molecular Formula: $C_4H_2F_6$
Structural Formula:



II. CHEMICAL AND PHYSICAL PROPERTIES (Chemours, 2016; DuPont, 2013, 2007a, 2007b, 2007c, 2015; Siemens AG, 2015)

Molecular Weight: 164.05 g/mol
Physical State and Appearance: Gas, odorless to ammonia-like
Conversion Factors: 1 mg/m^3 = 0.149 ppm (25 °C (77 °F) and 760 mm Hg); 1 ppm = 6.7 mg/m^3 (25 °C (77 °F) and 760 mm Hg)
Melting Point: < -20 °C (-4 °F)
Boiling Point: 7.51 °C (45.52 °F)
Vapor Pressure: 163.52 kPa (1226.5 torr; 23.7 psia) @ 20 °C (68 °F)
Vapor Density: 5.3 (Air = 1)
Flammability Limits: Not flammable
Flash Point: Not applicable
Autoignition Temperature: Not applicable
Density: 1.3099 g/cm^3 @ 20 °C (68 °F)
Log K_{ow}: 2.5 at 40 °C (104 °F)
Solubility in Water: 0.280 g/L at 25 °C (77 °F) and 760 mmHg
Stability: Stable under recommended storage conditions
Reactivity and Incompatibilities: Decomposes on heating. Not compatible with alkali metals alkaline earth metals, powdered metals, powdered metal salts.

III. USES

HFO-1336mzz-E is used as a foam expansion agent, heat transfer fluid, and specialty gas (Chemours, 2016).

IV. ANIMAL TOXICITY DATA

A. Acute Toxicity and Irritancy

1. Lethality Data

Data not available for oral and dermal routes as substance is a gas.

Species	Species, Route, Duration	LC_{50}
Rat	Inhalation (4-hr)	> 17,000 ppm (DuPont, 2012)
Rat	Inhalation (4-hr)	25,400 - 49,000 ppm (Charles River Laboratory, 2010)
Rat	Inhalation (4-hr)	> 24,000 ppm (DuPont, 2009)
Rat	Inhalation (10-min)	> 33,000 ppm (DuPont, 2011)

1. Eye Irritation

The substance is a gas and has not been tested for eye irritation. However, in 3- and 13-week whole-body inhalation toxicity studies in rats with exposures up to 1.5% in air, 6-hours/day, 5 or 7 days/week, no signs of ocular irritation were observed (WIL Research Laboratories, 2015; TNO, 2016a).

2. Skin Absorption

The substance is a gas and has not been tested for dermal toxicity.

3. Skin Irritation

The substance is a gas and has not been tested for skin irritation. However, in 3- and 13-week whole-body inhalation

toxicity studies in rats with exposures up to 1.5% in air, 6-hours/day, 5 or 7 days/week, no signs of dermal irritation were observed (WIL Research Laboratories, 2015; TNO, 2016a).

4. Skin Sensitization

The substance is a gas and has not been tested for skin sensitization.

5. Inhalation Toxicity

A GLP acute inhalation study was conducted according to OECD test guideline 403. Groups of (5 per sex) Crl:CD®(SD IGS) rats were exposed via whole-body inhalation exposure for up to 4 hours to 17,000 or 23,000 ppm HFO-1336mzz-E. Approximately 6 minutes after the 17,000-ppm exposure started, the rats displayed decreased activity, which continued throughout the exposure; however, the rats' startle responses were normal throughout the 4-hour exposure. There were no deaths during the exposure or the 14-day post-exposure observation period. No clinical signs of toxicity were observed after the exposure was terminated or during the observation period. Within 2 minutes of initiating the test substance vapor flow for the 23,000-ppm exposure group, the rats displayed decreased activity. The rats began to display muscular spasms by approximately 5 minutes into the exposure, followed by violent convulsions, which occurred approximately 8 minutes into the exposure. The exposure was terminated for humane reasons. Within 17 minutes of when the test substance vapor was shut off, the rats displayed normal startle response and had no abnormal clinical signs of toxicity. There were no clinical signs of toxicity observed throughout the 14-day recovery period (DuPont, 2012).

A second GLP acute inhalation study was conducted according to OECD test guideline 403. Groups of (5 per sex) Crl:CD®(SD IGS) rats were exposed via nose-only inhalation exposure for up to 4 hours to 14,600, 25,400, 49,000 or 122,000 ppm HFO-1336mzz-E. Severe clinical signs and mortality were observed in animals exposed to concentrations of 49,000 and 122,000 ppm, and exposures were terminated after 4 minutes and 20 minutes, respectively. All animals died in the 122,000 ppm exposure group (9 of 10 animals died during the exposure, with 1 animal being sacrificed for humane reasons at approximately 20 minutes after exposure). Two animals died in the 49,000 ppm group. The remaining 8 animals exhibited severe clinical signs of toxicity but showed good recovery and were normal by 5 hours after exposure. At 25,400 ppm, 1 animal died during approximately 1-hour exposure and no additional deaths occurred throughout the remaining 3-hours exposure or during the 14-day post-exposure observation period. The surviving animals exhibited severe clinical signs of toxicity but at a lower incidence compared with higher concentration groups and showed recovery at approximately 24-hours post-exposure. No mortality or adverse effects were observed in animals exposed to 14,600 ppm for 4 hours. Based on the findings, the 4-hour LC_{50} was estimated to be between 25,400 ppm and 49,000 ppm (Charles River Laboratories, 2010).

In a non-GLP, non-guideline acute inhalation toxicity screen, 5 male Crl:CD®(SD) rats were exposed to 24,000 ppm HFO-1336mzz-E via whole-body inhalation for approximately 1 hour. There were no deaths during the exposure or 14-day recovery period. During exposure, the rats exhibited labored breathing and decreased activity levels. No clinical signs of toxicity were observed in any rat during the recovery period. Under the conditions of this study, the 1-hour approximate lethal concentration (ALC) was greater than 24,000 ppm (DuPont, 2009).

In a non-GLP, non-guideline acute inhalation toxicity screen, (2 per sex) Crl:CD®(SD) rats were exposed to 33,000 ppm HFO-1336mzz-E via whole-body inhalation for approximately 10 minutes. During the first 5 minutes of the exposure, the animals displayed decreased activity levels, but had normal startle response. As the exposure proceeded, the animals began to show tremors and convulsions. Approximately 10 minutes after the exposure began, the rats displayed muscle spasms and severe convulsions and the exposure was terminated for humane reasons. There were no deaths during the exposure or 14-day recovery period. There were also no abnormal clinical signs during the post exposure period. Under the conditions of this study, 10 minutes of exposure to 33,000 ppm of HFO-1336mzz-E produced tremors, muscle spasms, convulsions and decreased activity levels during exposure (DuPont, 2011).

A GLP cardiac sensitization study was conducted using a titrated epinephrine challenge study design. One group of up to six male Beagle dogs was exposed via muzzle-only to inhalation exposures of 70,000, 80,000, or 90,000 ppm HFO-1336mzz-E. Each animal had a minimum of 48 hours of separation between exposures. Each dog served as its own control. Baseline responses to epinephrine challenge doses were collected for each animal approximately 5 days prior to exposure to the test substance and a predetermined challenge dose was established during this baseline period. This challenge dose determination was made based on the maximum level of epinephrine that would not cause a cardiac arrhythmia. The dogs were then exposed to the test substance for a total of

approximately 10 minutes. After the first five minutes of exposure, each dog received an injection of epinephrine at the pre-determined maximum sub-arrhythmia dose (8 μg/kg epinephrine). During the next five minutes of exposure, the dogs were monitored for the development of a cardiac arrhythmia. There were no arrhythmias at 70,000 ppm, but two of the six dogs displayed continuous convulsions. Higher concentrations could not be tested due to the presence of adverse clinical signs (convulsions). Under the conditions of this study, the NOAEL for cardiac sensitization was 70,000 ppm (WIL Research Laboratories, 2010).

B. Subacute Toxicity

1. Inhalation

A 3-week non-GLP inhalation toxicity range-finding study was conducted wherein three groups of male and female Wistar rats (5 per sex) were exposed to 0, 7500, or 15,000 ppm HFO-1336mzz-E via whole-body inhalation for 6 hours/day, 7 days/week for a 3-week period (21-consecutive days). There were no test substance-related effects on survival, organ weights, or macroscopic findings. Test substance-related tremors were noted in males and females during exposure to 15,000 ppm HFO-1336mzz-E. At the end of the exposure period, mean body weights in the 7500 and 15,000 ppm males were 3.2% and 4.5% lower, respectively, than the control group. There were no test substance-related effects on body weight for the female groups. Based on the results of this study, exposure of Wistar rats to concentrations of 7500 and 15,000 ppm HFO-1336mzz-E via whole-body inhalation for 6 hours/day for 21 consecutive days was well-tolerated. Test substance-related effects were limited to lower body weight gains in males from both exposure groups and test substance-related tremors and/or repetitive movement of the mouth and jaws in males and females from the 15,000-ppm exposure group (WIL Research Laboratories, 2016).

A 28-day GLP inhalation toxicity study was conducted according to OECD test guideline 412 wherein four groups of male and female Wistar rats (10 per sex) were exposed to 0, 1000, 10,000, or 15,000/20,000 ppm HFO-1336mzz-E via nose-only inhalation for 6 hours/day, 5 days/week for a 4-week period. The top exposure concentration was dropped to 15,000 ppm after the first week of exposure due to the premature death of two males during the 3rd day of exposure and moderate body weight loss in several males in this group. At day 23 of exposure, two more males died during the last hour of exposure in the 15,000-ppm group. In total, four male animals died during the treatment period. The cause of these deaths could not be established. Body weight gain was dose-dependently reduced in males of the 10,000 and 15,000 ppm exposure groups during the treatment period. Recovery was noted thereafter. In addition, transient body weight loss was noted in females of the 15,000 ppm exposure group. There was no effect on mean animal body weights during the course of the study. No test substance-related adverse effects were observed in micronucleus, clinical, neurobehavioral, or gross and anatomical pathology assessments. Based on the reduction in body weight gain and mortality in the highest exposure group, the NOAEL was 10,000 ppm (Harlan Laboratories, 2013).

2. Oral

No data available. Substance is a gas.

C. Subchronic Toxicity

1. Inhalation

A 90-day GLP inhalation toxicity study was conducted according to OECD test guideline 413 wherein five groups of male and female Wistar rats (10 per sex) were exposed via whole-body inhalation to 0, 1000, 5000, 7500 or 15,000 ppm HFO-1336mzz-E for 6 hours/day, 5 days/week. In addition, two recovery groups, also consisting of 10 male and 10 female animals each, were simultaneously exposed with the main study animals to the control or 15,000 ppm test atmospheres, and were sacrificed after a 4-week recovery period following the last exposure. There were no test substance-related adverse findings in ophthalmology, hematology, or urinalysis parameters. Three male animals in the 15,000 ppm group died during exposure. These animals were found dead during the second half of the 6-hour exposure period on days 12, 29 and 90. Clinical signs, primarily consisting of restlessness, blepharospasm and myoclonic jerks were observed in animals during exposure to 15,000 ppm. Clinical signs were mainly detected shortly after the start of exposure; animals adjusted during the 6-hour exposure period and no longer displayed any signs at the end or after exposure. The clinical signs were transient and after about a month of treatment, observation of these signs significantly decreased. A transient decrease in growth was observed in male animals of the 15,000 ppm exposure group during the first month of treatment, which was no longer seen during the remainder of the study. Necropsy of the three male animals found dead during exposure to 15,000 ppm HFO-1336mzz-E did not reveal an obvious cause of death. Microscopic evaluation, which was difficult because most tissues were partly autolytic, did not reveal any remarkable histopathological lesions. Macroscopic examination of all remaining animals at their scheduled termination revealed no

exposure-related gross pathology. In addition, microscopic examination did not reveal any histopathological changes which were attributable to HFO-1336mzz-E exposure. Under the conditions of this study, the NOAEL was 7500 ppm (TNO, 2016a).

2. Oral

No data available. Substance is a gas.

D. Chronic Toxicity/Carcinogenicity

No chronic toxicity studies have been conducted with this material. *In vitro* and *in vivo* genotoxicity test results presented below suggest that HFO-1336mzz-E is not likely to be carcinogenic.

E. Reproductive/Developmental Toxicity

A GLP prenatal developmental study was conducted according to OECD test guideline 414 wherein groups of 24 time-mated nulliparous female Wistar rats were exposed to 0, 1000, 5000, 7500 and 15,000 ppm HFO-1336mzz-E via whole-body inhalation for 6 hours/day beginning on gestation day (GD) 6 up to and including GD 19. No maternal, embryo, or fetal lethality or fetal structural malformations were observed at any exposure concentration. A lower maternal body weight gain during gestation was observed and a reduced food intake after the start of exposure from GD 6-12 was noted in the 15,000 ppm exposure group. Mean fetus weight and mean placenta weight were decreased in the 15,000 ppm exposure group for both male and female fetuses. Skeletal examination showed reduced ossification in the fetuses in the 15,000 ppm exposure group, which was indicative of growth retardation and considered to be related to the lower fetus weight in this group. Under the conditions of this study, the NOAEL for maternal and fetal effects was 7500 ppm (TNO, 2016a).

In the 90-day repeated dose inhalation systemic toxicity study (described above), no adverse effects were noted in the reproductive systems of either males or females. No adverse exposure-related macroscopic, weight, or histopathological changes were noted in the respective reproductive organs.

F. Genotoxicity/Mutagenicity

1. In vitro

A GLP Bacterial Reverse Mutation Assay (Ames) assay with HFO-1336mzz-E was conducted according to OECD test guideline 471 using *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* strain WP2 uvrA both in the presence and absence of an Aroclor-induced rat liver S9 activation system. The dose levels tested were a range of concentrations separated by a half-log10 dose interval up to 100% (i.e., the maximum practical level) using the plate incorporation method. The plates were exposed to HFO-1336mzz-E gas in Tedlar bags. Since no evidence of genotoxicity and only slight toxicity was seen in this initial test, the study was repeated using a narrower (approximately 2-fold) dose interval to confirm the results. Under the conditions of this study, HFO-1336mzz-E exhibited no mutagenic responses in either the presence or absence of metabolic activation (DuPont, 2010a).

A GLP *in vitro* mammalian chromosome aberration assay with HFO-1336mzz-E was conducted according to OECD test guideline 473 using cultured human peripheral blood lymphocytes both in the presence or absence of an Aroclor-induced rat liver S9 activation system. Human peripheral blood lymphocytes were stimulated into division in culture then treated with the gaseous test substance at a range of concentrations separated by a 2-fold dose interval up to 50% v/v, (i.e., the maximum practical level not expected to result in anoxia). Cultures were treated for 4 hours in the absence and presence of rat S9 mix and for 21 hours in the absence of rat S9 mix; appropriate concurrent vehicle and positive controls were included for each treatment regime. Metaphases from cultures treated with the three highest dose levels of test substance not producing excessive toxicity (together with appropriate vehicle and selected positive control cultures) were subjected to detailed examination for the presence of chromosomal aberrations using light microscopy. Cultures treated with HFO-1336mzz-E at levels up to 50% v/v in air did not show any statistically significant increases in the incidence of aberrant metaphases in either the absence or presence of S9 mix. Based on the findings of this study, it was concluded that HFO-1336mzz-E was negative for the induction of structural and numerical chromosome aberrations in cultured human peripheral blood lymphocytes in the presence or absence of metabolic activation. Under the conditions of this study, HFO-1336mzz-E was not clastogenic in either the presence or absence of metabolic activation (DuPont, 2010b).

A GLP *in vitro* mammalian gene mutation assay with HFO-1336mzz-E was conducted according to OECD test guideline 476 using cultured Chinese hamster ovary cells both in the presence or absence of an Aroclor-induced rat liver S9 activation system. In this assay, HFO-1336mzz-E was evaluated for its ability to induce forward mutations at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus. The

substance was evaluated in a preliminary dose range-finding assay at concentrations of 0.195, 0.391, 0.781, 1.56, 3.13, 6.25, 12.5, 25.0, 50.0 and 100% (v/v, in air) (the highest concentration evaluated was the limit dose for this assay). No visible precipitate was observed at the beginning or end of treatment, and the test substance did not have an adverse impact on the pH of the cultures. Based on these results, HFO-1336mzz-E was evaluated in the definitive mutagenicity assay at concentrations of 7.91, 15.8, 31.6, 42.2, 56.3, 75.0 and 100% (v/v, in air) with and without S9. No significant increases in mutant frequency, as compared to the concurrent vehicle controls, were observed at any concentration evaluated with or without S9. In contrast, the positive controls induced a significant increase in mutant frequency. Under the conditions of this study, HFO-1336mzz-E was not mutagenic in either the presence or absence of metabolic activation (BioReliance Corporation, 2015).

2. In vivo

A GLP rat micronucleus test was conducted according to OECD test guideline 474 and was included as part of a 28-day inhalation toxicity study (described above), in which four groups of male and female Wistar rats (10 per sex) were exposed nose-only to 0, 1000, 10,000, or 15,000/20,000 ppm HFO-1336mzz-E. The animals were exposed for 6 hours/day, 5 days/week during a 28-day period. Peripheral blood was collected from three to five males and five females per exposure concentration following the fourth exposure and at the time of final sacrifice for micronucleus evaluation. No increase in the number of micronucleated polychromatic erythrocytes was observed at any time point or concentration tested in this study. Under the conditions of this study, HFO-1336mzz-E did not induce micronuclei in peripheral blood cells of the Wistar rat (Harlan Laboratories, 2013).

G. Metabolism/Pharmacokinetics

Detailed information on the metabolism of pharmacokinetics of 1336mmzz-E is not available.

V. HUMAN USE AND EXPERIENCE

HFO-1336mmzz-E is a new chemical and is not in general use.

VI. RATIONALE

HFO-1336mzz-E has low acute inhalation toxicity, did not induce cardiac sensitization at concentrations up to 70,000 ppm, and was not genotoxic. Pregnant dams exposed to 15,000 ppm HFO-1336mzz-E had reduced body weight gain. A subsequent reduction in fetal and placental weight along with delayed ossification was noted and considered to be indicative of growth retardation and due to the reduced maternal weight at 15,000 ppm. The NOAEL for maternal and fetal effects in the developmental toxicity study was 7500 ppm (TNO, 2016b). In the 13-week inhalation toxicity study, effects noted at 15,000 ppm included lethality and clinical signs of toxicity including tremors which were transient and decreased in frequency of occurrence over time. No specific target organs were identified.

The WEEL for HFO-1336mzz-E is based primarily on the 13-week inhalation toxicity study in rats (TNO, 2016b). The point of departure is the NOAEL of 7500 ppm. This was also the NOAEL for the developmental toxicity study where developmental effects were only observed at maternally toxic levels. The subchronic inhalation NOAEL was adjusted to account for extrapolation from animal to human, inter-individual variability, subchronic to chronic duration of exposure, and residual uncertainty. The WEEL value of 400 ppm is expected to provide a significant margin of safety against the production of any potential adverse health effects in workers exposed to HFO-1336mzz-E.

VII. RECOMMENDED WEEL GUIDE

8-Hour Time-Weighted Average (TWA): 400 ppm

VII. REFERENCES

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