WORKPLACE ENVIRONMENTAL EXPOSURE LEVEL®

OARS WEEL

(E)-1,2-Difluoroethylene (HFO-1132E) (2022)

I. IDENTIFICATION (PubChem, 2021)

Chemical Name: (E)-1,2-Difluoroethylene

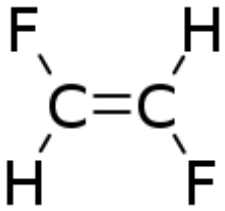
Synonyms: trans-1,2-Difluoroethylene; HFO-1132E; TKN-1

CAS Number: 1630-78-0

UN Number: 3161

Molecular Formula:$C_2H_2F_2$

Structural Formula:



II. CHEMICAL AND PHYSICAL PROPERTIES (Covance Laboratories Limited, 2020; Daikin Industries, Ltd., 2020; DEKRA, 2021; PubChem, 2021)

Molecular Weight: 64.03 g/mol

Physical Description: Colorless gas

Odor Detection: None

Boiling Point: -53ºC (-63.4ºF)

Flash Point: No flashpoint

Flammability Limits: 4% (vol) in air

Autoignition Temperature: 356ºC

Water Solubility: 2.0 g/mL

Vapor Density: 2.2 (relative to air = 1)

Vapor Pressure: 1.66 MPa (converted to 1600 kPa) at 25°C

Specific Gravity: 0.9 at 25°C

Partition Coefficient (Log P_{ow}): 0.9

Stability: Stable under recommended storage conditions

Reactivity/Incompatibilities: Decomposes on heating; not compatible with strong oxidizing agents, strong bases, alkali and alkaline earth metals, powdered metals, powdered metal salts.

Conversion Factor: 1 mg/m^3 = 0.371 ppm (20°C and 760 mm Hg)

1 ppm = 2.70 mg/m^3 (20°C and 760 mm Hg)

III. USES

HFO-1132E is a refrigerant.

IV. ANIMAL TOXICITY DATA

A. Acute Toxicity

1. Inhalation

Table 1. Acute Toxicity

Species	Route	LD_{50} or LC_{50}
Rat	Inhalation	4-h LC_{50} = >100,000 ppm (LSI Medience, 2019)

2. Oral

No data as the substance is a gas at room temperature.

3. Eye Irritation

No data as the substance is a gas at room temperature.

4. Skin Absorption

No data as the substance is a gas at room temperature. Based on physical and chemical characteristics, skin contact with the gas may result in burns consistent with frostbite.

5. Skin Irritation

No data as the substance is a gas at room temperature. Based on physical and chemical characteristics skin contact with the gas may result in frostbite.

6. Skin Sensitization

An *in vivo* study has not been conducted due to the physical form of the compound.

An *in silico* computational model was performed for skin sensitization utilizing TOPKAT, VEGA, Derek, Toxtree and Organization for Economic Co-Operation and Development (OECD) QSAR Toolbox (Covance Laboratories Limited, 2021a). HFO-1132E contains a mono-constituent substance such that the model predictions were collectively of poor reliability. There was disagreement for the classification of HFO-1132E between the models, and most predictions were of low reliability. In a read-across within the OECD QSAR Toolbox (v 4.3) model, there were two chlorinated, structurally similar substances that were predicted to be strong sensitizers. Overall, there was some evidence that HFO-1132E may likely be a skin sensitizing substance.

7. Inhalation Toxicity

An acute inhalation study was conducted in accordance with OECD test guideline 403 and in compliance with Good Laboratory Practice (GLP) regulations (LSI Medience Corporation, 2019). Groups of 6 male and 6 female Crl:CD®(BR) rats were exposed *nose-only* by inhalation exposure for 4 h to target vapor concentrations of 5000, 20,000 or 100,000 ppm. Measured vapor concentrations were 5160, 20,940 and 105,600 ppm. No mortalities occurred and no clinical signs of toxicity were reported following exposure during the study. All animals gained weight and there were no apparent effects on mean body weights during the study. No gross necropsy abnormalities were reported at the end of the study.

A cardiac sensitization study was conducted using a titrated epinephrine challenge design as previously described (Brock et al., 2003). The study was

conducted in compliance with Good Laboratory Practice (GLP) regulations. Six male Beagle dogs were exposed once via *muzzle-only* inhalation to escalating vapor concentrations of 10,000, 20,000, 50,000, 75,000, 100,000 or 150,000 ppm (Labcorp Early Development Laboratories Inc., 2022). The mean measured concentrations were variable. At 10,000 and 100,000 ppm, the measured concentrations were 137% and 116% of target, respectively. At 20,000 and 150,000 ppm, the measured concentrations were 72% and 66% of target, respectively.

There was a dose-related increase in the number of junctional escape complexes. However, the incidence and type of arrhythmias observed during the HFO-1132E exposure sessions generally remained comparable with the amount and type of arrhythmias observed during the air-only exposure session. Preliminary results indicate no cardiac sensitization response was observed at exposure levels of up to 100,000 ppm (measured concentration 116,000 ppm).

B. Short-term Repeat Dose Toxicity

1. Inhalation

A 14-day inhalation toxicity study was conducted with HFO-1132E using groups of 5 male and 5 female Sprague-Dawley rats (Covance CRS, LLC, 2020). The rats were exposed *nose-only* to levels of 0 (control), 4903 ppm (target 5000 ppm), 14,218 ppm (target 15,000 ppm) or 47,942 ppm (target 50,000 ppm), 6 h/day, 5 days/week for 2 weeks. The study resulted in no test material-related effects on clinical observations, mortality, body weights, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis or macroscopic observations. Heart weights (absolute, and relative to brain and body weight) were higher in males exposed to 50,000 ppm compared to controls. This finding correlated microscopically with left ventricular cardiomegaly seen in 2 of 5 males in the 50,000 ppm group and 1 of 5 males in the 15,000 ppm group. In addition, the vomeronasal organ (VNO), a sensory organ involved in detecting reproductive pheromones, had moderate to severe neuronal cell degeneration at >5000 ppm (Table 2). These effects were not observed following a 2-week recovery period. The VNO is well-developed in rats and mice and experimental impairment has been shown to adversely affect mating behavior and reproduction (Doving and Trotier, 1998). The VNO is poorly developed or absent in humans (Meredith, 2001).

Table 2. Test Item-related Findings in the Nose/Turbinates in Rats dosed with HFO-1132E for 14 days

Group/sex	**1M**	**2M**	**3M**	**4M**	**1F**	**2F**	**3F**	**4F**
Dose (ppm)	**0**	**5000**	**15,000**	**50,000**	**0**	**5000**	**15,000**	**50,000**
Vomeronasal Organ, Degeneration, Neuronal cell								
Minimal	0	0	0	0	0	2	0	0
Slight	0	2	0	0	0	3	0	0
Moderate	0	2	1	0	0	0	4	1
Marked	0	1	4	4	0	0	1	4
Severe	0	0	0	1	0	0	0	0
Total	0	5	5	5	0	5	5	5
Number of tissues examined	5	5	5	5	5	5	5	5

Source: Covance CRS, LLC., March 2020

In an OECD 412 study conducted in compliance with GLP, groups of either 10 male and 10 female (or only at the low exposure level 5 male and 5 female) Sprague-Dawley rats were exposed *nose-only* to levels of 0 ppm (control), 3,000 ppm, 10,000 ppm or 15,000 ppm for 6 h/day, 5 days/week for 4 weeks (Covance Laboratories Inc., 2020). Five rats per sex from the 0, 10,000 ppm and 15,000 ppm groups were held for an additional 2-week non-exposure period; all the others were sacrificed at the conclusion of the exposure phase. The study resulted in no test material-related effects on clinical observations, mortality, body weights, food consumption, ophthalmology, hematology, clinical chemistry, urinalysis or macroscopic observations. This study resulted in no test item-related adverse effects on mortality, clinical observations including Functional Observational Battery Motor Activity (FOB/MA), body weights, food consumption, ophthalmology, hematology, coagulation, clinical chemistry, urinalysis, bronchoalveolar lavage fluid (BALF), macroscopic necropsy observations or organ weights. Neuronal cell degeneration in the VNO was observed at exposures ≥-3,000 ppm (Table 3). The VNO is a sensory organ involved in detecting reproductive pheromones. It is well-developed in rats and mice and experimental impairment has been shown to adversely affect mating behavior and reproduction (Doving and Trotier, 1998). Moderate neuronal cell degeneration at ≥10,000 ppm could potentially impair its function and may be an adverse finding in this species. The VNO is poorly developed or absent in humans and its functionality has not been definitively established (Meredith, 2001).

Table 3. Test Item-related Findings in the Nose/Turbinates in Rats dosed with HFO-1132E for 4 weeks

Group/sex	**1M**	**2M**	**3M**	**4M**	**1F**	**2F**	**3F**	**4F**
Dose (ppm)	**0**	**3000**	**10,000**	**15,000**	**0**	**3000**	**10,000**	**15,000**
Degeneration, Neuronal Cell, Vomeronasal Organ								
Minimal	0	1	0	0	0	1	1	0
Slight	0	4	3	3	0	1	3	3
Moderate	0	0	2	2	0	0	1	2
Total	0	5	5	5	0	2	5	5
Number of tissues examined	5	5	5	5	5	5	5	5

Source: Covance Laboratories Inc., October 2020

Dogs from the cardiac sensitization study were exposed by facemask to 20,500 ppm HFO-1132E for 4 h/day for up to 4 days (Labcorp Early Development Laboratories Inc., 2022). No adverse effects were noted during the first 3 exposures. However, about 90 minutes into the 4th exposure, 4 of the 6 dogs exhibited clinical signs including increased activity, hypersensitivity to external stimuli, vocalization, aggression, panting, salivation and increased temperature. Further evaluation determined these animals were exposed to a much higher concentration of HFO-1132E. Findings reported during the study above included the observation of frost on the test substance cylinder and regulator, leakage of the gas sample at the inlet of the flowmeter and the doubling of sample consumption during days 3 and 4 compared to days 1 and 2. Additional work conducted by the study sponsor with results indicating frost was only observed when the test sample was taken from the liquid phase rather than the gas phase resulting in animals being inappropriately exposed to aerosols of the test substance at higher concentrations rather than to gas exposure of 20,500 ppm (Daikin Industries, Ltd., 2021a). The results of this dog study should be considered as invalid due to the exposure generating system utilizing the liquid instead of the gas phase of the test substance.

To determine if the deaths noted in the previous repeated exposure dog study were due to a potential issue in the exposure system, a new study was undertaken in which groups of 2 male dogs were exposed to 7500 and 20,000 ppm for 2 h per day for 5 consecutive days (Labcorp Early Development Laboratories Inc., 2021a). Exposure to HFO-1132E did not result in any adverse findings related to the test substance in the dogs. Parameters evaluated included body weight, organ weight (heart, lungs and bronchi), macroscopic and microscopic evaluation.

2. Oral Toxicity

No short-term oral toxicity data are available.

C. Subchronic Toxicity

1. Inhalation

In an OECD Guideline 413 study conducted under GLP, Sprague-Dawley CD rats (20/sex/group in the control and high exposure group; 10/sex/group in the low and mid- exposure groups) were exposed via *nose-only* inhalation to 0 (air control), 3000, 10,000 or 15,000 ppm HFO-1132E gas once daily for 6 h/day, 5 days/week, for 13 consecutive weeks (Labcorp Early Development Laboratories Inc., 2021b). At the end of the treatment period, 10 animals/sex/group were euthanized and necropsied. At the end of a 4-week recovery period, 10 animals/sex/group in the control and high exposure groups were euthanized and necropsied. Parameters evaluated during the study were: viability, clinical observations, ophthalmology, body weights, food consumption, MA and FOB (Week 12), clinical pathology (Week 14 and at end of recovery period), organ weights, macroscopic observations, and microscopic pathology. This study resulted in no test item-related adverse effects on mortality, clinical observations (including FOB/MA), body weights, food consumption, ophthalmology, hematology, coagulation, clinical chemistry, urinalysis, macroscopic necropsy observations or organ weights. At the terminal necropsy, HFO-1132E-associated degeneration (minimal to moderate) of the neuronal cells of the VNO was present in the nose/turbinates in animals exposed to ≥3066 ppm. The finding persisted following a 4-week recovery period in animals exposed to 14,972 ppm but was decreased in incidence and severity compared to the end of the exposure period. Thus, the No-Observed-Effect-Level (NOEL) for nasal pathology was determined to be 10,439 ppm. The No-Observed-Adverse-Effect-Level (NOAEL) was otherwise determined to be 14,972 ppm.

2. Oral

No subchronic oral toxicity data are available.

D. Chronic Toxicity/Carcinogenicity

No chronic toxicity studies have been conducted with this material.

E. Reproductive/Developmental

A GLP Reproductive/Developmental Toxicity study with HFO-1132E was conducted according to OECD test guideline 421 (Covance Laboratories Limited, 2021b). Briefly, Sprague Dawley rats (10 per sex) were exposed *nose-only* to targeted concentrations of 0, 3000, 9000 or 27,000 ppm HFO-1132E. Males were exposed daily prior to mating and continuing until at least 4 weeks of consecutive exposure. Females were exposed 2 weeks prior to mating, throughout pairing, gestation, and until day 12 of lactation. The F1 generation was not directly exposed to the test substance; any exposure occurred *in utero* or via lactation. The following parameters were evaluated: clinical condition, body weight, food consumption, thyroid hormone analysis, estrous cycle, pre-coital interval, mating performance, fertility, gestation length, organ weight, macroscopic pathology, and histopathology. The clinical condition, litter size and survival, sex ratio, body weight, anogenital distance, thyroid hormone analysis and macroscopic pathology for all offspring were also

assessed. Nipple counts were performed on male offspring on day 13 of age.

The achieved chamber concentrations were 3080, 9490 and 26,900 ppm. HFO-1132E did not show any evidence of reproductive/developmental toxicity nor of being an endocrine disruptor. The only effects observed were lowered body weight gain in the high exposure group parental animals. The NOAEL was considered to be 26,900 ppm for systemic, reproductive and developmental toxicity.

F. Genotoxicity/Mutagenicity

1. In Vitro

A GLP Bacterial Reverse Mutation Assay (Ames) assay with HFO-1132E was conducted according to OECD test guideline 471 using histidine-dependent auxotrophic mutants of *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 and a tryptophan-dependent mutant of *Escherichia coli* strain WP2 *uvr*A (pKM101) both in the presence and absence of a phenobarbital/5,6-benzoflavone-induced rat liver S9 activation system (Envigo CRS Limited, 2018). Nominal exposure concentrations of HFO-1132E were 0.3125, 0.65, 1.25, 2.5, 25, 50, and 70% volume per volume (v/v). HFO-1132E was diluted in sterile atmospheric air which was also used as an untreated control. Three seeded plates per treatment were exposed to HFO-1132E gas in stainless steel vessels. No evidence of toxicity was observed following exposure to HFO-1132E. Evidence of mutagenic activity was observed as clear concentration-related increases in the numbers of revertant colonies with strains TA98, TA100, TA1535 and SP2 *uvr*A (pKM101) in the absence and presence of S9 mix. Under the conditions of this study, it was concluded that HFO-1132E exhibited clear evidence of mutagenic activity.

A GLP *in vitro* mammalian chromosome aberration assay with HFO-1132E was conducted according to OECD test guideline 473 using cultured human peripheral blood lymphocytes both in the presence or absence of a phenobarbital/5,6-benzoflavone-induced rat liver S9 activation (Covance CRS Limited, 2020a). Human peripheral blood lymphocytes were stimulated into division in culture then treated with the gaseous test substance at concentrations from 5 to 70% v/v (the maximum practicable concentration). Sterile air was used as the vehicle control. Following a preliminary test to determine exposure concentrations for the main test, duplicate cultures were treated for 3 h in the absence and presence of S9 mix and for 21 h in the absence of S9 mix; appropriate concurrent vehicle and positive controls were included for each treatment regime. Two hours before the cells were harvested, mitotic activity was arrested, and cells were fixed onto slides. Mitotic cells were counted, and metaphases were subjected to detailed examination for the presence of chromosomal aberrations using light microscopy. Cultures treated with HFO-1132E at levels up to 70% v/v in air did not show any statistically significant increases in the mean proportion of aberrant metaphases in either the absence or presence of S9 mix when compared with the vehicle control. Further, no statistically significant increases in the proportion of polyploid or

endoreduplicated metaphase cells were observed during metaphase analysis under any treatment condition when compared with the vehicle control. Based on the findings and under the conditions of this cytogenetic study, it was concluded that HFO-1132E has shown no evidence of causing an increase in the frequency of structural and numerical chromosome aberrations in cultured human peripheral blood lymphocytes in the presence or absence of metabolic activation. A GLP *in vitro* mammalian cell micronucleus test with HFO-1132E was conducted according to OECD test guideline 487 using cultured human peripheral blood lymphocytes both in the presence or absence of a phenobarbital/5,6-benzoflavone-induced rat liver S9 activation system (Covance CRS Limited, 2020b). Human peripheral blood lymphocytes were stimulated into division in culture then treated with the gaseous test substance at concentrations from 0.05 to 70% v/v (the maximum practicable concentration). Sterile air was used as the vehicle control. Following a preliminary test to determine exposure concentrations for the main test, duplicate cultures were treated for 3 h in the absence and presence of S9 mix and for 20 h in the absence of S9 mix; appropriate concurrent vehicle and positive controls were included for each treatment regime. Cells were subjected to detailed examination for the incidences of mononucleate, binucleate and polynucleate cells using fluorescence microscopy. Cultures treated with HFO-1132E at levels up to 70% v/v in air did not show any statistically significant increases in the number of binucleated cells containing micronuclei in either the absence or presence of S9 mix when compared with the vehicle control. Based on the findings and under the conditions of this study, it was concluded that HFO-1132E did not show evidence of causing an increase in the induction of micronuclei in cultured human lymphocytes in the presence or absence of metabolic activation (Covance CRS Limited, 2020b).

2. In Vivo

A combined rat comet and micronucleus test was conducted according to OECD test guidelines 489 and 474, respectively (Covance Laboratories Limited, 2021c). Four groups of 6 male Sprague-Dawley CD rats (10/sex) were exposed via *nose-only* inhalation to targeted concentrations of 0 (sterile air), 30,000, 60,000, or 120,000 ppm HFO-1132E. Mean achieved atmosphere concentrations of HFO-1132E were 31,100, 63,700, and 122,000ppm. The animals were exposed for 6 h/day for 3 consecutive days. Animals were sacrificed and sampling was performed approximately 3 h after the completion of the 6-h exposure on day 3. Slides from a separate study of rats treated with 20 mg/kg cyclophosphamide (CPA) were stained and coded and used as the positive control for the micronucleus phase of this study. For the micronucleus phase, bone marrow was extracted from one femur per animal and micronucleus slides were prepared from the bone marrow smears. A positive control group (comet phase) was included whereby 3 male rats were exposed to sterile air and were dosed orally with 200 mg/kg ethyl methanesulfonate (EMS) on one occasion 3 h prior to termination. For the comet phase, sections of the liver, kidney, urinary bladder,

and lungs were collected, and comet slides were prepared from single cell suspensions of each tissue. HFO-1132E showed evidence of causing an increase in DNA strand breaks in the kidney at exposure concentrations of 60,000 ppm and above, and in the bladder, lung, and liver at 120,000 ppm. Hedgehog cells were also observed in the lung and liver at 120,000 ppm and in the bladder and kidney at 60,000 and 120,000 ppm and were considered to be directly correlated to the increases in the percent tail intensity. According to the authors, the exact etiology of hedgehog cells is ambiguous. Hedgehog cells are heavily damaged cells which exhibit a specific morphology (a small or non-existent head and a large diffuse tail). These cells have been widely assumed to represent apoptotic cells, however, there is evidence to suggest that the level of DNA damage in these comets is far less than the massive fragmentation that occurs in apoptosis. Evidence has shown that hedgehog cells can correspond to one level on a continuum of genotoxic damage, are not diagnostic of apoptosis, and should not necessarily be regarded as an indicator of cytotoxicity (Lorenzo et al., 2013). HFO-1132E did not show any evidence of causing an increase in the induction of micronucleated polychromatic erythrocytes or bone marrow toxicity at any concentration tested in this study. Under the conditions of this study, HFO-1132E did not induce micronuclei in male rat bone marrow cells but did induce DNA strand breaks in male rat liver, kidney, urinary bladder, and lungs (Covance Laboratories Limited, 2021c).

G. Metabolism/Pharmacokinetics

No specific *in vitro* or *in vivo* metabolism data are available for HFO-1132E. The chemical structure of HFO-1132E, however, would imply that metabolism at the terminal carbon, hydrogen, fluorine group (CHF) would occur resulting in potentially toxic intermediates or metabolites.

An *in vitro* chemical reactivity study was undertaken with HFO-1132E and utilized a structurally similar fluorocarbon that contained a CF2 group at a terminal carbon (Daikin Industries, Ltd., 2021b). Both substances were incubated at room temperature in a dimethylacetamide/water solvent for 21 h with glutathione. No dehydrofluorinated reaction products were observed for HFO-1132E, whereas a yield of 0.5% was seen with the structurally similar CF2 substance. In addition, the structurally similar CF2 fluorocarbon led to generation of a S-conjugated substance that was not found for HFO-1132E. The study authors suggested that this conjugation product could potentially lead to the generation of monofluoroacetate, a substance known to be toxic. The results revealed that HFO-1132E is not likely metabolized with glutathione. These *in vitro* results have not been demonstrated in a cell-based system (e.g., microsomes), or *in vivo*. Therefore, the relevance of this study to inform the metabolism of HFO-1132E is uncertain.

H. Other Information

Regarding observed effects on the VNO in rats, the studies summarized above indicate there is a temporal relationship with exposure to HFO-1132E and the

occurrence of vacuolation in cells of the VNO, i.e., exposures of 2 weeks were necessary to induce this reversible effect. No more severe effect on VNO tissues (i.e., necrosis, hyperplasia) was observed. Therefore, whether vacuolation in this tissue should be regarded as adverse or an adaptive response is unknown.

The VNO is a chemical sensory receptor in the nasal septum associated with the perception of pheromones and odor of food. This structure is a paired tubular diverticulum located in the vomer bone in the ventral portion of the nasal septum. The sensory cells have axons that extend forming the vomeronasal nerves, which connect this organ with the accessory olfactory bulb. This innervated structure is present in most mammals, e.g., dogs, cats, rodents, and rabbits (Harkema, 1991). In humans, the VNO develops *in utero* and appears to be associated with the early development of the reproductive system. However, as postnatal development occurs, the VNO begins to regress as other tissues become the primary source for hormonal secretion. After this initial development, the VNO regresses, leaving only a few vestiges in adults (Bhatnagar et al., 2001; Trotier, 2011). In humans, this structure has been described as a blind ending diverticulum in the septal mucosa (the VNO pit). Histologic examination finds an internal canal of variable length, extending back and covered with ciliary epithelium with numerous underlying glands. Compared to rodents, (e.g., mice) the general structure shows signs of regression, most notably the absence of vasculature. Furthermore, immunohistochemistry confirms the absence of epithelial receptor neurons and even of underlying nerve fibers that might allow neural information to be transported to the brain (Trotier et al., 2000). In addition, no synaptic junctions occur in the VNO pit of humans. Therefore, there is no nervous system connection with the brain. In contrast, a sensory axon connection is present in other species, (e.g., rat) that have a well-developed VNO (Meredith, 2001). Overall, there is compelling evidence that the vestigial VNO pit does not possess neural connection to the brain or chemosensory receptors. Therefore, despite the occurrence of histopathological changes in VNO in the rat following HFO-1132E exposure, a comparable anatomic structure is absent in humans and, hence, of limited relevance to humans.

V. HUMAN USE AND EXPERIENCE

No data are available.

VI. MECHANISM OF TOXICITY

No data are available.

VII. RATIONALE

HFO-1132E is a colorless gas with workplace exposures expected to be by inhalation exposure. The substance has low acute inhalation toxicity as indicated by a 4-h inhalation LC_{50} value of 100,000 ppm. HFO-1132E is a gas at ambient temperature, and as such a skin sensitizing study was not practical. A suite of *in silico* computational models were used to predict the skin sensitization potential of HFO-1132E. Although some models predicted that HFO-

1132E may likely be a skin sensitizing substance, there was general disagreement on the classification of HFO-1132E among the models utilized. In addition, most predictions were of low reliability and were based on two chlorinated, structurally similar substances that were predicted to be strong sensitizers. Overall, HFO-1132E is not expected to be a skin sensitizer in humans and no firm conclusion can be drawn from the *in silico* prediction.

No cardiac sensitization was observed in dogs exposed up to 116,000 ppm. Dogs exposed up to 20,000 ppm for 5 days did not exhibit any signs of toxicity including histopathology of the heart, lungs and bronchi. Repeated inhalation exposure in rats for 2 weeks resulted in cardiomegaly in males starting at 15,000 ppm and neuronal cell degeneration in the vomeronasal organ (VNO) at all exposure levels. Cardiomegaly was not observed in any other study including the 4-week and 13-week repeated inhalation toxicity studies in rats. The only effect noted in rats exposed for 4 weeks by inhalation to exposure levels up to 15,000 ppm was neuronal cell degeneration in the VNO which was reversible following a 2-week recovery period. Similar results were obtained in the 13-week inhalation study in rats, except the neuronal cell degeneration was still present but with lower incidence and severity following a 4-week recovery period. No reproductive or developmental toxicity was observed in rats exposed by inhalation to exposures up to 27,000 ppm in an OECD 421 reproductive/developmental screening study. The VNO is poorly developed or absent in humans and its significance is unclear. Its impairment may impact mating behavior and reproduction in rats; however, no evidence of impairment was noted in the OECD 421 reproductive/developmental screening study in rats. Several genetic toxicity studies were conducted, and the results were not consistent. HFO-1132E was not genotoxic in an *in vitro* chromosome aberration study or in *in vivo* or *in vitro* micronucleus assays but was genotoxic in 4 of 5 strains with and without S9 in the Ames assay. In an *in vivo* comet assay, statistically significant increases in DNA strand breaks were observed in the liver, bladder, lung and kidney. An increase in hedgehog cells was also noted at these concentrations which confounded the comet response as their presence could be associated with apoptosis or could correspond to one level on a continuum of genotoxic damage. No information was available on the metabolism of HFO-1132E to address potential species differences in metabolism and toxicity.

The critical study for the development of the WEEL was the 13-week inhalation toxicity study. As noted above, rats were exposed by *nose-only* inhalation to HFO-1132E at exposures up to 15,000 ppm. Neuronal cell degeneration of the VNO was observed at all concentrations. The VNO is poorly developed or absent in humans. Its impairment may impact mating behavior and reproduction in rats; however, no impairment was noted in the OECD 421 reproductive/developmental screening study in rats. The relevance of the VNO to humans is limited. The NOAEL for the 13-week study is 15,000 ppm.

The inhalation NOAEL was adjusted to account for exposure duration and also by application of appropriate uncertainty factors to account for interindividual variability, subchronic to chronic exposure, the uncertainty regarding the genotoxicity results, and the lack of metabolism data. A WEEL value of 350 ppm is expected to provide an acceptable margin of safety for potential adverse health effects in workers exposed to HFO-1132E.

VII. RECOMMENDED WEEL GUIDE

350 ppm, 8-hour Time Weighted Average

VIII. REFERENCES

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