2,3,3,3-Tetrafluoropropene

MAK value (2014)	**200 ml/m³ ≙ 950 mg/m³**
Peak limitation (2014)	**Category II, excursion factor 2**
Absorption through the skin	**–**
Sensitization	**–**
Carcinogenicity	**–**
Prenatal toxicity (2014)	**Pregnancy Risk Group C**
Germ cell mutagenicity	**–**
BAT value	**–**
Synonyms	2,3,3,3-tetrafluoropropylene
Chemical name	2,3,3,3-tetrafluoropropene
CAS number	754-12-1
Structural formula	$H_2C=CF–CF_3$
Molecular weight	114 (Honeywell International 2008)
Melting point	–152.2°C (Honeywell International 2008)
Boiling point	–30°C (Honeywell International 2008)
Vapour pressure at 21°C	6067 hPa (Honeywell International 2008)
log K_{OW} water/octanol partition coefficient	2.0 (Honeywell International 2008)
Solubility	198.2 mg/l water (at 24°C) (Honeywell International 2008)
1 ml/m³ (ppm) ≙ 4.73 mg/m³	**1 mg/m³ ≙ 0.21 ml/m³ (ppm)**

2,3,3,3-Tetrafluoropropene is a flammable, colourless gas with a weak characteristic odour. Unlike hydrocarbons containing chlorine, it is not ozone depleting and does not damage the ozone layer. At 4.4, its greenhouse potential is low (related to 100 years; carbon dioxide = 1). In this it differs from other fluorohydrocarbons such as tetrafluoroethane, which has a global warming potential of 1430. This is due to

the rapid degradation of 2,3,3,3-tetrafluoropropene; its average atmospheric lifetime is only about 12 days. It reacts mainly with reactive hydroxy radicals in the atmosphere; initially, it degrades to trifluoroacetyl fluoride and finally, by hydrolysis, to stable trifluoroacetic acid. 2,3,3,3-Tetrafluoropropene forms flammable gas-air mixtures and is classified as being extremely flammable. Its auto-ignition temperature is about 400°C. On burning, toxic and corrosive hydrogen fluoride is released (Nielsen et al. 2007; Papadimitriou et al. 2008; UBA 2013).

1 Toxic Effects and Mode of Action

2,3,3,3-Tetrafluoropropene has only very low acute toxicity. Repeated administration to rats induced infiltrates in the nose, lungs and kidneys and effects on haematological parameters at 50 000 ml/m^3 and above. The rabbit seems to be the most sensitive species. After 28-day inhalation exposure of female and male rabbits to 1000 ml/m^3, myocardial inflammation and necrosis were observed. These effects were characterized by the infiltration of lymphocytes, macrophages and neutrophilic granulocytes and were associated with focal degenerative tissue changes. Myocardial inflammation was detected in pregnant rabbits at 2500 ml/m^3 and above.

A 13-week inhalation study in rats did not reveal any irritating effects up to 50 000 ml/m^3, but there are no data available for irritation of the skin or eyes. The major fraction of 2,3,3,3-tetrafluoropropene is exhaled unmetabolized; only less than 1% is metabolized.

There are no long-term studies suitable for the evaluation of the carcinogenicity of the substance. 2,3,3,3-Tetrafluoropropene was mutagenic in Salmonella mutagenicity tests; chromosomal aberrations and micronuclei were not detected either in vitro or in vivo.

Effects on fertility, the foetuses or skeletal development were not observed in a 2-generation study in rats after inhalation exposure up to the highest concentration of 50 000 ml/m^3. Likewise, a study of the toxic effects on prenatal development did not reveal any adverse effects on the development of rats after inhalation exposure up to the highest concentration of 50 000 ml/m^3. In an inhalation study of the toxic effects on prenatal development in rabbits, effects on the heart and large vessels were observed in the foetuses at 5500 ml/m^3 and above.

There are no data available for sensitization.

2 Mechanism of Action

After myocardial inflammation and necrosis had been observed in rabbits in the developmental toxicity study, the creatine kinase activity in blood was determined as a clinico-chemical correlate in the 28-day inhalation study. Creatine kinase (CK) is an intracellular enzyme that transfers an N-phosphoryl group from phosphocrea-

tine to adenosine diphosphate (ADP) toand thus regenerates adenosine triphosphate. There are 4 isoenzymes: creatine kinase BB is found primarily in the brain, creatine kinase MB occurs above all in the heart, creatine kinase MM is detected in the skeletal muscle and creatine kinase MiMi in the mitochondria. Increased isoenzyme activity in the blood is evidence of leakage from the cell and thus of damage in this area. For example, creatine kinase MB increases if there is myocardial damage and creatine kinase MM increases in the case of skeletal muscle disorders. Increased creatine kinase MB and MM activities were determined in the 28-day inhalation study in rabbits (Table 4; Honeywell International 2013 a). The formation of fluoroacetate would be a possible mechanism of action. Fluoroacetate inhibits the aconitate hydratase of the citric acid cycle via the formation of fluorocitrate. This would particularly affect cells that have a high energy requirement, such as heart cells. It is not known whether the metabolite 3,3,3-trifluoro-2-propanol, which only forms in rabbits, is responsible for the heart effects or whether this metabolite results in the formation of fluoroacetate. To date, the mechanism of aconitate hydratase inhibition is known for such compounds as sodium fluoroacetate and 1,3-difluoro-2-propanol. This is contradicted by the fact that the central nervous effects described after the administration of sodium fluoroacetate were not observed in either dogs or rats. The dog has been shown to be the most sensitive species for this mechanism of action. It is interesting that effects on the heart predominated over central nervous effects in rabbits after the administration of sodium fluoroacetate (Goncharov et al. 2006). In rats, heart effects occurred after exposure to 1,3,3,3-tetrafluoropropene and 1,1,1,2,2-pentafluoropropane (Rush et al. 2013), while histological heart effects were observed after exposure to 1,3,3,3-tetrafluoropropene and 1,1,1,3,3-pentafluoropropane, but not after 1,1,1,2,2-pentafluoropropane and 2,3,3,3-tetrafluoropropene (Rush et al. 2013). Thus a propane or propene structure with one or several fluorine atoms at the C1 atom and 3 fluorine atoms at the C3 atom was regarded as a prerequisite for damage to the heart in rats. Direct cardiotoxic effects and indirect vasoactive properties have been suggested (Rush et al. 2013). However, 1,1,1,2,3,3,3-heptafluoropropane, which would be expected to cause heart effects if this were the case, did not induce any damage to the heart in rats up to 105 000 ml/m^3 in a 90-day study (ECHA 2014).

A possible explanation for the pronounced sensitivity of rabbits is their known high susceptibility to stress caused by exposure to various compounds. The stress hypothesis is supported by the fact that there were no effects on the skeletal muscles, only on the heart muscle; the effect was possibly caused by an increased base level of catecholamines, the low concentration of β2-adrenergic receptors in the heart (Bristow and Feldman 1992) and the very high heart rate in rabbits of 130 to 300 beats per minute compared with that in minipigs of 50 to 116 beats (Milani-Nejad and Janssen 2014), which is similar to the human heart rate. Rabbits react sooner to substances that affect the heart than other species and at markedly lower concentrations.

All in all, the actual mechanism of action for the effects on the hearts of rabbits and rats caused by some representatives of this class of substances is not clear, nor are the reasons known why rabbits are sensitive to 2,3,3,3-tetrafluoropropene.

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution and elimination

3.1.1 Absorption

There are no data available for absorption. From an assumed alveolar respiratory minute volume of 0.169 l/min, it was calculated that 15.27 mmol of 2,3,3,3-tetrafluoropropene was absorbed after the exposure of rats to 10 000 ml/m^3 for 6 hours. Studies showed that about 1% of this amount was eliminated with the urine and the remainder was exhaled. The 2,3,3,3-tetrafluoropropene concentration in the blood was not determined (Schuster et al. 2008).

Experimental data for absorption via the skin are not available. 2,3,3,3-Tetrafluoropropene is a gas; therefore, absorption of the compound via the gaseous phase should be taken into consideration. From the solubility in water of the substance, its molecular weight and vapour pressure, a Henry's constant of 3.44 atm × m^3/mol is calculated. The resulting concentration in the aqueous phase is 3.3×10^{-6} g/l at an external concentration of 100 ml/m^3. Using the models of Fiserova-Bergerova et al. (1990), Guy and Potts (1993) and Wilschut et al. (1995) and assuming 8-hour exposure of the whole body (surface area of skin 18 000 cm^2), the maximum amount absorbed at this concentration is 0.08 mg.

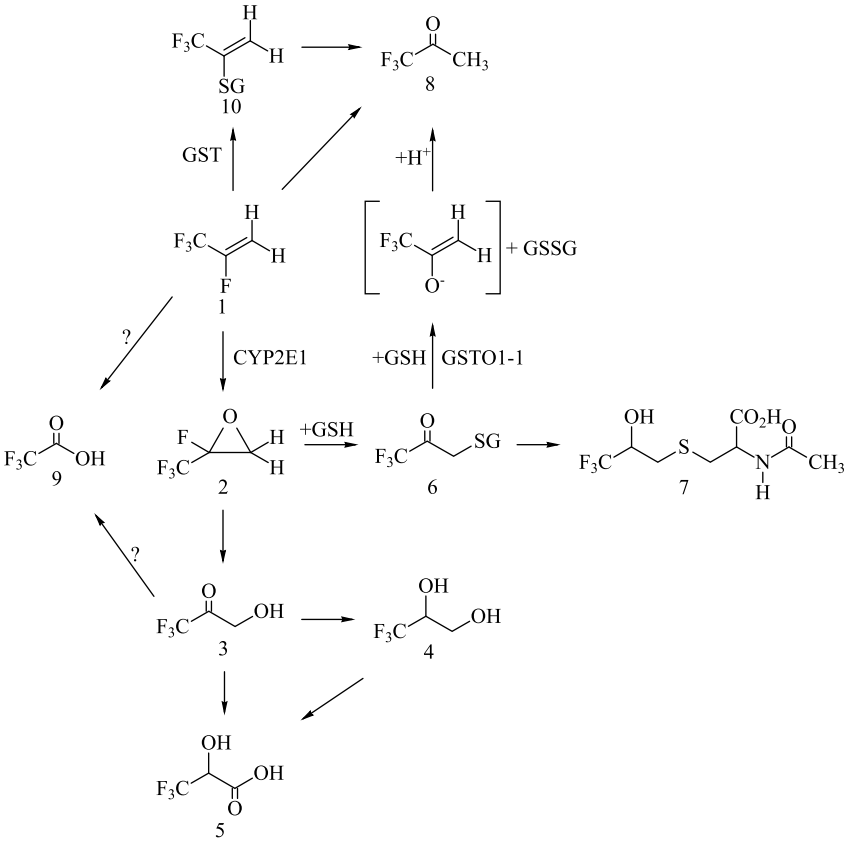
3.1.2 Elimination

The main metabolite N-acetyl-S-(3,3,3-trifluoro-2-hydroxy-propyl)-L-cysteine was eliminated with the urine by both rats and mice with a half-time of about 6 hours (Schuster et al. 2008, 2010).

3.2 Metabolism

Groups of 5 male Sprague Dawley rats were exposed for 3.5 hours via inhalation to 2,3,3,3-tetrafluoropropene concentrations of 2000, 10 000 or 50 000 ml/m^3 and 5 male B6C3F1 mice to 2,3,3,3-tetrafluoropropene concentrations of 50 000 ml/m^3. At the end of the exposure, urine was collected for 48 or 60 hours at 6-hour or 12-hour intervals and the metabolites were determined (Schuster et al. 2008).

N-acetyl-S-(3,3,3-trifluoro-2-hydroxy-propyl)-L-cysteine was identified as the main metabolite in the urine of both rats and mice (see Figure 1). Metabolism was



GST(O1-1) = Glutathionetransferase(omega1-1), GSH = Glutathione, GSSG = oxidized Glutathione

Figure 1 The biotransformation of 2,3,3,3-tetrafluoropropene in liver microsomes from rats, rabbits and humans and after inhalation in rats and mice (Schuster et al. 2008)

GST(O1-1) = glutathione transferase (omega1-1); GSH = glutathione; GSSG = oxidized glutathione

1: 2,3,3,3-tetrafluoropropene
2: 2,3,3,3-tetrafluoro-1,2-epoxypropane
3: 3,3,3-trifluoro-1-hydroxyacetone (detected in the urine)
4: 3,3,3-trifluoro-1,2-dihydroxypropane (detected in the urine)
5: 3,3,3-trifluoro lactic acid (detected in the urine)
6: S-(3,3,3-trifluoro-2-oxopropyl)glutathione (detected in rat, rabbit and human microsomes)
7: N-acetyl-S-(3,3,3-trifluoro-2-hydroxypropyl)-L-cysteine (detected in the urine; main metabolite)
8: 3,3,3-trifluoroacetone (detected in the urine)
9: trifluoroacetic acid (detected in the urine)
10: S-(3,3,3-trifluoropropenyl)-2-glutathione (detected in rat and female rabbit microsomes)

similar in rats and mice. However, only less than 1% of the amount absorbed by inhalation was eliminated with the urine in the form of this metabolite; the major fraction was exhaled unmetabolized. Conjugation with glutathione at the double bond followed by the formation of a reactive nephrotoxic thioketene via β-lyase, the pathway typical of other fluorolefins, is not possible as there is no fluorine atom available for cleavage at the C atom, where the glutathione residue is bound. The authors concluded that this might explain the absence of nephrotoxicity in the 90-day study in rats (see Section 5.2.1). They also suggested that only very a small amount (about 0.1%) of the possibly reactive epoxide 2,3,3,3-tetrafluoro-1,2-epoxypropane is formed, which is efficiently eliminated via glutathione conjugation and epoxide hydrolase resulting in the absence of liver toxicity after inhalation. This assumption was confirmed in the 90-day inhalation study in which no effects on the liver of rats were observed (Schuster et al. 2008).

Furthermore, rat liver microsomes and cytosol and S9 fraction and liver microsomes from humans were incubated with 2,3,3,3-tetrafluoropropene to investigate the metabolism. Rats were given daily intraperitoneal injections of pyridine for 5 days in doses of 100 mg/kg body weight to induce CYP2E1. If incubation was performed without NADPH, no metabolites were formed. In the presence of NADPH and glutathione, the metabolites S-(3,3,3-trifluoro-2-oxopropyl)glutathione, S-(3,3,3-trifluoro-2-hydroxypropyl)glutathione and 3,3,3-trifluoro-1-hydroxyacetone were identified in liver microsomes from humans and male and female rats. S-(3,3,3-trifluoropropenyl)-2-glutathione, which is formed via the binding of glutathione at the C atom of the double bond, was formed in rat liver microsomes to the extent of 1%, but not in human liver microsomes. In rat liver microsomes, the oxidation of 2,3,3,3-tetrafluoropropene was completely inhibited by diethyldithiocarbamate suggesting that metabolism mainly takes place at CYP2E1 (Schuster et al. 2008).

Groups of 3 female rabbits were exposed to 2,3,3,3-tetrafluoropropene concentrations of 2000, 10 000 or 50 000 ml/m^3 by inhalation for 3.5 hours. N-acetyl-S-(3,3,3-trifluoro-2-hydroxypropyl)-L-cysteine and S-(3,3,3-trifluoro-2-hydroxypropyl)mercaptolactic acid were detected in the urine of the rabbits as the main metabolites; 3,3,3-trifluoro-1,2-dihydroxypropane, 3,3,3-trifluoro-2-propanol (small amounts) and inorganic fluoride were also found. The following amounts of N-acetyl-S-(3,3,3-trifluoro-2-hydroxypropyl)-L-cysteine were determined in the urine after 6-hour exposure to 50 000 ml/m^3 and after adjustment for the body weight: 101 (rabbits), 11.7 (rats) and 184 (mice) µmol/kg body weight. Considering that this metabolite accounts for 44% of all metabolites detected in the urine of rabbits, 90% of those in rats and 32% of those in mice, there is not only a qualitative, but also a quantitative difference in the metabolism of 2,3,3,3-tetrafluoropropene in the 3 species (Schuster et al. 2010). 3,3,3-Trifluoro-1-hydroxyacetone was detected neither in the urine nor in vitro after incubation with liver S9 fraction. Consequently, the metabolites 3,3,3-trifluoroacetic acid and 3,3,3-trifluorolactic acid are not formed in rabbits (Figure 2). Rabbits are able to metabolize the possibly reactive 2,3,3,3-tetrafluoro-1,2-epoxypropane epoxide only via glutathione transferase. However,

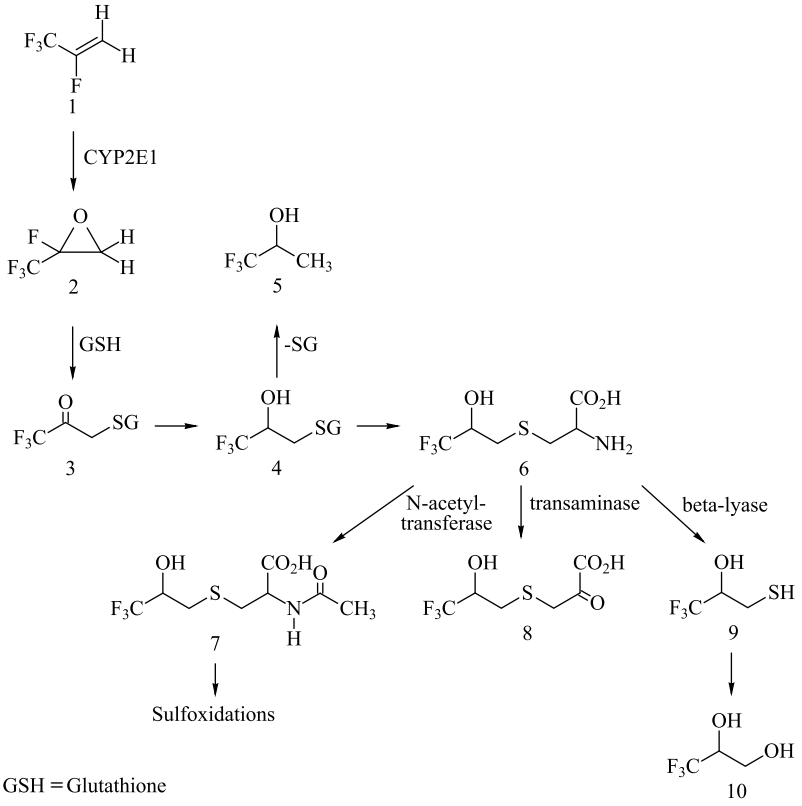


Figure 2 The biotransformation of 2,3,3,3-tetrafluoropropene after inhalation exposure in rabbits (Schuster et al. 2010)

1: 2,3,3,3-tetrafluoropropene

2: 2,3,3,3-tetrafluoro-1,2-epoxypropane

3: S-(3,3,3-trifluoro-2-oxopropyl)glutathione

4: S-(3,3,3-trifluoro-2-hydroxypropyl)glutathione

5: 3,3,3-trifluoro-2-propanol (detected in the urine)

6: S-(3,3,3-trifluoro-2-hydroxypropyl)-L-cysteine

7: N-acetyl-S-(3,3,3-trifluoro-2-hydroxypropyl)-L-cysteine (main metabolite; detected in the urine)

8: S-(3,3,3-trifluoro-2-hydroxypropyl)mercaptopyruvic acid (S-(3,3,3-trifluoro-2-hydroxypropyl) mercaptolactic acid detected in the urine as a reduction product)

9: 3,3,3-trifluoro-2-hydroxypropane-1-thiol

10: 3,3,3-trifluoro-1,2-dihydroxypropane (detected in the urine)

GSH = glutathione

liver toxicity was not observed in rabbits in the 28-day inhalation study (see Section 5.2.1). S-(3,3,3-trifluoro-2-hydroxypropyl)glutathione, 3,3,3-trifluoro-2-propanol and inorganic fluoride were detected after the incubation of liver microsomes from female rabbits. After the incubation of rabbit liver microsomes, S-(3,3,3-trifluoro-2-oxopropyl)glutathione, S-(3,3,3-trifluoro-2-hydroxypropyl)glutathione and, albeit in small amounts, the metabolite S-(3,3,3-trifluoropropenyl)-2-glutathione were identified (Schuster et al. 2008, 2010).

Male, non-pregnant female and pregnant female rabbits were exposed to 2,3,3,3-tetrafluoropropene concentrations of 50 000 and 100 000 ml/m^3 for one hour. The study was carried out because pregnant rabbits were found to have an increased incidence of myocardial inflammation at 2,3,3,3-tetrafluoropropene concentrations of 2500 ml/m^3 and above and increased mortality at 5500 ml/m^3 and above (see Section 5.5.2). The aim of this study was to clarify whether the metabolism of 2,3,3,3-tetrafluoropropene differs in pregnant rabbits. Urine was collected 48 hours after exposure. S-(3,3,3-Trifluoro-2-hydroxypropyl)mercaptolactic acid and N-acetyl-S-(3,3,3-trifluoro-2-hydroxypropyl)-L-cysteine were detected as the major metabolites in all samples. The main metabolite N-acetyl-S-(3,3,3-trifluoro-2-hydroxypropyl)-L-cysteine was quantified, and there were no significant differences between pregnant (50.47 ± 19.72 µmol) and non-pregnant rabbits (43.1 ± 22.35 µmol). Likewise, the metabolite pattern did not differ between these two groups. The increased mortality can thus not be attributed to changes in metabolism during pregnancy (Schmidt et al. 2012).

Table 1 Comparison of the metabolites in rats, rabbits and humans

Metabolites	Rats	Rabbits	Humans[a)]
urine	inhalation ♂	inhalation ♀	
N-acetyl-*S*-(3,3,3-trifluoro-2-hydroxypropyl)-L-cysteine (main metabolite)	x (90%)	x (44%)	
S-(3,3,3-trifluoro-2-hydroxypropyl) mercaptolactic acid	–	x	
3,3,3-trifluoro-1,2-dihydroxypropane	x	x	
3,3,3-trifluoro-2-propanol	–	x	
3,3,3-trifluoro-1-hydroxyacetone	x	–	
3,3,3-trifluorolactic acid	x	–	
3,3,3-trifluoroacetic acid	x	–	
3,3,3-trifluoroacetone	x	–	
liver microsomes	♂/♀	♂/♀	♂/♀
S-(3,3,3-trifluoro-2-oxo-propyl)glutathione	x/x	x/x	x/x
S-(3,3,3-trifluoro-2-hydroxypropyl)glutathione	x/–	x/x	x/x
S-(3,3,3-trifluoropropenyl)-2-glutathione	x/x	–/x	–/–
3,3,3-trifluoro-2-propanol	–/–	x/x	–/–

[a)] urine not examined; x = found; – = not found

Summary
There is both a qualitative and a quantitative difference in the metabolism of 2,3,3,3-tetrafluoropropene among the 3 species rabbit, rat and humans (see Table 1). On the basis of the metabolite 3,3,3-trifluoro-1-hydroxyacetone detected in vitro in human liver microsomes and in the urine of rats, the reactive metabolite 2,3,3,3-tetrafluoro-1,2-epoxypropane, catalyzed by CYP2E1, is assumed to be formed. The nephrotoxicity assumed to be caused by this metabolite was not confirmed in the 90-day inhalation study because this metabolite is formed in only very small amounts and is very efficiently inactivated via glutathione and epoxide hydrolase. Rabbits detoxify the reactive metabolite 2,3,3,3-tetrafluoro-1,2-epoxypropane only via conjugation to glutathione. Furthermore, the metabolites 3,3,3-trifluoro-1-hydroxyacetone, 3,3,3-trifluorolactic acid, 3,3,3-trifluoroacetic acid and 3,3,3-trifluoroacetone were detected only in the urine of rats, but not in rabbits. 3,3,3-Trifluoro-2-propanol is formed only in rabbits. Reactive thioketenes cannot be formed after glutathione conjugation.

4 Effects in Humans

There are no data available.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

5.1.1 Inhalation

Groups of 5 male and 5 female Sprague Dawley rats were exposed to 2,3,3,3-tetrafluoropropene concentrations of 0, 200 000 or 400 000 ml/m^3 by inhalation for 4 hours. The respiratory rate of 2 male and 2 female rats was reduced during the last 2 hours of exposure to 2,3,3,3-tetrafluoropropene concentrations of 200 000 ml/m^3; likewise, it was decreased in all rats of the high concentration group during hours 3 and 4 of exposure. Furthermore, laboured breathing was observed in the animals. Grey-coloured lungs were found in 1 male and 1 female of the group exposed to 200 000 ml/m^3 and in 3 males and 1 female of the group exposed to 400 000 ml/m^3. No other effects were observed (Honeywell International 2006 a).

On day 12 of gestation, 1 non-pregnant and 1 pregnant rabbit were exposed for 1 hour to 2,3,3,3-tetrafluoropropene concentrations of 100 000 ml/m^3 in whole-animal exposure chambers and observed for 2 days. Subsequently, they were sacrificed, clinical effects and body weights were established and the foetuses were examined for viability. In phase 2 of the study, groups of 5 male and 6 pregnant rabbits were exposed to 2,3,3,3-tetrafluoropropene concentrations of 0, 10 000, 50 000 or 100 000 ml/m^3 for 1 hour on day 12 of gestation and observed for 14 days. No

clinical signs, effects on body weights, or gross-pathological or histopathological changes in the heart, kidneys, liver or lungs were observed (Honeywell International 2011 a).

Sensitization of the heart to adrenaline was investigated in 6 beagle dogs. Each dog was exposed to 2,3,3,3-tetrafluoropropene concentrations of 20 000, 60 000 or 120 000 ml/m^3 by inhalation via the snout only at an interval of at least 48 hours. Each dog was simultaneously its own control. The pattern of exposure was as follows: first, an electrocardiogram was recorded followed 2 minutes later by an intravenous injection of adrenaline; exposure to the specific 2,3,3,3-tetrafluoropropene concentration took place after another 5 minutes. The dog was given another intravenous injection of adrenaline after another 5 minutes. The study ended after 10 minutes overall exposure to 2,3,3,3-tetrafluoropropene. Sensitization of the heart to adrenaline was not found in any dog in this study (Honeywell International 2006 b).

5.1.2 Oral administration

There are no data available.

5.1.3 Dermal application

There are no data available.

5.2 Subacute, subchronic and chronic toxicity

5.2.1 Inhalation

Rats

Groups of 5 male and 5 female Sprague Dawley rats were exposed nose-only to 2,3,3,3-tetrafluoropropene concentrations of 0, 5 000, 20 000 or 50 000 ml/m^3 for 6 hours a day, on 5 days per week, for 2 weeks. Body weights, feed consumption, haematological and clinico-chemical parameters, organ weights and the organs (necropsy) were investigated. Selected organs and tissues, including the respiratory tract and nose, were examined histopathologically. No effects were found up to the high concentration. A NOAEC (no observed adverse effect concentration) of 50 000 ml/m^3 was reported (Honeywell International 2005 a).

Groups of 5 male and 5 female Sprague Dawley rats were exposed nose-only to 2,3,3,3-tetrafluoropropene concentrations of 0, 5 000, 15 000 or 50 000 ml/m^3 for 6 hours a day, on 5 days per week, for 4 weeks. The rats were observed for 2 weeks after exposure. Body weights, feed consumption, haematological and clinico-chemical parameters, organ weights and the organs (necropsy) were investigated. Selected

organs and tissues, including the respiratory tract and nose, were examined histopathologically. In female rats, the urea concentration in the blood was significantly increased in the low and high concentration groups. The blood creatinine level was also increased at the high concentration. After the 2-week recovery period, the values were again in the range of the control animals. At the end of the 2-week recovery period, the absolute and relative liver weights and the blood urea level were significantly increased in the male rats of the high concentration group. No other effects were observed. Therefore, a NOAEC of 15 000 ml/m^3 was derived from this study for 2,3,3,3-tetrafluoropropene (Honeywell International 2006 c).

Groups of 10 male and 10 female Sprague Dawley rats were exposed to 2,3,3,3-tetrafluoropropene concentrations of 0, 5000, 15 000 or 50 000 ml/m^3 for 6 hours a day, on 5 days per week, for 13 weeks. Body weights and feed consumption remained unaffected. Clinical effects were not observed. Ophthalmoscopy did not yield any findings. Histopathological examination of all the organs was carried out only in the animals of the high concentration group. After exposure to the high concentration of 50 000 ml/m^3, plasmacytosis was detected in the cervical lymph nodes of 3 males and 1 control animal and of 4 female control animals and 1 exposed animal. Focal mononuclear cell infiltrates were found in the kidneys of 3 males compared with in 1 animal in the control group. This effect was observed in 1 female control animal, but not in any exposed females. Focal mononuclear cell infiltrates were found in the lungs of 5 male and 4 female rats compared with in 2 and none of the 10 rats of the control group, respectively. Furthermore, 4 male rats had focal mononuclear cell infiltrates in the nasal cavities, whereas none of the 10 control animals was affected. All infiltrates were reported to be very slight to slight. As only the high concentration group was examined, it was not possible to derive a dose–response relationship or a NOAEC for local effects. Therefore, a treatment-related effect on the respiratory tract cannot be ruled out. Isolated or multiple acidophilic foci were found in the pancreas of 3 females, but not in any animal of the control group. This effect was not observed in the males.

No effects on the heart were observed. Creatine kinase was not determined, nor were the skeletal muscles examined histopathologically. The erythrocyte volume was significantly reduced only in the males of the low and high concentration groups, but there was no clear concentration–response relationship. Therefore, this effect cannot be regarded as substance-induced. In all groups of females, the prothrombin time was significantly increased and the reticulocyte count (5000 ml/m^3: 17%, 15 000 ml/m^3: 17% and 50 000 ml/m^3: 20.3%) was significantly reduced. However, these effects were only very slight. As the erythrocyte count was not markedly reduced, which could result in increased erythropoiesis, this effect was probably caused by an increased number of reticulocytes in the female rats of the control group. The effects on the prothrombin time were contrary in males and females. The increase in the treated females of all 3 concentration groups was almost the same. A treatment-related effect seems questionable. There are no other supportive findings that provide evidence of a clotting disorder. A relationship with the treatment is questionable because of the absence of a concentration–response relationship.

A NOAEC of 50 000 ml/m³ was derived from this study on the grounds that there were no concentration-dependent changes in the haematological parameters and microscopic effects were only sporadically observed in a few animals (Honeywell International 2007)

Rabbits

Groups of 25 male and 25 female rabbits were exposed to 2,3,3,3-tetrafluoropropene concentrations of 0, 500, 1500/1000 or 5500/4500 ml/m³ in whole animal exposure chambers for 6 hours a day, for 28 days. Five animals per sex and group were sacrificed on days 8 and 15, 10 animals per sex and group were sacrificed on day 29 after the beginning of exposure and 5 animals were observed for 28 days. Because of the increase in the total creatine kinase activity and the histopathological changes in the heart muscle after exposure for 7 and 14 days, the concentration in the group exposed to 1500 ml/m³ was lowered from 1500 to 1000 ml/m³ in the last 2 weeks. As 2 animals of the group exposed to 5500 ml/m³ had not survived after the first 7 exposure days, the animals were subsequently exposed only on 6 days per week instead of 7 days. The cause of death of the 2 animals is unclear. On day 8, the concentration of 5500 ml/m³ was reduced to 4500 ml/m³ (exposure pattern: Table 2) (Honeywell International 2013 a).

Table 2 Exposure pattern of the 28-day inhalation study with 2,3,3,3-tetrafluoropropene in rabbits (Honeywell International 2013 a)

Group	Exposure [ml/m³]	Number of animals ♂/♀	Interim sacrifices ♂/♀			Recovery period (28 days)
			day 8	day 15	day 29	
1	0	25/25	5/5	5/5	10/10	5/5
2	500	25/25	5/5	5/5	10/10	5/5
3	1500/1000	25/25	5/5	5/5	10/10	5/5
4	5500/4500	25/25	4/4	5/4	10/ 9	5/5

Clinical symptoms were not observed, nor were there any changes in body weights or feed consumption. Likewise, blood values, coagulation, blood gases and urinary values were unaffected. On day 8 of exposure, the relative liver weights were significantly increased in male rabbits. After 28 days recovery, the liver weights were again in the range of those of the control animals.

At 1000 ml/m³ and above, both the females and males of the interim sacrifice groups were found to have subacute/chronic myocardial inflammation that was characterized by small lymphocyte aggregates, macrophages and neutrophilic granulocytes and associated with foci of degeneration and necrosis. Degeneration and necrosis were considered to be a result of the inflammatory process rather than a separate finding. The incidence and severity of the findings did not increase with the exposure period (Table 3). After 28 days recovery, myocardial inflammation

Table 3 Incidences of myocardial inflammation and skeletal muscle necrosis in rabbits after exposure to 2,3,3,3-tetrafluoropropene by inhalation (Honeywell International 2013 a)

	2,3,3,3-Tetrafluoropropene concentration [ml/m³]			
	0	500	1500/1000	5500/4500
	myocardial inflammation			
day 8	♂ 0/ 5	0/ 5	1/ 5 (20%)	5/ 5 (100%)
	♀ 0/ 3	0/ 3	0/ 5	2/ 6 (33%)
day 15	♂ 0/ 5	0/ 5	0/ 5	2/ 5 (40%)
	♀ 0/ 5	0/ 5	2/ 5 (40%)	0/ 4
day 29	♂ 0/10	0/10	1/10 (10%)	6/10 (60%)
	♀ 0/10	0/10	0/10	4/10 (40%)
recovery period	♂ 0/ 5	0/ 5	0/ 5	0/ 5
	♀ 0/ 5	0/ 5	0/ 5	0/ 5
	skeletal muscle necrosis			
day 8	♂ 3/ 5 (60%)	3/ 5 (60%)	3/ 5 (60%)	0/ 4
	♀ 3/ 5 (60%)	2/ 5 (40%)	2/ 5 (40%)	0/ 5
day 15	♂ 2/ 5 (40%)	2/ 5 (40%)	4/ 5 (80%)	3/ 5 (60%)
	♀ 1/ 5 (20%)	2/ 5 (40%)	4/ 5 (80%)	2/ 4 (50%)
day 29	♂ 3/10 (30%)	2/10 (20%)	3/10 (30%)	5/10 (50%)
	♀ 3/10 (30%)	2/10 (20%)	3/10 (30%)	6/10 (60%)
recovery period	♂ 1/ 5 (20%)	3/ 5 (60%)	0/ 5	1/ 5 (20%)
	♀ 3/ 5 (60%)	5/ 5 (100%)	0/ 5	3/ 5 (60%)

was no longer observed in any rabbit. As necrosis was detected histopathologically during treatment, the finding is considered to be a sign of persistent defect after healing .

Acute skeletal muscle necrosis was found in control group animals and exposed animals at all times of exposure and irrespective of sex. It was characterized by myofibrillar loss, hyaline degeneration in the sarcoplasm and the loss of transverse striation. Signs of inflammation, fibrosis or the transformation of myofibrils to adipose tissue were not observed. There was no concentration-related increase in the incidence or severity of muscular necrosis (Table 3). After 28 days recovery, the incidence and severity of muscular necrosis in the exposed rabbits was similar to that in the control animals. Unlike rats and mice, rabbits react very sensitively to a diet that is not balanced exactly as regards vitamin E and selenium. This may be the case especially during pregnancy. Therefore, small (multi)focal foci of necrosis or histiocytic reactions may often be observed in both heart and skeletal muscles. Moreover, intermediate stages of parasitic diseases are more common in the muscles of rabbits. For all these reasons, muscular findings are not unusual in rabbits.

The total creatine kinase activity in female and male rabbits was significantly increased on day 29 at 1000 ml/m³ and above. This effect was observed in females as early as after 8 days of exposure. In male rabbits, the total creatine kinase activity

was increased even at 500 ml/m³ after 28 days recovery. However, this increase was not related to the concentration. As the creatine kinase activity in the group exposed to 500 ml/m³ was not increased at any other time of investigation, a NOAEC of 500 ml/m³ was derived from this study (Table 4). As the concentration in the 2 higher exposure groups was reduced during the study, it is difficult to assess whether the effects increased with the exposure period. In the middle concentration group, the prevalence of myocardial inflammation increased in both sexes from 10% to 20% in the period from days 8 to 15; subsequently, after the concentration had been lowered to 1000 ml/m³, the prevalence was 5%. The same applies to skeletal muscle necrosis; its prevalence was no longer increased after the concentration was lowered to 1000 ml/m³. This is evidence that the exposure period has less influence than the concentration, at least at concentrations in the range of the NOAEC. However, the prevalence of myocardial inflammation in the animals of the high concentration group increased from 20% to 50% from day 15 to day 29. Therefore, after prolonged exposure, the severity can be expected to increase markedly at concentrations that cause effects.

Table 4 Clinico-chemical effects in rabbits after exposure to 2,3,3,3-tetrafluoropropene by inhalation (Honeywell International 2013 a)

Day of examination	Exposure [ml/m³]	Findings (determined in the blood)
day 8	500: ♂, ♀:	no clinico-chemical effects
	1500: ♂:	total protein ↓; albumin ↓
	1500: ♀:	creatine kinase MM ↑; fatty acid binding protein ↑
	5500: ♂:	alanine aminotransferase ↑; urea ↑; total creatine kinase ↑; creatine kinase MM ↑; myoglobin ↑
	5500: ♀:	total creatine kinase ↑; creatine kinase MM ↑; alanine aminotransferase ↑; fatty acid binding protein↑; myoglobin ↑
day 15	500: ♂, ♀:	no clinico-chemical effects
	1500: ♀:	total creatine kinase ↑; myoglobin ↑; creatine kinase MM ↑
	4500: ♂:	total creatine kinase ↑; Ca^{2+} ↓; creatine kinase MM ↑; myoglobin ↑
	4500: ♀:	aspartate aminotransferase ↑; prothrombin time ↓; total creatine kinase ↑; creatine kinase-MB ↑
day 29	500: ♂, ♀:	no clinico-chemical effects
	1000 and above: ♂:	total creatine kinase ↑; Ca^{2+} ↓; creatine kinase MB ↑
	1000 and above: ♀:	total creatine kinase ↑; creatine kinase MM ↑; fatty acid binding protein↑; myoglobin ↑
	4500: ♂:	creatine kinase MM ↑; fatty acid binding protein ↑
	4500: ♀:	aspartate aminotransferase ↑; alkaline phosphatase, glucose ↑
recovery period	500 and above: ♂:	total creatine kinase increased but not in a concentration-dependent manner↑; phosphate ↑

creatine kinase MM: skeletal muscle; creatine kinase MB: heart

Minipigs

Groups of 3 male and 3 female minipigs (Göttingen minipigs) were exposed to 2,3,3,3-tetrafluoropropene concentrations of 0, 5500 or 10 000 ml/m³ in whole-animal exposure chambers for 6 hours a day, for 14 days. On days 7 and 14, the animals were examined clinically and on day 14, all organs, particularly the heart and muscles, were investigated histopathologically. Mortality was not increased, and there were no changes in body weights, organ weights or feed consumption. Haematological and clinico-chemical parameters (myoglobin, troponin, creatine kinase, aspartate aminotransferase and alanine aminotransferase) were unchanged. Histopathological effects on the heart and muscles consisted of minimal cellular infiltrates, pigmented macrophages and minimal myofibrillar degeneration in both the control and exposed animals. As these effects were not concentration-dependent and had no consistent pattern, they were not regarded as substance-induced. The authors therefore derived a NOAEC of 10 000 ml/m³ for 2,3,3,3-tetrafluoropropene (Honeywell International 2013 b).

Groups of 8 male and 8 female minipigs (Göttingen minipigs) were exposed to 2,3,3,3-tetrafluoropropene concentrations of 0, 5000 or 10 000 ml/m³ in whole-animal exposure chambers for 6 hours a day, for 28 days. Mortality was not increased, and there were no changes in body weights or feed consumption. Haematological and clinico-chemical parameters (myoglobin, troponin, aspartate aminotransferase and alanine aminotransferase) were unchanged. Total creatine kinase and creatine kinase MM (skeletal muscle) were minimally increased in 3 of 8 females of the high concentration group; the authors did not consider this finding to be substance-induced as the values were still within the range of biological variability. The relative liver weights were significantly increased at 5000 ml/m³ and above, and the absolute liver weights were significantly increased in the high concentration group. Only the heart and muscles were examined histopathologically. Minimal cellular infiltrates, pigmented macrophages and minimal myofibrillar degeneration were evidence of effects on the heart and muscles in both the control and exposed animals. As these effects were not concentration-dependent and had no consistent pattern, they were not regarded as substance-induced. Therefore, a NOAEC of 10 000 ml/m³ was derived for 2,3,3,3-tetrafluoropropene (Honeywell International 2013 b).

Summary

At 1000 ml/m³ and above, both the female and male rabbits of the interim sacrifice groups were found to have subacute/chronic myocardial inflammation that was characterized by small lymphocyte aggregates, macrophages and neutrophilic granulocytes and associated with foci of degeneration and necrosis. Effects on the heart were not observed in a 13-week inhalation study in rats and a 28-day inhalation study in minipigs. As minipigs are very similar to humans with regard to heart anatomy, cardiovascular physiology (heart rate in rabbits: 130–300, pigs/humans: 50–116 beats/minute), ventricular flow, electrophysiology and coronary artery pattern, this species is favoured in pharmacology (Milani-Nejad and Janssen2014; Svendson

2006; Swindle et al. 2012;). Furthermore, rabbits are very susceptible to stress and myocardial inflammation is often caused by viral or bacterial infections. The stress hypothesis is supported by the fact that the skeletal muscles, unlike the heart muscle, were not affected; this effect was possibly caused by an increased base level of catecholamines and the low concentration of β2-adrenergic receptors. The localization and histopathological findings that were observed in rabbits after exposure to 2,3,3,3-tetrafluoropropene coincide well with the effects described by Gruber et al. (1933) after intravenous injection of, for example, theophylline, adrenaline and ephedrine.

In the 28-day inhalation study in rabbits, no viral or bacterial infections occurred, however. Considering that the mechanism of action for heart effects in rabbits is not exactly known and effects on the heart were observed in rats after exposure to 1,3,3,3-tetrafluoropropene, the rabbit is used as the most sensitive species for the evaluation of heart effects induced by 2,3,3,3-tetrafluoropropene.

5.2.2 Oral administration

There are no data available.

5.2.3 Dermal application

There are no data available.

5.3 Local effects on skin and mucous membranes

There are no data available.

5.4 Allergenic effects

There are no data available.

5.5 Reproductive and developmental toxicity

5.5.1 Fertility

In a 2-generation study, 28 Wistar rats per sex and group were exposed to 2,3,3,3-tetrafluoropropene concentrations of 0, 5000, 15 000 or 50 000 ml/m^3 by inhalation for 6 hours a day, on 5 days per week, beginning 10 weeks prior to mating (♂; F0: head-only exposure; ♀: head-only exposure until GD 19 and whole-body exposure on PND 5 and thereafter) until the end of lactation. At the end of lactation,

offspring were selected as the F1 parental animals and exposed in the same way as the F0 generation in whole-animal exposure chambers beginning in postnatal week 4 until the end of lactation for the F2 generation. There was a statistically significant decrease in feed consumption, body weights and body weight gains during the first 3 weeks after direct exposure of the F1 animals (PND 28, 35 and 42). The body weights of the F1 females decreased by 13%, 15% and 14% in the 5000, 15 000 and 50 000 ml/m^3 concentration groups. The authors did not consider this decrease to be an adverse effect because it was transient, feed consumption decreased to the same extent and body weights varied by only within 3% of those of the controls at the end of treatment and prior to mating. In the low and middle concentration groups, decreased cycle lengths in the female F0 animals were not regarded as adverse because they were not observed in the high concentration group of the F0 animals or in any group of F1 animals. The duration of gestation was extended in the high concentration group, but was not longer than 22 days.

The statistically significant increase in mortality of 8.5% in the offspring of the high concentration group of the F1 generation up to postnatal day 4 was within the historical control range (0%–20%) and was therefore not regarded as adverse. Two dams of the high concentration group had 3 offspring with malformations (one pup: no tail and anal atresia; one pup: polypodia of the right hind leg; one pup: no tail). The malformations that occurred were not regarded as substance-induced because no malformations were observed in the developmental toxicity study. Substance-induced effects on body weights or on the body weight gains of the offspring were not observed in either generation. Vaginal opening was retarded by up to 6 days in the female F1 animals of the middle and high concentration groups. Because of the reduced feed consumption and reduced body weights, the retardation in vaginal opening is regarded as a secondary effect. Adverse findings were not obtained when the ribs of the F1 offspring were screened from postnatal days 21 to 23 for skeletal abnormalities that were observed in the developmental toxicity study (see Section 5.5.2). The gross-pathological and histopathological examinations of the F1 and F2 offspring revealed no abnormalities. Therefore, the decrease in the absolute and relative thymus weights of the F2 offspring in the high concentration group was not regarded as substance-induced. No other organ weight changes were detected.

A NOAEC of 50 000 ml/m^3 was derived for the systemic toxicity of the parental animals, for fertility and foetotoxicity and for the screening of the ribs of the F1 offspring for skeletal changes on postnatal days 21 to 23 (Honeywell International 2011 c).

5.5.2 Developmental toxicity

In a nose-only inhalation study carried out according to OECD Test Guideline 414, groups of 25 female Wistar rats were exposed to 2,3,3,3-tetrafluoropropene concentrations of 0, 5000, 15 000 or 50 000 ml/m^3 for 6 hours a day, from days 6 to 19 of

gestation. The body weights of the sacrificed dams were reduced compared with the body weights in all groups, including the control group, on day 6 of gestation. Effects on the reproductive organs were not observed. The hearts of the dams were not examined histopathologically. There were no effects on fertility or the pregnancy index, the number of implantations, pre and post-implantation losses, the number of live foetuses, resorptions or the sex ratio. A statistically significant increase in the number of corpora lutea was recorded in the middle concentration group. The visceral examination revealed no unusual findings. Skeletal variations and reduced ossification were observed at 2,3,3,3-tetrafluoropropene concentrations of 5000 ml/m^3 and above, but these effects were not concentration-dependent and were not regarded as adverse. The authors derived a NOAEC of 50 000 ml/m^3 for developmental toxicity and maternal toxicity (Honeywell International 2006 d). The Commission considered the number of foetuses with reduced weights (< 75% of the mean body weights of the control group) to be significantly increased at 15 000 ml/m^3 and above on both a foetus and litter basis (0, 5000, 15 000, 50 000: 0%, 1.8%, 3.2% and 3.4% on a foetus basis, and 0%, 14%, 21% and 28% on a litter basis). This effect made a decisive contribution to the increase in the total incidence of external abnormalities in the foetuses at 15 000 ml/m^3 and above, compared with the incidence in the control group. A statistically significant increase in this incidence was recorded also at 5000 ml/m^3, but it was due to a significant increase in large foetuses (6 of 224 foetuses compared with 0 of 267 foetuses). As it was observed only in this concentration group, it is not regarded as substance-induced. This finding indicates delayed development after prenatal administration.

The 2-generation study described above (see Section 5.5.1), which was carried out by the same testing laboratory using the same doses and the same strain of rat, did not reveal any effects on the number of foetuses with reduced weights in the F1 and F2 generations. This 2-generation study included a longer exposure period, and because this effect was not observed in either generation, the Commission considered the delayed development in the developmental toxicity study to be reversible. Therefore, the NOAEC for developmental toxicity and maternal toxicity is 50 000 ml/m^3 for rats.

Groups of 24 rabbits were exposed to 2,3,3,3-tetrafluoropropene concentrations of 0, 2500, 4000, 5500 or 7500 ml/m^3 in whole-animal exposure chambers for 6 hours a day, from days 6 to 28 of gestation. The effects on the dams are described in Tables 5 and 6. The subacute inflammation of the heart was characterized by the infiltration of lymphocytes, degeneration of myocardial fibres, extension of the basophilic ground substance of the connective tissue and fibrosis. The lesions were multifocal at the left and right ventricles and sporadically also at the papillary muscles of the cardiac wall. The severity of the inflammation of the heart increased with the concentration and was the cause of death in the group exposed to 7500 ml/m^3. The number of live foetuses, the sex ratio and other litter parameters were unaffected. The foetuses were not found to have any significant visceral or skeletal changes compared with the control group. However, effects on the heart and large vessels (aortic bulb, stenosis in the pulmonary vessel, absence of cardiac septum,

Table 5 Developmental toxicity after administration of 2,3,3,3-tetrafluoropropene in rabbits (Honeywell International 2011 b)

Species, strain, number per group	Exposure	Findings
rabbits, New Zealand White 24 ♀	**GD 6–28**, 0, 2500, 4000, 5500, 7500 ml/m³, examination on GD 28	**2500 ml/m³and above**: dams: myocardial inflammation: concentration-dependent increase in incidence and severity, multifocal foci in the cortical renal tubules, pigmented alveolar macrophages with haemosiderin; **4000 ml/m³**: dams: body weights significantly ↓; foetuses: NOAEC; **5500 ml/m³**: dams: mortality: 4/24, feed consumption significantly ↓, body weights significantly ↓; foetuses: aortic bulb, stenosis in the pulmonary vessel and absence of cardiac septum (1 foetus; 0.8% per litter), interrupted aortic arch (1 foetus; both foetuses from the same litter) not significant; **7500 ml/m³**: dams: mortality: 7/24, body weights not significantly ↓; foetuses: aortic bulb, stenosis in the pulmonary vessel and absence of cardiac septum (3 foetuses in 2 litters; 2.4% per litter), absence of tricuspid valve (2 foetuses in 1 litter) not significant

Table 6 Maternal toxicity in the developmental toxicity study with 2,3,3,3-tetrafluoropropene in rabbits (Honeywell International 2011 b)

		Concentration [ml/m³]			
	0	2500	4000	5500	7500
number of animals examined	24	24	24	24	24
histopathological findings					
myocardial inflammation		8	12	10	15
minimal	0	3	1	2	2
mild	0	2	2	5	6
moderate	0	3	8	3	6
severe	0	0	1	0	1
kidneys: tubular necrosis	0	0	0	3	1
lungs					
congestion	0	2	5	3	3
haemorrhage	2	6	3	3	1
inflammation	4	8	8	3	4
alveolar macrophages	2	10	7	2	3

absence of tricuspid valve or interrupted aortic arch) were evident in 2 foetuses of 2 litters of the group exposed to 5500 ml/m^3 and in 3 foetuses of 2 litters exposed to 7500 ml/m^3. These findings were not statistically significant compared with the incidence in the control group, but they were outside the incidence of the historical controls (0.5%) for aortic bulb and stenosis in the pulmonary vessel (0.8% and 2.4% per litter for 5500 and 7500 ml/m^3, respectively). The other findings were not observed in the historical controls. In view of the litter data and the similarity of the multiple findings obtained in the cardiovascular system, these findings must be regarded as substance-induced. A NOAEC of 4000 ml/m^3 was derived for the developmental toxicity of 2,3,3,3-tetrafluoropropene, whereas no NOAEC was obtained for maternal toxicity; a LOAEC (lowest observed adverse effect concentration) of 2500 ml/m^3 was established for 2,3,3,3-tetrafluoropropene (Honeywell International 2011 b).

5.6 Genotoxicity

5.6.1 In vitro

In the Salmonella mutagenicity test, 2,3,3,3-tetrafluoropropene concentrations of 100 000 to 750 000 ml/m^3 air in the incubation chamber were investigated according to OECD Test Guideline 471 using the strains TA98, 100, 1535, 1537 with and without the addition of a metabolic activation system and the Escherichia coli strain WP2 uvrA. 2,3,3,3-Tetrafluoropropene induced base substitutions in the Salmonella strain TA100 and in Escherichia coli with metabolic activation (> 2-fold increase at 2,3,3,3-tetrafluoropropene concentrations of 200 000 ml/m^3 and above); the increase was concentration-dependent and the substance was therefore found to be mutagenic. Cytotoxicity was observed in the strain TA100 at 100 000 ml/m^3 and above without metabolic activation, but the cytotoxicity was not concentration-dependent (Honeywell International 2005 b).

2,3,3,3-Tetrafluoropropene was not clastogenic in chromosomal aberration tests in human lymphocytes either with or without the addition of a metabolic activation system. The 2,3,3,3-tetrafluoropropene concentrations in the air of the incubation chamber were 100 000 to 750 000 ml/m^3. Mild cytotoxicity was observed after 48 hours with metabolic activation at the high 2,3,3,3-tetrafluoropropene concentration of 750 000 ml/m^3 (Honeywell International 2005 c).

A chromosomal aberration test using a Chinese hamster lung cell line (CHL/IU) yielded negative results after incubation with 2,3,3,3-tetrafluoropropene concentrations of 200 000 to 800 000 ml/m^3 air for 6, 24 and 48 hours both with and without the addition of a metabolic activation system. Cytotoxicity was found at 2,3,3,3-tetrafluoropropene concentrations of 400 000 ml/m^3 and above (Honeywell International 2001). Both studies were carried out according to OECD test guidelines.

5.6.2 In vivo

Groups of 5 male and 5 female Sprague Dawley rats were exposed nose-only to 2,3,3,3-tetrafluoropropene concentrations of 0, 5000, 15 000 or 50 000 ml/m³ for 6 hours a day, on 5 days per week, for 4 weeks. The livers of male rats were perfused and the hepatocytes were subsequently examined for the induction of DNA repair synthesis. The micronucleus test in bone marrow cells revealed neither the induction of DNA repair synthesis nor an increased number of micronuclei. Cytotoxicity was not observed in the micronucleus test (Honeywell International 2006 a).

Groups of 5 to 12 male mice were exposed nose-only to 2,3,3,3-tetrafluoropropene concentrations of 0, 12 500, 50 000 or 200 000 ml/m³ for 4 hours according to OECD Test Guideline 474. After sacrifice, bone marrow cells were removed from 5 control animals, 5 exposed animals and 5 positive control animals 24 hours after the last exposure; the remaining 5 animals of the high concentration group and another 5 animals of the control group were sacrificed and bone marrow cells were removed 48 hours after the exposure. The 5 animals of the positive control group were given a single intraperitoneal mitomycin C injection of 0.75 mg/kg body weight. The incidence of polychromatic erythrocytes and micronuclei was not significantly increased in mice exposed to 2,3,3,3-tetrafluoropropene after 24 and 48 hours, compared with that in the control animals. The incidence of micronuclei was significantly increased in the positive control animals compared with that in the negative control animals. Cytotoxicity was not detected (Honeywell International 2005 d).

5.7 Carcinogenicity

5.7.1 Short-term studies

Groups of 10 to 12 female B6C3F1 mice, the most sensitive species for the development of liver carcinomas, and groups of 10 to 12 male F344 rats, the most sensitive species for the development of renal carcinomas, were exposed for 13 weeks to 3 substances that had induced liver carcinomas in mice and renal carcinomas in rats in 2-year studies, to 4 substances that had not caused any carcinomas and to 2,3,3,3-tetrafluoropropene concentrations of 10 000 and 50 000 ml/m³ by inhalation (see Tables 7 and 8 for test procedure). Tetrafluoroethene was included as a carcinogenic substance because of its structural similarity. A feed control group, a corn oil control group and an ambient air control group were used concurrently.

After 13 weeks, the animals were sacrificed, the rat kidneys and mouse livers were examined histopathologically and a genome-wide microarray analysis was carried out. The SVM (support vector machine) algorithm was used to classify the microarray data in order to be able to predict the carcinogenic potential of 2,3,3,3-tetrafluoropropene.

Table 7 Test procedure in male rats in the 13-week study (Honeywell International 2006 e)

Substance	Administration	Dose/ concentration	Carcinogenic to the rat kidney
corn oil	gavage[1)]	0[2)] mg/kg body weight/day	–
iodoform	gavage[1)]	142[2)] mg/kg body weight/day	no
trichlorofluoromethane	gavage[1)]	977[2)] mg/kg body weight/day	no
control	diet	0[3)] mg/kg diet	–
1-amino-2,4-dibromoanthra-quinone	diet	10 000[3)] mg/kg diet	yes
tris(2,3-dibromopropyl) phosphate	diet	100[3)] mg/kg diet	yes
N-(1-naphthyl)ethylenediamine dihydrochloride	diet	1000[3)] mg/kg diet	no
control	inhalation	0[4)] ml/m^3	–
tetrafluoroethylene	inhalation	625[4)] ml/m^3	yes
tetrafluoroethane	inhalation	50 000[4)] ml/m^3	no
2,3,3,3-tetrafluoropropene	inhalation	10 000[4)] ml/m^3	no
2,3,3,3-tetrafluoropropene	inhalation	50 000[4)] ml/m^3	no

[1)] corn oil as vehicle; [2)] 5 days/week, 13 weeks; [3)] 7 days/week, 13 weeks; [4)] 6 hours/day, 5 days/week, 13 weeks

At the end of the exposure period, the body weights of the **rats** were significantly reduced in both the low and the high exposure groups. The relative kidney weights were significantly increased in the low concentration group. The SVM model classified both 2,3,3,3-tetrafluoropropene concentrations as non-carcinogenic; with a sensitivity of about 70% if tetrafluoroethylene was used as reference substance for the algorithm or with a sensitivity of between 90% and 100% without tetrafluoroethylene as reference substance. However, also tetrafluoroethylene, which is carcinogenic, was classified wrongly. The authors concluded that this was because of its low carcinogenic potential. They reported that tetrafluoroethylene was not considered to be carcinogenic to rat kidneys until after a comprehensive histological evaluation in the NTP study and is therefore probably quite a weak carcinogen. The use of male rats may have been another reason why negative results were obtained with tetrafluoroethylene in this study. In the 2-year inhalation study, the female rats developed renal carcinomas and the male rats renal adenomas. Significant changes in gene expression were found in 12 genes in the low concentration group, in 21 genes in the high concentration group and in 3 genes in both concentration groups (Table 9). The functions of the regulated genes were mainly assigned to the regula-

Table 8 Test procedure in female mice in the 13-week study (Honeywell International 2006 e)

Substance	Administration	Dose/ concentration	Carcinogenic to the mouse liver
corn oil	gavage[1)]	0[2)] mg/kg body weight/day	–
iodoform	gavage[1)]	3925[2)] mg/kg body weight/day	no
trichlorofluoromethane	gavage[1)]	93[2)] mg/kg body weight/day	no
control	diet	0[3)] mg/kg diet	–
1-amino-2,4-dibromoanthra-quinone	diet	20 000[3)] mg/kg diet	yes
tris(2,3-dibromopropyl) phosphate	diet	1000[3)] mg/kg diet	yes
control	inhalation	0[4)] ml/m^3	–
tetrafluoroethene	inhalation	1250[4)] ml/m^3	yes
tetrafluoroethane	inhalation	50 000[4)] ml/m^3	no
2,3,3,3-tetrafluoropropene	inhalation	10 000[4)] ml/m^3	no
2,3,3,3-tetrafluoropropene	inhalation	50 000[4)] ml/m^3	no

[1)] corn oil as vehicle; [2)] 5 days/week, 13 weeks; [3)] 7 days/week, 13 weeks; [4)] 6 hours/day, 5 days/week, 13 weeks

tion of endocrine mechanisms, detoxification and oxidative defence. The authors concluded that neither the classification nor the functions of the regulated genes support a carcinogenic effect of 2,3,3,3-tetrafluoropropene on the kidneys of rats at the two tested concentrations of 10 000 and 50 000 ml/m^3.

The body weights of the **mice** were significantly increased in the high concentration group. The two tested concentrations of 10 000 and 50 000 ml/m^3 were likewise classified as non-carcinogenic to the livers of mice. In the livers of female mice, the expression was not changed in any of the genes investigated in the low concentration group. In the high concentration group, only the expression of Akr1b7 (aldoketoreductase family 1) and Qrsl1 (glutaminyl-tRNA synthase) was increased (Honeywell International 2006 e).

On the basis of the currently available data for the molecular modes of action of carcinogenicity, the regulated genes do not provide evidence of carcinogenic processes. The small number of regulated genes is surprising.

Table 9 Rat: gene names and function according to UniProt and PANTHER (Protein ANalysis THrough Evolutionary Relationships) (Honeywell International 2006 e)

Gene symbol	Protein name	Function	Regulation
RGD1311126 (predicted)(Ttc39a)	tetratricopeptide repeat protein 39A	unknown	↑
Eif4g1	eukaryotic translation initiation factor 4 gamma 1	translation	↓
LOC501546	LOC501546 protein (HUWE1)	ubiquitin ligase	↓
Cpe	carboxypeptidase E	metallopeptidase activity	↑
Akap2	A-kinase anchor protein 2	signal transduction	↓
Polr2a mapped	DNA-directed RNA polymerase II subunit RPB1	transcription	↓
RGD1305179 (predicted)	N4bp1 protein	negative regulation of protein ubiquitination	↓
Tmed5	transmembrane emp24 domain-containing protein 5	intracellular protein transport	↓
Gpd2	glycerol-3-phosphate dehydrogenase, mitochondrial	metabolism	↑
Apoh	apolipoprotein H	coagulation	↑
RGD1310433 (predicted) Setd5	Setd5 protein	methyltransferase	↓
RGD1560873 (predicted)	Ccdc66 protein	unknown	↑
RGD1561030 (predicted)	deptor protein	apoptosis	↓
Hpgd	15-hydroxyprostaglandin dehydrogenase	oxidoreductase activity	↑
Eaf2	ELL-associated factor 2	transcription	↑
Sah	SA rat hypertension-associated gene; acyl-coenzyme A synthetase ACSM3, mitochondrial	medium-chain fatty acid: CoA ligase activity, body mass index ↑, triglycerides ↑, cholesterol ↑	↑
Cyp1a1	cytochrome P-450 Cyp1a1	oxidoreductase-activity; Ah receptor activation	↑
Angptl4	angiopoietin-related protein 4	receptor binding	↓

5.7.2 Long-term studies

There are no long-term studies available.

6 Manifesto (MAK value/classification)

2,3,3,3-Tetrafluoropropene has effects on the heart, lungs, kidneys and nose.

Carcinogenicity. There are no long-term animal studies available from which the carcinogenicity of the substance can be evaluated. In a 13-week inhalation study in rats and mice, the gene expression patterns did not indicate carcinogenic potential. However, it should be pointed out that also tetrafluoroethylene, which is carcinogenic to the kidneys of rats and livers of mice, yielded negative results in this study. The reasons for this may be that male rats instead of female rats, which have a higher incidence of renal carcinomas, were investigated in the gene expression study, and that a different strain of rat was used.

2,3,3,3-Tetrafluoropropene was mutagenic in the Salmonella strain TA100 and Escherichia coli. Chromosomal aberration tests yielded negative results in vitro. The negative results obtained in micronucleus tests in vivo in rats and mice cannot invalidate the positive results obtained in mutagenicity tests in vitro. Mutagenicity tests or genotoxicity studies in the target organs the kidneys and liver are not available. As the metabolite 3,3,3-trifluoro-1-hydroxyacetone was detected in vitro in human liver microsomes and in the urine of rats, the reactive metabolite 2,3,3,3-tetrafluoro-1,2-epoxypropane is assumed to be formed, although only in very small amounts. Liver toxicity suspected to be caused by this metabolite was not confirmed in the 13-week inhalation study in rats. In spite of the positive mutagenicity test results in vitro, overall there are insufficient data to be able to classify 2,3,3,3-tetrafluoropropene in one of the carcinogen categories.

MAK value. There are no data available from humans. Irritation was not observed after inhalation exposure of rats for 13 weeks to 2,3,3,3-tetrafluoropropene concentrations of up to 50 000 ml/m^3. Mononuclear cell infiltrates in the kidneys, lungs and nose were observed at this concentration. After 28-day inhalation exposure to concentrations of up to 10 000 ml/m^3, no adverse effects on the heart or muscles of minipigs were found. The concentration-dependent occurrence of focal myocardial necrosis and inflammation was recorded in pregnant rabbits at the lowest concentration tested of 2500 ml/m^3 and above. A NOAEC of 500 ml/m^3 was derived from a 28-day inhalation study in rabbits for the effects on the heart and the increase in creatine kinase activity. As the rabbit is the most sensitive species and it has not been demonstrated that the findings obtained in the heart are a species-specific effect, the NOAEC of 500 ml/m^3 serves as the basis for the derivation of the MAK value. Although it was only a 28-day inhalation study, the concentration in the

range of the NOAEC probably has a greater influence than the period of exposure. Therefore, the concentration is not converted from 7-day exposure per week to 5-day exposure at the workplace and only a slight decrease in the NOAEC is expected after long-term exposure. As no effects on the heart were observed in rats up to 50 000 ml/m^3 and in minipigs up to 10 000 ml/m^3, rabbits are assumed to have a markedly higher sensitivity as regards effects on the heart, although the mechanism is not exactly known. Therefore, a MAK value of 200 ml/m^3 has been established taking into account the preferred value approach. In view of the higher sensitivity of rabbits, the Commission does not consider it necessary to lower the MAK value any further.

Peak limitation. As the MAK value was derived from systemic effects and the substance does not have irritating effects, 2,3,3,3-tetrafluoropropene has been classified in Peak Limitation Category II. Elimination half-times for the parent substance are not available. Therefore, a default excursion factor of 2 has been established according to the procedures of the Commission (see documentation "Spitzenbegrenzung" 2011, available in German only).

Prenatal toxicity. In a developmental toxicity study in rats with inhalation exposure, the number of foetuses with lower weights and thus the total incidence of external abnormalities was significantly increased at 15 000 ml/m^3 and above. This finding suggests delayed development after prenatal administration.

In a 2-generation study in rats that was carried out by the same testing laboratory using the same doses and the same strain of rat, a NOAEC of 50 000 ml/m^3 was derived for the systemic toxicity of the parental animals, for foetotoxicity and for screening the ribs of the F1 offspring for skeletal changes on postnatal days 21 to 23. There were no effects on the number of foetuses with reduced weights in the F1 or F2 generation although the exposure period in the 2-generation study was longer and 2 generations were examined. The Commission therefore considered the delayed development in the developmental toxicity study to be reversible. The NOAEC for developmental toxicity and maternal toxicity is thus 50 000 ml/m^3 for rats.

In a developmental toxicity study in rabbits, effects on the heart and large vessels were observed in foetuses after inhalation exposure at 5500 ml/m^3 and above. The NOAEC for developmental toxicity was 4000 ml/m^3. Maternal toxicity in the form of myocardial inflammation was found at the low concentration of 2500 ml/m^3 and above.

The NOAECs for developmental toxicity in rats and rabbits are thus higher by a factor of 250 and 20, respectively, than the established MAK value of 200 ml/m^3. Therefore, 2,3,3,3-tetrafluoropropene is classified in Pregnancy Risk Group C.

Germ cell mutagenicity. 2,3,3,3-Tetrafluoropropene was found to be mutagenic in the Salmonella strain TA100 and Escherichia coli. Chromosomal aberration tests in vitro and micronucleus tests in vivo yielded negative results. As no appropriate

studies in germ cells are available and negative genotoxicity data were obtained in vivo, 2,3,3,3-tetrafluoropropene has not been classified in any of the germ cell mutagen categories.

Absorption through the skin. Under normal conditions, 2,3,3,3-tetrafluoropropene is a gas. There are no experimental data available for the dermal absorption of the compound from the gaseous phase. Model calculations for absorption through the skin at the level of the MAK value yielded negligible levels of absorption compared with absorption after inhalation exposure. For isoflurane, which is a similar compound in terms of its molecular weight and lipophilia, animal studies demonstrated that the contribution of dermal absorption from the gaseous phase to systemic exposure is negligible compared with absorption via the respiratory tract (McDougal et al. 1990). The other physico-chemical properties suggest the proportion of 2,3,3,3-tetrafluoropropene absorbed through the skin is even lower compared with the total amount absorbed under MAK conditions. Therefore, the substance is currently not designated with an "H" (for substances which are absorbed through the skin).

Sensitization. There are no data available for sensitization in animals, nor is sensitization known in humans; therefore, 2,3,3,3-tetrafluoropropene is not designated with either "Sa" or "Sh" (for substances which cause sensitization of the airways or skin).

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