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ORIGINAL INVESTIGATION

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Sensory irritation mechanisms investigated from model compounds: trifluoroethanol, hexafluoroisopropanol and methyl hexafluoroisopropyl ether

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Abstract Quantitative structure-activity relationships (QSAR) have suggested the importance of hydrogen bonding in relation to activation of the sensory irritant receptor by nonreactive volatile organic chemicals. To investigate this possibility further, three model compounds with different hydrogen bond acidity, trifluoroethanol, hexafluoroisopropanol and methyl hexafluoroisopropyl ether, were selected for study. The potency of each chemical is obtained from the concentration necessary to reduce respiratory rate in mice by 50% (RD50). The RD50 values obtained were: methyl hexafluoroisopropyl ether ($\geqslant$160 000 ppm), trifluoroethanol (11 400–23 300 ppm), and hexafluoroisopropanol (165 ppm). QSAR showed that trifluoroethanol and methyl hexafluoroisopropyl ether behaved as predicted as nonreactive sensory irritants, whereas hexafluoroisopropanol was much more potent than predicted. The higher than predicted potency of hexafluoroisopropanol could be due to a coupled reaction, involving both strong hydrogen bonding and weak Brönsted acidity. A concerted reaction could thus be more efficient in activation of the receptor. Hydrogen bonding properties and concerted reactions may be important in the activation of the sensory irritant receptor by nonreactive volatile organic chemicals.

Key words Sensory irritation · Hydrogen bonding · Trifluoroethanol · Hexafluoroisopropanol · Methyl hexafluoroisopropyl ether · Mice · Receptor · Brönsted acid · Coupled reaction

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Introduction

Irritation of eyes and upper respiratory tract by airborne chemicals, termed sensory irritation (Alarie 1973), is a common reaction in occupational settings. Sensory irritation is also caused by indoor and outdoor exposures to airborne pollutants (Alarie 1973; Nielsen 1991; Nielsen and Alarie 1992). Furthermore, sensory irritation is the aim of tear gases (Alarie 1973). Sensory irritation can also be caused by application of pharmaceutical products in eyes and nose. The irritation effect of gases and vapours is believed to be caused by their direct interaction with one or more proteins (receptors) on trigeminal nerve endings in the cornea and nasal mucosa (Alarie 1973; Nielsen 1991). The chemosensitive trigeminal nerves are C-fibres and possibly also Aδ-fibres. They are part of the somatic sensory system which conveys peripheral impulses to the central nervous system (CNS). Stimulation of the trigeminal nerve endings results in a stinging sensation, which can increase to a burning and painful sensation (Alarie 1973; Cain 1990; Cometto-Muñiz and Cain 1991; Silver and Finger 1991; Nielsen and Hansen 1993).

Several investigations have dealt with the importance of hydrogen bonding in relation to chemical activation of the trigeminal system (Nielsen and Bakbo 1985; Abraham et al. 1990; Nielsen 1991). The effect of hydrogen bonding in upper respiratory tract irritation of male Swiss OF_1 mice has been assessed in detail (Abraham et al. 1990), using a quantitative structure-activity relationship (QSAR) which is now facilitated by a large collection of data (e.g. Schaper 1993). It was shown to involve nonreactive irritants as hydrogen bond acids, i.e. the receptor site or phase is a hydrogen bond base. However, in the QSAR study, the range of hydrogen bond acidity of the nonreactive irritants was

not very large, the most hydrogen bond acidic compounds being the simple alcohols. The range could not be extended using carboxylic acids, as these compounds are also reactive irritants (Brönsted acids). In order to increase the range of hydrogen bond acidity of the studied irritants, we chose the fluorinated alcohols trifluoroethanol and hexafluoroisopropanol as model compounds. These have hydrogen bond acidities, as $\Sigma\alpha_2^H$, of 0.57 and 0.77, respectively, as compared to that of 0.37 for a primary alcohol such as ethanol (Abraham 1993).

In this study, we observed that the sensory irritation of trifluoroethanol was as estimated from recent QSARs, but that hexafluoroisopropanol was much more potent than calculated. Such an increase in potency could be due to either the presence of the $(CF_3)_2$ group, or to the alcohol now being so strong a hydrogen bond acid that it can react with the receptor. The former possibility was investigated by synthesizing and determining the sensory irritation of methyl hexafluoroisopropyl ether, $(CF_3)_2CH$-O-Me. This substance contains the $(CF_3)_2$ group but has little hydrogen bond acidity.

Materials and methods

Chemicals

Trifluoroethanol (> 99% pure) was obtained from Merck-Schuchardt, Germany and was used without further purification. Hexafluoroisopropanol (> 99% pure), used in the biological testing, was also obtained from Merck-Schuchardt, Germany. The high purity, which was found to be 99.7%, was confirmed by gas chromatography on a 4-m column with 10% Carbowax 1500 at 87°C. Hexafluoroisopropanol used for some of the experiments was stored over $NaHCO_3$ overnight and decanted before use. If acidic compounds, for example HF, were present they would be neutralized and their salts possibly also removed from the liquid phase. If a salt was dissolved in the solvent it would have given rise to a solid deposit in the aerosol generator used for the generation of the exposure concentration in the biological experiments. However, this was not seen.

Synthesis and purity of methyl hexafluoroisopropyl ether

This compound was prepared from hexafluoroisopropanol by methylation with methyl sulfate and aqueous potassium hydroxide solution, using a modification of a procedure described in the literature (Croix 1975). The crude product was purified by distillation from potassium hydroxide. The ^1H NMR spectrum ($CDCl_3$, 400 MHz) showed that in addition to the required compound (δ 3.74s, 3H and 3.94 sept, 1H), the product contained 3.6% hexafluoroisopropanol (δ 4.40m) and 0.2% methanol (δ 3.32s). This finding was confirmed by observation of the ^{19}F NMR spectrum ($CDCl_3$, 376 MHz) which showed a major doublet (J5.7 Hz) with 4.2% of a second doublet at 1.6 ppm to lower field due to hexafluoroisopropanol. Further purification was effected by shaking the product with aqueous potassium hydroxide solution followed by distillation to give a product which contained no observable hexafluoroisopropanol (GC) and only a trace of methanol. The GC analysis was carried out at ambient temperature using the stationary phases Fomblin Z-Dol and poly(vinyl tetradecanol) on which the ether, methanol, and hexafluoroisopropanol were completely separated, and using OV202 on which the two alcohols eluted together, but well separated from the ether.

Experimental

A 500 cm^3 round bottom three-neck flask was fitted with a thermometer, dropping funnel, still head and condenser. KOH (36 g, 0.64 mol) was added to the flask followed by water (100 cm^3). With magnetic stirring and ice cooling, hexafluoro-2-propanol (Aldrich Chemical Co., 100 g, 0.59 mol) was added so that the temperature did not exceed 20°C. The mixture was then warmed to 70°C and methyl sulfate (82 g, 0.65 mol) added at such a rate to keep the temperature in the range 68–70°C. The product distilled (still head temperature 50–54°C) and was collected in an ice cooled flask to give a cloudy liquid (82 g). This product was stirred with powdered KOH (0.5 g) for 0.5 h and then distilled to give a colourless liquid (77.8 g, b.p. 49–50°C). This liquid was shaken with ice-cold potassium hydroxide solution (1 M, 50 cm^3), then separated (it is the lower layer) and distilled through a 15-cm silvered vacuum jacketed column, discarding the first 0.6 g (b.p. 47.5–49°C), to give the product (68.3 g, b.p. 49–51°C).

Animals and housing

Ssc:CF-1 male mice, mean weight 25 ± 3 g (number of mice, $n = 124$) supplied by Statens Seruminstitut, Denmark, were used. The mice were placed in wire mesh cages placed inside polycarbonate cages with sawdust bedding. Food (Altromin No. 1324) and tap water were available ad lib. The light:dark cycle was 12:12 h, with light on from 0700 hour.

For exposure of mice via a tracheal cannula, the animals were anaesthetizes with 50 mg/kg body wt of sodium pentobarbital given i.p., supplemented if required. A tracheal cannula was inserted, secured by a suture, and the skin incision was closed with cyanoacrylate glue. The mice were allowed to recover prior to exposure.

Generation of gas-air mixtures

Dynamic exposure conditions were used with trifluoroethanol and hexafluoroisopropanol. Briefly, the solvents were evaporated, diluted with room air, and led to a 3.3-l exposure chamber. Each nominal concentration was calculated from the amount of evaporated chemical and the total gas-air flow (set between 18 and 25 l/min). Chamber concentrations were monitored continuously by infrared (IR) spectroscopy (Nielsen and Alarie 1982; ASTM 1984). The differences between the nominal and the monitored exposure concentrations were normally less than 10%. Air concentrations are given in ppm: ml gas per m^3 gas-air mixture (Kristiansen et al. 1989).

Static exposure conditions were used with investigation of methyl hexafluoroisopropyl ether due to the large quantities of the chemical required to elicit response. The ether was evaporated in a 34.6-l stainless steel tank. Tank, exposure chamber, IR analyser and a pump were connected in a closed loop system (Hansen et al. 1991; Nielsen and Alarie 1992). The exact exposure concentrations were obtained from IR spectroscopy.

Exposure conditions

Each mouse was placed in a body plethysmograph attached to the exposure chamber so that the head of the mouse protruded into the

exposure chamber which was fitted with four plethysmographs. The respiratory rate and the relative tidal volume of each mouse were obtained continuously by attaching a pressure transducer to each plethysmograph. Data were recorded on a dynograph and collected on a computer. At each exposure experiment, a preexposure period of 10 min was used for collecting base line values. The exposure was 30 min with the dynamic conditions and 10 min with the static conditions. Then the exposure chamber was flushed with room air for a 20-min recovery period (Nielsen and Alarie 1982; ASTM 1984; Nielsen et al. 1993).

Determination of effects

Sensory irritation of the upper respiratory tract causes a pause before exhalation and thereby a decrease in respiratory rate due to stimulation of the trigeminal nerve endings in the nasal mucosa (Alarie 1973). This produces the characteristic sensory irritation pattern on the dynograph recordings, as the small leak at the rubber dam around the neck of the mouse results in a decrease in the pressure in the plethysmograph during the pause. The leak, however, does not interfere with the determination of the relative tidal volume if determined from the maximum amplitude of the respiratory patterns (Nielsen at al. 1993). The exposure effect of each mouse is expressed as the percentage decrease in respiratory rate from the preexposure rate (baseline value set equal to 100%). Concentration-effect relationships are obtained by plotting the percentage decrease versus the logarithm of the exposure concentration (Nielsen and Alarie 1982; ASTM 1984).

Airborne substances inducing bronchoconstriction increase duration of inspiration and particularly duration of expiration resulting in a decrease in respiratory rate. No characteristic change is occurring in tidal volume. This effect is best quantitated from the airflow limitation wave (Vijayaraghavan et al. 1993), a parameter not measured in this investigation.

At the alveolar level vagal C-fibres (previously termed J receptors) may be directly stimulated by airborne chemicals, thereby inducing reflex effects. In this case, cessation of exposure results in a rapid recovery. Other chemicals (often called pulmonary irritants), which induce inflammatory reactions, congestion and edema, stimulate the same vagal endings. In this case the chemicals act slowly and the recovery is slow. At higher concentrations, both types of substance reflexly decrease the respiratory rate in mice due to a pause between the end of expiration and the beginning of the following inspiration (Vijayaraghavan et al. 1993). As both types of substance induce the same respiratory pattern on the dynograph recordings, the pattern will for simplicity be termed "pulmonary irritation". The pattern is not specific, as it is also seen with anesthesia (Nielsen et al. 1985b). Interference from anesthesia can be revealed from the absence of "escape attempts" on dynograph recordings. Non-anesthetized mice will, from time to time, move in the plethysmographs and thereby create characteristic pressure changes. Tidal volume can decrease particularly with effects on the lungs (Vijayaraghavan et al. 1993) or by anesthesia (Nielsen et al. 1985b), and has sometimes been observed with an effect on the upper respiratory tract (Hansen et al. 1992).

Statistical analysis

Least-squares linear regression analysis was used for investigation of the log concentration-effect relationships. The rectilinear part of the relationships is selected from a visual inspection of the figures. The selected range is equal to the range covered by each line and the number of mice used for the construction of a line is given in Table 1. The concentration depressing the respiratory rate by 50% is termed RD50 and the concentration depressing the tidal volume by 50% VTD50.

Table 1 Log concentration-effect relationship for the decrease in respiratory rate and tidal volume

Substance	Period[a]	Type of response[b]	n[c]	Range of concentration used in the regression analysis (ppm)	Slope[d] (%/log conc.)	RD50[e] (ppm)	Cause of the effect[f]
Trifluoroethanol	16–30 s (n)	RD	20	3792–11 678	85.2 ± 20.1	11 438 (8353–15 661)	U
	0–2 min (n)	RD	20	3792–11 678	55.1 ± 18.0	23 323 (9768–55 689)	U
	9–10 min (n)	RD	24	7751–23 644	82.4 ± 13.6	19 671 (16 181–23 914)	L
	9–10 min (c)	RD	12	11 597–15 938	445.0 ± 64.4	14 558 (13 846–15 308)	L
	9–10 min (n)	VTD	20	9147–23 644	94.2 ± 16.3	20 799 (17 307–24 997)	U
Hexafluoroisopropanol	0–2 min (n)	RD	24	69–263	81.7 ± 7.6	165 (146–185)	U
	0–10 min (n)	RD	24	69–263	82.3 ± 7.8	165 (146–186)	U
Methyl hexafluoro-isopropyl ether	0–10 min (n)	RD	16	2568–24025	38.7 ± 3.6	31 294 (23 137–42 326)	L
	9–10 min (n)	RD	16	2446–18 346	46.8 ± 4.0	22 825 (17 509–29 754)	L
	9–10 min (n)	VTD	16	2446–18 346	34.3 ± 6.7	31 998 (15 512–66 007)	U

[a] Normal mice (n) and cannulated mice (c)
[b] The decrease in respiratory rate is indicated by RD and the decrease in tidal volume by VTD
[c] Number of mice used for construction of the regression line
[d] The ± value is the SD value
[e] The 95% confidence interval is given in bracket
[f] Effects from the upper respiratory tract (U) and effects from the lower respiratory tract (L)

Results

Trifluoroethanol

In normal mice, trifluoroethanol induced a rapid decrease in respiratory rate, immediately followed by a fade in this response. The decrease is seen as downward spikes in the period 0–1 min of the exposure period (Fig. 1, upper part). Subsequently, a new decrease in respiratory rate was seen. The first decrease is highlighted in Fig. 2. The presence of the characteristic sensory irritation patterns (Fig. 2), the negligible effect on the tidal volume (Fig. 1, lower part), and the lack of decrease in effects in cannulated mice indicate that the first decrease was caused by a trigeminal effect.

A few minutes after the onset of the exposure, the second decrease in respiratory rate was seen both in normal and in cannulated mice at the higher concentrations (Fig. 1), which indicates that the effect occurred beyond the upper respiratory tract. The respiratory patterns in normal mice were equivalent to those of pulmonary irritation or anesthesia, perhaps superimposed with unresolved bronchoconstriction (Fig. 3). The respiratory pattern in cannulated mice was exclusively indicative of pulmonary irritation or anesthesia (Fig. 3).

Simultaneously with the second decrease in respiratory rate, the tidal volume also decreased in a concentration dependent manner in normal mice, contrary to the lack of effect in cannulated mice (Figs 1, 3 and 4c). This suggests that the decrease in volume was due to an effect on the upper respiratory tract. Furthermore, lack of effect on tidal volume in cannulated mice suggests that anesthesia could not explain the finding. In normal mice prominent escape attempts, excluding anesthesia, were seen within the first 10 min of the exposure periods, except at the highest concentration where prominent escape attempts only were seen within the first 5 min of the exposure period.

Quantitative comparison of sensory irritation effects in the period 16–30 s with lung effects in the period 9–10 min shows (Fig. 4a, Table 1) that the slopes of the log concentration effect curves were the same. However, the sensory irritation induced decrease in respiratory rate was the most potent as also seen from the RD50 values (Table 1). The log concentration-effect curve, 9–10 min, in cannulated mice was significantly steeper compared to that in normal mice (Fig. 4b and Table 1). The lines intersect but potencies taken at the middle of the curves were approximately equal. The tidal volume decreased in normal mice (Fig. 4c),

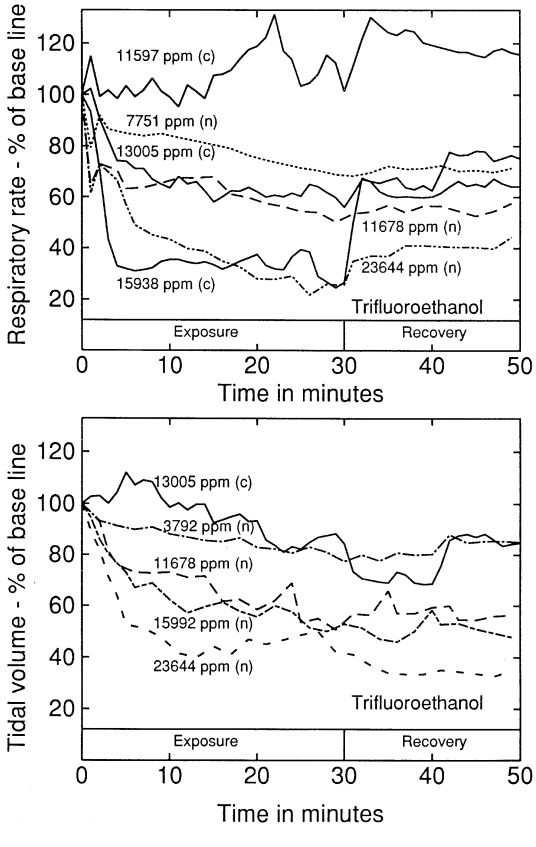


Fig. 1 Trifluoroethanol exposures: representative examples of time-frequency (*upper part*) and time-volume (*lower part*) relationships in normal (*n*) and cannulated (*c*) mice. Each point represents the average respiratory rate of a group of four mice

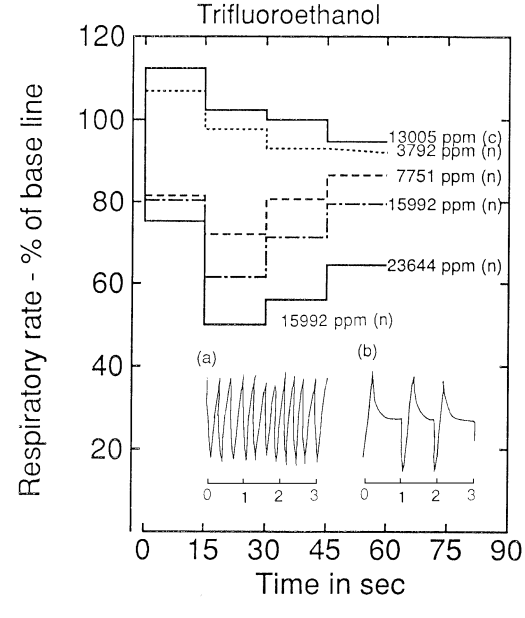


Fig. 2 Trifluoroethanol exposures: representative curves of the mean respiratory rate of groups of four mice within the first minute of the exposure period. Values are obtained for each consecutive 15-s period. The initial decrease in respiratory rate in normal mice (*n*) was due to sensory irritation, illustrated from the characteristic sensory irritation pattern (*b*) in the 16–30 s period of a representative mouse exposed to 15 992 ppm trifluoroethanol. The preexposure pattern of the same mouse (a) is also shown. Each of the tracings represents a 3-s period. When prominent decreases occurred, the maximum decrease was seen within the first 16–30 s of the exposure period. Results from a group of cannulated mice (*c*) are also included

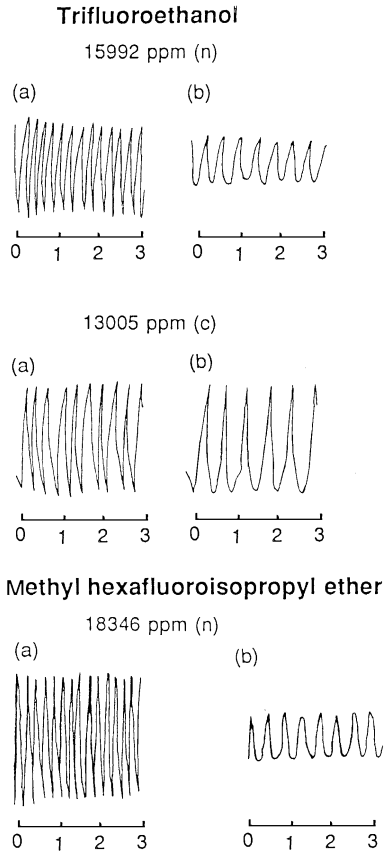


Fig. 3 Representative respiratory patterns in normal (*n*) and cannulated mice (*c*) exposed either to trifluoroethanol or to methyl hexafluoroisopropyl ether. The preexposure patterns are indicated by *a* and the patterns seen with exposure by *b*. The *b* tracings are from the 9–10 min of the exposure period. The effects are caused by interaction between the vapours and the lower respiratory tract or CNS. Each tracing represents a 3-s period

whereas no significant effect was found in cannulated mice.

Hexafluoroisopropanol

A rapid decrease in respiratory rate was seen while exposing normal mice to hexafluoroisopropanol (Fig. 5). The response reached a maximum within the first 2 min of the exposure period. It was then followed by a slow desensitization. This is also seen from the equal RD50 values obtained from the 0–2 and 0–10 min of the exposure period (Table 1). The characteristic sensory irritation patterns appeared on the dynograph recordings at the same time. No decrease was seen in cannulated mice (Figs 5 and 6). These findings show that the decrease was exclusively caused by sensory irritation and that interfering effects from lungs and CNS were absent. This was further substantiated from the lack of a concentration-related effect on tidal volume in normal as well as in cannulated mice.

The concentration-effect relationship for the sensory irritation induced decrease in respiratory rate is shown in Fig. 6. The maximum decrease in respiratory rate is seen to be about 60%. As the two highest concentrations were outside the rectilinear part of the log concentration-effect curve, these values have been excluded when calculating the RD50 values given in Table 1. Sensory irritation was not caused by acidic impurities as hexafluoroisopropanol stored over $NaHCO_3$ clearly was just as irritating as the untreated substance (Fig. 6).

Methyl hexafluoroisopropyl ether

A short-lasting, 5 to 10-s, period with very faint patterns of sensory irritation was seen immediately after onset of the highest exposure concentration (24 025 ppm) in normal mice. No sustained decrease in respiratory rate in the first minute of the exposure period was observed (Fig. 7). The respiratory rate in normal and cannulated mice showed the same slowly developing decrease (Fig. 7), indicating that the major effect occurred beyound the upper respiratory tract. The decreases reached roughly a stable level about 5 min after the onset of the exposure. The same general type of response was seen with the decrease in tidal volume in normal mice, whereas no effect was found in cannulated mice. A recovery was not apparent after the end of exposure, either for the frequency nor for the tidal volume. The respiratory pattern indicated pulmonary irritation or anesthesia (Fig. 3). Body movements were present at exposure up to 15 740 ppm. They decreased at higher concentrations but were still occurring even at the highest exposure concentration. Thus, anesthesia could not account for the decrease in respiratory rate and tidal volume, which is in agreement with the lack of effect on the tidal volume in cannulated mice.

Concentration-effect relationships for the decrease in respiratory rate and tidal volume in the last minute of the exposure period are shown in Fig. 8. The RD50, an effect not caused by sensory irritation, and the VTD50 are given in Table 1.

Discussion

Biological effects

Trifluoroethanol induced a number of conspicuous effects. An initial short-lasting sensory irritating effect was seen which decreased the respiratory rate with the tidal volume unaffected. The lack of effect on the tidal volume is in agreement with other reports (Nielsen and Yamagiwa 1989; Vijayaraghavan et al. 1993). A very short-lasting sensory irritating effect has previously been found with propyl ether (Nielsen et al. 1985a). The

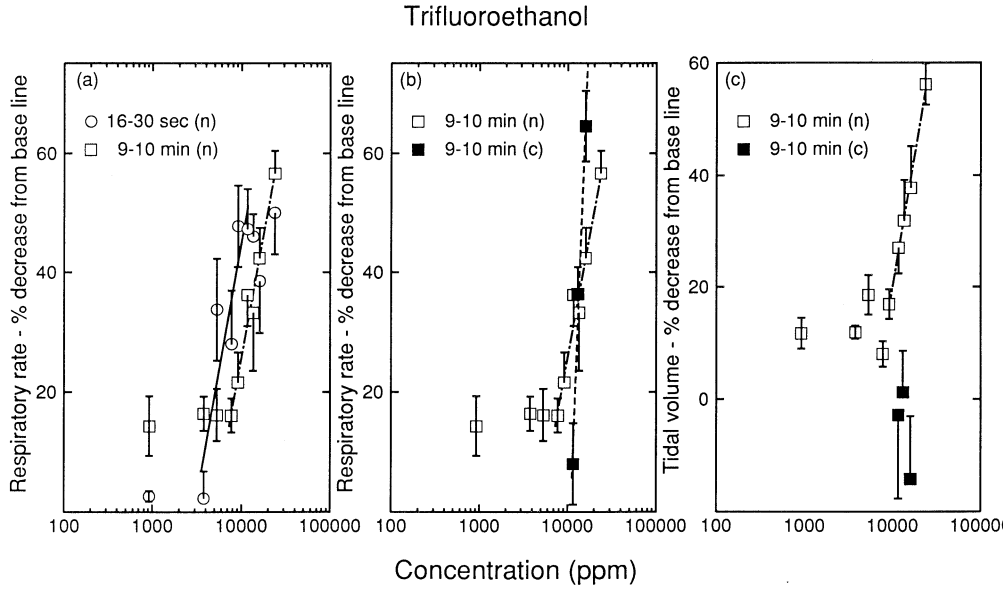


Fig. 4 a Concentration-effect relationships for decreases in respiratory rate in normal mice caused by exposure to trifluoroethanol. Decreases in the first 16–30 s of the exposure period were due to sensory irritation. Sensory irritation disappeared very fast. It was followed by a new decrease due to an effect on the lungs, giving rise to the concentration-effect relationship in the period 9–10 min. **b** Concentration-effect relationships in normal (*n*) and cannulated mice (*c*) in the 9–10 min of the exposure period. In this period the decreases were caused by effects on the lower respiratory tract. **c** Concentration-effect relationships of trifluoroethanol's effect on the tidal volume in normal and cannulated mice. Each point is the mean ± SEM of a group of four naive mice

two lower alcohols, *n*-propanol (Kristiansen et al. 1986) and *n*-butanol (Kristiansen et al. 1988), also gave rise to short-lasting sensory irritation effects, but the desensitization rate was not nearly as fast as seen in this case.

In general, RD50 values are obtained from the maximum decrease of a 1-min period within the first 10 min of the exposure period. As a rapid desensitization was found with trifluoroethanol, two RD50 values, one from a 15-s period and one from the entire first min of the exposure period, have been given. They deviates approximately by a factor of 2. As the desensitization rate only had a limited influence on the RD50 values, the desensitization cannot have introduced an invalidating bias in the evaluations below.

After the sensory irritation response, a new decrease in respiratory rate was seen in normal mice which was caused by an effect on the lungs. The difference in slopes in the period 9–10 min in normal and in cannulated mice (Fig. 4b and Table 1) could most likely be explained by the difference between nose breathing versus breathing through a tracheal cannula. Simultaneously, the tidal volume decreased in normal mice which was caused by an effect on the upper respiratory tract as no effect was found in cannulated mice. A similar decrease in tidal volume in normal mice has also been reported for *n*-propanol (Kristiansen et al. 1986) and *n*-butanol (Kristiansen et al. 1988).

Comparing with trifluoroethanol, hexafluoroisopropanol was a more specific sensory irritant. Neither pulmonary effects nor anesthesia was seen with the concentrations investigated. Furthermore, it was by far the most potent irritant of these two substances. The potency ratio, taken as the ratio between the RD50 values (Table 1), is 69–140. Impurities could not account for the sensory irritating properties.

Methyl hexafluoroisopropyl ether had negligible sensory irritating effect at the concentrations investigated and no RD50 value can actually be obtained from this effect. However, assuming that the threshold response was just reached at the highest exposure concentration, as a very faint sensory irritation pattern was seen on the dynograph recording, this allows estimation of a minimum RD50 value. In general, the threshold concentration should be divided by 0.15 for reaching the RD50 value (Nielsen et al. 1985a) and thus RD50 is expected to be 160 000 ppm or higher.

The observed pulmonary irritation of the ether, seen from the decrease in respiratory rate in normal and cannulated animals, showed very little recovery after end of exposure. Thus, a direct interaction with the vagal nerve endings is not a likely reaction. Rather, lack of recovery is expected to be caused by an inflammatory reaction, oedema or congestion. It is not likely that the pulmonary irritation pattern was caused by anesthesia. The ether induced light anesthesia in mice at 5% and deep anesthesia in 30 s at 10%, followed by recovery within 30 s after cessation of administration (Croix 1975).

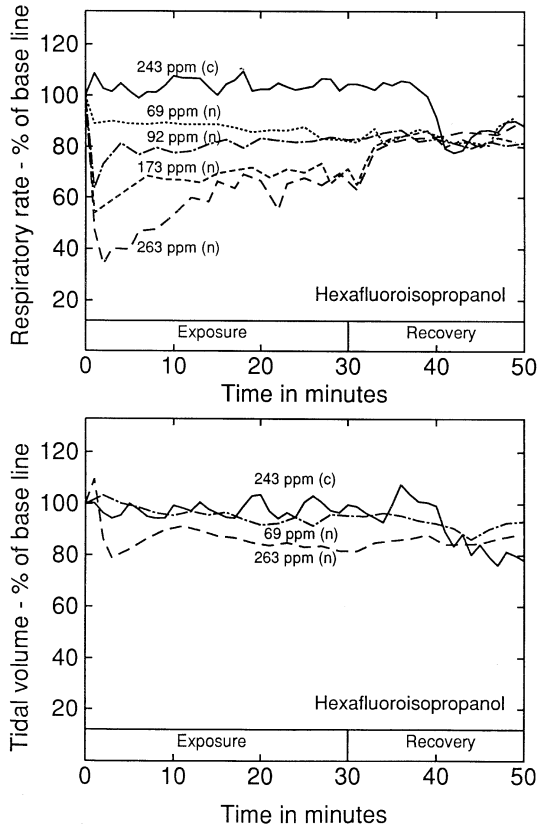


Fig. 5 Hexafluoroisopropanol exposure: representative examples of time-frequency (*upper part*) and time-volume (*lower part*) relationships in normal (*n*) and cannulated (*c*) mice. The characteristic sensory irritation patterns were seen on the dynograph recordings, indicating that the decrease in respiratory rate was due to sensory irritation. Each point represents the average respiratory rate of a group of four naive mice

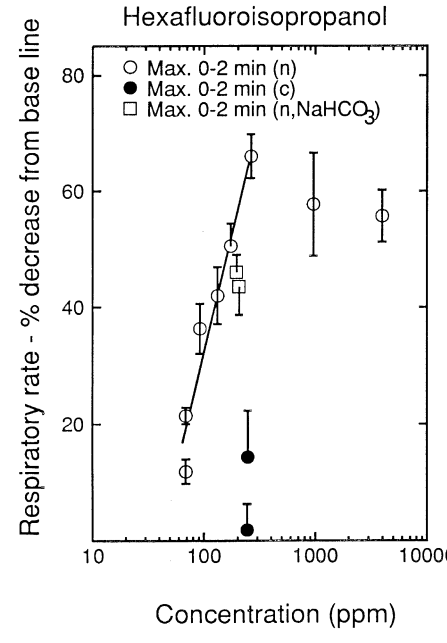


Fig. 6 The *open circles* show the concentration-effect relationships for the decrease in respiratory rate in normal mice (*n*) exposed to hexafluoroisopropanol in the first 2 min of the exposure period. The corresponding regression line has been constructed leaving out the responses from the two highest exposure levels. The line was used for calculation of the RD50 value given in Table 1. Decreases induced from exposures where the hexafluoroisopropanol had been stored over $NaHCO_3$ to remove possible acidic impurities are indicated by *open squares*. Effects in cannulated mice (*c*) are shown as *filled circles*. Each point is the mean ± SEM of a group of four naive mice

Receptor activation mechanisms

Investigating QSARs of nonreactive sensory irritants, Alarie et al. (1995) found the relationship between the sensory irritation potency, i.e. RD50, and the gas-hexadecane partition coefficients (L^{16}) to be: log RD50 (ppm) = 5.24 − (0.602 · log L^{16}), $n = 75$ and $r^2 = 0.71$. Except for hexafluoroisopropanol, which is much more potent than expected, the equation can account for the potency of the investigated compounds (Table 2). Being biologically significant in relation to mechanistic investigations of sensory irritation requires a deviation between measured and estimated potencies by a factor of about 10 (Nielsen and Yamagiwa 1989). This is the case for hexafluoroisopropanol. The log L^{16} term is related to two physicochemical processes, the volume involved in the interaction between an irritant and the receptor, and the interaction caused by the general dispersion interactions (Abraham et al. 1994a). As taken into account in the QSAR, these two processes cannot account for the excess potency of hexafluoroisopropanol.

As shown (Abraham et al. 1990, 1994a,b) linear solvation energy relationship (LSER) models, using physicochemical descriptors of the irritants, allow further insight in the activation mechanisms of nonreactive irritants:

$$\log \mathrm{RD50} = \mathrm{c} + \mathrm{rR_2} + \mathrm{s}\pi_2^{\mathrm{H}} + \mathrm{a}\Sigma\alpha_2^{\mathrm{H}} + \mathrm{b}\Sigma\beta_2^{\mathrm{H}} + \mathrm{llog}L^{16}$$

The descriptor R_2 accounts for an excess molar refraction of the irritant, π_2^{H} for the irritant dipolarity/polarisability, $\Sigma\alpha_2^{\mathrm{H}}$ for the hydrogen-bond acidity, and $\Sigma\beta_2^{\mathrm{H}}$ for the hydrogen-bond basicity. Thus "r" is the ability of the receptor to interact with π- and n-electron pairs, "s" is the receptor dipolarity/polarisability, "a" is the receptor hydrogen-bond basicity, "b" is the receptor hydrogen-bond acidity, and "l" is the ability of a receptor to distinguish between homologous substances.

The first investigation, dealing exclusively with results in OF_1 mice (Abraham et al. 1990), and a recent follow-up investigation (Alarie et al. 1995), using the 75 RD50 values from different stocks and strains in the QSAR and their LSER descriptors, gave the same general results. Only the terms including π_2^{H}, $\Sigma\alpha_2^{\mathrm{H}}$ and log L^{16} were the independent variables which were statistically significant. The Alarie et al. (1995) equation

reads:

$$\log \text{RD50 (ppm)} = 6.90 - 1.49\pi_2^{\text{H}} - 2.37\Sigma\alpha_2^{\text{H}} - 0.761\log \text{L}^{16} \quad n = 75 \; r = 0.938$$

For the substances in Table 3, the calculated contribution from the term $1.49\pi_2^{\text{H}}$ deviates by a maximum of 0.37, corresponding to a difference in RD50s by a factor of about 2. The $2.37\Sigma\alpha_2^{\text{H}}$ value deviate up to 1.42, corresponding to a difference in potency by a factor of 25. The $0.761 \cdot \log \text{L}^{16}$ term deviates by a maximum of 0.70, corresponding to a factor of 5 in RD50 values. These results clearly highlight the importance of hydrogen-bonding acidity of the irritant molecules as well as the hydrogen-bond basicity of the receptor. The ratio 9.52 for hexafluoroisopropanol indicates that an additional explanation for the high potency should be sought, other than its high hydrogen-bond acidity.

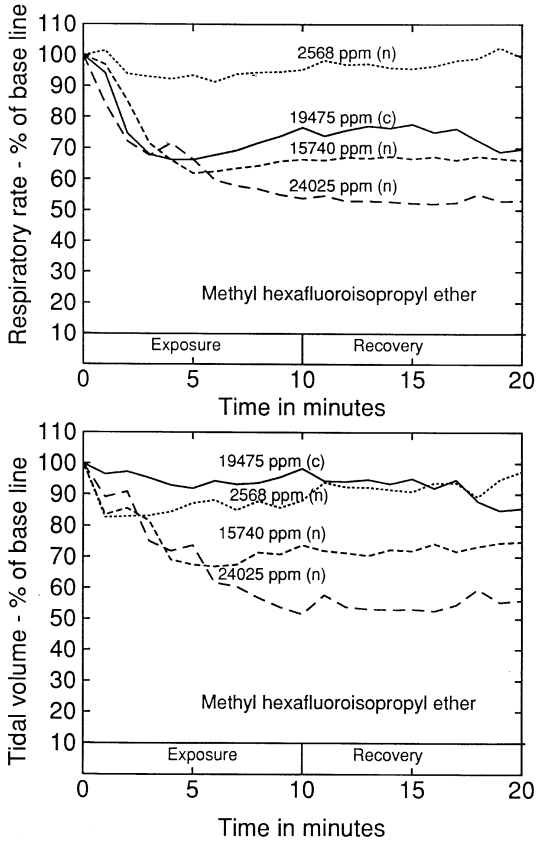


Fig. 7 Methyl hexafluoroisopropyl ether: curves are representative examples of time-frequency (*upper part*) and time-volume (*lower part*) relationships in normal (*n*) and cannulated (*c*) mice. Each point represents the average respiratory rate of a group of four mice

The $\Sigma\alpha_2^{\text{H}}$ and the pKa values in water (Table 3) are correlated and thus the Brönsted acidity of the irritants may be important. One possibility is that hexafluoroisopropanol is a strong enough Brönsted acid to react with the receptor. Such a reaction may occur if the receptor contains a very basic group, e.g. ionic groups as alkoxide or thiolate, or a free amino group. Fluorinated alcohols are weak Brönsted acids in that they have little tendency to take part in full proton transfer; cf. the pKa values in water (Table 3). Also, no CO_2 was seen to be liberated with the $NaHCO_3$ purification, and a 10% solution in water had a pH $\approx$ 6.2. The postulation of a free alkoxide or thiolate ion at a pH of living organisms is not a very attractive explanation.

However, a coupled reaction including both hydrogen bonding and proton transfer driving the receptor activation process in the same direction offers an attractive explanation for the high potency of hexafluoroisopropanol. Coupled reactions may also be attractive to explain the effect of other highly irritating substances. First, these types of reactions are known from chemistry (Larson and McMahon 1987; Rabold et al. 1995) and second, they are generally involved in enzyme reactions. Proposed, reversible reactions which may explain the excess sensory irritation effect of hexafluoroisopropanol are shown in Fig. 9.

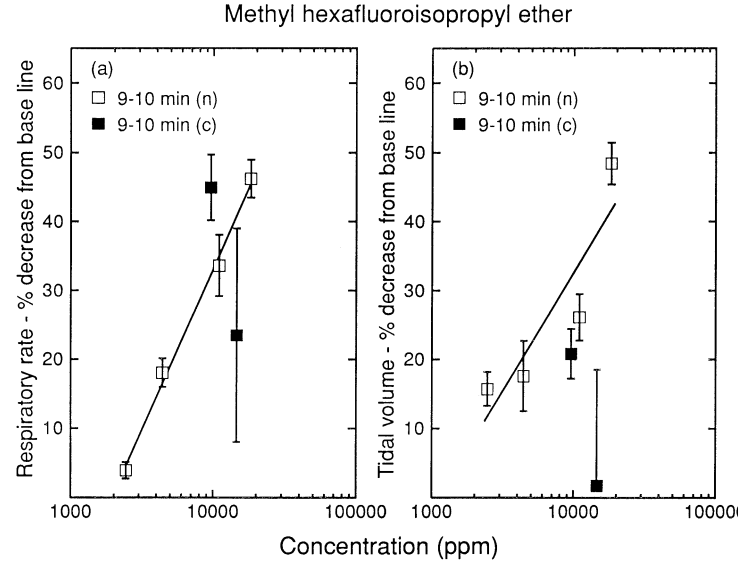


Fig. 8 Methyl hexafluoroisopropyl ether: concentration-effect relationships for the decrease in respiratory rate and tidal volume in normal (*n*) and cannulated mice (*c*). Results, mean $\pm$ SEM of groups of mice, are from the last min of a 10-min exposure period. Each group consists of four naive mice. Least squares regression lines are also included

Table 2 Comparison of experimentally determined and estimated potencies of sensory irritants. Estimated values are obtained from log L^{16} values

Substance	log L^{16} [a]	log L^{16} [b]	(A) RD50 (ppm)[c]	(B) RD50 (ppm)[d]	B/A
n-Propanol	2.031	2.080	22 080	10 407	0.47
Trifluoroethanol	1.224	1.248	23 323	31 853	1.37
Hexafluoroisopropanol	1.392	1.173	165	25 236	153
Methyl hexafluoroiso-	–	1.115	$\geqslant$ 160 000	37 048	$\leqslant$ 0.23
propyl ether		1.334		27 348	$\leqslant$ 0.17

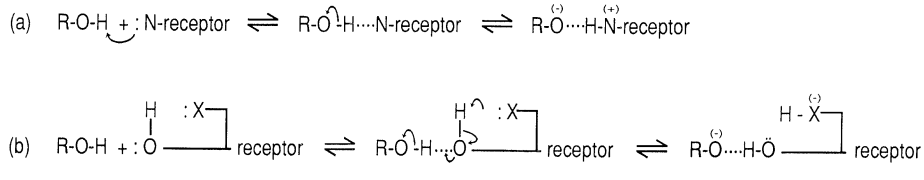
[a] Experimentally determined log solute partition coefficients in the gas-hexadecane system at 298°K from Abraham et al. (1994a)
[b] Log L^{16} values calculated from fragmental constants (Havelec and Ševčík 1994); the value 1.334 is obtained from hexafluoroisopropanol subtracting the fragmental constant for "OH" and adding the constants for "-O-" and "-CH_3"
[c] Experimentally determined RD50 values. The *n*-propanol value is from Kristiansen et al. (1986). Generally, RD50 values are obtained from log concentration-effect relationships of maximum decreases during 1-min periods and thus the value 23 323 ppm is used for trifluoroethanol. As methyl hexafluoroisopropyl ether showed negligible sensory irritation effect under the test conditions, no exact RD50 value can be obtained from the irritation effect. For explanation of the estimate, see the Discussion section
[d] Calculated RD50 values are obtained from Alarie et al. (1995): $\log RD50 = 5.24 - 0.602 \cdot \log L^{16}$. Experimental log L^{16} values were used when available

Table 3 Comparison of experimentally determined and estimated potencies of sensory irritants. Estimated values are obtained from a QSAR using the linear solvation energy relationship method

Substances	$\pi_2^{H(a)}$	$\Sigma\alpha_2^{H(a)}$	Log $L^{16(b)}$	pKa[c]	(C) RD50 (ppm)[d]	C/A (Table 2)
n-Propanol	0.42	0.37	2.031	16.10	7 107	0.32
Trifluoroethanol	0.60	0.57	1.224	12.40	5 292	0.23
Hexafluoroisopropanol	0.55	0.77	1.392	9.39	1 571	9.52
Methyl hexafluoroiso-	0.67	0.17	1.115	–	44 698	$\leqslant$ 0.28
propyl ether			1.334		30 453	$\leqslant$ 0.19

[a] From Abraham et al. (1994a); the value for methyl hexafluoroisopropyl ether is taken from methoxyfluorane ($CHCl_2$-CF_2-O-CH_3)
[b] The log L^{16} values are the experimentally determined values (Abraham et al. 1994a) except for the ether where the calculated values (Table 2) are used
[c] The pKa value for *n*-propanol is from Takahashi et al. (1971) and the values for the fluorinated alcohols are from Arrowsmith et al. (1991)
[d] The estimated RD50 values are obtained from $\log RD50 = 6.90 - 1.49 \cdot \pi_2^H - 2.37 \cdot \Sigma\alpha_2^H - 0.761 \cdot \log L^{16}$ according to Alarie et al. (1995)

Fig. 9 Examples of coupled reactions which may be involved in the activation of the sensory irritant receptor



In conclusion, this study experimentally confirmed the important role of hydrogen bonding as a mechanism to activate the sensory irritant receptor and brings into consideration the possibility of coupled reactions to explain the high potency of chemicals as sensory irritants.

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