# MUSK AMBRETTE AND MUSK XYLENE

## 1. Exposure Data

### 1.1 Chemical and physical data

#### 1.1.1 *Nomenclature*

**Musk ambrette**

*Chem. Abstr. Serv. Reg. No.*: 83-66-9

*Chem. Abstr. Name*: 1-(1,1-Dimethylethyl)-2-methoxy-4-methyl-3,5-dinitrobenzene

*IUPAC Systematic Name*: 6-*tert*-Butyl-3-methyl-2,4-dinitroanisole

*Synonyms*: Amber musk; artificial musk ambrette; 5-*tert*-butyl-1,3-dinitro-4-methoxy-2-methylbenzene; 4-*tert*-butyl-3-methoxy-2,6-dinitrotoluene; 2,6-dinitro-3-methoxy-4-*tert*-butyltoluene; synthetic musk ambrette

**Musk xylene**

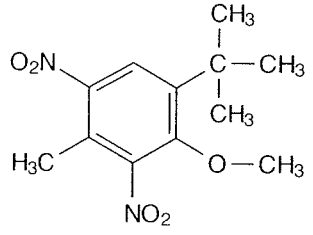
*Chem. Abstr. Serv. Reg. No.*: 81-15-2

*Chem. Abstr. Name*: 1-(1,1-Dimethylethyl)-3,5-dimethyl-2,4,6-trinitrobenzene

*IUPAC Systematic Name*: 5-*tert*-Butyl-2,4,6-trinitro-*meta*-xylene

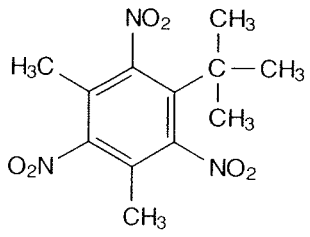
*Synonyms*: 1-*tert*-Butyl-3,5-dimethyl-2,4,6-trinitrobenzene; musk xylol; 2,4,6-trinitro-1,3-dimethyl-5-*tert*-butylbenzene; 2,4,6-trinitro-3,5-dimethyl-*tert*-butylbenzene; xylene musk

#### 1.1.2 *Structural and molecular formulae and relative molecular mass*




Musk ambrette

$C_{12}H_{16}N_2O_5$ Relative molecular mass: 268.30




Musk xylene

$C_{12}H_{15}N_3O_6$ Relative molecular mass: 297.30

1.1.3 *Chemical and physical properties of the pure substance*

**Musk ambrette**

(*a*) *Description*: Pale yellowish, whitish yellow or yellow granular crystals, leaves (from alcohol) or pale yellow powder with a sweet, heavy floral-musky odour (Lide, 1993; Flavor and Extract Manufacturers' Association, 1995a; Penta Manufacturing Co., 1995a)

(*b*) *Boiling-point*: > 200 °C (Flavor and Extract Manufacturers' Association, 1995a)

(*c*) *Melting-point*: 84–86 °C (Lide, 1993)

(*d*) *Spectroscopy data*: Infrared (prism [18719], grating [8877]), ultraviolet (UV) [6029], nuclear magnetic resonance (proton [2085]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980)

(*e*) *Solubility*: Virtually insoluble in water; soluble in 95% ethanol (3.3 g/100 g), methyl carbitol (16.4 g/100 g), benzyl benzoate (50.0 g/100 g), diethyl phthalate (36.7 g/100 g) and diethyl ether (Lide, 1993; Research Institute for Fragrance Materials, 1994a; Penta Manufacturing Co., 1995a)

**Musk xylene**

(*a*) *Description*: Yellow crystals, plates or needles (from alcohol) with a musky odour (Lide, 1993; Penta Manufacturing Co., 1995b)

(*b*) *Boiling-point*: > 200 °C (Penta Manufacturing Co., 1995b)

(*c*) *Melting-point*: 110 °C (Lide, 1993)

(*d*) *Spectroscopy data*: Infrared (prism [1479], grating [250]), UV [422], nuclear magnetic resonance (proton [6497], C-13 [4212]) and mass spectral data have been reported (Sadtler Research Laboratories, 1980).

(*e*) *Solubility*: Virtually insoluble in water; soluble in diethyl ether and ethanol (Lide, 1993; Research Institute for Fragrance Materials, 1994b)

(*f*) *Volatility*: Vapour pressure, < 0.001 mm Hg [0.13 Pa] at 20 °C (Flavor and Extract Manufacturers' Association, 1995b)

(*g*) *Octanol/water partition coefficient (P)*: log P, 5.20 (Helbling *et al.*, 1994)

### 1.1.4 *Technical products and impurities*

Musk ambrette and musk xylene are available commercially (Penta Manufacturing Co., 1995a,b).

### 1.1.5 *Analysis*

Several methods for the determination of nitro musks in fragrance products have been developed based on gas chromatography (GC) (electron capture detection (ECD)/GC, Betts *et al.*, 1982; capillary GC, Spanedda *et al.*, 1986; ECD/GC, Porcu & Spanedda, 1988), liquid chromatography (Bruze *et al.*, 1985) and thin-layer chromatography (Bruze *et al.*, 1985; Goh & Kwok, 1986). Wisneski *et al.* (1994) described a method for the determination of musk ambrette in fragrance products by ECD/GC.

Capillary GC with atomic emission detection using programmed temperature vaporization has been used to detect nitro musks in human fat. The limits of detection for the nitro musks using this method were 1.0–1.6 ng (Linkerhägner *et al.*, 1994).

Nitro musks have been analysed in human adipose and fish tissues and human milk samples by capillary GC/ECD with confirmation by mass spectrometry (MS). The detection limit was 10 µg/kg fat (Rimkus & Wolf, 1993a,b; Rimkus *et al.*, 1994; Rimkus & Wolf, 1995).

Liebl and Ehrenstorfer (1993) used a similar GC/ECD method for the analysis of nitro musks in human milk samples.

Helbling *et al.* (1994) described a capillary GC/MS method for the determination of musk xylene in blood. The detection limit for musk xylene was 5 pg/g plasma or 1 ng/g lipids.

Similar methods have been developed for the determination and quantitation of nitro musks in cosmetics and detergents (Sommer, 1993).

## 1.2 Production and use

### 1.2.1 *Production*

Probably the earliest report on the synthesis of compounds having musk-like odour appeared in 1759 in the *Actes de l'Académie de Berlin* which contained Morggraf's statement that, when oil of amber is treated with fuming nitric acid, a resinous material is obtained that possesses a musk odour (Bedoukian, 1986).

Although Kelbe was probably the first to prepare a synthetic nitro musk and characterize it, Baur is credited with the discovery and commercialization of nitrated compounds having strong musk odours. In his 1889 German patent, Baur described a process whereby toluene was butylated with butyl halide in the presence of aluminium chloride and the product, boiling at 170–200 °C, was nitrated to give a crystalline substance possessing a strong musk odour (Bedoukian, 1986).

In 1892, Baur obtained another patent in which he identified his original musk as being trinitro-butyltoluene. At the same time he described a new product obtained by nitrating butylated *meta*-cresol methyl ether. This compound later became known as

musk ambrette. In 1894, Baur patented another very important musk compound known today as musk ketone. This compound was prepared by nitrating acetylated *tert*-butyl-*meta*-xylene (Bedoukian, 1986).

Musk ambrette is now prepared commercially by the following multistep synthesis. The potassium salt of *meta*-cresol is methylated with dimethyl sulfate to give the methyl ether, which is then butylated using isobutyl chloride in the presence of aluminium chloride. The resulting *tert*-butylcresyl methyl ether is obtained in yields of 55–60% and is purified with fractionation. Nitration at temperatures below 0 °C with fuming nitric acid leads to the formation of the dinitro derivatives in yields of 45–60%. The pure product is obtained by crystallization from 95% ethanol. The by-products in this case consist of the mononitro derivative, 4,6-dinitro-*meta*-cresol methyl ether and smaller amounts of trinitro derivatives (Bedoukian, 1986).

Musk ambrette can also be produced by the methylation of 5-methyl-2-*tert*-butyl-phenol to the corresponding anisole (ambrogen), which on nitration gives musk ambrette (Reed, 1978).

Musk xylene is prepared by the nitration of *tert*-butyl-*meta*-xylene. The *tert*-butyl-*meta*-xylene is prepared by the Friedel-Crafts alkylation of *meta*-xylene with *tert*-butyl or isobutyl chloride in the presence of anhydrous aluminium chloride. The yield is typically around 70–80%. The product is nitrated with fuming nitric acid or with a mixture of sulfuric and nitric acids (70 : 30), and crude musk xylene crystallizes from the heated reaction mixture upon cooling. The crystals are filtered and washed with water and dilute sodium carbonate, and the dried product is purified by recrystallizing from 95% ethanol, with a yield (based on *tert*-butyl-*meta*-xylene) of about 88% (Bedoukian, 1986).

The aromatic class of musks consists of macrocyclics, polycyclics and nitro musks. In 1987, nitro musks constituted about 35% of the worldwide production volume of about 7000 tonnes per year of aromatic musk chemicals. Most musk compounds were produced in western Europe, where capacity exceeded demand by about 25%. The United Kingdom ranked number one in aromatic musk production, with 28% of the total worldwide. In 1987, demand for musk in the United States of America exceeded domestic production by 100%; almost 60% of the volume consumed was imported, with about 40% of nitro musk imports coming from China. Until the mid-1980s, China and India produced only nitro musks (Anon., 1988; Barbetta *et al.*, 1988).

By the early 1990s, annual worldwide production of nitro musks had declined to approximately 1000 tonnes, of which 67% was musk xylene, 21% musk ketone and 12% musk ambrette (Qinghua, 1993; Ippen, 1994). Musk ambrette was produced mainly in China and India for internal markets (Topfer, 1992).

### 1.2.2 *Use*

Since the mid-1980s, nitro musks have begun to be replaced in many uses by other aromatic musks, notably the polycyclics. This is due to the superior fragrance qualities of the newer materials and concerns about potential toxicity of the nitro musks (Anon., 1989; Topfer, 1990, 1992).

Musk ambrette was a fragrance ingredient used for a wide variety of applications. However, by 1992, it was reportedly no longer used in the United States and its use was very limited in Europe (Topfer, 1992). Musk ambrette has been used as a fragrance in products at the following typical concentrations (%): soap, 0.03 (max., 0.2); detergent, 0.003 (max., 0.02); creams/lotions, 0.01 (max., 0.07); and perfume, 0.2 (max., 2.0) (Opdyke, 1975). Musk ambrette also has been used in certain beverages and foods at the following concentrations (ppm) (mg/kg): alcoholic beverages, 0.10; non-alcoholic beverages, 0.18 (max., 0.42); gelatin pudding, 0.45 (max., 1.32); chewing gum, 36.0; and hard candy, 423.0 (Flavor and Extract Manufacturers' Association, 1995a).

Musk xylene is a fragrance ingredient used in fragrance compounds for a wide variety of applications. It has been in use since the early 1900s and its use in the European Union is in the region of 200 tonnes per annum. In a survey of major fragrance companies, the Research Institute for Fragrance Materials found the estimated upper 90th percentile concentrations of musk xylene in cosmetic products to be (%): toilet soap, 0.04; shampoo, 0.01; skin cream, 0.0075; deodorant, 0.0075; aftershave, 0.03; cologne/toilet water, 0.075; and fine fragrance, 0.05–0.1 (Research Institute for Fragrance Materials, 1994b).

## 1.3 Occurrence

### 1.3.1 *Natural occurrence*

None of the nitro musks (musk ambrette and musk xylene) are known to occur as natural products.

### 1.3.2 *Occupational exposure*

The National Occupational Exposure Survey conducted between 1981 and 1983 indicated that 22 735 employees in the United States were potentially exposed to musk ambrette and 134 410 were potentially exposed to musk xylene. The estimate is based on a survey of companies and did not involve measurements of actual exposure (United States National Institute for Occupational Safety and Health, 1995).

### 1.3.3 *Environmental occurrence*

#### (*a*) *Water*

In recent studies, nitroaromatic compounds, including nitro musks, were detected in unfiltered water samples of the North Sea (German Bight) and the Rivers Elbe and Stör. The highest concentrations of nitro musks were found in the effluents of a wastewater treatment plant of the city of Hamburg. These values were about one order of magnitude higher than those in the River Elbe near Hamburg. The concentrations in water from various sampling points in the Rivers Elbe and Stör were about 1 ng/L musk xylene (Rimkus & Wolf, 1995). The lowest contamination levels were found in water from various stations in the North Sea, with musk xylene ranging from < 0.03 to 0.17 ng/L (Gatermann *et al.*, 1995).

Musk xylene was detected in the River Tama (river water and dam water) in Japan in 1981 at a mean concentration of 4.1 ng/L (18 samples), in the flowing water in the tributaries which discharged into the River Tama at a mean concentration of 15 ng/L (13 samples) and in the wastewater from the sewage of three treatment plants at a mean concentration of 32 ng/L (3 samples) (Yamagishi *et al.*, 1983).

(*b*) *Other*

Musk xylene was detected (by GC/flame ionization) as one of the components of Japanese incense sticks and was attributed to the synthetic perfumes used in the sticks (Takiura *et al.*, 1973).

Nitro musks are used as fragrance ingredients in products commonly used both at home and in the laboratory. In the early 1980s, when musk xylene had not yet been reported as a contaminant in foods or the environment, it was detected in one of three fish samples caught in a particular lake. However, the sample was suspected to have been contaminated outside the aquatic environment; qualitative ECD/GC or GC/MS analyses of a sample of soap and three samples of hand lotions used in the laboratory showed the presence of musk xylene in each product. Musk ambrette was also found in two of the three hand lotions examined (Yurawecz & Puma, 1983).

Goh and Kwok (1986) analysed 32 men's colognes for the presence of nitro musks using thin-layer chromatography. The concentrations of musk ambrette varied from 0.02% to 0.39% w/v in 14 colognes and that of musk xylene from 0.02% to 0.78% w/v in 11 colognes.

Using GC coupled with thermal energy analysis, Nair *et al.* (1986) reported that, during analysis of extracts of betel quid with tobacco and of saliva of chewers of betel quid with tobacco for *N*-nitrosamines, two unknown compounds were detected. These were subsequently identified as musk ambrette and musk xylene by GC/MS and Fourier transform nuclear magnetic resonance spectroscopy. In samples of betel quid and tobacco, musk ambrette concentrations ranged from 0.82 to 1.44 mg/g wet weight and musk xylene concentrations from 0.45 to 0.79 mg/g wet weight; in samples of perfumed chewing tobacco, the concentrations ranged from 11.22 to 23.51 mg/g wet weight and 'not detected' to 0.60 mg/g wet weight, respectively.

Nitro musks have been identified and quantified in cosmetics and detergents. In a study in Germany, a total of 60 cosmetic products and 41 detergents were analysed; 53% of them contained nitro musks, with musk xylene being present mainly in detergents and musk ambrette detected in only two samples. Results from the various categories of products (number of samples with detectable levels of the nitro musk/number of samples analysed, maximum concentration found) were as follows: perfume — musk xylene (4/23, 13 mg/kg), musk ambrette (none detected); shampoo — musk xylene (2/13, 300 mg/kg), musk ambrette (1/13, 18 mg/kg); lotion and creme samples — musk xylene (1/24, 16 mg/kg), musk ambrette (none detected); liquid and powder detergents — musk xylene (14/30, 100 mg/kg), musk ambrette (1/30, 5.3 mg/kg); fabric softener — musk xylene (3/11, 7.2 mg/kg), musk ambrette (none detected) (Sommer, 1993).

The United States Food and Drug Administration screened 125 finished fragrances in 1985 and 1986 and found that, in both years, over 40% of the products contained musk ambrette. Perfume and cosmetic products in the market-place were also surveyed for the presence of musk ambrette in 1989, 1990 and 1992. In this study, musk ambrette was detected in 41% (29/41) of the products assayed in 1989, 8% (3/36) of the products assayed in 1990 and 11% (2/18) of the products assayed in 1992. Musk ambrette levels in the products ranged between 0.045% and 0.35% (Anon., 1987; Jackson, 1993).

### 1.3.4 *Food*

Musk xylene was detected in 40 samples collected from several sampling stations along the River Tama at a dam and in Tokyo Bay, Japan, during July and October 1980 and 1981 (three species of freshwater fish and four species of marine shellfish). The average concentrations of musk xylene were 53.9 µg/kg (ppb) in the viscera of freshwater fish, 16.0 µg/kg (ppb) in the fish muscle and 2.7 µg/kg (ppb) in marine shellfish (Yamagishi *et al.*, 1983).

In 1991–92, residues of musk xylene were identified in tissues of farmed fish (mainly trout) and in tissues of fish from the River Lauchert, Germany. The residue levels ranged from 5 to 82 µg/kg fresh weight in 40/44 samples (when the concentration of the river water was measured at the same time as that in fish). The author noted that the river water contamination was caused by musk xylene that was added to washing powders as a perfuming agent (Hahn, 1993).

During the German Food Contamination Monitoring Programme in 1990–92 and in an extension of that study, 142 samples of fish, mussels and shrimp were analysed for nitro musks. Low levels of musk xylene (0.01–0.04 mg/g fat) were detected in the mussel samples. In trout samples from aquaculture ponds in Schleswig-Holstein, low musk xylene levels (max., 0.1 mg/kg fat) were determined, although some samples of imported trout contained high concentrations of musk xylene (max., 1.06 mg/kg fat or 0.048 mg/kg fish). Very low levels of musk ambrette were also tentatively identified, but not confirmed, in a small number of samples. Fish samples from waters in northern Germany were found to contain concentrations of musk xylene that varied with the pollution level of the water source (max., 0.35 mg/kg fat) (Rimkus & Wolf, 1993b, 1995).

### 1.3.5 *Biological monitoring*

Musk xylene has been found in human adipose tissue and breast milk. The quantity found in human fat (32 samples, 13 in women, 19 in men) varied between 0.02 and 0.09 mg/kg fat in men and 0.02 and 0.22 mg/kg fat in women. The quantity present did not vary with age as did the quantities of other substances investigated. The quantity in human breast milk (23 samples) varied between 0.02 and 0.19 mg/kg fat. The average fat content of the milk (where given) was 2.2% (range, 0.1–5.1%) (Rimkus & Wolf, 1993a; Rimkus *et al.*, 1994).

Using GC/ECD, Liebl and Ehrenstorfer (1993) analysed 391 milk samples (48 in 1991, 343 in 1992) of nursing mothers living in southern Bavaria, Germany, for nitro musks. Musk ambrette was detected at concentrations ranging from < 0.01 to 0.29 mg/kg

fat (mean concentration, 0.04 mg/kg fat). Concentrations of musk xylene were about two to three times higher, ranging from 0.01 to 1.22 mg/kg fat, with a mean content of 0.1 mg/kg fat.

Helbling *et al.* (1994) reported on the levels of musk xylene in 11 blood samples from three individuals. Musk xylene concentrations ranged from 66 to 270 pg/g plasma or 12 to 49 ng/g blood lipids. Potential laboratory sources of contamination during analysis, including paper tissues, latex gloves, the surface of a worker's hands and laboratory solvents, contributed about 50 pg/g to plasma levels and 10 ng/g to blood lipid levels.

### 1.4 Regulations and guidelines

Spurred by reports in 1979 and 1980 that musk ambrette was a photosensitizer, the International Fragrance Association (IFRA) in Geneva, Switzerland, issued non-enforceable guidelines in 1981 limiting the use of musk ambrette to 4% in new fragrance compounds (Anon., 1983). Since 1983, IFRA has recommended that musk ambrette should not be used in fragrance products for cosmetics, toiletries or other products which under normal conditions of use will come into contact with the skin. This includes rinse-off products. For other applications, musk ambrette should not be used as a fragrance ingredient at a level over 4% in fragrance compounds. This restriction should not be exceeded irrespective of the end-use concentration. The low-level use (< 1%) of fragrance compounds in those products in which use is allowed would result in final product concentrations of less than 0.04% (Research Institute for Fragrance Materials, 1994a).

Musk ambrette was removed from the Generally Recognized As Safe (GRAS) list in the United States in 1984 and does not have any other food use status. Musk xylene does not have food use status (Oser *et al.*, 1984; Research Institute for Fragrance Materials, 1994a). For this reason, they should not be used in lip products or flavours for oral hygiene products (Research Institute for Fragrance Materials, 1994a,b).

Due to its toxicity profile, musk xylene has not been used in Japanese products on the basis of a voluntary restriction since 1982 (Minegishi *et al.*, 1991).

## 2. Studies of Cancer in Humans

No data were available to the Working Group.

## 3. Studies of Cancer in Experimental Animals

### Musk ambrette

No data were available to the Working Group.

## Musk xylene

### 3.1 Oral administration

*Mouse*: Groups of 50 male and 50 female B6C3F1 mice, six weeks of age, were administered 0, 0.075 or 0.15% musk xylene (purity, > 96%) in the diet for 80 weeks, after which they were maintained on basal diet until week 90 when all survivors were killed. Dietary intakes were 0.091 (range, 0.07–0.125) and 0.170 (0.141–0.228) g/kg bw per day for males and 0.101 (0.080–0.143) and 0.192 (0.166–0.259) g/kg bw per day for females in the low- and high-dose groups, respectively. Musk xylene intake had a significant inhibitory effect on growth in high-dose males, and this was apparent from week 4 to week 80. By the end of the study, there was no longer any difference between the groups. In females, no significant difference in growth occurred throughout the experiment. There was no significant difference in cumulative mortality between controls and treated males or females. Complete histopathological examination was carried out on all animals. The overall tumour incidences (number of mice with tumours) in treated males and females at both dose levels were significantly higher than those in controls (males — 22/49 in controls; 37/50 at the low dose and 40/47 at the high dose; females — 9/46 in controls; 30/50 at the low dose; and 30/49 at the high dose. Increased tumour incidences were observed in the liver and Harderian gland (see Table 1) (Maekawa *et al.*, 1990).

**Table 1. Summary of main neoplastic lesions in B6C3F1 mice given musk xylene in the diet for 80 weeks**

| Tumour site and type | Number of male mice with tumours | | | Number of female mice with tumours | | |
|---|---|---|---|---|---|---|
| Dose | 0% | 0.075% | 0.15% | 0% | 0.075% | 0.15% |
| Effective number of mice | 49 | 50 | 47 | 46 | 50 | 49 |
| Liver | | | | | | |
| Adenoma | 9 | 19* | 20** | 1 | 14*** | 13*** |
| Carcinoma | 2 | 8* | 13** | 0 | 1 | 2 |
| Adenoma/carcinoma | 11 | 27** | 33*** | 1 | 15*** | 15*** |
| Harderian gland | | | | | | |
| Adenoma | 2 | 9* | 10* | 3 | 3 | 5 |
| Carcinoma | 1 | 1 | 0 | 0 | 0 | 0 |
| Adenoma/carcinoma | 3 | 10* | 10* | 3 | 3 | 5 |

From Maekawa *et al.* (1990)

*$p < 0.05$, **$p < 0.01$; ***$p < 0.001$; $\chi^2$-test

# 4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

## 4.1 Absorption, distribution, metabolism and excretion

### 4.1.1 *Humans*

Musk xylene ($^{15}$N-labelled) was given to three volunteers and the elimination from blood plasma was followed for up to 162 days. The elimination half-life ranged from 63 to 107 days (Kokot-Helbling *et al.*, 1995).

Musk xylene has been found in human fatty tissue (Rimkus & Wolf, 1993a; Rimkus *et al.*, 1994) and breast milk (Liebl & Ehrenstorfer, 1993; Rimkus & Wolf, 1993a; Rimkus *et al.*, 1994).

### 4.1.2 *Experimental systems*

When musk xylene ($^{3}$H-labelled in the 5-*tert*-butyl group) was administered intragastrically to male Wistar rats, approximately 50% of the dose was excreted into urine and faeces by 24 h and almost 87% by seven days. The proportion of the dose excreted into urine and faeces was 10.3% and 75.5%, respectively. The main metabolites observed were derived from the reduction of the 2-nitro group (2-amino-5-*tert*-butyl-4,6-dinitroxylene; 2-amino-5-*tert*-butyl-1-methyl-3-hydroxymethyl-4,6-dinitrobenzene; 2-amino-5-*tert*-hydroxybutyl-4,6-dinitroxylene) while reduction at the 4-nitro position proceeded less effectively (4-amino-5-*tert*-butyl-2,6-dinitroxylene; 4-amino-5-*tert*-butyl-1-methyl-3-hydroxymethyl-4,6-dinitrobenzene) (Minegishi *et al.*, 1991).

## 4.2 Toxic effects

### 4.2.1 *Humans*

Musk ambrette can cause photoallergic contact dermatitis (Raugi *et al.*, 1979). Most cases were in men, although contact dermatitis in a woman whose husband used a cologne containing musk ambrette has also been reported (Fisher, 1995). Patients presented with patches of eczema on the cheeks, chin and neck — the light-exposed areas on which their aftershaves had been applied. A few individuals have a more widespread eczematous reaction. There are several probable reasons why men are particularly affected: the concentration of musk ambrette in aftershaves was previously very high (as high as 15%), and the aftershave was applied in relatively large volumes to the thin skin of the face, which was often freshly abraded by shaving and is usually a maximally light-exposed area (Wojnarowska & Calnan, 1986).

Patients exhibited a positive photopatch test, which is provoked by UVB and sometimes also by UVA radiations (Ramsay, 1984). In addition, some of the patients displayed patch-test positivity (without light) to musk ambrette (Wojnarowska & Calnan, 1986) and, in one case, to its photodecomposition products as well (Bruze & Gruvberger, 1985).

Some patients develop a persistent light reaction/chronic actinic dermatitis (pruritic dermatitis with lichenification on the light-exposed areas), which can persist for years, in spite of the patient having removed the exposure (Cronin, 1984). The mechanism behind this reaction is not known.

Over a six-year period (1985–90) in New York City, United States, photopatch tests were carried out on 187 patients (76 males and 111 females) with a history of photosensitivity. Ten of the relevant responses were due to musk ambrette (DeLeo *et al.*, 1992). Other musks are less sensitizing than musk ambrette, although positive photopatch tests in patients have also been obtained with musk xylene and moskene, but not with musk ketone or musk tibetine (Cronin, 1984).

### 4.2.2 *Experimental systems*

#### Musk ambrette

An oral $LD_{50}$ of 339 mg/kg bw was reported for musk ambrette in rats (Jenner *et al.*, 1964); a value of 4.8 g/kg has also been reported. The acute dermal $LD_{50}$ of musk ambrette exceeded 2 g/kg in rabbits (Opdyke, 1975).

Musk ambrette induced photosensitivity in guinea-pigs after application to abraded skin or by using occlusion (Kochever *et al.*, 1979; Jordan, 1982; Bueler *et al.*, 1985); it was also positive in the mouse ear-swelling model (Gerberick & Ryan, 1990a). UVB irradiation did not enhance the photoallergic reaction of mouse ear to musk ambrette caused by UVA (as it did for the model photoallergen, 6-methylcoumarin) (Gerberick & Ryan, 1990b); musk ambrette did not elicit a positive photoallergic response in the local lymph node assay (as did the strong photoallergens tetrachlorosalicylanilide and fentichlor) (Scholes *et al.*, 1991).

No effect was observed in rats after a 12-week feeding of 0.76 mg/kg musk ambrette in the diet (Bär & Griepentrog, 1967). After feeding 0.5–4 mg/g of diet musk ambrette to rats, growth retardation, testicular atrophy (at 2.5 mg/g) and progressive paralysis of hind limbs (at 1.5 mg/g) were observed after 12–15 weeks. At the higher doses, complete hind limb paralysis was observed within 16–40 weeks. In female rats, depressed erythrocyte counts and haemoglobin values were observed at $\geq$ 1.5 mg/g musk ambrette and icterus, indicating haemolysis, at all dose levels. Histopathological investigation revealed muscular and testicular atrophy in males and enlarged adrenal glands in females (Davis *et al.*, 1967 (abstract); Spencer *et al.*, 1984). Neuropathological changes included primary demyelination and distal axonal degeneration (Ford *et al.*, 1990).

In rats, musk ambrette induced CYP1A2 but much less CYP1A1. At a daily intraperitoneal dose of 0.1 mmol (28 mg)/kg bw for five days, it caused a 50% increase in the hepatic activity of UDP-glucuronosyl transferase but did not affect the activities of DT-diaphorase or glutathione *S*-transferase (Iwata *et al.*, 1993a).

#### Musk xylene

The acute oral $LD_{50}$ of musk xylene was reported to exceed 10 g/kg, and the acute dermal $LD_{50}$ in rabbits to exceed 15 g/kg (Opdyke, 1975).

Musk xylene was a weak inducer of contact hypersensitivity (occluded patch test) in guinea-pigs; the reaction was not affected by exposure to UV irradiation (Parker *et al.*, 1986).

Of five male and five female B6C3F1 mice given a single oral dose of 4000 mg/kg bw musk xylene, one female mouse died within 14 days. When musk xylene was added to the diet of B6C3F1 mice (8 males and 8 females) at concentration levels of 0.3, 0.6, 1.25, 2.5 or 5% for 14 days, all mice given 0.6% musk xylene, except one female, died within two to four days. All animals given 0.3% musk xylene survived to the end of the experiment. Histological examination revealed haemorrhagic erosions in the glandular stomach only. In a 17-week study with dietary levels of 0.0375, 0.075, 0.15, 0.3 or 0.6% musk xylene, all mice at the highest-dose level and all females and 8/10 males given 0.3% musk xylene died; no death occurred in the other groups. At dose levels of 0.15% and less, no difference in body-weight development or organ weights between treated and control animals was observed. Enlargement and irregularity of liver cells were observed in animals fed 0.15% musk xylene (Maekawa *et al.*, 1990).

No excess mortality, decrease in body-weight gain, clinical chemistry abnormality or gross or microscopic change were observed in Sprague-Dawley rats after daily dermal applications of 240 mg/kg bw musk xylene for 90 days. The only abnormal finding was an increase in the relative liver weight (Ford *et al.*, 1990).

Musk xylene increased the liver weight and the total cytochrome P450 content, and induced CYP1A2 and, to a far lesser extent, CYP1A1 in male Wistar rats (Iwata *et al.*, 1992, 1993a,b). At a daily intraperitoneal dose of 0.1 mmol [30 mg]/kg bw for five days, musk xylene did not affect the hepatic activities of DT-diaphorase, glutathione *S*-transferase or UPD-glucuronosyl transferase (Iwata *et al.*, 1993b). At higher doses, induction of these enzyme activities was observed (Iwata *et al.*, 1993a).

In male B6C3F1 mice, musk xylene given at 200 mg/kg bw per day by gavage for seven days increased liver weight by 40%, caused hepatocellular hypertrophy and increased cytochrome P450 content with a concomitant induction of CYP1A1, 1A2 and 2B proteins. However, while the activities of CYP1A1 and 1A2 were elevated, that of 2B was not. This was explained by the fact that it inhibited murine CYP2B enzymes *in vitro* ($IC_{50}$ approx 1 μmol [300 μg]/L) (Lehman-McKeeman *et al.*, 1995). As reported in an abstract, a single oral dose (200 mg/kg bw) of musk xylene given to B6C3F1 mice pretreated with phenobarbital decreased the measurable CYP2B activity by 90%; this inhibition was not seen in mice also treated with a combination of neomycin, tetracycline and bacitracin. The authors interpreted this to mean that the inhibition of CYP2B was not due to musk xylene itself but to a metabolite formed by intestinal microflora (Caudill *et al.*, 1995; Lehman-McKeeman *et al.*, 1995).

## 4.3 Reproductive and developmental effects

No data were available to the Working Group.

### 4.4 Genetic and related effects

4.4.1 *Humans*

No data were available to the Working Group.

4.4.2 *Experimental systems* (see also Table 2 and Appendices 1 and 2)

Musk ambrette was mutagenic in *Salmonella typhimurium* TA100 requiring metabolic activation by rat-liver S9.

In *Drosophila*, musk ambrette induced sex-linked recessive lethal mutations in mature sperm. After intraperitoneal injection or after oral dosing musk ambrette did not induce micronuclei in the bone marrow of male or female NMRI mice.

Musk xylene gave uniformly negative results in a series of short-term genotoxicity tests that included the *S. typhimurium* mutation test, the mouse lymphoma assay, an in-vitro cytogenetics assay in Chinese hamster ovary (CHO) cells, the in-vitro unscheduled DNA synthesis assay in primary rat hepatocytes and an in-vivo unscheduled DNA synthesis assay.

## 5. Summary of Data Reported and Evaluation

### 5.1 Exposure data

Musk ambrette and musk xylene are nitro musks, which are prepared by nitration of *tert*-butylcresol methyl ether and *tert*-butyl-*meta*-xylene, respectively. Musk xylene and, in lower amounts, musk ambrette have been used since the early 1900s as fragrance ingredients in perfumes, soaps, detergents and cosmetics. Musk ambrette has also been used at low levels in foods such as candy, chewing gum and beverages. Nitro musks have been detected in surface waters and in fish and shellfish.

### 5.2 Human carcinogenicity data

No data were available to the Working Group.

### 5.3 Animal carcinogenicity data

No data were available on the carcinogenicity of musk ambrette.

Musk xylene was tested for carcinogenicity in mice by oral administration in the diet in one experiment and induced increased incidences of hepatocellular adenomas and carcinomas and Harderian gland tumours in males and hepatocellular adenomas in females.

**Table 2. Genetic and related effects of nitro musks**

| Test system | Result[a] | | Dose[b] (LED/HID) | Reference |
|---|---|---|---|---|
| | Without exogenous metabolic system | With exogenous metabolic system | | |
| **Musk ambrette** | | | | |
| SA0, *Salmonella typhimurium* TA100, reverse mutation | – | + | 200 | Wild *et al.* (1983) |
| SA0, *Salmonella typhimurium* TA100, reverse mutation | – | + | 100 | Nair *et al.* (1986) |
| SA0, *Salmonella typhimurium* TA100, reverse mutation | – | – | 128 | Zeiger *et al.*(1987) |
| SA5, *Salmonella typhimurium* TA1535, reverse mutation | – | – | 128 | Zeiger *et al.* (1987) |
| SA7, *Salmonella typhimurium* TA1537, reverse mutation | – | – | 128 | Zeiger *et al.* (1987) |
| SA9, *Salmonella typhimurium* TA98, reverse mutation | – | – | 200 | Nair *et al.* (1986) |
| SA9, *Salmonella typhimurium* TA98, reverse mutation | – | – | 128 | Zeiger *et al.* (1987) |
| DMX, *Drosophila melanogaster*, sex-linked recessive lethal mutations | (+) | | 2680 adult feeding | Wild *et al.* (1983) |
| MVM, Micronucleus test, mouse bone-marrow cells *in vivo* | – | | 1072 ip × 2 | Wild *et al.* (1983) |
| MVM, Micronucleus test, mouse bone-marrow cells *in vivo* | – | | 2948 po × 1 | Wild *et al.* (1983) |
| **Musk xylene** | | | | |
| SA0, *Salmonella typhimurium* TA100, reverse mutation | – | – | 200 | Nair *et al.* (1986) |
| SA0, *Salmonella typhimurium* TA100, reverse mutation | – | – | 100 | Api *et al.* (1995) |
| SA5, *Salmonella typhimurium* TA1535, reverse mutation | – | – | 100 | Api *et al.* (1995) |
| SA7, *Salmonella typhimurium* TA1537, reverse mutation | – | – | 100 | Api *et al.* (1995) |
| SA8, *Salmonella typhimurium* TA1538, reverse mutation | – | – | 100 | Api *et al.* (1995) |
| SA9, *Salmonella typhimurium* TA98, reverse mutation | – | – | 200 | Nair *et al.* (1986) |
| SA9, *Salmonella typhimurium* TA98, reverse mutation | – | – | 100 | Api *et al.* (1995) |
| URP, Unscheduled DNA synthesis, rat primary hepatocytes *in vitro* | – | 0 | 30 | Api *et al.* (1995) |
| G5T, Gene mutation, mouse lymphoma L5178Y cells, tk locus | – | – | 400 | Api *et al.* (1995) |
| CIC, Chromosomal aberrations, Chinese hamster CHO cells *in vitro* | – | – | 30 | Api *et al.* (1995) |
| UPR, Unscheduled DNA synthesis, rat hepatocytes *in vivo* | – | | 5000 | Api *et al.* (1995) |

[a] +, positive; (+), weak positive; –, negative; 0, not tested; ?, inconclusive (variable response within several experiments within an adequate study)

[b] LED, lowest effective dose; HID, highest ineffective dose. In-vitro tests, μg/mL; in-vivo tests, mg/kg bw

### 5.4 Other relevant data

Application of musk ambrette on the skin may cause photocontact dermatitis and chronic actinic dermatitis.

Musk ambrette was mutagenic in *Salmonella* and *Drosophila*. It did not induce micronuclei in the bone marrow of mice *in vivo*.

In humans, musk xylene is absorbed from the gastrointestinal tract. It is distributed to the adipose tissue and its half-time in blood plasma is two to three months. It is excreted in human milk.

Musk xylene is metabolized in the rat by nitroreduction. Musk xylene is a phenobarbital-type inducer of cytochromes P450 in rats and mice.

Musk xylene did not induce genetic damage in bacteria, cultured mammalian cells or, in one study, in mammals *in vivo*.

### 5.5 Evaluation[1]

There is *inadequate evidence* in humans for the carcinogenicity of musk ambrette and musk xylene.

There is *inadequate evidence* in experimental animals for the carcinogenicity of musk ambrette.

There is *limited evidence* in experimental animals for the carcinogenicity of musk xylene.

### Overall evaluation

Musk ambrette and musk xylene are *not classifiable as to their carcinogenicity to humans (Group 3)*.

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[1]For definition of the italicized terms, see Preamble, pp. 24–27.

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