European Chemicals Bureau

Institute for Health and Consumer Protection

European Chemicals Bureau

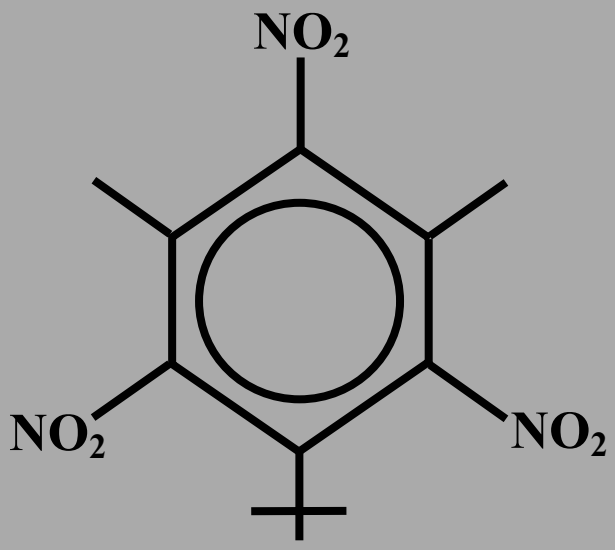
Existing Substances

European Union Risk Assessment Report

CAS No: 81-15-2

EINECS No: 201-329-94

5-tert-butyl-2,4,6-trinitro-m-xylene (musk xylene)



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European Union Risk Assessment Report
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European Union Risk Assessment Report

5-TERT-BUTYL-2,4,6-TRINITRO-M-XYLENE (MUSK XYLENE)

CAS No: 81-15-2

EINECS No: 201-329-4

Risk Assessment

Cataloguing data can be found at the end of this publication
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5-TERT-BUTYL-2,4,6-TRINITRO-M-XYLENE
(MUSK XYLENE)

CAS No: 81-15-2

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RISK ASSESSMENT

Final report, 2005

The Netherlands

Rapporteur for the risk assessment of 5-tert-butyl-2,4,6-trinitro-m-xylene (musk xylene) is the Ministry of Housing, Spatial Planning and the Environment (VROM) in consultation with the Ministry of Social Affairs and Employment (SZW) and the Ministry of Public Health, Welfare and Sport (VWS). Responsible for the risk evaluation and subsequently for the contents of this report, is the rapporteur.

The scientific work on this report has been prepared by the Netherlands Organisation for Applied Scientific Research (TNO) and the National Institute for Public Health and the Environment (RIVM), by order of the rapporteur.

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Date of Last Literature Search:	**January 2003**
Review of report by MS Technical Experts finalised:	**September 2002**
Final report:	**2005**

Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups.

The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 tonnes per year.

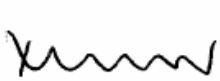
There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.

The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2], which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a Meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Health and Environmental Risks (SCHER) which gives its opinion to the European Commission on the quality of the risk assessment.

If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.

The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992.

This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals.



Roland Schenkel
Acting Director-General
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[1] O.J. No L 084, 05/04/1993 p.0001 – 0075

[2] O.J. No L 161, 29/06/1994 p. 0003 – 0011

[3] Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

0 OVERALL RESULTS OF THE RISK ASSESSMENT

CAS No:	81-15-2
EINECS No:	201-329-4
IUPAC Name:	1-Tert-butyl-3,5-dimethyl-2,4,6-trinitrobenzene

Environment

Conclusion (i) There is a need for further information and/or testing

This conclusion is reached, because the substance is considered a PBT candidate chemical. A further PBT- testing strategy is proposed.

Human health effects assessment

Workers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Consumers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Humans exposed indirectly via the environment

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

Combined exposure

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those which are being applied already.

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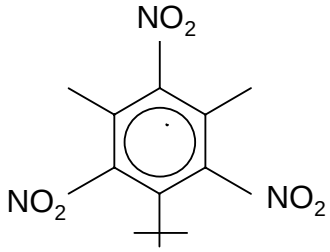
TABLES

1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS No: 81-15-2
EINECS No: 201-329-94
IUPAC Name: 1-tert-butyl-3,5-dimethyl-2,4,6-trinitrobenzen
Synonyms: 5-tert-butyl-2,4,6-trinitro-m-xylene, musk xylene, musk xylol
Molecular formula: $C_{12}H_{15}N_3O_6$
Molecular weight: 297.3
Structural formula:



1.2 PURITY/IMPURITIES, ADDITIVES

Purity: >99%
Impurities: unidentified impurities, <1%
Additives: none

1.3 PHYSICO-CHEMICAL PROPERTIES

In **Table 1.1** the physico-chemical properties of musk xylene are summarised.

Table 1.1 Physico-chemical properties of musk xylene.

Property	Result	Comment	References
Physical state	solid, powder		
Melting point	112-114°C	#, *	Treff, 1926; Le Fèvre and Le Fèvre, 1935; Opfer-Schaum and Piristi, 1944
Boiling point	not applicable	**	Tas and Van de Plassche, 1996; Givaudan, 1990
Relative density	0.77, 0.85 g/cm^3 Recommended: 0.77 g/cm^3	* $	BAM, 1994; RVO-TNO, 1974
Vapour pressure	0.0097 Pa at 40°C, 0.47 Pa at 74.5°C 0.00003 Pa (calculated) at 20°C Recommended: 0.00003 Pa at 20°C	*	Grain, 1990; Tas and Van de Plassche, 1996
Surface tension	not applicable	&	-
Water solubility	0.15 mg/l (measured) 0.49 mg/l (calculated) Recommended: 0.15 mg/l	*	Tas and Van de Plassche, 1996; Schramm et al., 1996
Solubility in other solvents	-	-	-
Partition coefficient n-octanol/water (log value)	4.9, 4.4, 3.4 (measured) 3.7, 4.45 (calculated) Recommended: 4.9	* $	Rudio, 1996; Schramm et al., 1996; Tas and Van de Plassche, 1996; Johnson et al., 1984
Flash point	168°C	*	Aroma Chemicals
Flammability	Flammable	*	BAM, 1986; RVO-TNO, 1974; Cutler, 1988
Autoflammability temperature	305-341°C	*	BAM, 1994
Explosive properties	initiated by shock and heat, propagation depends on packaging size and characteristics, and is limited in typical transport packaging	*	Cutler, 1988; Clancey, 1977; RVO-TNO, 1974; BAM, 1994; Givaudan, 1990
Oxidising properties	not oxidising	***	-
Granulometry	100% v/v < 100 μm 21.8% v/v < 10 μm 14.4% v/v < 4 μm	*	Rodriquez, 1998

The substance has an unstable form melting at 105-106°C or 107°C, and a stable form melting at 112-114°C. When the unstable form is allowed to resolidify, it will convert to the stable form.
$ Recommended value based on test report.
& The low water solubility renders further determinations as superfluous.
* One or several values found in literature, all in the same range, not all methods are specified.
** Not applicable, decomposition will start at 270°C.
*** Conclusion based on theoretical, and/or structural considerations.

Data on boiling point, surface tension, and oxidising properties were not provided. In view of the nature of the substance, determination of these parameters is considered to be irrelevant. All other required physico-chemical data were submitted. Most of these data are based on

information from databases, material safety data sheets, or general published information. Only the particle size distribution and one measured value for both the relative density and the water/octanol coefficient are based on test reports. With respect to the selection of the recommended values for several physico-chemical properties the following remarks should be made:

Relative density

The density of 0.77 g/cm^3 described in detail by RVO-TNO (1974) is preferred over the bulk density of 0.85 g/cm^3.

Vapour pressure

The vapour pressure is calculated with the Modified Grain method. As the source of the other values is unknown (probably from a handbook) and has been measured at high temperatures, the calculated value is preferred.

Water solubility

For the calculated value a QSAR using log K_{ow}, melting point and molecular weight is used (Tas and Van de Plassche, 1996). Schramm et al. (1996) measured the water solubility using HPLC resulting in 0.15 mg/l. The measured and calculated water solubility is in the same range. Yet, the measured value is preferred over the calculated one.

Log K_{ow}

Log K_{ow} has been measured once using the shake-flask procedure and twice by the reverse-phase HPLC method. Johnson et al. (1984) report that a log K_{ow} of 3.4 has been measured using the shake-flask procedure. However, for hydrophobic compounds with a log K_{ow} of > 4.5 the classical shake-flask procedure leads to considerable errors. Rudio (1996) applied the HPLC method according to OECD Test-Guideline 117, resulting in a log K_{ow} of 4.9. Schramm et al. (1996) measured a value of 4.4 using HPLC. Log K_{ow} can also be calculated based on the structural formula. Two databases are used for the calculation of log K_{ow}: ClogP and Syracuse (SRC), giving a log K_{ow} of 3.72 and 4.45, respectively. The measured values using HPLC are preferred over the calculated values and the shake-flask value. As more details on the test by Schramm et al. (1996) are not available, the value of 4.9 described in detail by Rudio (1996) is preferred.

Experimental investigations indicate explosive properties that can be initiated by shock and heat. Based on these data, the CMR Working Group decided to classify the substance as explosive (symbol E).

Summary of physico-chemical properties

All data are considered as sufficiently reliable to fulfil the Annex VIIA requirements. The substance should be classified as explosive, E. The following R-sentence is applicable based on the physico-chemical properties; R2.

1.4 CLASSIFICATION AND LABELLING

Classification and labelling according to the 29th ATP of Directive 67/548/EEC[4]:

Classification

Carc. Cat.3; R40	Limited evidence of a carcinogenic effect
E; R2	Risk of explosion by shock, friction, fire or other sources of ignition
N; R50-53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

Specific concentration limits: None

Labelling

E; Xn; N

R: 2-40-50/53

S: (2-)36/37-46-60-61	Keep out of the reach of children – Wear suitable protective clothing and gloves - If swallowed, seek medical advice immediately and show this container or label - This material and its container must be disposed of as hazardous waste - Avoid release to the environment. Refer to special instructions/Safety data sheets.

[4] The classification of the substance is established by Commission Directive 2004/73/EC of 29 August 2004 adapting to technical progress for the 29th time Council Directive 67/548/EEC on the approximation of the laws, regulations and administrative provisions relating to the classification, packaging and labelling of dangerous substances (OJ L 152, 30.04.2004, p.1).

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION

There is no production of musk xylene in the European Union (EU). Several European companies have terminated their productions in the last decade. Producers in China are now the most important source for the European imports.

Production process

Musk xylene is manufactured in a batch process by Friedel-Crafts alkylation of m-xylene with tert.butyl chloride using aluminium chloride as a catalyst. The resulting tert.butyl-m-xylene is subsequently nitrated to yield musk xylene, which is purified by recrystallisation (Bedoukian, 1986).

2.2 USE PATTERN

The imported crystalline solid is used as an ingredient in fragrance compositions. Fragrances are complex mixtures, prepared by blending many fragrance ingredients in varying concentrations. They are nearly always liquids, in which musk xylene has to be dissolved. Musk xylene is partly used in cosmetic products and partly in detergents, fabric softeners, household cleaning products and other fragranced products. All these products can be classified as follows:

- Main category: wide dispersive use
- Industrial category: category 5: personal/domestic use and/or;
  category 6: public domain;
- Use category: category 9: cleaning/washing agents and additives;
  category 15: cosmetics and or;
  category 36: odour agents.

Musk xylene is received by fragrance companies from suppliers in and outside the EU or through the intervention of brokers importing the substance. Subsequently, fragrance compounds are supplied to customers for incorporation in the consumer products mentioned above. These products are sold on the EU market or exported to countries outside the EU.

The following data (**Table 2.1**) have been taken from the IUCLID data sheets and additional, more recent sources (industry information).

Table 2.1 Import volumes per major site (> 10 tonnes in 1990-1994 period).

Site	Import volume	Year
Site 1	12	1996
Site 2	4	1996
Site 3	18	1996
Site 4	6.5	
Site 5	16	1997
Site 6	0.65	1997

The Research Institute of Fragrance Materials (RIFM) has carried out surveys in 1993, 1996, 1997 and 2002 on the total usage of fragrances by fragrance compounding facilities in Europe. For musk xylene the results are shown in **Table 2.2**.

Table 2.2 Results of RIFM surveys: import of musk xylene for Europe in tonnes (IFRA, 1999).

1992 (survey carried out in 1993)	174 tonnes
1995 (survey carried out in 1996)	110 tonnes
1996 (survey carried out in 1997)	105 tonnes
1998	86 tonnes
2000	67 tonnes

According to RIFM the 1995 use volumes account for approximately 90% of the total use as 32 companies involved in fragrance compounding responded to the survey, which included the entire major fragrance producers world wide. No details are available on the survey carried out in 1997 resulting in the use volumes for 1996.

Although, there may be some uncertainty in the data from the RIFM the import and use of musk xylene seems to be decreasing in the EU (see **Table 2.2**). This is likely to be a consequence of a recommendation in 1993 by the Association of the German Toiletries and Detergents Industry (IKW) to replace musk xylene by another substance.

There is a discrepancy between the volumes reported by RIFM and the total volume reported in the IUCLID data sheets, because only companies importing over 10 tonnes/year between 1990 and 1994 had to submit information to the European Chemicals Bureau according to the Council Regulation 793/93. In the EU there are many companies using musk xylene in amounts well below the reporting level of 10 tonnes/year. It should be noted that RIFM reported the import volumes for entire Europe and not only for the EU.

For the exposure calculations for the life-cycle parts ‘end product formulation’ and private use’ the volume reported by RIFM for 2000 of 67 tonnes in **Table 2.2** will be used. This might be an overestimation of the real use as export to non-EU countries is included. Industry sources estimate that 20-30% of their production is exported outside the EU as finished fragrance compounds or in consumer products. On the other hand the RIFM survey covered only 90% of the total use and no data are available on import into the EU of fragrance compounds. According to industry this import is negligible compared to the export. The same is expected to be true for imported products containing musk xylene.

3 ENVIRONMENT RISK ASSESSMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General discussion

3.1.1.1 Degradation

Abiotic degradation

Studies on hydrolysis of musk xylene are not available. Based on the structure of the compound it is assumed that hydrolysis does not take place. According to Lyman et al. (1990) aromatic nitro compounds contain functional groups that are resistant to hydrolysis.

Photolysis of musk xylene was studied by Butte et al. (1999). Under laboratory conditions using an UV immersion lamp, photolysis of musk xylene was observed in which an initial phase where the reaction followed first order kinetics (k: 0.344 minutes^{-1} and $t_{1/2}$: 2.0 minutes) was followed by a phase with a longer half life. Using GC/MS the metabolites 3,3,5,7-tetramethyl-4,6-dinitro-3H-indole and 3,3,5,7-tetramethyl-4,6-dinitro-2-indolinone were identified. Degradation was slower in an outdoor experiment in midsummer at midday under cloudless conditions (no results presented). Model estimation (SRC AOPWIN) of photodegradation for reaction with OH-radicals results in a half life of approximately 19 days when using the TGD OH concentration ($5 \cdot 10^5$ molec.cm^{-3}/24 hours).

It can be concluded on structural grounds that photolysis of musk xylene occurs. However, extrapolation of these results to a field situation is difficult, e.g. UV radiation intensity decreases with the depth of the water. In addition, in eutrophic surface waters algae and humic substances will adsorb most of the UV radiation (Kalf et al., 1995). The estimated DT50 for photodegradation for reaction with OH-radicals also indicates that this is not a major degradation route. Therefore, in the environmental risk assessment no photodegradation will be assumed.

Biotic degradation

Ready biodegradability of musk xylene was tested in the MITI I test (OECD Guideline 301C). The Biological Oxygen Demand (BOD) was measured during a 28-day test with 30 mg/l activated sludge and a concentration of 107 mg/l musk xylene. Throughout the test the level of BOD in the sample with musk xylene was identical to the sample without test compound. It was therefore concluded that musk xylene was not readily biodegradable under the test conditions (Calame and Ronchi, 1989).

Biodegradation of ^{14}C-musk xylene was also tested in another test with activated sludge (amount of inoculum not given). Concentrations of 10 and 100 µg/l (in triplicate) musk xylene were tested in a 28-d test. $^{14}CO_2$ was trapped and analyzed by LSC. The amount of trapped $^{14}CO_2$ was comparable to flasks in which HCl was added to kill the micro-organisms. It was concluded that musk xylene was not biodegradable under the tested conditions (Marks and Marks, 1987).

Simonich et al. (1998) measured fragrance material removal during activated sludge and trickling filter sewage treatment. From influent and effluent measurements they calculated a total removal of 98.7% for musk xylene. Simonich et al. (2000) and Sabaliunas et al. (2001) confirmed that the removal musk xylene within a STP is high i.e. app. 95%. The calculated removal is (again) based on influent and effluent measurements within both an activated sludge and trickling filter sewage treatment plant. This high removal rate indicates that besides adsorption also a biotransformation route (or routes) may be present (see Section 3.1.1.2). A plausible explanation for this could be that during an anaerobic phase of the sewage treatment a reduction of one or more of the nitro groups occur (expert judgement RIVM). Recently, Gatermann et al. (1998) and Rimkus et al. (sub.) presented measurements in influent and effluent of STPs, surface waters and biota for metabolites of musk xylene assuming that nitro musks will be transformed to the corresponding amino compounds. They analysed and detected the 2-amino and 4-amino metabolites (chemical names: 1-tert-butyl-3,5-dimethyl-2-amino-4,6-dinitrobenzene and 1-tert-butyl-3,5-dimethyl-4-amino-2,6-dinitrobenzene), but were unable to detect diamino-musk xylene. Herren and Berset (2000) also detected amino metabolites of musk xylene in STP water. These data support in a qualitative way the findings of Simonich et al. (1998 and 2000) and Sabaliunas et al. (2001).

Reduction of the nitro group is a well known transformation route for nitroaromatic compounds (Higson, 1992). It has for example been shown for the related chemical structure 2,4,6-trinitrotoluene (TNT), those white rot fungi or ectomycorrhizal basidiomycetes can degrade TNT (Gorontzy et al., 1994; Meharg et al., 1997). For musk xylene no such experimental data are available. However, the measurements described above show that reduction of nitro groups occurs for musk xylene in sewage treatment plants and fish.

Subsequently, for the environmental risk assessment:

- the results of the measurements of these metabolites in water and fish will be described in sections 3.1.2.4 and 3.1.5.1, respectively;
- ecotoxicological data have been looked for in view of a risk characterisation for the detected substances;
- based on the test results from Calame and Ronchi (1989) and Marks and Marks (1987) a biodegradation rate constant of 0 hr^{-1} could be assumed as musk xylene is not readily biodegradable. The use of the BIOWIN model (TGD, 2002) for estimating aerobic biodegradability also points to the lack of biodegradation of musk xylene. However, the amino reaction products have been measured in substantial amounts in effluents showing that primary degradation of musk xylene occurs in an STP (see Section 3.1.2.4). In principle the measured 2-amino and 4-amino metabolites in effluent can also be degradation products of other substances than musk xylene, but this is not likely to be the case. However, as the formation of these metabolites has not yet been shown in laboratory experiments and there are no quantitative data on biodegradation kinetics, the PECs for musk xylene will be calculated assuming a biodegradation rate constant of 0 hr^{-1}. It is realised that this is a conservative approach for the aquatic exposure assessment (see next section).

3.1.1.2 Distribution

Using a vapour pressure of $0.03 \cdot 10^{-3}$ Pa and a water solubility of 0.15 mg/l a Henry's law constant of 0.0595 $Pa.m^3/mol$ are calculated.

Using the measured log K_{ow} of 4.9 a log Koc of $1.17 \cdot 10^4$ L/kg can be estimated using the equation for predominantly hydrophobics[5] from the TGD:

$Koc = 1.26 \cdot K_{ow}^{0.81}$ **(1)**

This results in the following partition coefficients:

- $K_{soil\text{-}water}$: 352 m^3/m^3;
- $K_{susp\text{-}water}$: 294 m^3/m^3;
- $K_{sed\text{-}water}$: 294 m^3/m^3.

The calculated solids-water partition coefficient for suspended matter is 1,170 l/kg (organic carbon content: 10%).

No experimental data are available on the partitioning of musk xylene between water and soil, sediment or sludge. On the other hand Winkler et al. (1998) determined partition coefficients between water and suspended matter collected from the river Elbe during a summer flood (background concentration not reported although in earlier measurements musk xylene could not be determined). In an experiment of desorption 25 mg suspended matter (organic carbon content: 7.4%), spiked to a concentration of circa 10 mg/kg, was vigorously shaken with 1 litre distilled water for 48 hours. The partition coefficient from this laboratory experiment was 16,300 l/kg. For a number of compounds Winkler et al. presented in their study also field experimental Kp values. For musk xylene no field data are available. The difference between the laboratory Kp and the corresponding mean field Kp for the other compounds in the Winkler study was found to be around a factor 10. Applying the factor of 10 (see above) on the laboratory Kp of 16,300 l/kg would result in a 'theoretical field' value of 1,630 l/kg. This value is more or less equal to the default value of 1,170 l/kg. The default value will be used in the environmental risk assessment.

EUSES (SimpleTreat) estimates the following default distribution for musk xylene in a STP: air: 0%, water: 43% and sludge: 57%. The results of Simonich et al. (1998 and 2000) and Sabaliunas et al. (2001) indicated that the musk xylene removal within a STP can be very high i.e. 95-98% (see Section 3.1.1.1). As these data do not allow making a clear, quantitative distinction between sorption to sludge and (bio)degradation, the default STP distribution will be used in the present RAR. This implies that the aquatic emission load of musk xylene may be overestimated, whereas the load to sludge may be underestimated.

Bioaccumulation

The BCF can be calculated using the QSAR mentioned in the TGD: log BCF(wet weight) = $0.85 \cdot \log K_{ow}$ - 0.70. Using a log K_{ow} of 4.9 a BCF of 2900 L/kg is obtained.

In addition to the calculated BCF a number of experimental data are available for musk xylene. These bioaccumulation studies, including their technical shortcomings, are discussed below.

Musk xylene was tested in bluegill sunfish (*Lepomis macrochirus*) (Paradice and Suprenant, 1984). Details of this test are given in **Table 3.1.** The radioactivity was determined in edible and non-edible portions of the fish, and a whole body concentration was calculated. The whole body concentration in fish was stable between 7 and 16 days of exposure, while the

[5] An alternative option would be to use the QSAR for nitrobenzenes for calculating the Koc from the Kow. Owing to the chemical structure of musk xylene this QSAR may be more accurate than the general one for hydrophobics. The log Kow for musk xylene, however, falls outside the domain for this QSAR.

concentration in water showed some fluctuation. The uptake rate constant has not been calculated from the concentration in fish due to the rapid stabilisation of the concentration. The log-transformed elimination shows a slightly bent curve. However, the correlation coefficient is sufficiently high to assume first order elimination. Depuration half-lives were approximately 2.5 days. The elimination rate constants correspond nicely for the two tested concentrations. The bioconcentration factor has been derived from C_{fish} /C_{water}, with C_{fish} determined between day 3 to 16, and C_{water} as the overall mean. There was no attempt to identify whether the radiolabel was parent compound or metabolite. In the test a solubiliser (DMF, Tween) was used to prepare the stock solution, but the test-concentrations were well below the water solubility. The test was carried out using a radiolabel, without identification of the parent compound in the fish. In water the parent compound is identified by HPLC for musk xylene. It should be realised that the BCF based on parent material will be lower than the current value of 1,600 l/kg.

Table 3.1 Bioconcentration of musk xylene (low and high refer to high and low dose of 0.98 and 13 µg/l, respectively) (Paradice and Suprenant, 1984).

^{14}C-radiolabel identified	No
species	bluegill sunfish
low dose [µg/l]	0.98 ± 0.26
high dose [µg/l]	13 ± 11
period of exposure [d]	16
period of elimination [d]	12
uptake rate constant [l/kg/day]	not determined
elimination rate constant [d^{-1}]	0.26 (low), r^2=0.84
	0.29 (high), r^2=0.98
$t^{1/2}$ elimination [d^{-1}]	2.7[a] (low); 2.4[a] (high)
bioconcentration factor (whole fish, wet weight) [l/kg]	1,600[b]

a Recalculated from the original data;
b Based on radio labelled residue in fish.

In Yamagishi et al. (1983) a mean concentration ratio between fish-muscle and water of 4,100 l/kg for musk xylene is reported. These values were obtained by dividing the concentration in fish by the concentration in water in the environment. The reliability of bioconcentration factors obtained from actual concentrations measured in the environment is questionable, since it is unknown whether a steady state has occurred. (Industry recalculated the BCF with the original data of Yamagishi et al. (Industry e-mail dated 2 May 2002). Their conclusion was that the BCF of 4,100 l/kg could not exactly be reproduced (median value of 2,778 and average value of 3,146).

Geyer et al. (1994) cites a reference (MITI, 1992) describing that in carp with 3.4% lipid contents, the BCF was between 640-5,820 l/kg ww when exposed to 10 µg/l for 10 weeks and between 1,440-6,740 l/kg ww when exposed to 1 µg/l. The test was carried out in a flow through system and analytics were performed based on the parent compound. The test was carried out according to "305C. Bioaccumulation: Degree of Bioconcentration in Fish, stipulated in the OECD Guidelines for testing of Chemicals (May 12, 1981)". There is no information whether a steady state was reached in the test and, additionally, the relatively large variability in BCF values was not discussed. A recent study by Boleas et al. (1996) on the bioaccumulation of musk xylene in rainbow trout (44 g) was carried out under semi-static conditions with daily water renewal. Musk xylene was solved in ethanol and concentrations

were 1, 10 and 100 µg/l. The plateau level was reached within a week and bioconcentration factors were between 10 and 60 l/kg for the edible portion. The analytical method used to measure musk xylene in the fish samples was the one as described by Fernandez et al. (1996). In this method the fat containing extract of the fish samples is injected directly - without further clean-up - into the GC/MS.

The study of Boleas et al. (1996) has been criticised by Rimkus et al. (1997):

- as no clean-up procedure is used after some injections the injector, column and the MS system including the detector will be contaminated;
- a static system instead of a flow-through system was used;
- water concentrations were not measured, but calculated from the dilution of the added stock solutions;
- relatively high concentrations in control fish up to 10 µg/kg fw were measured. This may be due to contamination in the laboratory during the fish experiment or analytical procedures. Helbling et al. (1994) has shown that organic solvents, paper tissues, rubber gloves and the hands of laboratory analysts can be potential sources of laboratory contamination with musk xylene.

These arguments are considered convincing reasons to reject the BCF values obtained by Boleas et al. (1996).

Rimkus et al. (1997) report a study of Kuhlmann et al. (in press) in which rainbow trouts were exposed for several months to average water concentrations of 22.5 ng/l. The BCF was estimated at 4,400 l/kg ww. Further calculations on the same data resulted in BCF values of 4,200-5,100 l/kg ww and 115,000-122,000 l/kg on the basis of fat. No musk xylene could be detected in fish after 140 days in tests with spiked feed using concentrations of 1 and 10 µg/kg feed. No further details were presented in Rimkus et al. (1997). Information on the lipid content of the fish during the study and the stability of the very low test concentration during the test phase is for example not available. According to one of the principal co-authors, Dr. Rimkus, the study meets all requirements, including the analytics, for a reliable estimate of the BCF of musk xylene in fish (pers. comm. Dr. Rimkus).

Conclusion BCF fish

The experimental bioaccumulation studies for musk xylene showed a number of uncertainties (see above). However, based on a weight of evidence approach, with a number of studies (MITI, Kuhlmann and Yamagishi) pointing at BCF values around 4,000 to 5,000 l/kg, and taking into account the calculated BCF of 2,900 l/kg, it is proposed to use the value of 4,400 l/kg of the Kuhlmann study in the current risk assessment on musk xylene.

Accumulation in earthworms

No experimental data are available on accumulation in earthworms. Therefore, the BCF is estimated according to the following QSARs given in the TGD:

$BCF_{earthworm} = 0.84 + 0.012\ K_{ow} / RHO_{earthworm}$
where for $RHO_{earthworm}$ by default a value of 1 ($kg_{wwt}.L^{-1}$) can be assumed.
The formula for the $BCF_{earthworm}$ in $kg_{soil}/\ kg_{worm}$ then becomes:
$(0.84 + 0.012\ K_{ow} \cdot RHO_{soil})/(K_{soil\text{-}water} \cdot CONV_{water})$

Using a log K_{ow} of 4.9 gives a BCF_{worm} of 4.6 kg/kg.

3.1.2 Aquatic compartment

3.1.2.1 Emission during production, fragrance compounding and end product formulation

Production

There is no production of musk xylene in the EU.

Fragrance compounding

Emissions of musk xylene in fragrance compounding facilities depend on the standard operating procedures of these facilities. Fragrance compounding should be regarded as the first formulation step of musk xylene. Several emission scenarios can be distinguished:

1. Blending vessels and other equipment, in which the substance was stored, are cleaned with an organic solvent, which is collected and disposed off by incineration. Emission to waste water does not occur in this case.
2. Blending vessels are washed with steam and/or water and trace amounts of musk xylene present in the remaining fragrance oils are discharged with the wastewater.

PECs are calculated for sites 1 to 6. Site-specific emission data are submitted for these sites. As they cover approximately 70% of the total EU compounding tonnage, the exposure assessment for this life cycle stage is assumed to be represented sufficiently. Therefore no additional generic scenario is carried out. (Note: The exposure assessment furthermore shows that the emissions from this life cycle stage are very minor compared to those from end use formulation and private use. Furthermore the PEC/PNEC ratios for compounding sites were all shown to be far below 1 (Section 3.3 risk characterisation).

Site 1

The tonnage per year for fragrance compounding is 12 tonnes/year. The estimated weight percent loss to wastewater due to washing procedures is 0.01%. The number of emission days per year is unknown. According to the TGD this can be calculated from the fraction of the main source and the tonnage. In order to avoid unrealistic values for musk xylene the tonnage has to be corrected by multiplying the tonnage with the tonnage divided by the percentage of musk xylene in a formulation. According to EFFA (1997) this is 3.5%, being equal to the 50^{th} percentile of thousands of fragrance formulations. The corrected tonnage is: $100 / 3.5 \cdot 12 = 342$ tonnes/year. The number of emission days is: $0.6^{6} \cdot 342 = 206$ days (see **Table B2.1** of Appendix I of the TGD). The release for the emission episode is: $0.0001 \cdot 12 / 205 = 0.6 \cdot 10^{-5}$ ton/day = $0.6 \cdot 10^{-2}$ kg/day = 6 g/day.

A pre-treatment facility treats the on-site wastewater consisting of sedimentation, oil-skimming and pH control by neutralisation. The removal in the pre-treatment facility of this plant is estimated to be between 49 and 73%. These percentages are based on the removal efficiency of TOC (and COD) in the local pre-treatment facility.

6 Although the fraction of the main source should in fact be 1, a value of 0.6 is used for the calculation of the number of emission days (default). The formula f*T used in **Table 2.1** of Appendix I of the TGD should only be regarded as a 'calculation rule' to estimate the number of emission days. Using a fraction of main source of 1 in this formula would have resulted in an unrealistic number of emission days (342). The value of 0.6 is used also in the calculations for the other site/companies (site 2 and 4).

The effluent from the pre-treatment facility goes to a municipal STP in which a removal rate of 57% (TGD) is assumed. The remaining load of musk xylene after these two steps is (0.27 to 0.51) · 0.43 = 12 to 22% of the amount originally emitted. This means that 12 to 22% of 6 g/day is 0.7 to 1.3 g/day is finally emitted to surface water. The effluent flow of the STP is 60,000 m^3/day, so the concentration in the effluent is: PEC_{STP}: 0.01-0.02 µg/l.

As the dilution factor for this site is 1 the C_{local} in water, calculated according to the TGD, becomes 0.011 to 0.021 µg/l.
Adding the $PEC_{regional}$, calculated in section 3.1.2.3 gives a $PEClocal_{water}$ (dissolved) = 0.011/0.021 + 0.18 = 0.19/0.20 (rounded off) µg/l.

Subsequently, based on the equilibrium partitioning theory, the $PEClocal_{sed}$ = 0.13 mg/kg dw .

Site 2

The tonnage per year for fragrance compounding is 4 ton/year. The weight percent loss to wastewater due to washing procedures is 0.01%. The number of emission days per year is calculated in the same manner as for site 1: the corrected tonnage is: 100 / 3.5 · 4 = 114 tonnes/year. The number of emission days is: 0.6 · 114 = 69 days (see Table B2.1 of Appendix I of the TGD). The release for the emission episode is: 0.0001 · 4 / 68 = 5.8 g/day.

A pre-treatment facility treats the on-site wastewater consisting of flocculation, coagulation, filtration and pH control by neutralisation. Based on the measured removal percentage of oil and assuming that all musk substances are in the oil, the removal percentage for musk xylene for that particular treatment facility is > 99%. This site does not (yet) have an STP which means that <1% of musk xylene is emitted to water, that is <0.01·5.8 = < 0.058 g/day. The flow rate of the river is 4.3 · 10^6 m^3/day. The C_{local} water for site 2 then becomes 0.013 ng/l.

Adding the $PECregional_{water}$ calculated in paragraph 3.1.2.3 this gives a $PEClocal_{water}$ (dissolved) = 0.000013 + 0.18 = 0.18 µg/l.

Subsequently, based on the equilibrium partitioning theory, the $PEClocal_{sed}$ = 0.12 mg/kg dw.

Site 3

This site stated to have no emissions to water. Compounding tanks are rinsed with solvent and the rinsings are recycled by using in cheap perfumes. Aqueous residues derived from washing operations in the compounding area are drummed and disposed of by an authorised waste disposal company.

Site 4

The tonnage per year for fragrance compounding is 6.5 ton/year. The weight percent loss to wastewater due to washing procedures is 0.05%. The number of emission days per year is calculated in the same manner as for site 1: the corrected tonnage is: 100 / 3.5 · 6.5 = 186 tonnes/year. The number of emission days is: 0.6 · 186 = 111 days (see Table B2.1 of Appendix I of the TGD). The release for the emission episode is: 0.0005 · 6.5 / 111 = 29 g/day.

A pre-treatment facility treats the wastewater consisting of sedimentation and oil-skimming. As the removal percentage in this facility is unknown the lowest value from the other sites is taken, i.e. 49% (site 1). Effluent from the pre-treatment facility goes to a municipal STP with a flow rate of 2,700 m^3/day. The removal percentage in the STP is assumed to be 57%

(default) which means that the total load passing both treatment steps is 0.51 · 0.43 · 29 g/day = 6.4 g/day. With an STP flow rate of 40,000 m^3/day, the effluent concentration of the STP becomes 6.4/40,000 = 0.16 µg/l (PEC STP). The C_{local} is calculated according to the TGD with a default dilution factor of 10, resulting in a value of 0.016 µg/l.

Adding the $PECregional_{water}$ calculated in Section 3.1.2.3 this gives a $PEClocal_{water}$ (dissolved) = 0.016 + 0.18 = 0.19 µg/l.

Subsequently, based on the equilibrium partitioning theory, the $PEClocal_{sed}$ = 0.13 mg/kg dw.

Site 5

The tonnage per year for fragrance compounding is 16 ton/year. The use of musk xylene is evenly distributed over all working days (250, actual figure) and the weight percent loss to wastewater is assumed to be 0.2%. This is a rather worst-case approach for the aquatic compartment as the figure of 0.2% refers to overall emissions during the entire process. These total emissions may also include emissions to air. The exact split-up between water and air is unknown. The release for the emission episode is: 0.002 · 16 / 250 = 128 g/day. A pre-treatment facility treats the wastewater consisting of sedimentation, oil-skimming, pH-neutralisation, coagulation, flocculation and filtration. On average the percentage of oil separation is roughly 90% which is assumed to be equal to the removal percentage of musk xylene. Effluent from the pre-treatment facility goes to a municipal STP with a flow rate of 2,700 m3/day. The removal percentage in the STP is assumed to be 57% (default) which means that the total load passing both treatment steps is 0.1 · 0.43 · 128 g/day = 5.5 g/day. The effluent concentration of the STP thus becomes 5.5/2700 = 2 µg/l. The excess sludge of the biological treatment is delivered to a factory where it is digested. The residue of the digestor is incinerated. Therefore none of the sludge is used on agricultural soils.

The effluent is discharged into a river with a flow of 9.5 · 10^9 l /day. The resulting dilution factor is (9.5 · 10^9 + 2.7 · 10^6) / 2.7 · 10^6 = 3,520, leading to a $Clocal_{water}$ for the emission episode of: 0.57 ng/l. Adding the $PECregional_{water}$ calculated in Section 3.1.2.3 this gives a $PEClocal_{water}$ (dissolved) = 0.00057 + 0.18 = 0.18 µg/l.

Subsequently, based on the equilibrium partitioning theory, the $PEClocal_{sed}$ = 0.12 mg/kg dw.

Site 6

The tonnage per year for fragrance compounding is 0.65 ton/year. The use of musk xylene is evenly distributed over all working days (250, actual figure) and the weight percent loss to wastewater is assumed to be 0.2%. This is a rather worst-case approach for the aquatic compartment as the figure of 0.2% refers to overall emissions during the entire process. These total emissions may also include emissions to air. The exact split-up between water and air is unknown. The release for the emission episode is: 0.002 · 0.65 / 250 = 5.2 g/day. The daily wastewater flow of the STP is 1.4 · 10^9 litres, resulting in a concentration of 5.2 / 1.4 · 10^9 = 3.7 ng/l. Removal is assumed to be 57% (default). The resulting effluent concentration is 0.43 · 3.7 = 1.6 ng/l. The effluent is discharged into a river with a flow of 7.8 · 10^9 l/day. The resulting dilution factor is (7.8 · 10^9 + 1.4 · 10^9) / 1.4 · 10^9 = 6.6, leading to a $Clocal_{water}$ for the emission episode of: 0.241 ng/l (dissolved concentration). Adding the $PECregional_{water}$ calculated in paragraph 3.1.2.3 this gives a $PEClocal_{water}$ (dissolved) = 0.000241 + 0.18 = 0.18 µg/l.

Subsequently, based on the equilibrium partitioning theory, the $PEClocal_{sed}$ = 0.12 mg/kg dw.

End product formulation

Fragrance compounding (first formulation step) is followed by the formulation of musk xylene in end products (cosmetics, detergents, fabric softeners etc). Industry submitted some general statements that major detergents companies are not using nitromusks any longer. Information on smaller sites that are still using nitromusks, either in detergent or cosmetics, was recently submitted by industry. In addition, the TGD contains an emission scenario document (ESD) "Assessment of the environmental release of soaps, fabric washing, dish cleaning and surface cleaning substances". This scenario document[7] comprises Personal/domestic use (no.5) and Public domain (no 6) and use category Cleaning/washing agent (no. 9) and cosmetics (no.15). According to the ESD the emission factor "washing liquid"[8] for wastewater is 0.0009 and 0.00002 for air (Table 2). The site-specific emission factor to wastewater for a cleaning agent formulating company (a smaller one) amounts to 0.002, which is about a factor of 2 higher than the ESD value for water. The loss to air for this site is stated to be minimal. Site-specific data for larger formulators point to emission factors that are two to three times lower than the ESD value for water emissions. As the formulation of nitromusks is expected to take place nowadays mainly at the relatively smaller sites, the site-specific emission factor of 0.002 (water) will be used in the present risk assessment. For air the ESD default of 0.00002 is taken.

Neither the fraction of main source nor the number of days is given in the emission scenario document. Therefore defaults could be derived from the B-tables (TGD). The fraction of main source is 0.4 (Table B2.1) and the number of emission days are 300[9]. For obtaining the former data from the B-table the tonnage of formulated end product should be calculated. Therefore, data on the percentage of musk xylene in compounded fragrances and end product is needed. In the first formulation step, 3.5% musk xylene is used in fragrance compounds (EFFA, 1997). The second formulation step, 0.02% (see Section 4.1.1.3) musk xylene was selected for the formulated end products (household detergents). It should be noted that a higher percentage of musk xylene in cosmetics (0.02-0.59%) and air fresheners (1%) could be selected. However, the outcome of the calculation for the number of days and fraction of main source remains unchanged. On top of that, for air fresheners no use category is available within the TGD. The number of emission days (300) can be overruled in the current exposure assessment, however, by a site-specific value (250 days) for the specific smaller formulating company. The dult fraction of main source of 0.4, literally meaning that 40% of the formulation in the region may take place at one site, may be considered as over-conservative in combination with the site-specific emission factor for the smaller formulators. This because industry submitted information about the number (n=225) of musk xylene formulating sites (soaps, detergents, cosmetics) in Europe. A further (realistic) assumption is that the geographical locations of the formulators are more or less equally spread over the EU, justifying the use of the 10% rule. Applying the 10% rule for calculating a 'theoretical'

[7] The emission scenario document does not include air fresheners and/or odour agents.

[8] Other release factors (**Table 2** of ESD in TGD) are available for regular washing powders and compact powder. The column of "washing liquid" was selected because fragrances are complex mixtures which are nearly always liquids, in which musk xylene has to be dissolved (see Section 2.2). The category washing liquid also represents a worst-case approach concerning the % emission to water (factor 9 higher than the other two categories).

[9] Calculation of tonnage end product which will be used as input (B-tables) for the derivation of the number of emission days and fraction of main source: Musk xylene is present at 3.5% in fragrance compounds and 0.02% in formulated end products (households detergents). 67 tonnes in the EU gives 1,914 tonnes of fragrance compounds (100/3.5 · 67 = 1,914 tonnes) and 122,850,00 tonnes of formulated end product (100/0.02 · 1,914 = 9,570,000 tonnes). Applying the 10% rule this leads to a tonnage of 957,000 tonnes at regional scale. This tonnage of 957,000 is used as input for the B-tables (B2.1).

regional volume a factor of 0.04 (1/(225/10) = 1/22.5) should therefore be seen as a realistic average alternative for the fraction of main source of 0.4 for the smaller sites. For the larger sites, i.e. 'large' among the smaller ones, a factor of $1/22.5 \cdot 5 = 0.2$ may hold, where the additional factor 5 is a rule of thumb that is earlier agreed upon by the TM for compensating for differences in size of production/processing sites. In the current RA both factors, i.e. 0.04 and 0.2 will be used for comparison purposes. It should be borne in mind, however, that the combination of the higher 'fraction of main source' factor of 0.2 with the high site-specific emission of 0.002 factor (small sites) may be too conservative. Actual data on use volumes per sites are lacking, however, to verify this statement, but this (possibly) over-conservative aspect will be referred to in the risk characterisation.

The total volume of musk xylene used end product formulation in Europe for 2000 is assumed to be 67 tonnes (see Section 2.2). Applying the 10% rule leads to a local emission to wastewater of $(0.04\text{-}0.2 \cdot 0.002 \cdot 0.10 \cdot 67$ tonnes/year)/250 days = 0.0021-0.01 kg/day. Using a default sewage flow of 200 l/eq/day gives a concentration in untreated wastewater of $1.07\text{-}5 \cdot 10^{-3}$ mg/l. The fraction (default) of musk xylene in the STP directed to air, water and sludge is $4.6 \cdot 10^{-4}$, 0.43 and 0.57, respectively. These defaults will be used for the assessment (see Section 3.1.1.2). The resulting effluent concentration is $0.43 \cdot 1.07\text{-}5 \cdot 10^{-3}$ = 0.46-2.3 µg/l using a dilution factor of 10 the $Clocal_{water}$ is 0.046-0.23 µg/l (dissolved). Adding the $PECregional_{water}$ (dissolved) = 0.046-0.23 + 0.18 = 0.22-0.41 µg/l.

Subsequently, based on the equilibrium partitioning theory, the $PEClocal_{sed}$ = 0.06-0.1 mg/kg ww.

3.1.2.2 Local emissions from private use

After use of the fragranced consumer products mentioned in Section 2.2 (cosmetics, detergents, fabric softeners etc.) most of the musk xylene will be emitted with the wastewater of households. Now it is assumed that the total volume of musk xylene used in compounding fragrances in Europe for 2000, i.e. 67 tonnes, is released to wastewater going to an STP. This 100% release is a rather worst-case assumption that is due to the fact that the split-up between the use of musk xylene in detergents and in cosmetics is unknown at present. Now the entire chemical is attributed to use in detergents, whereas the use of cosmetics is known to have (somewhat) lower default aquatic emission factors than detergents. In reality the release can also be lower for reasons given already in Section 2.2 (a.o. export outside EU) and additionally because some musk xylene will probably remain on the fabric. For the latter factor no quantitative data are available.

The 10% rule will be used for estimating the regional use volume from the continental use volume (TGD). This results in a regional volume of 67/10 = 6.7 tonnes/year. It may be argued that this approach does not sufficiently take into account that differences in use of fragrance products may occur between EU regions. In fact this is known to be the case for cosmetics and detergents (COLIPA, 2001 and HERA, 2002). In some EU countries, in particular Southern European countries, the use of these products is higher than in Northern Europe. The difference between the country with the highest use, i.e. Italy, and the European average, amounts to a factor of 1.9. From the total EU use volume, a *per capita* amount of $67/370 \cdot 10^6$ (number of EU citizens) = 0.18 g/year can be calculated. For a theoretical EU region with 20 million inhabitants this would lead to a regional use volume of 3.6 tonnes/year ($20 \cdot 10^6 \cdot$ 0.18 g/year). Multiplying this average EU region with a factor of 1.9 (see above) results in a volume of 6.8 tonnes/year for a Southern European region. As this volume of 6.8 tonnes/year

equals the volume calculated with the 10% rule (6.7 tonnes/year) the followed approach in the present risk assessment covers a conservative, 'high use' region.

According to the TGD a fraction of 0.002 is emitted to the main local source. Applying the 10% rule leads to a local emission to wastewater of (0.002 · 67 · 0.10 tonnes/year) / 365 days = 0.037 kg/day. Using a sewage flow of 200 l/eq[7]/day gives a concentration in untreated wastewater of 0.018 mg/l. The fraction of musk xylene in the STP directed to air, water and sludge is $4.6 \cdot 10^{-4}$, 0.43 and 0.57, respectively. The resulting effluent concentration is 0.43 · 0.018 = 7.8 µg/l using a dilution factor of 10 the $Clocal_{water}$ is 0.8 µg/l (dissolved). Adding the $PECregional_{water}$ calculated in Section 3.1.2.3 this gives a $PEClocal_{water}$ (dissolved) = 0.8 + 0.18 = 0.98 µg/l.

Subsequently, based on the equilibrium partitioning theory, the $PEClocal_{sed}$ = 0.64 mg/kg dw. The rapporteur is aware that the current private use scenario for musk xylene is a rather worst-case scenario. This is because of the reasons already mentioned above, but also due to the fact that a relatively high fraction of the emissions to wastewater is directed to water (default).

3.1.2.3 Regional emissions

For calculating the PECs at the regional scale only the emissions due to private use are taken into account. At such scale emissions from compounding sites and end product formulation are negligible compared to those from private use. Assuming the whole EU tonnage of 67 tonnes/year to be released to water (rather worst-case scenario; see also private use scenario) results in a continental aquatic emission of 67/365= 184 kg/day. Applying the 10% rule the regional emission becomes 18.4 kg/day. In the EUSES the input for regional emissions is 18.4 kg/day and for continental emissions 166 kg/day (0.9 · 184). Assuming a split up of 30% discharge directly to surface water and 70% to an STP, results in emission values of 12.9 (indirect) and 5.5 kg/day (direct) for the regional scale and 116 (indirect) and 49.6 kg/day (direct) for continental emissions. EUSES calculates a PEC regional of 0.18 µg/l for surface water and 0.2 mg/kg dwt for sediment.

3.1.2.4 Monitoring data

In the last decade, the presence of musk xylene has been investigated in several environmental compartments. In Table 3.2 concentrations of musk xylene measured in wastewater, surface water and suspended matter are presented. Some details on these data are given below.

In a study by Eschke et al. (1994) influent and effluent concentrations of musk xylene were measured in 25 community sewage treatment plants along the River Ruhr in Germany. Influent concentrations were at the level of 0.09 to 1.7 µg/l and the median effluent level was 0.12 µg/l using the median influent and effluent concentrations the removal is 82%. Surface water concentrations in the Ruhr were generally at a level of 0.01 µg/l. Based on these figures, the mean dilution factor for effluents in the Ruhr seems to be approximately 10.

Lower influent levels of musk xylene were found in Southern Germany in 1992, where a hospital using musk xylene containing detergents produced wastewater with 0.053 µg/l musk xylene. The effluent of the STP contained 0.022 µg/l. From these data a percentage removal of 58% can be estimated which fits well the estimated value using EUSES. At an earlier date

(1991) the peak measured concentration in the River Lauchert at the point of discharge was 39 ng/l. Upstream and downstream of the STP, river water concentrations were at a level of <1 and 1 to 3 ng/l, respectively (Hahn et al., 1993).

In Japan, in 1981, mean concentrations in effluent from three treatment plants along the river Tama were 0.035 µg/l with a median level in the River Tama of 0.004 µg/l. Highest concentrations both in effluents and in the river were within a factor of 2 of the mean. Concentrations were also measured in four tributaries which discharge into the river Tama without any treatment: the median value was 4 times higher than in the main river (Yamagishi et al., 1983).

Musk xylene was detected in the effluents of the three largest sewage treatment plants in Sweden in levels between 1 and 5 µg/l (Paxéus, 1996). In a Swiss river, which is influenced by the outlet of a STP, the concentration of musk xylene was 0.00062 µg/l (Müller et al., 1996).

Simonich et al. (2000) and Sabaliunas et al. (2001) recently analysed musk xylene and musk ketone in the influent and effluent of two different communal STPs within USA and UK, and in river water in Yorkshire (north of England). The measured STP effluent concentrations of musk xylene (UK and USA) showed concentrations between 0.005-0.031 µg/l. The river water concentrations for musk xylene immediately downstream of the effluent discharge were 0.007 µg/l. The river water concentration for musk xylene upstream of the effluent discharge was 0.002 µg/l.

In a study on the main rivers in The Netherlands musk xylene was neither detected at levels above the detection limit in the water samples (0.01 µg/l, 1994-1996) nor in most of the samples of suspended matter (1990-1996, detection limit 0.05 mg/kg) (Breukel and Balk, 1996). Only in three suspended matter samples of 1990 and 1994 in the river Meuse musk xylene was detected at levels of 0.06, 0.08 and 0.1 mg/kg. Based on an empirical relationship, the concentration in sediment is taken to be half of the concentration in suspended solids in the Netherlands resulting in sediment concentrations below 0.025 mg/kg based on a median of <0.05 mg/kg. The water analyses refer to filtered samples, contrary to the study in Germany (Eschke et al., 1994) where total concentrations in water were measured.

Samples were taken 10 metre below the water surface in the German Bight and in the eastern part of the North Sea north of The Netherlands near Denmark and Germany. Median (total) concentrations were near the detection limit of 0.03 ng/l. Relatively high levels of 0.8 ng/l were found near the River Elbe (Gatermann et al., 1995).

Gatermann et al. (1998) measured musk xylene and the possible transformation products 1-tert-butyl-3,5-dimethyl-2-amino-4,6-dinitrobenzene and 1-tert-butyl-3,5-dimethyl-4-amino-2,6-dinitrobenzene at three locations in the river Elbe. Concentrations of the 2-amino metabolite were <1 ng/l and for the 4-amino metabolite 2-9 ng/l (n = 1-2 for each location). From the STP Hamburg also a 24 hour composite influent sample and effluent sample was taken. Concentrations of the 2-amino metabolite were <0.5 ng/l, 10 ng/l and <0.5 ng/l (n = 1) for influent, effluent and river Elbe water, respectively. For the 4-amino metabolite these concentrations were <0.5 ng/l, 34 ng/l and 1-9 ng/l, respectively.

Sludge was sampled in six communal STPs in The Netherlands (Blok, 1998). These STPs can be considered as representative for the Dutch situation and were also used in an earlier monitoring study on surfactants (Feijtel and Van de Plassche , 1995). One of the STPs had no combined thickener or anaerobic digester, while another one had no anaerobic digester but a thickener with a retention time of several days. Also a compost facility treating digested

activated sludge from several STPs was sampled. Two grab samples were taken with an interval of one week. Recovery in a sample of digested sludge with a dry weight percentage of 2.8 was only 31%. In all 31 samples of primary, secondary or digested sludge musk xylene concentrations were lower than the reporting level of 1 mg/kg. The authors considered concentrations in sludge below 1 mg/kg dw irrelevant and did not determine detection levels.

Herren and Berset (2000) reported musk xylene and musk xylene metabolite levels in (communal) sewage sludge samples from different catchment areas in Switzerland. Musk xylene was detected in just one sludge at a concentration of 32.5 µg/kg dwt. The metabolite amino musk xylene was found in much more sludge samples and also at higher levels (1-50 µg/kg dwt). Further specification of the metabolite (location of amino-group) could not be given due to the technique that was used (mass spectrometry).

Table 3.2 Monitoring results of musk xylene in the aquatic environment.

Sample		N	concentration (µg/l)	Reference
Effluent		18	median: 0.035 (0.025-0.036)	Yamagishi et al., 1983
Tama River, Japan		18	median: 0.0035 (0.0017-0.023)	
wastewater:	influent	19	median: 0.68 (0.09-1.7)	Eschke et al., 1994
	effluent	36	median: 0.12 (0.03-0.31)	
River Ruhr, Germany		34	mean: 0.01 (0.01-0.03)	
Hamburg	influent	1	0.15	Gatermann et al., 1998
	effluent	1	0.010	
Elbe		5	<0.0005-0.002	Gatermann et al., 1998
Lauchert River		16	median: 0.002 (<0.001-0.039[a])	Hahn, 1993[c]
Local STP	influent	-[b]	0.053	
	effluent	-[b]	0.022	
Danube		1	1	
River Glatt, Switzerland		-[b]	0.00062	Müller et al., 1996
River Rhine, Netherlands		31	<0.01	Breukel and Balk, 1996
River Meuse, Netherlands		34	<0.01	
Elbe, Germany		31	not detectable (<0.002)	Winkler et al., 1998
Surface waters, Berlin		30	0.18 (1 of 30 samples)	Heberer, 1999
North Sea		30	median: 0.00003; max	Gatermann et al., 1995
			0.00017	
Sweden STP	effluent		1-5	Paxéus, 1996

Table 3.2 continued overleaf

Table 3.2 continued Monitoring results of musk xylene in the aquatic environment

Sample	N concentration (µg/l)	Reference
STP (communal), USA	-[d]	Simonich et al., 2000
Influent (AS)*	0.376	
Effluent	0.005	
STP (communal), USA	-[e]	
Influent (TF)*	0.339	
Effluent	0.031	
STP (communal), UK	-[d]	Sabaliunas et al., 2001
Influent (AS)*	0.22	
Effluent	0.01-0.02	
STP (communal), UK	-[e]	
Influent (TF)*	0.339	
Effluent	0.01-0.02	
Rivers in Yorkshire, UK	-[f] 0.002 (upstream)	
	0.007 (downstream)	
Suspended matter		
River Rhine, Netherlands	14 <0.05 mg/kg	Breukel and Balk, 1996
River Meuse, Netherlands	14 median: <0.05 mg/kg (<0.05-0.1)	
Sediment		
Elbe, Hamburg Germany	3 0.185-0.297 (µg/kg wwt)	Rimkus et al., 1999
Elbe , Wedel-Schulau Germ.	1 0.263 (µg/kg wwt)	
STP sludge (communal)		
Switzerland	n.d – 32.5 ug/kg dwt	Herren and Berset, 2000
Netherlands	31 < 1 mg/kg	Blok, 1998

a Value of 39 ng/l measured at point of discharge;
b Number of samples unknown.
c original values of Hahn (1993) were corrected from µg/l into ng/l according to Käfferlein et al., 1998.
d Samples were collected hourly over 3-day period from an Activated Sludge Wastewater Treatment. The samples were composited into three daily samples, based on plant flow.
e Samples (duplicate) were collected hourly over 3-day period from a Trickling Filter Wastewater Treatment. The samples were composited into three daily samples, based on plant flow.
f Triplicate grab river water samples were taken at distances 20 m., 0.5 km and 3.5 km downstream from the effluent discharge point. Another set of water samples was taken about 50 m. upstream from the wastewater plant discharge point.
* AS: Activated Sludge Wastewater Treatment; TF: Trickling Filter Wastewater Treatment.

3.1.2.5 Comparison of PECs with monitoring data

The following remarks should be made when comparing calculated with measured concentrations:

- with respect to private use measured as well as calculated concentrations are direct reflections of the use volume of musk xylene. However, these volumes vary from country to country and do vary in time. Since the use of musk xylene has decreased over the last

years[10], only recent measured concentrations can be compared with predicted concentrations based on the most recent use volumes;

- measured concentrations in Table 3.2 are sometimes total, sometimes dissolved concentrations. However, considering the height of the partition coefficient between water and suspended matter this does not seem to be an important factor: less than 10% will be sorbed to suspended solids.

Effluent

Concentrations have been determined by Eschke et al. (1994) in effluents from STPs along the Ruhr. The median value of 0.12 µg/l is about two orders of magnitude lower than the calculated local effluent concentration for private use of 7.8 µg/l. The measured concentrations by Gatermann et al. (1998) are even lower: 0.010 µg/l. On the other hand the data from Paxéus (1996) in Sweden, 1-5 µg/l, are fairly well in line with the estimated PEC effluent. The recent analysis of Simonich et al. (2000) and Sabaliunas et al. (2001) in the UK and USA showed measured (communal) STP effluent concentrations for musk xylene between 0.005-0.031 µg/l. These data also confirm that measured concentrations are much lower than the calculated local effluent concentration for private use (7.8 µg/l).

Surface water

Measured surface water concentrations vary from <0.0005 to 0.18 µg/l, being respectively a factor > 2,500 and 6 lower than the $PEClocal_{water}$ for private use and a factor > 360 and 1 lower than the $PECreg_{water}$. The concentration of 0.18 µg/l (Heberer, 1999) should be considered as an extreme value as it refers to water highly influenced by STP effluents. The representativity of this sampling point is discutable. Recently measured river water concentrations of musk xylene in the UK (Sabaliunas et al., 2001) vary from 0.002 to 0.007 µg/l, being respectively a factor > 500 and 140 lower than the calculated $PEClocal_{water}$ for private use (1 µg/l) and a factor > 115 and 30 lower than the $PECreg_{water}$ (0.18 µg/l).

Sediment

From the measured concentrations in suspended matter in the rivers Rhine and Meuse a concentration of less than 0.025 mg/kg dw has been calculated in Section 3.1.2.4. This is a factor 10 lower than the $PECreg_{sed}$. Sediment data from the Elbe river (Rimkus et al., 1999) of 0.185-0.296 µg/kg wwt are more than two orders of magnitude lower than the calculated PECs for sediment.

STP sludge

A sludge concentration of 26 mg/kg is calculated for the private use scenario. Measured data from communal (private use) STPs in Switzerland and the Netherlands were all (much) lower. The maximum measured concentration (Blok, 1998) of < 1 mg/kg is at least factor 30 lower than the calculated value.

Conclusion

As the amount of aquatic monitoring data for the EU is rather limited, the current risk assessment will predominantly be based on the calculated exposure data. However,

[10] In Germany the Soap and Detergents Association (IKW) advised in 1993 to avoid the use of musk xylene in detergents and other cleaning products.

monitoring data will be taken into account as well. Available monitoring data point to much lower concentrations than the calculated ones which may be due to a number of conservative assumptions in the calculations. On the other hand it may also be due to the fact that the available monitoring data come from countries (mostly NL, D, UK and Switzerland) of which it is known that active measures were taken in the recent past to reduce nitromusk usage. See also introduction of Section 3.3 Risk characterisation.

The PECs in soil and worm were calculated both with the calculated sludge concentration and the maximum measured value of 1 mg/kg (see Sections 3.1.4 and 3.1.5.1). Both values will be addressed in the corresponding risk characterisation sections.

3.1.3 Atmosphere

No site specific data are available on the emission of musk xylene to the atmosphere. In Section 3.1.2 it is mentioned that for sites 5 and 6 the total emission (i.e. water and air) during the process amounts to 0.2%. This site-specific figure is used for estimating the aquatic emissions and the rapporteur realises that using the TGD default of 0.0025 (see below and considered equal to the site-specific value of 0.002) for atmospheric emissions results in exceeding of the total emissions for both plants. The following generic local scenarios are used:

- fragrance compounding using the individual processing volumes (see Section 3.1.2.1). Emission factor (TGD default) is 0.0025;
- end product formulating using a regional volume of 6.7 (= 10% of 67 tonnes) tonnes/year (see Section 3.1.2.1). Emission factor (ESD in TGD) is 0.00002;
- private use using a regional volume of 6.7 (= 10% of 67 ton) ton/year (see Section 3.1.2.2). Emission factor (TGD default) is 0.

The regional scenario for the atmospheric compartment is based on a regional volume of 6.7 tonnes/year (10% of 67 tonnes).

Results are presented in **Table 3.3**.

Table 3.3 PECs for musk xylene in air.

	PEC (µg/m^3)
Site 1	$2.28.10^{-2}$
Site 2	$7.66.10^{-3}$
Site 3	$3.43.10^{-2}$
Site 4	$1.24.10^{-2}$
Site 5	$3.05.10^{-2}$
Site 6	$1.28.10^{-3}$
End product formulation	$4.3\text{-}5.9.10^{-5}$
private use	$4.41.10^{-5}$
Regional	$3.9.10^{-5}$

A measured value in outdoor air of 8-54 pg/m3 has been reported in Norway (Kallenborn et al., 1999). About 500 pg/m^3 were detected in indoor air. Further details are lacking, but the regional calculated PEC in air is more or less of the same order of magnitude.

3.1.4 Terrestrial compartment

The terrestrial compartment will be exposed to musk xylene due to deposition and application of sewage sludge on agricultural land. The following scenarios are used:

- fragrance compounding for the sites described in Section 3.1.2.1;
- end product formulation using a regional volume of 6.7 tonnes/year (see Section 3.1.2.1);
- private use using a regional volume of 6.7 tonnes/year (see Section 3.1.2.2).

Results are presented in **Table 3.4.** As mentioned in Section 3.1.2.5 PECs in soil were calculated both with the default value for the sludge concentration and the maximum measured value of 1 mg/kg. The alternative scenario, as is mentioned in **Table 3.4** and **Table** 3.5, is used for calculating the PEC_{soil} en $PEC_{oral,worm}$. The basis of this alternative scenario is a concentration in sewage sludge of 1 mg/kg_{dwt}[11]. This sludge concentration is only used for calculating the soil concentrations for the local private use scenario and the regional scenario. The latter because emissions from private use fully determine the regional scenario.

For the local private use scenario the concentration in sewage sludge of 1 mg/kg_{dwt} can directly be entered in EUSES. For the regional scenario this is not possible. Indirectly the sewage sludge concentration can be used in the EUSES program via the TGD equations 21 and 22 (TGD, 1996). With these equations and the known sewage sludge concentration regional and continental emissions to wastewater and surface water can be recalculated. These emissions are entered in the EUSES program for calculating the soil concentrations on a regional and continental scale in the alternative scenario.

Table 3.4 PECs for the terrestrial compartment.

	PEC in soil (mg/kg dw) Default scenario	PEC in soil (mg/kg dw) Alternative scenario (maximum sludge level of 1 mg/kg sludge
Site 1	0.0048-0.0054	0.0033-0.0039
Site 2	0.0025	0.00096
Site 3	0.0054	0.0040
Site 4	0.051	0.052
Site 5	0.005	0.0036
Site 6	0.0017	0.00033
End product formulation	0.02-0.1	0.02-0.1
private use	0.43	0.017
Regional (natural)	0.0015	0.000097
Regional (agricultural)	0.15	0.003

Measured concentrations in soils are not available.

[11] Based on measured data from communal (private use) STPs in the Netherlands.

3.1.5 Non compartment specific exposure relevant to the food chain

3.1.5.1 Calculation of PECs

The measured BCF for fish is 4,400 l/kg. The BCF for earthworms is estimated from the log K_{ow} applying relationships as presented in the TGD, resulting in a value of 4.6 kg/kg. The following scenarios are used:

- fragrance compounding for the sites described in Section 3.1.2.1;
- end product formulation using a regional volume of 6.7 tonnes/year (see Section 3.1.2.1);
- private use using a regional volume of 6.7 tonnes/year (see Section 3.1.2.2).

The revised TGD prescribes the use of a biomagnification factor (BMF) for the aquatic route. Musk xylene falls in the category ($logK_{ow}$ 4.5-<5 and BCF (fish) 2,000-5,000) where a BMF of 2 is applicable. This factor is used on the calculated $PEC_{oral, fish}$.

Results are presented in **Table 3.5**.

Table 3.5 PECs for fish and earthworms.

	$PEC_{oral, fish}$ (mg/kg ww)	$PEC_{oral, worm}$ (mg/kg ww) Default scenario	$PEC_{oral, worm}$ (mg/kg ww) Alternative scenario (maximum sludge level of 1 mg/kg)
Site 1	1.6	0.3	0.02
Site 2	1.6	0.3	0.01
Site 3	1.6	0.3	0.01
Site 4	1.6	0.4	0.1
Site 5	1.6	0.3	0.01
Site 6	1.6	0.3	0.01
End product formulation	1.7-2.2	0.4-0.5	0.05-0.2
Private use	5	1.2	0.04

3.1.5.2 Monitoring data

Concentrations found in fish and shellfish are summarised in **Table 3.6**.

Median concentrations in fish from natural waters in Germany were 0.07 mg/kg fat (Rimkus and Wolf, 1993) and 0.6 mg/kg fat (Eschke et al., 1994) with a highest level of 1.0 mg/kg fat (Eschke et al., 1994). The median level found in carp in the Tama River in Japan was slightly below the levels found by Eschke et al. (1994) in the Ruhr. The same is true for the median surface water concentrations in both rivers. Levels for shellfish were in the same range as for fish (Yamagishi et al., 1983).

Wiertz (1995) measured concentrations in yellow eel in the Netherlands in lakes and rivers and in cod livers in the Southern North Sea. The median concentration in cod livers was <0.004 mg/kg wet weight. The median concentration in yellow eel in rivers and lakes was 0.13 mg/kg fat. Concentrations were lower in lakes than in rivers like Rhine and Meuse, e.g. <0.02 mg/kg fat at two sample sites in the IJssel Lake. The highest concentration was measured in the river Rhine near Lobith: 0.43 mg/kg fat. None of the sample sites was influenced by local STPs (Tas and Van de Plassche, 1996).

In a second sampling program in 1996 the median level in eel was 0.069 mg/kg fat. On most locations concentrations were lower by a factor 2 or more. In freshwater pike-perch, mussels and shrimps from coastal areas and the North Sea, concentrations were below the detection limit (0.5 µg/kg fresh weight for fish with a relatively low fat content to 10-20 µg/kg fresh weight for fish with a high fat content) (De Boer and Wester, 1996). This was also the case for whiting, sole, mackerel, twaite and liver of codfish from the North Sea.

A RIVO research (1997) in the Netherlands showed musk xylene levels in fish ranging from < 3 to 22 µg/kg (fresh weight). Fish samples were from major Dutch rivers and other large fresh water bodies.

Rimkus et al. (sub.) measured musk xylene and their monoamino en diamino metabolites in fish in the rivers Elbe and Stör, in aquacultures in Denmark, Spain and Austria and in ponds of an STP. Mean concentrations of 1-tert-butyl-3,5-dimethyl-4-amino-4,6-dinitrobenzene were 54 (8-161), 109 (11-332), 74 (5-151) µg/kg fat in fish. In mussels in the STP pond the concentration was 152 µg/kg fat. For 1-tert-butyl-3,5-dimethyl-2-amino-2,6-dinitrobenzene these data were 7.3 (<3-14), 1.7 (<1-3), 9.2 (2-18) and 13 µg/kg fat. Fish sampled were pike-perch, pike and bream (Elbe and Stör; fat contents of 0.3-1.7%), trout (aquacultures; fat contents: 2.4-4.2%), tench, crucian carp and eel (ponds STP; fat contents: 0.8-1.4% for tench; 1.1-4.3% for crucian carp and 15.7-17.9% for eel). Fat content of mussels was 1.4%. Of the STP also influent and effluent concentrations were measured. Concentrations of musk xylene in influent and effluent were 2 and 1 ng/l, respectively. For the 4-amino metabolite these were 8 and 10 ng/l, respectively. In none of the fish, mussel and water samples the diamino metabolite was detected.

Fromme et al. (1999) measured levels of nitromusks in eel in the Berlin area. The mean values of musk xylene during the measurement periods 1995 and 1996 were, respectively, 24 and 12 µg/kg fresh wt. Maximum levels were 170 µg/kg fresh wt (1995) and 79 µg/kg fresh wt (1996).

In one laboratory, contamination of the samples was suspected (Yurawecz and Puma, 1983). Analysis of soaps and hand lotions used in the laboratory showed that musk xylene was present in soap and one hand lotion. The authors did not identify the source of musk xylene detected in their analysis of fish samples, and therefore did not publish the concentrations.

Kallenborn et al. (2001) recently investigated synthetic musk levels in marine fish samples collected in the vicinity of densely populated areas in Norway. Sampling sites around Trondheim and Tromsø were selected close to municipal sewage treatment plants assuming that high levels would be found close to sewage treatment outlets. Possible primary industrial sources were covered by samples from the Oslofjord and Greenland fjord areas. The measured MX concentrations in 25 different fish samples ranged from < 5 to 178 µg/kg lipid. The highest MX level (178 µg/kg lipid) was found in haddock filet from the inner harbour of Trondheim. Haddock is a predator fish, so these data are representative for musk xylene levels in animals that are ‘high’ in the marine food chain.

Table 3.6 Monitoring data for musk xylene in aquatic biota.

Sample	N	Concentration (µg/kg)	Reference
Japan			Yamagishi et al., 1983
-carp	31	median: 15 (1.5-41) muscle ww	
		median: 2 (1.4-140) viscera ww	
-shellfish	9	median: 1.7-53 ww	
Germany			Rimkus and Wolf, 1993
-freshwater fish	26	median: 70 (10-350) fat	
-mussels	9	10-20 fat	
-trout (fish farm)	46	mean: 330 (90-1,060) fat	
Germany			Eschke et al., 1994
-fish (river Ruhr)	9	median: 6 (2-95) muscle ww	
		median: 530 (nd-1,000) fat	
-fish (effluent pond)	13	median: 51 (21-753) muscle, ww	
		median: 2,400 (1,800-3,600) fat	
Germany			Hahn, 1993
-trout (pond and	44	mean: 680 (340-1,800) fat	
Lauchert river)		mean: 26 (11-82) ww	
-trout (other ponds)	44	mean: 390 (300-780) fat	
		mean: 15 (<5-300) ww	
Germany			Rimkus and Wolf, 1995
-fish farm	50	mean: 20 (10-100) fat	
-fish (German rivers)	31	mean: 80 (<10-350) fat	
-shrimps	3	10 fat	
Germany			Rimkus et al. (sub)
-fish (German rivers)	7	mean: 49 (10-99) fat	
-fish (effluent pond)	11	mean: 295 (205-395) fat	
-mussels (effluent pond)	1	121 fat	
trout (fish farms 3 countries)	7	109 (11-332) fat	Rimkus et al. (sub)
The Netherlands			Wiertz, 1995
-eel	13	median: 30 (<4-80) ww	
		median: 130 (<20-430) fat	
The Netherlands			De Boer and Wester, 1996
-other fish	7	nd	
-shellfish	4	nd	
the Netherlands, large waters		<3-22 ww	RIVO, 1997
Berlin, Germany		mean: 24(1995) – 12(1996) ww	Fromme et al. 1999

Table 3.6 continued overleaf

Table 3.6 continued Monitoring data for musk xylene in aquatic biota

Sample	N	Concentration (μg/kg)	Reference
		max: 170(1995)-79(1996) ww	
Norway; harbour and fjord areas			
-fish	25	<5 – 178 ww fat	Kallenborn et al, 2001
Germany, baden Wurtenberg area			
-fish	17	0 – 248	Janda et al. 2000
Italy (Lago Verbano)			
-fish	270	med. 0.8 (0.5-1.1) ww	Ceschi et al. 1996

3.1.5.3 Comparison of PECs with monitoring data

As only measured concentrations for fish are available, only the $PECoral_{fish}$ can be compared with monitoring data. On a wet weight basis the calculated concentration is 2.5 mg/kg for private use (Note that the BMF of 2 is not taken into account here in the comparison with actual measured data. This is because the 'trophic level status' of the corresponding 'field' fish is not always known. The differences between the calculated data versus the measured ones would, however, even be larger when focussing on the calculated value of 5 mg/kg (see below). Assuming a fat content of 5% this is equal to 50 mg/kg fat. Measured concentrations cannot be compared with the $PECoral_{fish}$ for formulation as no fish has been monitored near fragrance compounding sites.

Median or maximum measured concentrations in several rivers in the EU presented in **Table** 3.6 are lower than the calculated one. The median concentration measured in the river Ruhr by Eschke et al. (1994) is a factor 90 lower than the $PECoral_{fish}$ on a fat basis. Median concentrations measured in eel in the Netherlands by Wiertz (1995) and De Boer and Wester (1996) on a wet weight basis are a factor 80 and 160, respectively, lower than the $PECoral_{fish}$. Median concentrations for fish from more recent studies in the Netherlands and Germany point to more or less similar factors. Maximum measured concentrations presented by the authors given above are circa a factor 2-20 higher than the median concentration, so also these concentrations are lower than the calculated values.

3.2 EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION) - RESPONSE (EFFECT) ASSESSMENT

3.2.1 Aquatic compartment

3.2.1.1 Toxicity data

Results of toxicity tests with aquatic organisms are presented in **Table 3.7**.

Schramm et al. (1996) tested the acute toxicity of musk xylene with photoluminescent bacteria (*Vibrio fischeri*), *D. magna* and *Scenedesmus subspicatus*. They found no effects up to the highest concentration tested, i.e. 80% of the water solubility of 0.15 mg/l for bacteria and the water solubility for algae and daphnids.

From the tests on ready biodegradability it can be concluded that musk xylene was not toxic to the inoculum used at test concentrations up to 107 mg/l resulting in an NOEC of > 107 mg/l (Calame and Ronchi, 1989). This NOEC is much higher than the water solubility of 0.15 mg/l for musk xylene.

Algae (*Selenastrum capricornutum* and *Microcystis aeruginosa*) by Hughes and Krishnaswami (1985a and 1985b) showed very low sensitivity to musk xylene. In the test with *S. capricornutum* population density was reduced by 34% at 5 mg/l while no effects were observed at lower concentrations. In the test with *M. aergunisoa* population density was reduced by 13% at 10 mg/l while no effects were observed at lower concentrations. In both tests a white precipitate was observed at concentrations of 1.0 mg/l for *S. capricornutum* and 1.8 mg/l for *M. aeruginosa* and higher, test-concentrations which are much higher than the water solubility of 0.15 mg/l. It is therefore concluded that the NOEC is equal to the highest test concentrations in which no precipitate was observed.

Adema and Langerwerf (1985a and 1985b) carried out tests with *Daphnia magna*: a 48-hour static toxicity test and a 21-day reproduction test. For the acute toxicity test the EC50 was higher than the highest tested concentration (5.6 mg/l), and a NOEC for swimming behaviour could be determined. In the reproduction test with *D. magna* reproduction was absent at 0.56 mg/l, while dead young born occurred at 0.1 mg/l. The condition of the parent daphnia was affected at 0.1 mg/l. At the end of the test 40% mortality occurred at 0.56 mg/l and 80% at 1.0 mg/l. An LC50 of 0.68 mg/l was estimated, while the NOEC was 0.056 mg/l.

In the acute fish test with bluegill sunfish (*Lepomis macrochirus*) by Sousa and Suprenant (1984) the lowest concentration of 0.78 mg/l showed 30% mortality, and the highest concentration of 6 mg/l 100%. The oxygen concentration in the control declined to 6.0 mg/l, while in the solvent control and the other concentrations a dramatic decline to 2.3-0.8 mg/l was observed. This could be caused by leaving dead fish in the aquarium, but no explanation was given in the test.

In the 14-day fish test with *Brachydanio rerio* test according to OECD Guideline 204 by Adema and Langerwerf (1985c) 30% mortality occurred at 0.32 mg/l, 100% at 1.0, 1.8 and 3.2 mg/l, and one fish survived in the highest concentration of 5.6 mg/l. The estimated LC50 declined from 0.76 at day 2 to 0.40 mg/l at day 14. Since the 7-d LC50 is 0.42 mg/l, it is assumed that the value at day 14 represents the incipient LC50. The NOEC for swimming behaviour was 0.1 mg/l. Growth was affected in all concentrations: in the lowest concentration of 0.1 mg/l, the weight of the fish was 80% of the weight of the controls, while at the highest concentration of 0.56 mg/l this was 60%.

In a 96 hour-screening test with musk xylene on rainbow trout alevins (*Oncorhynchus mykiss*) mortality, clinical symptoms or abnormal behaviour were not observed even at the highest concentration of 1,000 mg/l (Boleas et al., 1996). In a 21 day-test with rainbow trout the effect on cytochrome P-450 related parameters was studied. In concentrations of 1, 10 and 100 µg/l, both the EROD activity and plasma retinol levels were not significantly different from the controls (Boleas et al., 1996).

Table 3.7 Toxicity data for aquatic organisms.

Species	Test	Result (mg/l)	Remark[a]	Reference
Vibrio fischeri	30 minute	EC50 = >0.12	DIN 38412, part 34	Schramm et al., 1996
Selenastrum capricornutum	5-day static	NOEC > 0.56	Method Payne and Hall (1979); carrier: acetone; range: 0.1-5.6 mg/L (n=6) nominal concentrations	Hughes and Krishnaswami, 1985a
Mycrocystus aeruginosa	5-day static	NOEC > 1.0	Method Payne and Hall (1979); carrier: acetone; range: 0.1-10 mg/l (n=7) nominal concentrations	Hughes and Krishnaswami, 1985b
Scenedesmus subspicatus	72-hour static	EbC50 = > 0.15	OECD TG 201	Schramm et al., 1996
Daphnia magna	48-hour static	EC50 = >0.15	OECD TG 202	Schramm et al., 1996
Daphnia magna	48-hour static	EC50 > 5.6 NOEC = 0.32 (swimming behaviour)	EEC Dir. 79/831, Annex V part C.2; static; carrier: DMSO; range: 0.18-5.6 mg/L (n=6); nominal concentrations	Adema and Langerwerf, 1985a
Daphnia magna	21-day semi-static	LC50 = 0.68 NOEC = 0.056 (reproduction)	EEC Ring test method 1985; carrier: DMSO; range: 0.01-1.0 mg/l (n=9); nominal concentrations	Adema and Langerwerf, 1985b
Bluegill sunfish *Lepomis macrochirus*	96-hour static	LC50 = 1.2 (0.55-1.7)	US-EPA-660/3-75-009; fish weight: 0.45 (0.17-0.77) g; length: 32 (23-39) mm; carrier: DMF; range: 0.78-6.0 mg/l (n=5); nominal concentrations; O_2 declined in test below 60%	Sousa and Suprenant, 1984
Zebrafish *Brachydanio rerio*	14-day semi-static	LC50 = 0.4 (0.32-0.5) NOEC <0.1 (growth)	OECD TG 204; 3x weekly renewal; carrier: DMSO; range 0.1-5.6 mg/l (n=8); nominal concentrations; fish weight; 94±26 mg; length: 22.4±2.1 mm	Adema and Langerwerf, 1985c
Rainbow trout alevins *Oncorhynchus mykiss*	96-hours	LC50 = >1,000	APHA 1980; 5-10 g; range: up to 1,000 mg/l; screening test	Boleas et al., 1996
Rainbow trout *Oncorhynchus mykiss*	21-day semi-static	no effects on EROD activity in hepatic S9 fractions and retinol levels in hepatic plasma samples	fish weight: 44.2 $\pm$ 2.8 g; range: 1-100 μg/l; solvent: ethanol	
Fish Zebra fish	48-hour 96-hour	LC50 = 3.75 LC50 $\approx$ 0.2 mg/l	no information DMSO sonicated dispersions	MITI (1992) Unilever, 1995

a the number of concentrations tested (n) is without control and solvent control

In the TGD several QSARs for non-polar narcosis are given for calculating toxicity data for aquatic data. Results for musk xylene are presented in **Table 3.8** The QSAR estimates for

algae and for acute toxicity to fish and *D. magna* are all at or near the water solubility of musk xylene of 0.15 mg/l which is in agreement with the experimental data. The QSAR estimate for the 16-d NOEC for *D. magna* is almost equal to the 21-day experimental NOEC. The QSAR estimate of 0.058 mg/l for the 28-32-day NOEC for fish agrees reasonably well with the 14-day NOEC of <0.1 mg/l from a test with *B. rerio*. These results indicate that musk xylene probably acts by non-polar narcosis.

An embryo larvae (Zebra fish) toxicity test according to Swedish Standard (SS 02 81 93) was performed with musk xylene (Carlsson and Norrgren 2002 *in press*). Six concentrations between 0.1 and 33 µg/l of musk xylene were used, with six replicates per concentration. The musk was first dissolved in DMSO, resulting in a concentration of 0.5 % in the final solution. Newly hatched embryos were exposed to the musk in beakers. The water was renewed every day until all embryos and larvae were dead and the number of living and dead eggs and larvae in each beaker were recorded. This gave a median hatching time and a median survival time for each beaker. The results showed a LOEC of 33 µg/l and a NOEC of 10 µg/l on larvae survival time. The ecological significance of this test is however questionable as the larvae were not fed during the experiment. It is felt therefore that this survival time test (control survival time is around 13 days) is more a multi-stress experiment comprising starvation and the impact of the toxicant. For this reason this endpoint will not be used for the PNEC derivation.

The study also includes a test where newly fertilised Zebrafish eggs were exposed in 96-well microtiter plates to a series of musk xylene concentrations. The embryo development was studied until 48 hours after fertilisation. A number of parameters were investigated including spontaneous movement, circulation, coagulation of eggs and heartbeat. The resulting NOEC and LOEC for the inhibition of the heartbeat frequency were found to be 3.3 µg/l and 10 µg/l respectively. The relationship of the parameter heartbeat with population dynamics is unknown and the test result is thus not useful for the PNEC derivation.

Table 3.8 Toxicity data using QSARs from TGD (Chapter 4) for non-polar narcosis (mg/l) using a log K_{ow} of 4.9 and a MW of 297.3 g/mol.

Species	Endpoint	Result (mg/l)
Pimephales promelas	96-hour LC50	0.83 (0.36-1.9)
Brachydanio rerio/*Pimephales promelas*	28-32-day NOEC (ELS)	0.058 (0.027-0.12)
Daphnia magna	48-hour EC50 16-day NOEC	0.31 (0.14-0.69) 0.030 (0.012-0.074)
Selenastrum capricornutum	72-96-hour EC50	0.22 (0.15-0.33)

Results of an *in vitro* competitive estrogen receptor binding study (Chou and Dietrich, 1999) with musk xylene and musk xylene metabolites are discussed in Section 3.3.5.

3.2.1.2 PNEC for the aquatic compartment

For the determination of the PNEC both short and long-term toxicity test results studies are available for musk xylene. The 5 day-growth test with algae and the 21 day-reproduction test with *Daphnia magna* are considered long term tests. The 14day-fish test was considered too short for a long-term test, leaving two-long term studies for this substance. Subsequently, an

assessment factor of 50 is applied to the long-term NOEC for *Daphnia magna* giving a $PNEC_{water}$ of 1.1 µg/l.

The 14day-fish toxicity data may be used to support this PNEC. That is, if the LOEC of the 14day-fish growth study is extrapolated using a factor of 10 to a chronic NOEC and an assessment factor of 10 is used, the resulting PNEC is almost identical to the PNEC obtained without using the fish data.

Applying the equilibrium partitioning theory, a $PNEC_{sed}$ of 0.3 mg/kg ww is calculated as described below:

$$PNEC_{sed} = \frac{K_{susp-water}}{RHO_{susp}} \cdot PNEC_{water}$$

$PNEC_{sed}$:PNEC for sediment-dwelling organisms (kg/kg_{wwt})
$PNEC_{water}$:PNEC for aquatic organisms (kg/m^3)
$K_{susp\text{-}water}$:suspended matter-water partition coefficient (294 m^3/m^3)
RHO_{susp}:bulk density of suspended matter (1,150 kg_{wwt}/m^3)

For micro-organisms one test was available with bacteria where no effect was observed at the highest test concentration of 0.12 mg/l. However, according to the TGD these tests with photoluminescent bacteria cannot be used for deriving a $PNEC_{STP}$. From the test on inherent biodegradability a NOEC of > 107 mg/l could be derived. Applying an assessment factor of 10 leads to a minimum $PNEC_{STP}$ of >10.7 mg/l. It is realised that this value is much higher than the water solubility of musk xylene of 0.15 mg/l.

3.2.2 Atmosphere

No toxicity data are available for organisms exposed via the air.

3.2.3 Terrestrial compartment

3.2.3.1 Toxicity data

The toxicity of musk xylene to earthworms was studied in a 14-day test according to OECD TG 207 in artificial soil (initial weight of the worms: 0.4 g; soil pH: 6.1-8.1; 3.4% o.c.). No effects were observed on survival up to the highest test concentration of 50 mg/kg dw (Downing, 1994).

3.2.3.2 PNEC for the terrestrial compartment

For musk xylene a 14d-toxicity study was carried out for earthworms resulting in a '> value', because no effects were found up to the highest concentration. Therefore the $PNEC_{soil}$ was derived from the $PNEC_{water}$ using the equilibrium partitioning theory, leading to a value of 0.26 mg/kg dw.

3.2.4 Non compartment specific effects relevant to the food chain

No toxicological data are available for (top-)predators. No specific toxicological data are available on e.g. (fish-eating) birds. The PNEC for secondary poisoning will therefore be based on mammalian toxicity data for musk xylene. The oral NOAEL of 7.5 mg/kg bw/day for peri/postnatal toxicity in rats is used for this purpose (see Section 4). As toxicity is expressed on the P-generation (rats > 6 weeks) a food conversion factor of 20 has to be used. As this study equals a 28 days test, applying an AF of 300 on the ground of the exposure time should be considered here (TGD, 1996). However, a number of arguments can be adduced why the use of such factor of 300 may be over-protective in this case. One reason is that even at the next concentration in the test, i.e. 22.5 mg/kg bw/day, only marginal (6%) effects were seen on the body weight gain of the pups. This makes this LOAEL, and, implicitly, the selected NOAEL, rather conservative. Additionally, an 80 weeks mice oral carcinogenicity study is available (Maekawa et al., 1990) showing no effects on reproductive organs. However, no NOAEL could be derived from this study and other, ecologically relevant, effects were not addressed. A semi-chronic dermal rat study is further available (Ford et al., 1990) from which an oral NOAEL of 9.6 mg/kg bw/day can be calculated (route-to-route). This value is in line with the value of 7.5 mg/kg bw/day indicating that the extrapolation step from sub-acute to semi-chronic does not necessarily demand an additional uncertainty factor. A weak point here is that the TGD is clear in that only oral or dietary exposures should be used to derive a PNEC for secondary poisoning (and thus not an extrapolated dermal exposure).

From the above it is clear that the data set contains more useful information than 'just' the results of the 28-days test (AF <300), but that this extra information is not sufficient to fully equate this test with a semi-chronic NOAEL from a feeding study (AF > 90). When using the NOAEL of 7.5 mg/kg bw/day as a starting point for the $PNEC_{oral}$ derivation of musk xylene makes it is therefore suggested to use an AF of 150 as a reasonable 'compromise' between 90 and 300. The $PNEC_{oral}$ then becomes: $7.5 \cdot 20/150 = 1$ mg/kg food.

$PNEC_{oral} = 1$ mg/kg food

3.3 RISK CHARACTERISATION

In Chapter 2 some uncertainties were mentioned about the total volume of musk xylene being used in the EU. This among others because of unknown amounts of musk containing products imported into the EU. According to industry such volumes are expected to be very low compared to the figures for the 'isolated' substance. Furthermore it should be kept in mind in the risk characterisation that the available monitoring data 'implicitly' comprise the overall emissions from the use of musk xylene in the EU, thus both from products formulated inside and outside the EU. The monitoring data will be taken into account in the risk characterisation (see below).

It is further emphasised that the monitoring data set comprises various EU regions (especially musk xylene levels in biota) and that the set also contains data from before 1994. Such 'old' data may be representative for those EU regions where at present no legal restrictions on the use of nitromusks have been taken.

3.3.1 Aquatic compartment

The PEC/PNEC ratios for the STP and surface water are presented in **Table 3.9**.

Table 3.9 PEC/PNEC ratios for the aquatic environment.

	STP	Surface water
Site 1	< 0.01	0.2
Site 2	< 0.01	0.2
Site 3	n.r.	0.2
Site 4	< 0.01	0.2
Site 5	< 0.01	0.2
Site 6	< 0.01	0.2
end product formulation	<0.01-<0.01	0.2-0.4
Private use	<0.01	0.9
Regional	n.r.	0.2

From **Table 3.9** it can be seen that all PEC/PNEC ratios are below 1: **conclusion (ii)**.This conclusion is confirmed by measured data as all the available aquatic monitoring data are below the calculated PEC.

Sediment dwelling organisms

PEC/PNEC ratios for sediment based on calculated PECs are similar to those for surface water. In addition, however, also measured concentrations are available. Sediment levels in the rivers Elbe, Rhine and Meuse, being comparable to a regional scale, lead to a PEC/PNEC less than one: **conclusion (ii)**. For fragrance compounding (formulation), end product formulation (local scale) and private use (local scale) no monitoring data were available.

3.3.2 Atmosphere

A risk characterisation for the atmosphere is not considered relevant for this purpose as there are no experimental data and also no indications of either biotic or abiotic effects.

3.3.3 Terrestrial compartment

The PEC/PNEC ratios for soil are presented in **Table 3.10**.

Table 3.10 PEC/PNEC ratios for soil.

	PEC/PNEC Default scenario	PEC/PNEC Alternative scenario (maximum sludge level of 1 mg/kg)
Site 1	0.02	0.01
Site 2	< 0.01	< 0.01
Site 3	0.02	0.02
Site 4	0.19	0.2
Site 5	0.02	0.01

Table 3.10 continued overleaf

Table 3.10 continued PEC/PNEC ratios for soil.

	PEC/PNEC Default scenario	PEC/PNEC Alternative scenario (maximum sludge level of 1 mg/kg)
Site 6	< 0.01	< 0.01
end product formulation	0.1-0.5	0.08 - 0.4
Private use	1.7	0.06
Regional	0.6	0.01

For sites 1-6, end product formulation scenario and the regional scenario the PEC/PNEC for soil is ≤ 1, both in the default and alternative scenario: **conclusion (ii)**. For the private use scenario the PEC/PNEC exceeds 1. In the alternative scenario, however, there is no potential risk for private use. The alternative private use scenario is considered more realistic than the default one (see Section 3.1.2.2). For this reason **conclusion (ii)** is considered as most relevant for the private use scenario.

3.3.4 Non compartment specific effects relevant to the food chain

The PEC/PNEC ratios for secondary poisoning are presented in **Table 3.11**.

Table 3.11 PEC/PNECs for the non compartment specific effects relevant to the food chain.

	PEC/PNEC fish	PEC/PNEC worm Default scenario	PEC/PNEC worm Alternative scenario (maximum sludge level of 1 mg/kg)
Site 1	1.6	0.3	<0.1
Site 2	1.6	0.3	<0.1
Site 3	1.6	0.3	0.1
Site 4	1.6	0.4	<0.1
Site 5	1.6	0.3	<0.1
Site 6	1.6	0.3	<0.1
end product formulation scenario	1.7 -2.2	0.4-0.6	< 0.1- 0.1
Private use	5	1.1	<0.1

All PEC/PNEC ratios for fish eating predators are found to be above 1. It is clear here that the regional PEC completely determines the PEC/PNEC ratios for the six compounding sites and the end product formulation scenario (low 'fraction of main source' factor). The TGD assumption is that 50% of the diet of the predator comes from the local area (PEC local) and 50% of the diet comes from a regional area (PEC regional). For the compounding sites and the end product formulation scenario (low 'fraction of main source' factor) the C local is negligible compared to the PEC regional and therefore the PEC local is (almost) equal to the PEC regional. The regional background plays an important, but somewhat less dominant role in the 'private use' scenario and the end product formulation scenario (high 'fraction of main source' factor).

The calculated PEC/PNECs for the private use and the compounding and end product formulation scenarios (fish-route), all being dominated by the calculated regional PEC, can be overruled however by using the rather large regional monitoring data set for fish from a number of different EU regions. All these measured values are much lower than the maximum calculated value of 5,000 µg/kg dwt (private use). The set also contains data from before 1994 which may represent those regions in which reduction measures were (possibly) not yet taken for this compound. Several data are also available for which both the sampling year (before 1994) and the location (effluent pond) reflect a worst-case situation (e.g Eschke et al. 1994 and Hahn, 1993). Even the maximum levels (e.g 300 µg/kg wwt) would then still yield PEC/PNEC ratios below 1.

Based on 1) the dominance of the (calculated) regional PEC in the local scenarios for secondary poisoning (fish route), 2) the relatively large fish database for various EU regions, including data from years in which the restriction measures were not yet (or just) taken in those countries, 3) the fact that PEC/PNEC ratios even for maximum measured values in worst-case environments are below 1, and 4) the uncertainties in the PEC calculation (a.o. uncertainties around the end product formulation scenario (use of higher 'fraction of main source' factor), it is considered that a **conclusion (ii)** is most appropriate for the private use, the six compounding and the end product formulation scenarios.

For worm-eating animals the PEC/PNEC ratios are <1, except for the default private use scenario. In the alternative scenario, however, there is no potential risk for private use. The alternative private use scenario is considered more realistic than the default one (see Section 3.1.2.2). For this reason **conclusion (ii)** is considered as most relevant for the private use scenario.

3.3.5 Metabolites of musk xylene

Mono and diamino metabolites have been measured in water and fish in Germany. Diamino metabolites were not detected. The monoamino metabolites were detected in all samples. In surface water the concentration of the 4-monoamino metabolite appeared to be up to a factor 3-10 higher than musk xylene, while in fish the mean concentrations were comparable. Gatermann (1998) reported a value of 2 ng/l 4AMX in the Elbe river. The 2-amino metabolite was detected at lower concentrations than musk xylene; for example in fish the difference was a factor 7.

In addition to the (limited) exposure data on metabolites, recently also ecotoxicity data have become available. Behechti et al. (1998) studied the acute toxicity of four musk xylene derivated on Daphnia magna. The test procedure followed the OECD guideline 202. The reduced amino derivative 1-tert-butyl-3,5-dimethyl-4-amino-2,6-dinitrobenzene exhibits a very high toxicity to Daphnia, with an EC50 of 0.00025 mg/l. The other metabolites showed less toxicity (2-amino-4,6-dinitro-metabolite: 1.07 mg/l; 2,4-diamino-6-nitro-metabolite: 23.3 mg/l and 2,4,6-triamino-metabolite: 58.8 mg/l). The suggestion is made by the authors that, because no clear relationship between K_{ow} and EC50 was found, the toxicity of the 4-amino metabolite must be based on a specific rather than an unspecific narcotic mechanism. In a more recent US Springborn Laboratories study (Putt, 1999) the results of the Behechti et al. study could not be replicated. The acute toxicity of Daphnia magna to the 4-amino metabolite was tested (OECD 202) under four different conditions (**Table 3.12**).

Table 3.12 Results of the acute Daphnia toxicity study (Putt, 1999) with 4-amino musk xylene metabolite under various test conditions.

Test conditions	EC50 (µg/l; 95% conf. Intervals)
Laboratory water, normal light	490 (400-600)
Laboratory water, dark	510 (470-540)
Natural water, normal light	370 (350-380)
Natural water, dark	440 (410-470)

The difference between the results of the Behechti study (0.00025 mg/l) and the Putt study (0.37-0.49 mg/l) is very great: a factor 1,500-2,000. A discussion has started between the two research groups about possible explanations for this difference. According to very recent information (Schramm, 2000 Water Research Author's reply) the high toxicity in the Behechti could not be repeated in an additional in-house study and the (preliminary) conclusion is therefore that the initial high toxicity could not be related to the 4-amino metabolite. Taking the EC50 of 370 µg/l from the Putt study and using an assessment factor of 1,000 on this acute test result, leads to a PNEC of 0.4 µg/l for the 4-aminometabolite.

Comparing the PNEC of 0.4 µg/l with the measured value of 2 ng/l in the Elbe river from Gatermann, indicates that there seems to be no direct reason for concern for the 4-aminometabolite.

Some additional information has become available on the ecotoxicity of musk xylene metabolites. Chou and Dietrich (1999) investigated the competitive binding capability of musk xylene and musk xylene metabolites to the estrogen receptor in trout (*Oncorhynchus mykiss*) and clawed frog (*Xenopus laevis*). No binding of the parent compound musk xylene was observed. In contrast, however, binding to the ER was noticed for 2-amino musk xylene (2X) and 4-amino xylene (4X) in both species, Xenopus being the most sensitive species. The IC50 (competitive binding at the ER) of 2X in rainbow trout was found to be 1.3 ± 1.1 mM. The 2X and 4X IC50 in Xenopus were 30.8 ± 28.5 and 12.9 ± 10.3 µM, respectively. Although competitive binding was demonstrated for the metabolites of MX, the relevance of such *in vitro* assays for the environmental situation is still unclear. IC-50 values for 2X and 4X are many orders of magnitude higher than those levels found at present in the environment. It should be emphasised, however, that for bioaccumulative compounds long term test results outweigh short term data. Despite of this, the difference between the IC50 for 4X (appr. 4 mg/l) and the above-mentioned PNEC for 4X (0.4 µg/l) seems to indicate that the PNEC of 0.4 µg/l is also protective for possible estrogen receptor binding by 4X.

Overall, a **conclusion (ii)** is therefore considered most relevant for musk xylene metabolites in the environment.

3.3.6 PBT assessment

Musk xylene is considered to be a PBT candidate substance. The Persistence criterion seems to be fulfilled with the results of two biodegradation tests clearly showing no (ready) biodegradability, accompanied by some inconclusive influent/effluent studies. In addition, the use of the BIOWIN model for estimating aerobic biodegradability also points to the lack of biodegradation of musk xylene. The Bioaccumulation criterion is fulfilled as the experimental BCF is above 2,000. The Toxicity-criterion would not be fulfilled for ecotoxicity with no

ecologically relevant NOECs less than 10 µg/l. However based on human health toxicity, musk xylene does fulfil the T-criterion (Carc. Cat. 3; R40).

Testing strategy

Further testing seems to be less relevant for refining the B- and T-criterion for musk xylene. A simulation test on biodegradability (half-life in the marine environment) should be considered here for refining the P-criterion (see TGD (2002)).

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

4.1.1 Exposure assessment

4.1.1.1 General introduction

See also Section 2.2 for use pattern of musk xylene.

The substance is not produced within the EU, but imported from China. Inside the EU the pure substance is used in fragrance compounding.

Synthetic musk compounds are widely used as fragrances and fragrance enhancers in body care products and household detergents. The industrially most important synthetic musks are derived of nitro benzenoid compounds (e.g. musk xylene and musk ketone) and of non-nitro polycyclic compounds. Musk xylene is widely used in consumer products like toiletries, colognes, shampoos, laundry detergents and cleaning agents. The concentration of musks in these end products varies up to 1% (Müller, 1997).

Data were received from six of the seven European facilities. The risk evaluation therefore may be considered as representative for Europe.

Table 4.1 Use of musk xylene.

Industrial category	Use category
Fragrance	Fragrance compounding
Personal and domestic use	Cosmetics, odour agents, air freshener systems, household and laundry cleaning agents

4.1.1.2 Occupational exposure

The substance, a crystalline material, is imported in plastic bags in 50 kg cardboard drums and added to other compounds on an ‘as needed’ basis to form a liquid fragrance compound. Musk xylene is added to the fragrance mixture in closed vessels, in relative small quantities. The batches are typically less than 1,000 kg of which less than 10% is musk xylene (Company A, 1998a). Per facility usually one batch per day is made. Batches are made in vessels with local exhaust systems. Exposure of workers to dust can not be excluded in the process of manual weighing and filling the vessels through dumping the substance from the drums. The end product is a liquid which is drummed and used in the cosmetic industry for the production of consumer products like toiletries or cleaning products. It is assumed that the major part of the liquid in which it is mixed, and in which it will dissolve, are fragrance oils. In the cosmetic industry, it is assumed that dosing to consumer products will be highly automated and exposure may be possible when the liquid fragrance is poured.

The following data are used for occupational exposure assessment:

- physico-chemical data, physical appearance and vapour pressure;

- data regarding the production process and use pattern of the products and amount of the substance in the product;
- exposure data from the HEDSET;
- measured data for musk compounds or analogues;
- results from exposure models (EASE-model).

In this part of the assessment, external (potential) exposure is assessed using relevant models and other available methods in accordance with the Technical Guidance Documents and agreements made at official Meetings of Competent Authorities. Internal dose depends on external exposure and the percentage of the substance that is absorbed (either through the skin or through the respiratory system).

The exposure is assessed without taking account of the possible influence of personal protective equipment (PPE). If the assessment as based on potential exposure indicates that risks are to be expected, the use of personal protective equipment may be one of the methods to decrease actual risks, although other methods (technical and organisational) are to be preferred. This is in fact obligatory following harmonised European legislation.

Knowledge of effectivity of PPE in practical situations is very limited. Furthermore, the effectivity is largely dependent on site-specific aspects of management, procedures and training of workers. A reasonably effective use of proper PPE for skin exposure may reduce the external exposure with 85%. For respiratory protection the efficiency depends largely on the type of protection used. Without specific information, a tentative reduction efficiency of 90% may be assumed, equivalent to the assigned protection factors for supplied-air respirators with a half mask in negative pressure mode (NIOSH, 1987). Better protection devices will lead to higher protection. Imperfect use of the respiratory protection will lower the practical protection factor compared to the assigned factor. These estimations of reduction are not generally applicable "reasonable worst-case" estimations, but indicative values based on very limited data. They will not be used directly in the exposure and risk assessment. Furthermore, the reduction of external exposure does not necessarily reflect the reduction of absorbed dose. It has to be noted, that the use of PPE can result in a relatively increased absorption through the skin (effect of occlusion), even if the skin exposure is decreased. This effect is very substance-specific. Therefore, in risk assessment it is not possible to use default factors for reduction of exposure as a result of the use of PPE.

In some specific situations the model estimates, with normal assumptions for input parameters in the assessed exposure scenarios, are expected not to lead to a reasonable assessment of exposure. For situations with high risk of direct acute effects, such as manual handling of corrosive substances and hot materials, or possible inhalation exposure of substances with severe acute effects on the respiratory tract, the total level of containment given by all exposure control measures is assumed to be higher than for similar scenarios with other substances. For estimating a single day exposure an extra protection is assumed, reducing exposure with 90%. The extra protection can be reached by a combination of technical and organisational control measures and personal protective equipment. If the extra protection is reached (mainly) by using personal protective equipment, this is an unwanted situation that should be changed by further technical and organisational control measures.

The estimate of repeated dermal exposure depends on the knowledge of a ‘maximum non-corrosive concentration’. If such a concentration can be estimated, this concentration will be used in estimating repeated dermal exposure. Otherwise the estimate for single day exposure will be used.

From the uses of musk xylene as mentioned in **Table 4.1** the following scenario's for exposure will be discussed:

Scenario 1: The production of fragrance compounds
Scenario 2: The use of liquid fragrance compounds
Scenario 3: The use of cleaning agents by professional cleaners

4.1.1.2.1 The production of fragrance compounds (Scenario 1)

Musk xylene is imported as a crystalline powder. Determination of the particle size showed that all material grains were smaller than about 0.1 mm (100 μm). The respirable fraction (particle size lower than 4 μm) was up to 14.4% (Company B, 1998).

At room temperature the substance has a very low vapour pressure: 3.10^{-8} kPa. Inhalation exposure to the vapour is probably negligible, but exposure to dust may be possible. The fragrance compounds are probably mixed on customer's demand and the amount of xylene musk added may vary from batch to batch. Exposure may occur during weighing and adding of the solid to the (liquid) mixture.

After production, the drums containing the (liquid) compounded musk will be used in the cosmetic industry for the production of toiletries and household detergents etc. Exposure will occur when the drums are opened and poured.

When evaporating, the fragrance oil may probably serve as a vehicle for evaporation of the musk. It is therefore assumed that, with a maximum of 10% in the liquid, the maximum concentration in the vapour may also be 10%. The vapour pressure of the fragrance oils may vary between 0.0001 and 13 Pa at 20°C. A worst-case vapour pressure of 10 Pa is chosen, which means that the assumed worst-case partial vapour pressure of musk xylene is 1 Pa (Company A, 1998b).

Measured data (and data for analogous substances)

Workplace monitoring data are available from two companies (Company A and E, 1997).

Table 4.2 Measured data.

Activity (Company)	Year	Number of measurements	TWA or short term value	Range of data (mg/m^3)
Fragrance compounding (A)	Occasionally	unknown	unknown	n.d.
Air monitoring program (A)	1988	25	unknown	n.d.
Fragrance mixing (E)	Unknown	unknown	unknown	n.d.

n.d. non detectable

Details of these measurements, such as activity during sampling, method (total or respirable dust, analysis for specific musks), duration, personal or static sampling, limit of detection etc. were not mentioned.

Models and analogue substances

Manual weighing and addition of powder may lead to the emission of dust, depending on the dustiness of the substance and on the proper use of adequate local exhaust ventilation. Exposure levels estimated by the EASE model, assuming the presence of proper local exhaust ventilation, are up to 2-5 mg/m^3 (reasonable worst-case estimate).

Published exposure data on manual weighing is rather scarce. Geometric means for total dust exposure in a number of studies ranged from 1.4 to 14 mg/m^3 (with LEV), while scooping from an almost empty drum and weighing without LEV is reported to lead to levels as high as 40 mg/m^3 (Lansink et al., 1996a).

Bag dumping is described several times in the literature. Total dust exposures reported vary from 0.1 to 15.9 mg/m^3, while respirable dust varies from <0.1 to 5 mg/m^3. These data are for situations with LEV. Without LEV exposures are stated to be much higher, but actual data to verify this were not reported by the available sources.

Comparing the reported data for analogues with the estimates by the EASE model it appears that the estimation with LEV does not represent a reasonable worst-case. This may be due to the use of not highly efficient ventilation systems in some of the sources studied. A reasonable worst-case estimate for total dust exposure levels due to weighing and dumping of powders, using more or less efficient LEV is 10 mg/m^3, based on literature data.

Dermal exposure during addition from plastic bags is assumed to involve non-dispersive use, direct handling with an intermittent contact level, leading to a predicted exposure by EASE of 0.1-1 mg/cm^2/day. Assuming the exposed area to be the half of two hands (approximately 420 cm^2), this leads to an estimated exposure level by EASE of 42-420 mg/day.

In a recent study in The Netherlands, skin exposure of hands and forearms to a powder in the paint industry (calcium carbonate) has been measured using cotton gloves. Exposure was measured for each separate operation. Dumping calcium carbonate for one batch of paint was for example considered to be one operation. Exposure levels were between 52 and 4,214 mg per two hands and part of forearms, for collecting raw materials, manual weighing, manual dumping from paper bags and removal of empty bags. The GM values varied from 215 to 890 mg per two hands and part of forearms (Lansink et al., 1996b). The field study mentioned did not include accumulation of exposure due to repeated operations. Since the gloves may give an overestimation of the exposure, the measured values are assumed to be total daily dermal exposures. Comparing the results of the study with the assessment of EASE, it seems as if EASE does not give a 'reasonable worst-case' assessment. Assuming that dumping from plastic bags results in lower exposure levels than dumping from paper bags and that there are more careful work practices compared to the paint industry, the GM for bag dumping (890 mg/day) calculated in the study may be used for the risk characterisation of dumping powders. It must be noted, however, that there was a clear relationship between the number of bags dumped and dermal exposure. In fragrance compounding per batch only one or two bags of musk xylene are weighed and poured. Information from industry on use practices of PPE (from 5 sites) indicates that gloves are regularly worn during tasks that involve direct handling of material. These gloves are mostly reported to be natural latex dispensable gloves that are changed after every use or before every break (Industry, 2000). One of the reasons for using gloves is the smell of the material. Extensive exposure will lead to prolonged strong odours coming from the exposed parts. Two publications (regarding the same experimental data) describe the effect of evaporation from the skin of fragrances. For nine fragrances it was shown that 25-75% of the applied amount was evaporated from the skin after 7 hours and

15 minutes. Two other fragrances were found to evaporate only for up to 7% (Vuilleumier et al., 1995, Hellewegen and Van Bergen, 2000). Hellewegen and Van Bergen (2000) suggest that these results are artefacts. However, the studied fragrances have a substantially higher vapour pressure than musk xylene and the descriptions of the study are such, that the relevance of the results for this assessment are unclear. The mentioned GM for bag dumping may, in this case, be an overestimate.

The drumming of the liquid fragrance compound may result in inhalation exposure and will involve dermal exposure through manual contact with contaminated surfaces.

The EASE model, assuming a partial vapour pressure of 1 Pa, non-dispersive use and LEV predicts an inhalation range of 0.5-3 ppm (6-37 mg/m^3) and a dermal exposure of 42 mg/day, assuming incidental contact, non-dispersive use and direct handling with exposure of the palms of both hands (420 cm^2). An alternative model for estimation of inhalation exposure to liquids is the USEPA transfer model (USEPA, 1991).

The USEPA transfer model is a model in which the equilibrium concentrations reached in a room during liquid transfer is calculated. These calculations actually consist of two parts. In the first part the generation of vapours by displacement of air from containers during liquid transfer is calculated. The generation rate of the vapour is then used as an input variable in a mass balance ventilation model. For several input parameters typical and worst-case default values have been established from empirical knowledge. If more specific information is lacking, the default values can be used to calculate concentrations. These concentrations are spatially averaged concentrations. To calculate exposure levels from these concentrations the time workers spend in this and other environments and the concentrations in the other environments should be known or estimated. As a worst-case assumption it can be assumed that workers spend a whole shift transferring liquids, since transferral is often the activity with the highest levels of emission. These estimations are for pure substances. For substances in mixtures, the partial vapour pressure should be used. As a rough estimate, the resulting exposure levels for substances in mixtures can be calculated by multiplying the result of the model by the fraction of the substance in the mixture. The operation considered in the model is filling of cans (50 l).

Estimation of concentration due to transfer operations - USEPA model:

$$Cm = 1{,}000 \cdot (f \cdot M \cdot V \cdot r \cdot P)/(R \cdot Tl \cdot Q \cdot k)$$

where:

Cm = calculated concentration (mg/m^3)
f = saturation factor
M = molar weight (g/mol)
V = volume of container (m3)
r = fill rate (/h)
P = vapour pressure of substance (Pa)
R = universal gas constant
Tl = temperature of liquid (K)
Q = ventilation rate (m^3/h)
k = mixing factor

The fixed parameters for the model are:

M = 297
V = 0.05

P = 1
R = 8.3144
Tl = 293

For the remaining parameters default values describe the typical and worst-case approach:

	Typical case	Worst-case
f	0.5	1.0
r	20	30
Q	5100	850
k	0.5	0.1

The calculated range for filling of cans of 50 l is 0.5 and 2 mg/m^3 (typical case worst-case range). It may be remarked that these calculations are only valid if displacement of vapour is the predominant route of emission of contaminant into the air. It is noted however that the effect of local exhaust ventilation is not estimated. Assuming an efficiency of LEV of 90% the values that may be used in the risk characterisation are 0.05-0.2 mg/m^3.

Conclusions

Due to the lack of information on the measured data, the results of the estimation with the EASE model and the analogous substances are used for estimating inhalation exposure due to compounding. The quantities of musk xylene that are used are relatively small. Per facility usually one batch per day of less than 1,000 kg is made, with less than 10% musk xylene. In this case, it seems reasonable to consider the value of the analogue substance as a short term value and the ranges of the EASE model as typical and worst-case values. Recalculated for an exposure of half an hour per day and negligible exposure during the remainder of the day, the typical value is approximately 0.1 mg/m^3, the worst-case value 0.3 mg/m^3 and the short term value remains 10 mg/m^3.

To estimate the dermal exposure for dumping only one or two bags per day the lower value of the EASE model is taken, 42 mg/day. This value corresponds well with the mentioned lower range for calcium carbonate: 52 mg/day. This value is considered to be a (substantial) overestimate of the actual exposure values. The substance is crystalline and therefore probably less dusty than general powders. The strong odour of the substance will induce the use of PPE (gloves) by workers that will lead to a reduction of actual exposure levels. The available information is too limited to quantify the reduction in exposure due to these factors.

The estimate of the EASE model for inhalation exposure seems to be too high. This is due to the fact that the model only works with discrete classes of vapour pressure. The estimate of the USEPA models, including the effect of LEV, is considered most relevant for drumming: <0.01-0.14 mg/m^3 (typical case, respectively worst-case).

For dermal exposure the result of the EASE model may be used: 42 mg/day.
In compounding fragrances inhalation exposure is higher than during drumming. But during drumming dermal exposure is estimated to be equal. For the risk characterisation the inhalation estimates during compounding are used: 0.3 mg/m^3 for inhalation and 42 mg/day for dermal exposure.

4.1.1.2.2 The use of liquid fragrance compounds (Scenario 2)

The drummed liquid fragrance is used in the cosmetic industry for production of toiletries, shampoos etc. Exposure may be possible during the handling of the drums, and during cleaning and maintenance. It is assumed that the rest of the production is a highly automated process, with little or no exposure to musks.

Measured data

No measured data are available.

Models and analogous substances

The EASE model estimates for the direct handling of liquids assuming non-dispersive use and incidental contact a dermal exposure of 0-0.1 mg/cm^2/day. With the palms of two hands exposed (420 cm^2) and a concentration of 10% in the liquid the exposure is 4 mg/day. Inhalation exposure with direct handling and non-dispersive use is estimated to be negligible.

For cleaning and maintenance it is assumed that there is previous rinsing of the equipment which lowers the concentration with 90%. With direct handling and non-dispersive use and extensive contact (up to 5 mg/cm^2/day) and exposure of both hands and part of the forearms (1,300 cm^2) the estimate is 650 mg/day. With 10% of the substance in the original liquid and 90% dilution of the original liquid the exposure is approximately $650 \cdot 0.1 \cdot 0.1 = 6.5$ mg/day. Inhalation exposure is estimated to be negligible.

Conclusions

For the risk characterisation it is estimated that inhalation exposure is negligible and dermal exposure is 4 mg/day on a daily basis. Cleaning and maintenance will probably be only once a week with an estimate of 6.5 mg/day. Comparable to Scenario 1, the smell of the estimated amount of fragrance mixture on the skin will induce the use of gloves to prevent extensive exposure. No pertinent information on the use of gloves is available for this scenario, so the assumed reduction is not quantified.

4.1.1.2.3 The use of cleaning agents by professional cleaners (Scenario 3)

The use of musks in consumer products is subject to changes. The general trend in detergents and cleaning products is to replace musks by other fragrances. One of the end products which may (still) contain musks, are household cleaning agents. Professional cleaners may be exposed to some extent. It is assumed that cleaning agents are diluted before use.

The available information indicates that the product types that contain muks xylene are not used by spraying.

Measured data

No measured data are available.

Models and analogue substances

For inhalation exposure the EASE model predicts with the assumption of no aerosol formation a negligible exposure. For dermal exposure assuming extensive contact and wide

dispersive use the exposure ranges from 5-15 mg/cm^2/day (cleaning agent with 1% musk xylene) and with exposure to both hands (840 cm^2) and assuming the detergent is diluted 50 times the exposure is 0.8-2.5 mg/day (5, respectively 15 · 840 · 0.01 ·1/50).

Conclusions

The values estimated by the EASE model are taken in the risk characterisation. Inhalation exposure is negligible and dermal exposure is estimated to be 2.5 mg/day.

Although also in this case the use of gloves is possible, it is not assumed that this is regularly done by the majority of the workers.

Table 4.3 Conclusions of the occupational exposure assessment.

Scenario	Exposure		Estimated inhalation exposure level (musk xylene; mg/m3)						Estimated skin exposure level (musk xylene; mg/day)
			Full shift (8 hour time weighted average)				Short-term		
	Duration (hr/day)	Frequency (day/year)	Typical	Method	Reasonable worst-case	Method	Level	Method	
1 The production of fragrance compounds	0-1	225	0.1	EASE	0.3	EASE	10	Analogy	42[1)]
2 The use of liquid fragrance compounds:									
- addition	0-1	225	negl.	EASE	negl.	EASE	negl.	Expert	4[1)]
- cleaning and maintenance	0-1	20-50	negl.	EASE	negl.	EASE	negl.	Expert	6.5[1)]
3 The use of cleaning agents by professional cleaners	4-6	225	negl.	EASE	negl.	EASE	negl.	Expert	2.5

EASE Calculation with the EASE model
Analogy Based on measured data for other substances used in similar exposure situations
Expert Expert judgement
Negl. Negligible
1) This is assumed to be an overestimate of true exposure levels due to the fact that the substance is crystalline and that workers will regularly wear gloves to prevent extensive exposure that will lead to unwanted strong smell of the skin

4.1.1.3 Consumer exposure

4.1.1.3.1 Introduction

Consumer exposure occurs from consumer products to which musk xylene is added intentionally.

Musk xylene was assessed by the EU Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCC, 1997; SCCNFP, 1999): musk xylene is widely used as a fragrance and fragrance enhancer in body care products such as toiletries, creams, lotions, soaps, shampoos etc. (SCC, 1997; SCCNFP, 1999).

Musk xylene may also be used in household detergents (HEDSET; Tas et al., 1997). In the HEDSET the amount of musk xylene in detergents is stated to be <0.02%. Müller (1997) reports the detection of musk xylene in laundry detergents (maximum value is 0.025% in several products, based on data from 1993 for Switzerland). In Germany the industry voluntarily decided not to use musk xylene in detergents (Käfferlein et al., 1998). According to the Consumentengids (1995) musk xylene was not found in detergents and softeners in The Netherlands.

4.1.1.3.2 Potential exposure to fragrances in cosmetics

The exposure table of the SCCNFP (1999) evaluation is given (see **Table 4.4**) for the exposure of consumers to musk xylene in cosmetics. The way the exposure was calculated by the SCCNFP (1999) is in accordance with the TGD (1996). SCCNFP considered that the range of products selected covers all those that are likely to be used in any one weekly period. Measured data derived from Müller (1997) are included as well.

Table 4.4 Overview of products and uses that can contain musk xylene following the SCCNFP (1999). Values between brackets are derived from Müller (1997).

Product type	Quantity in grams per application	Frequency of application per day	Retention factor[1]	Normal use in g/day	Maximum xylene concentration in product (in %)[6]	Dermal exposure to musk xylene in µg/kg bw/day during normal use[7]
Body lotion[2]	8	0.71	100%	5.68	0.0284	26.9
Face cream[3]	0.8	2	100%	1.6	0.0213	5.7
Fragrance cream[2]	5	0.29	100%	1.45	0.284	68.6
Eau de toilette[4]	0.75	1	100%	0.75	0.568 (0.9)	71
Other[5]						34.1
					TOTAL	206.3

1 Proportion of product remaining on the skin.
2 It is assumed that body lotion and fragrance cream will not be used on the same day; body lotion on 5 days per week (i.e. 0.71 times per day) and fragrance cream on 2 days per week (i.e. 0.29 times per day).
3 Includes make-up and foundation.
4 Includes all hydroalcoholic products (i.e. perfumes, after-shaves, colognes, etc.). These products are unlikely to be used on one occasion. As the quantity per application will be inversely related to the fragrance concentration in the product, the figure for eau de toilette covers all products.
5 Includes products such as anti-perspirant/deodorant, shampoo, bath products, shower gel, toilet soap and hair spray.
6 This concentration corresponds to the upper 97.5th percentile.
7 Consumer weight of 60 kg is taken.

According to the SCCNFP (1999) the dermal exposure to musk xylene can be estimated at 210 µg/kg bw/day (rounded off). This value must be regarded as conservative as it is most unlikely that a consumer will consistently use a number of different cosmetic products which are all perfumed with the upper 97.5th percentile level of the fragrance ingredient. In the exposure assessment of the SCCNFP (1999), the inhalation route is not taken into account. This is considered acceptable, as for all selected cosmetic products, including the spraying products, application is directly to the skin, resulting in the dermal route being the main route of exposure. Although a part of the applied dose will evaporate and thus lead to inhalation exposure, this part is considered to be very small compared to the dermal exposure part because of the low volatility of musk xylene (vapour pressure 0.00003 Pa and Henry coefficient 0.0595 Pa.m^3/mol). In a draft report by Hellewegen and van Bergen (draft 5, 2000) it was in fact shown that 2-benzylidene octanal, a substance with a vapour pressure and log K_{ow} similar to musk xylene, evaporation from the skin was only 7%.

It should be noted that the SCCNFP (1999), based on the retention of musk xylene in human fat and its excretion in human milk (see Section 4.1.1.4 below) and the findings in long-term studies with mice (see Section 4.1.2.7), recommended that the exposure of consumers due to the cosmetic use of musk xylene should be reduced by 50%. If this measure comes into effect, the exposure would drop to 206.3/2 = 103.2 µg/kg bw/day.

4.1.1.3.3 Potential exposure to fragrances in detergents

The total fragrance level in detergents is usually around 0.3%. In case musk xylene is used in fragrances the upper level of use (97.5 percentile) is reported as 7.1% (data from industry). This

corresponds to 213 mg musk xylene/kg washing powder. Using a dilution factor of 100 (e.g. 100 g of washing powder (TDG value) in one bucket of 10 L water) and assuming that 1 kg washing powder corresponds roughly with 1,000 cm^3, the skin is exposed to 0.00213 mg/cm^3 of product. The skin is only exposed to a 0.01 cm thickness of layer of product in contact with the skin. Therefore the exposure is 0.0000213 mg/cm^2. The exposed area is 840 cm^2 (hands both sides), therefore the total exposure will be 0.0179 mg/event. This latter value corresponds to 0.3 μg/kg bw (assuming a body weight of 60 kg and washing is done every day). This value is considered negligible compared to the cosmetic use (see Section 4.1.1.3.2).

4.1.1.3.4 Potential exposure to fragrances in air fresheners

According to industry musk xylene is used as a fragrance in air fresheners. Air freshener aerosol may contain up to 1% of fragrance. The amount of musk xylene in the fragrance is 7.1%. The estimated worst-case exposure to musk xylene in air freshener is 6.6 μg/kg bw/day, assuming 5 g air freshener/event (comparable to hair spray), one event/day, in a living room of 30 m^3, an exposure period of 4 hours, an inhalation rate of 20 m^3/day, a body weight of 60 kg and not taking into account ventilation and deposition. Air freshener will not be included as a separate scenario as the exposure to air freshener is very small compared to the exposure to cosmetics (see Section 4.1.1.3.2).

4.1.1.3.5 Other products

Musk xylene was also found in incense sticks in one kind of Chinese incense but not in others (Roveri et al., 1998), and it was found in betel quid and/or perfumed tobacco (Käfferlein et al., 1998). This exposure is considered minor compared to the exposure via cosmetics.

4.1.1.3.6 Summary

The dermal exposure of consumers to musk xylene via cosmetics amounts to 210 μg/kg bw/day. Compared to this value, the exposure of consumers to musk xylene in detergents, air fresheners and other products is negligible. Therefore only the figure of 210 μg/kg bw/day is taken forward to the risk characterisation.

4.1.1.4 Indirect exposure via the environment

<u>EUSES calculations</u>

In the EUSES model, a log K_{ow} value of 4.9 has been used as being representative for distribution in the environment. A measured fish bioconcentration factor of 4,400 L/kg (see Section 3.1.1.3) has been used in the EUSES model to estimate the concentration in wet fish. For other parts of the food chain, particularly root crops, leaf crops, meat and milk, EUSES estimates the concentrations in these food products using methods that, similar to the fish BCF, rely on log K_{ow} as no equivalent measured accumulation factors exist for these routes.

The concentrations given in **Table 4.5** for formulation, private use and regional exposure scenarios have been used to estimate the daily human intake in food. The estimated daily human intake using these figures is shown in **Table 4.6**.

Table 4.5 Estimated concentrations of musk xylene in food for humans.

Lifecycle step	Site	Estimated concentration in human intake media						
		Wet fish (mg/kg)	Root crops (mg/kg)	Leaf crops (mg/kg)	Drinking water (mg/l)	Meat (mg/kg)	Milk (mg/kg)	Air (mg/m³)
Formulation	Site 3	0.791	0.015	0.173	8.99e-5	0.0234	7.4e-3	3.43e-5
Private use		4.23	1.2	1.29e-3	1.85e-3	4.31e-4	1.36e-4	4.41e-8
Regional		0.791	0.405	5.59e-4	6.26e-4	2.64e-4	8.36e-5	3.95e-8

Table 4.6 Estimated human daily intake of musk xylene via environmental routes.

Lifecycle step	Site	Estimated human daily intake (mg/kg body weight/day)[1]							
		Wet fish	Root crops	Leaf crops	Drinking water	Meat	Milk	Air	Total
Formulation	Site 3	1.3e-3	8.22e-5	2.97e-3	2.57e-6	1.01e-4	5.93e-5	7.35e-6	4.52e-3
Private use		6.95e-3	6.56e-3	2.2e-5	5.28e-5	1.85e-6	1.09e-6	9.46e-9	0.0136
Regional sources		1.3e-3	2.22e-3	9.59e-6	1.79e-5	1.14e-6	6.7e-7	8.46e-9	3.55e-3

1 Daily intake of: drinking water 2 L/day, fish 0.115 kg/day, leaf crops 1.2 kg/day, root crops 0.384 kg/day, meat 0.301 kg/day, dairy products 0.561 kg/day. Inhalation rate: 20 m³/day. Bioavailability for oral uptake: 1. Bioavailability for inhalation: 0.75. Body weight of human: 70 kg.

For most fragrance compounding sites the estimated daily intake can be mainly attributed to the intake via leaf crops and fish. The leaf and root crops are solely exposed via air (almost 100%). The highest exposure via formulation is for site 3 (4.52e-3 mg/kg bw/day).

Private use gives a total daily intake of 0.0136 mg/kg bw; intake is mainly derived via fish and root crops. Musk xylene was not coming from air but from poor water concentrations and so via application of sludge on agricultural soil.

As private use shows the highest total daily intake of all life cycle steps the value of 0.0136 mg/kg bw will be taken further into the risk characterisation for local use.

End product formulation (local) is not further taken into consideration, because the total daily intake is lower than that for private use.

The regional concentrations are relatively high for root crops and fish and they attribute for 99% to the total daily intake of 3.55e-3 mg/kg bw. The musk xylene in crops is mostly derived from pore water (application of sludge) and less from air.

Although the EUSES calculations indicate that the consumption of fish is an important exposure route for musk xylene, Sönnichsen et al. (1999) and Käfferlein et al. (1998) did not find a correlation of fish consumption with levels of musk xylene in human milk and human plasma, respectively (see below).

Drinking water concentrations

No data on musk xylene in drinking water were available. The amounts of musk xylene in surface water are usually at or below the detection limit being around 0.01 µg/L (see Table 3.2 for details).

Literature data on food concentrations

Monitoring data for musk xylene in aquatic biota are summarised in **Table 3.6** Käfferlein et al. (1998) reported that musk xylene was not detected in meat, milk and eggs. No other data are available.

Exposure via mother's milk

A recent study on synthetic musk fragrances in human milk was carried out by Sönnichsen et al. in 1998. From 108 women milk was taken and analysed for several polycyclic musks and nitromusks. To avoid contamination of the milk sample by musk clinging to the skin, the breasts were cleaned three times with a cotton swab before sampling. After sampling, various measures were taken to minimise contamination of the milk samples with synthetic musks from the environment. The women were also asked to report on their use of fragranced cosmetics and household products as well as their fish consumption. A mean and a median fat content of 3.67 and 3.40%, respectively, were found in the mother's milk. The concentration of musk xylene in breast milk showed a mean value of 7.43 µg/kg milk fat with a standard deviation of 9.58. The minimum and maximum value found were close to zero (detection limit <1 µg/kg milk fat) and 68.3 µg/kg milk fat, respectively. There was no convincing correlation of musk xylene levels in the milk with maternal age, body mass, loss of body mass and weight and pregnancy and lactation variables. It was stated that there was no significant correlation with the use of skin products and with fish consumption (this could not be verified as the report was not complete).

Remark: More or less the same results were reported by Liebl et al. (2000), who investigated 40 human breast milk samples (taken in 1997/1998 from healthy nursing mothers at a paediatric hospital) under carefully controlled sampling and analytical procedures to avoid secondary contamination. The mean fat content of the milk was 3.69%, while the mean musk xylene concentration was 8.6 µg/kg milk fat (range 1.3-47.9 µg/kg milk fat). Given the similarity in results, also for other nitromusks and polycyclic musks (especially the maximum values found), and the same authors involved in both studies, it is very well possible that the 40 samples examined by Liebl et al. (2000) were part of the 108 samples examined by Sönnichsen et al. (1999).

Other literature data from the early to mid nineties are shown below in the sequence of years that the milk samples were taken. Milk samples (391) from nursing mothers living in Southern Bavaria (Germany) were analysed for musk xylene in 1991 (48 samples) and 1992 (343 samples). Levels of musk xylene varied from 10 to 1,220 µg/kg milk fat, with a mean of 100 µg/kg milk fat (one outlier of 1,220 µg/kg milk fat, rest <700 µg/kg milk fat; 90- and 95-percentile 210 and 290 µg/kg milk fat, respectively) (Liebl and Ehrenstorfer, 1993). In another study in 23 human milk samples from 1992/1993 in Northern Germany, musk xylene levels up to 190 (average 78) µg/kg milk fat were found by Rimkus et al. (1994). These milk samples showed fat contents of 0.1 – 5.1%. Käfferlein et al. (1998) showed the presence of musk xylene in mother's milk of various research-centres throughout Germany between 1992-1994. The mean concentrations varied from 11-90 µg/kg milk fat (with one extreme mean value of 245 µg/kg

milk fat due to an extremely high individual value of 3 mg/kg milk fat). Ott et al. (1999) found mean levels of 41 µg musk xylene/kg milk fat in human milk samples (n=55) from women in Middle Hesse, Germany in 1995 (maximum level approximately 250 µg/kg milk fat). In a 1993/5 survey of human milk (n=73 samples from different European countries), musk xylene levels varied from approximately 25-260 µg/kg milk fat, with a mean of approximately 50 µg/kg milk fat. The mean fat content of the milk was 3.1% (Ramseier et al., 1998). Käfferlein et al. (1998) also showed that the concentration of musk xylene decreased during the years 1992-1996/1997 in Bavaria and Baden-Württemberg. In 1996/1997 the average values shown were approximately 10 and 20 µg/kg milk fat for Baden-Württemberg and Bavaria, respectively.

Remark 1: It must be noted that in the above mentioned studies nothing has been reported on the measures taken to prevent contamination of the milk samples during collection, handling and processing.

Remark 2: The studies by Liebl and Ehrenstorfer (1993) and Rimkus et al. (1994) have been heavily criticised by Lammi-Keefe (1995) and Jensen (1995). They argue that the data by these authors are only of the screening type and cannot be used quantitatively. This because data on the milk sampling procedures are lacking, and there is no information on whether or not appropriate quality control steps have been taken in the collection, handling and analysis of the milk samples. Lammi-Keefe and Jensen therefore have doubts on the representiveness and the volumes of the milk samples and they question the extraction techniques employed given the very low milk fat concentrations reported by Rimkus et al. (in general, milk fat concentrations range between 3 and 4% and milk fat concentrations <2% are extremely rare or just not seen). Besides, environmental contamination (from contaminants on the breast, the milk container or in the laboratory) cannot be excluded.

The data from the early to mid ninety studies show somewhat higher musk xylene levels in human milk than the levels found by Sönnichsen et al. in 1999 (and Liebl et al. (2000)). This might be due to differences in the methods used when taking and processing the samples and the (presumable) lack of precautions against environmental contamination in the earlier studies, but it may also be indicative of reduced exposure. Although the data presented by Sönnichsen et al. are probably more accurate, for a worst-case estimate of the exposure of infants to mother's milk, the data from the early to mid ninety studies, despite their shortcomings, are taken for risk characterisation.

The exposure to babies is calculated according to the WHO (1998) and is described here. For the first three months in life, an infant consumes an average of 120 grams per day of human milk per kilogram of body weight. After three months of age, the volume consumed per unit weight of the infant decreases with increasing age. By multiplying the concentration (given as mg/kg or mg/l) of a particular substance in whole breast milk by a factor of 0.12, the approximate daily intake of the substance in mg/kg bw can be estimated. If the contaminant concentration in the milk fat is not reported it is assumed that the average fat content of the milk is 3.5%.

Exposure to musk xylene in mother's milk is based on the highest mean and maximum concentrations found for musk xylene in the early to mid ninety studies (100 and 1,220 µg/kg milk fat, respectively) and the consumption of 0.120 kg milk/day per kg bw containing 3.5% fat:

Mean

100 µg musk xylene/kg milk fat = 100 · 0.120 · 0.035 = 0.42 µg musk xylene/kg bw/day.

Maximum

1,220 µg musk xylene/kg milk fat = 1,220 · 0.120 · 0.035 = 5.12 µg musk xylene/kg bw/day.

The exposure (worst-case estimate) via mother's milk for infants thus varies between 0.42 and 5.12 µg musk xylene/kg bw/day.

Human plasma and human adipose tissue

There is some information on musk xylene found in human plasma samples taken in 1993: levels ranged between <0.1 and 1.12 µg/l, with a median level of 0.24 µg/l (Käfferlein et al., 1998). No significant differences in human plasma levels could be found between men and women, smokers and non-smokers, and between groups with different fish consumption in this study. When the same authors examined human plasma samples from 1998, a remarkable decrease in musk xylene levels was found (range <0.1-0.29 µg/l, median <0.1 µg/l). The number of positive samples was also much lower (12% in the 1998 samples as compared to 72% in the 1993 samples). Again no differences were found between men and women and smokers and non-smokers. The authors explained the decrease in plasma musk xylene levels by the discontinued use of musk xylene in detergents in Germany since 1993 (Käfferlein and Angerer, 2001).

Musk xylene was determined in 32 human adipose tissue samples (13 from females and 19 from males) in Northern Germany. All samples were from 1992/3. Levels of musk xylene in adipose tissue varied from 20 to 90 µg/kg fat for males and from 20 to 220 µg/kg fat for females (Rimkus et al., 1994). Musk xylene was also detected by Müller et al. (1996) in 15 samples (5 from males, 10 from females) of human adipose tissue of different age groups (3-100 years). The samples were collected in Switzerland in 1983/4 and 1994 and musk xylene levels varied from 6.7 to 69 µg/kg fat (two outliers of 106 and 288 µg/kg fat).

In both studies no information on the habits of the donors was available so no relation between the levels found and e.g. the use of cosmetics and fish consumption could be drawn.

4.1.1.5 Combined exposure

It is possible that humans are exposed to musk xylene under different circumstances, e.g. via the workplace and from consumer products, or indirectly via the environment. A worst-case estimate for this combined (external) exposure would be the sum of the worst-case estimates for the three individual populations, i.e. 0.6 mg/kg bw/day (dermal, workplace) + 0.043 mg/kg bw/day (inhalation, workplace) + 0.21 mg/kg bw/day (dermal, consumers) + 0.0136 mg/kg bw/day (oral, locally via the environment).

4.1.2 Effects assessment: Hazard identification and dose (concentration)-response (effect) assessment

4.1.2.1 Toxicokinetics, metabolism and distribution

4.1.2.1.1 Studies in animals

Oral

Three male rats (Wistar, 6 weeks old) were given a single oral dose of 70 mg 5-tert-butyl-^{3}H-musk xylene/kg bw in olive oil. Urine and faeces were collected up to 7 days. On day 7 after administration blood was collected, after which the animals were sacrificed and several organs and tissues were collected for analysis. Four other male rats, which were bile duct cannulated, received a single oral administration of musk xylene of 50 mg/rat in olive oil. Bile was collected for 48 hours.

Results

About 50% of the dose was excreted into urine and faeces by 24 hours and almost 86% of the dose was recovered by 7 days. The excretion into urine and faeces was about 10.3% and 75.5%, respectively. About 2% of the dose remained in the carcass after 7 days. It is stated that the major route of musk xylene excretion was the faeces via the bile. However, no quantitative data were available. Concentrations in adipose tissue and liver were 3.8 and 2.9 times the blood level, respectively. The levels in kidneys and lungs were somewhat higher than the blood level. The other tissues and organs had similar or lower levels than the blood level (concentrations or absolute figures were not available) (Minegishi et al., 1991).

Six male rats (Wistar, 6 week old) were given oral doses of 200 mg/kg bw of musk xylene in 0.5 ml olive oil for 2 weeks (5 days per week). Urine and faeces were collected for identification of metabolites. Bile from four bile duct cannulated rats (see study above) was also analysed for metabolites. Quantitative information with respect to relative importance of the various metabolites was not provided, but the authors (Minegishi et al., 1991) stated which the main excretion products via all three routes were. In the following text these are indicated by "[M]". Musk xylene itself [M], 2-acetylamino-5-tert-butyl-1-methyl-3-hydroxymethyl-4,6-dinitrobenzene [M], 2-amino-5-tert-butyl-1-methyl-3-hydroxymethyl-4,6-dinitrobenzene [M] and 2-Amino-5-tert-butyl-4,6-dinitro-m-xylene [M] were found in faeces, bile, and urine. 4-Amino-5-tert-butyl-4,6-dinitro-m-xylene and an unidentified metabolite were found in faeces and urine. 2-Amino-5-tert-hydroxybutyl-4,6-dinitro-m-xylene was found in bile and urine and 4-amino-5-tert-butyl-1-methyl-3-hydroxymethyl-2,6-dinitrobenzene was found in urine. Another metabolite was identified as either 5-tert hydroxybutyl-1,3-dimethyl-2,4,6 trinitrobenzene or 1-methyl-3-hydroxymethyl-5-tert-butyl-2,4,6 trinitrobenzene, which was excreted as unspecified conjugation product in the bile. Conjugation with other metabolites was not observed (Minegishi et al., 1991).

Remark: The last metabolite mentioned (1-methyl-3-hydroxymethyl-5-tert-butyl-2,4,6 trinitrotoluene) is hydroxymethyl musk xylene, which has been tentatively identified as a biliary metabolite in the rat by Hawkins et al. (1984) and Hawkins and Ford (1999).

Bioaccumulation in blood and tissues was measured by GC-ECD in adult and developing Long Evans rats. Males and females were fed a diet containing musk xylene at 1, 10, 33 or 100 mg/kg feed for 10 weeks before mating. Treatment continued during pregnancy and lactation. Pups were killed at postnatal days 1 or 14. Offspring exhibited dose dependent musk xylene accumulation with 1/2 - 3/4 of adult female or 3-4 times adult male body fat levels at 100 mg/kg feed. Musk xylene levels in milk were comparable to adult female adipose tissue levels. In rats fed musk xylene in adulthood, levels were highest in adipose tissue with significant amounts in other organs (ovary, adrenal). In females tissue levels were 3.7-6.8 times higher than in males. This sex difference is not explained, but according to the authors it was unrelated to body fat content and unlikely to be related to differences in rate of elimination. The sex difference was absent in the offspring (Suter-Eichenberger et al., 1998). The levels of musk xylene in various tissue samples are given in **Table 4.7**:

Table 4.7 Musk xylene levels in tissues and milk of adult male and female Long Evans rats and in offspring.

	Musk xylene level[1] (mg/kg tissue)					Musk xylene level[1] (mg/kg lipid)	
	CNS[2]	Kidney	Liver	Ovary / Testes	Adrenal	Fat	Milk
adult rats fed dietary musk xylene at 100 mg/kg feed for 10 weeks (pre-mating)							
males (n=4)	0.4 ± 0.1	1.0 ± 0.2	0.7 ± 0.1	0.4 ± 0.3	4.3 ± 2.8	36.9 ± 9.6	
females (n=4)	2.4 ± 0.3	3.8 ± 1.7	4.4 ± 0.5	17.4 ± 3.9	23.1 ± 3.8	162.0 ± 24.2	
one day old pups of dams fed dietary musk xylene at 100 mg/kg feed for 10 weeks pre-mating + gestation							
(n=4)							218.9 ± 52.3
14 day old pups of dams fed dietary musk xylene at 100 mg/kg feed for 10 weeks pre-mating + gestation and lactation							
males (n=8)						120.5 ± 39.4	
females (n=8)						115.3 ± 29.7	

1 Data are rounded means ± standard deviation.
2 CNS : central nervous system.

Dermal

The absorption, distribution and excretion of radioactivity have been measured after topical application of 0.5 mg ring-^{14}C-musk xylene/kg bw to the shaven backs of 16 CD Sprague-Dawley and 5 Long-Evans rats. The doses were prepared by dissolving ^{14}C-musk xylene (in a mixture of phenylethyl alcohol and ethanol). The dose was applied evenly over an area of 9 cm^2. The application rate was 0.01 mg/cm^2. The treated area was covered by occlusive dressing. After a 6 hour application the dressing was removed and the area of treated skin was wiped with cotton wool containing 1% ethanolic phenylethyl alcohol. The bile ducts of two male rats (one CD and one Long-Evans) were cannulated immediately before dosing. Urine, faeces and expired air were collected for rats killed 6 hours after dosing or later. Pairs of CD rats were killed at 1, 3, 6, 8, 24, 48, 96 and 120 hours after start of dosing and the Long Evans rats were killed at 6, 24, 48, 96 and 120 hours after start of dosing. Prior to sacrifice blood was withdrawn and analysed. Tissues were analysed for radioactivity.

Result: About 8% of the applied dose was absorbed from the shaven backs during 6 hours. About 14% of the dose remained in the skin after the washing which continued to be absorbed. A total of about 20% of the dose was absorbed during 48 hours with 2% remaining in the skin. The results are similar for both strains of rats.

In CD rats' means of 3.9% and 15.2% of the applied dose had been excreted in the urine and faeces, respectively, after 120 hours with only about 0.2% remaining in the carcass. In Long-Evans rats the rate of elimination was very similar with 4.0% and 14.0% of the dose excreted during 5 days in the urine and faeces, respectively. Most of the radioactivity was eliminated in the first 48 hours after start of dosing. Between 48 and 120 hours <0.5% and <3% of the dose was excreted in the urine and faeces, respectively. Radioactivity was not detected in expired air. Radioactivity was detected in nearly all the tissues of animals killed after 24 hours. Concentrations were highest at about 8 hours after start of dosing and then declined steadily. Highest concentrations after 8 hours were present in the gastro-intestinal tract (0.868 µg equivalents/g), adipose tissue (0.159 µg/g), liver (0.062 µg/g), and pancreas (0.0425 µg/g), kidneys (0.0265 µg/g) of CD rats. Levels of radioactivity in these tissues remained the highest throughout the study. Concentrations of radioactivity in the adrenals (0.0685 µg/kg after 8 hours) and the thyroid (0.0696 µg/kg) were also among the highest up to 24 hours but then declined rapidly. The distribution in the Long-Evans rats was very similar with the exception of the eyes. In the albino CD rats the concentration of radioactivity in the eyes reached a peak of 0.0044 µg/kg after 24 hours declining to below the level of determination after 48 hours, while in the pigmented Long-Evans rats the concentration of radioactivity in the eyes rose gradually throughout the 5 day period from 0.0041 µg/kg at 6 h to 0.0064 µg/kg after 120 hours. In the bile duct cannulated rats radioactivity was excreted in the bile at a steady rate of ca. 1.4% of the dose per hour in both CD and Long-Evans rats. The concentrations of radioactivity in the urine of the bile duct cannulated rat was only 0.21% of the dose during 24 hours and the cannulated Long-Evans rat excreted a total of 0.3% of the dose in urine during 48 hours, which suggests an enterohepatic cyclus. One metabolite, a glucuronic acid conjugate, presumably of hydroxymethyl-musk xylene, accounted for >50% of the radioactivity found in bile and was apparently deconjugated and further metabolised in the gastro-intestinal tract to at least four other chromatographically more polar compounds. Some of these components were at least partially reabsorbed giving rise to a complex profile of urinary metabolites (Hawkins et al., 1984; Hawkins and Ford, 1999).

Ring-labelled ^{14}C-musk xylene (in phenylethyl alcohol and ethanol) was applied under occlusion to the shaven backs (area about 9 cm^2) of 10 male Sprague-Dawley CD rats up to fourteen daily 24-hour doses of 0.5 mg/kg bw. The skin remained unrinsed between the applications. Two rats were killed for whole-body autoradiography, one 24 hours after the first dose, and the other 24 hours after the 14th dose. From the remaining 8 rats urine and faeces were collected at several time points, and at sacrifice samples of blood, treated skin, brain, kidney, liver, thyroid, and fat were taken.

Whole-body autoradiography showed that at 24 hours after the first dose radioactivity was not widely distributed throughout the body. Relatively high concentrations were present at the site of application and in the small intestine contents, large intestine contents and bile ducts. Lower levels were present in the nasal turbinate. Tissues of the rat killed at 24 hours after the 14th dose generally contained marginally more radioactivity, the highest concentrations still associated with the site of application and the gastro-intestinal tract and lower levels present in liver, thyroid, and nasal turbinate. Hence, the absorption of radioactivity was incomplete, given the large amounts of the applied radioactivity remaining at the site of application.

Means of 1.42 µg and 2.42 µg musk xylene equivalents were excreted in urine and faeces, respectively, in the 24 hours following the application of dose 1. The mean rate of excretion of radioactivity in urine was maximal (about 2.65 µg/day) in the 24-hour periods following the application of doses 10 and 14, but the data suggest that a steady state of urinary excretion was

reached at about 7 days after the administration of dose 1. The rate of excretion of radioactivity in faeces increased gradually being about 11-12 μg/day in the collections made after application of doses 12 and 14, but apparently did not reach a steady state.

At sacrifice, the concentration of radioactivity in treated skin was high, whereas the total radioactivity present in blood and selected tissues was only a very small proportion of the total of 14 applied doses (0.19-0.44% in fat, and even less in liver, blood, kidneys, brain, and thyroid) (Hawkins et al., 1989; Hawkins and Ford, 1999).

A bile duct cannulated rat received a single dose of 5.87 mg ring-labelled ^{14}C-musk xylene (in phenylethyl alcohol and ethanol)/kg bw on the shaved skin of the back. Bile was collected and samples were treated with β-glucuronidase and extracted with ethyl acetate. Extracts of β-glucuronidase treated rat bile showed one major metabolite, which was identified as hydroxymethyl-musk xylene (Hawkins et al., 1989).

Inhalation

No data available.

Intravenous

A group of four male Sprague-Dawley CD rats received a single intravenous administration of ring-labelled ^{14}C-musk xylene (0.5 mg/kg bw in polyethylene glycol, aqueous sodium chloride and ethanol). Blood samples were taken at 5, 30 and 90 min and at 3, 6, 24, 48, 72, 96, 120, 168 and 240 hours after dosing. The mean concentration of radioactivity in plasma 5 minutes after injection was 0.398 μg musk xylene equivalents/ml. The mean concentration of radioactivity in plasma of each rat then declined and increased again to a second peak of 0.101 μg/ml at 6 hours after dosing. The concentration of radioactivity subsequently declined in an apparently multi-exponential manner to levels below the limit of detection at 160-240 hours, with a mean half-life of 42.6 hours. The mean area under the curve was 3.22 μg.h/ml (Hawkins et al., 1989).

Special investigation

Musk xylene was administered by gavage to pregnant Charles River CD rats (n=15/group) at 2.5 and 25 mg/kg bw as a solution in corn oil, daily from day 14 of gestation up to day 21 *post partum*. Milk samples of ca. 0.5 ml were obtained manually from 3 dams per dose level per time point (after administration of oxytocin) at 1, 2, 4, 8 and 24 hours after dosing on days 7 and 14 *post partum*. Highest mean concentrations of musk xylene were found in the 8 hour samples, declining more than 7-fold by 24 hours after dosing, by which time musk xylene was still quantifiable in all animals (see **Table 4.8**). This suggests that the F_1 progeny is continuously exposed to musk xylene in the milk throughout the period of lactation investigated. The rate and extent of systemic exposure on days 7 and 14 increased approximately proportionally with dose (Brooker et al., 1998).

Table 4.8 Mean (n=3) concentrations in milk (in μg/ml).

Sample time (h)	Day 7 – Dose level (mg/kg bw)		Day 14 – Dose level (mg/kg bw)	
	2.5	25	2.5	25
1	0.80	26.12	0.89	34.15
2	1.46	22.40	2.17	47.47
4	2.32	42.87	2.10	77.87
8	8.12	84.94	6.44	80.93
24	0.67	2.48	0.96	1.60
AUC24 (μg.h/ml)	97	1,059	83	1,162

In vitro

Freshly obtained circles (1.7 cm diameter) of full thickness dorsal skin of male F344 rats were placed into flow-through diffusion cells of an *in vitro* skin absorption model. Skin surface temperature was maintained at 32°C by a water circulator, and a receptor fluid of 50% v/v aqueous ethanol flowed across the underside of the skin at a rate of 1.5 ml/h. ^{14}C-musk xylene (place of labelling not given) was applied to the skin surface in an ethanol:diethylphthalate (75:25) vehicle as 0.1% and 0.5% dose solutions (15 and 78 μg/cm^2, respectively), and the skin was either occluded with a teflon cap or left open to the atmosphere (unoccluded). Receptor fluid was collected every 2 hours for up to 72 hours. At the end of the experiment the skin surface was washed and swabbed, after which the skin was digested in methanolic sodium hydroxide. Radioactivity in receptor fluid, skin washes and skin was determined by liquid scintillation spectrometry.

Total recovery of radioactivity was low, although still better than 80%. After 24 hours, musk xylene was poorly absorbed through unoccluded skin, given that on average 0.61 ± 0.16% was found in the receptor fluid. Occluding of the skin did not really affect this absorption at 24 hours (1.84 ± 1.15% on average). Significant amounts of radioactivity were recovered from within the skin (at 24 hours, 43% in unoccluded skin and 30% in occluded skin). Over 48 hours, musk xylene continued to be absorbed into the receptor fluid and the total absorption at 48 hours was enhanced by occlusion (Ashcroft and Hotchkiss, 1996).

Remark: it is not clear for which dose solution the results are given. No data were presented for the 48-72 hour time period.

In another *in vitro* experiment to investigate the percutaneous absorption ^{14}C-musk xylene (approx. 3 μg/cm^2) was applied to hairless guinea pig skin or human skin in the form of an occluded thin film. Two vehicles were simultaneously evaluated: a methanol vehicle and an oil in water emulsion vehicle. After 24 hours the total absorption (skin and receptor fluid) of musk xylene in hairless guinea pig skin was 55% from the emulsion vehicle and 45% from the methanol vehicle. With human skin, permeation of musk xylene from both vehicles was 22% of the applied dose. When human studies were continued for an additional 6 days after skin surface washing only 6% of the applied dose remained in the skin. A permeability constant for musk xylene from isopropylmyristate permeation through hairless guinea pig skin was $6.86 \cdot 10^{-5}$ cm/hr, under steady state conditions (Hood et al., 1996).

To determine the penetration rate into and through skin, 3% and 10% solutions of ^{14}C-musk xylene in ethanol/acetone (1:1) (corresponding to doses of 180 and 600 μg/cm^2) and a 10%

solution of ^{14}C-musk xylene in benzoeacid benzyl ester (corresponding dose 600 µg/cm^2) were applied to explanted mini pig skin (area 5 cm^2) with intact as well as with removed stratum corneum. The contact time was maximally 16 hours for the intact skin and 6 h for the skin from which the stratum corneum was removed. After exposure of intact skin, the total penetration rate, as calculated separately from the total amounts in stratum corneum and the living skin layers (epidermis, corium and subcutis), was very low for all tested solutions. The penetration into living skin layers seems to be more dependent on the dose than on contact time and vehicle type (see **Table 4.9**). The stratum corneum acts as a very effective penetration rate limiting membrane: when it was removed before exposure, about 5 times more musk xylene was found in the living skin layers than in skin on which the stratum corneum was present during exposure (see **Table 4.10**) (Klecak, 1982).

Table 4.9 Penetration rate into layers of intact explanted mini pig skin.

Contact time	30 mg/ml in ethanol / acetone (1/1, v/v)		100 mg/ml in ethanol / acetone (1/1, v/v)		100 mg/ml in benzoeacid benzyl ester	
	Stratum corneum	Living skin layers	Stratum corneum	Living skin layers	Stratum corneum	Living skin layers
1 hour	5.4%	1.3%	2.6%	1.0%	1.9%	0.3%
6 hours	7.2%	1.9%	2.9%	1.1%	3.4%	0.4%
16 hours	8.7%	2.8%	3.1%	1.3%	4.1%	0.5%

Percentages in this table refer to the applied dose.

Table 4.10 Penetration rate into explanted mini pig skin layers with or without stratum corneum.

	30 mg/ml in ethanol / acetone (1/1, v/v)		100 mg/ml in ethanol / acetone (1/1, v/v)	
	Intact skin exposed *	Living skin layers exposed	Intact skin exposed*	Living skin layers exposed
stratum corneum (6 h)	7.2%	-	2.9%	-
living skin layers (6 h)	1.9%	9.0%	1.1%	5.1%

* Values taken from Table 4.9; percentages in this table refer to the applied dose.

4.1.2.1.2 Studies in humans

Oral

The pharmacokinetics of musk xylene was determined after a single oral intake of ^{15}N-labelled musk xylene by three male volunteers (0.05 to 0.14 mg/kg bw; dissolved in 30% ethanol). The plasma elimination half lives amounted to 60, 67, and 94 days, respectively. The values were found to be proportional to the body fat concentration of the volunteers. A biological half-life of 60 days corresponds to a body fat concentration of 15%, 67 days to 21% fat and 94 (107 is also reported) days to 25% fat. The human distribution pattern in blood was as follows: 10% in cells, 8% in VLDL and chylomicrons, 13% in LDL, 10% in HDL and 59% in the rest of the plasma most likely associated with proteins (Kokot-Helbling et al., 1995a/b).

A single dose of 0.3 mg/kg bw of ^{15}N-labeled musk xylene (> 99% ^{15}N) was given to six volunteers (three male and three female) by the oral route. Urine was collected for 96 hours after exposure. Blood samples were taken at intervals for up to 140 days after administration. The metabolite 1-tert-butyl-3,5-dimethyl-^{15}N-4-amino-2,6-dinitrobenzene ("^{15}N-p-NH$_2$-musk

xylene") in urine and ^{15}N-musk xylene in plasma were quantified by gas chromatography/electron-capture mass spectrometry.

Peak plasma concentrations of ^{15}N-musk xylene were reached at 6 hours post-dosing and amounted to 36-262 ng/ml plasma. Based on these plasma peak levels and an estimated total plasma volume of about 5% of the body weight it was estimated that 0.6 to 3.8% of the dose was absorbed after oral administration.

The elimination of ^{15}N-musk xylene in plasma could be described by a two-compartment kinetic model with an initial rapid decrease with a plasma half-life of about 11 hours over the first 30 hours which increased to a terminal plasma half-life of 70 days. The amount of ^{15}N-p-NH_2-musk xylene in recovered urine represented 0.1-0.5% of the oral applied dose of ^{15}N-musk xylene. After a short time of invasion the concentrations of ^{15}N-p-NH_2-musk xylene in urine reached a maximum 18-24 hours after administration. The elimination of the metabolite occurred by first-order kinetics with an average elimination half-life of 11.8 hours.

Urinary p-NH_2-musk xylene was the only metabolite which was reported. The rat metabolite N-acetyl musk xylene was not observed. Other rat metabolites (i.e. hydroxylation products) were not studied.

After the single oral dose of ^{15}N-musk xylene, ^{15}N-p-NH_2-musk xylene was not detected in hemoglobin. However, hemoglobin samples contained unlabeled p-NH_2-musk xylene (11.4-18.9 fmol/mg Hb), likely derived from chronic environmental exposures (Riedel and Dekant, 1999)

Remark: The estimates of oral uptake percentages may be too low, because they were based on plasma peak levels and an estimate of the total body plasma volume. However, for a lipophylic substance like musk xylene the volume of distribution is likely to be higher than the volume of the plasma compartment.

Dermal

Two healthy adult male subjects received an application of 1 mg ring-^{14}C-musk xylene (^{14}C-musk xylene was dissolved in a mixture of ethanol and phenylethyl alcohol at a concentration of 1 mg/ml) on the unshaven skin of the upper quadrant of the chest (area of 100 cm^2) for 6 hours. Then the dressing was removed from the test area and the treated skin was wiped. Urine and faeces were collected up to 120 hours after application. Blood samples were withdrawn before dosing and again at 16 times during 120 h after application. After 120 hours the treated area of skin was stripped with adhesive tape and the stripes were analysed.

^{14}C-Musk xylene was very poorly absorbed from the skin; 90% of the applied dose was recovered from the site of application after 6 hours. After 120 hours a mean of only 0.26% and <0.1% of the dose had been excreted in the urine and faeces, respectively. No radioactivity was detected in any of the plasma or whole blood samples or in the skin strips (Hawkins et al., 1984; Hawkins et al., 2002).

When urine samples of one of the volunteers from the study above were extracted with ethyl acetate the recovery of the radioactivity was low (about 15%). When urine samples were treated with β-glucuronidase and extracted with ethyl acetate the recovery was about 88%, indicating that a large proportion of the metabolites of musk xylene in human urine were present as glucuronide conjugates. Extracts of β-glucuronidase treated human urine contained a single major (unidentified) metabolite which was not present in rat bile extract (Hawkins et al., 1989; Hawkins et al., 2002).

Riedel and Dekant (1999) reported also the results of kinetic studies in human volunteers following a single dermal dose of 0.3 mg/kg bw of ^{15}N-labeled musk xylene (> 99% ^{15}N). The study was according to the same protocol as that used for its oral counterpart (see under "oral").

Peak plasma concentrations of ^{15}N-musk xylene were reached after 6 hours post dosing and amounted to 1.6-5.5 ng/ml plasma. Based on these plasma peak levels and an estimated total plasma volume of about 5% of the body weight it was estimated that 0.03 to 0.06% of the dose was absorbed after dermal administration.

The elimination of ^{15}N-musk xylene in plasma could be described by a two-compartment kinetic model with an initial rapid decrease with a plasma half-life of about 11 hours over the first 30 hours which increased to a terminal plasma half-life of 70 days. The amount of ^{15}N-p-NH_2-musk xylene in recovered urine represented, 0.02-0.16% of the dermally applied dose. After a short time of invasion the concentrations of ^{15}N-p-NH_2-musk xylene in urine reached a maximum 18-24 hours after administration. The elimination of the metabolite occurred by first-order kinetics with an average elimination half-life of 11.8 hours.

Urinary p-NH_2-musk xylene was the only metabolite which was reported. The rat metabolite N-acetyl musk xylene was not observed. Other rat metabolites (i.e. hydroxylation products) were not studied. Because p-NH_2-musk xylene was observed after both dermal and oral administration (described under "oral") the study authors hypothesised that in humans hepatic nitroreductases may have been responsible for these metabolites, rather than intestinal flora (cf the situation in rodents). However, a minor contribution of nitroreduction by gut flora was not completely ruled out.

After the single dermal dose of ^{15}N-musk xylene, ^{15}N-p-NH_2-musk xylene was not detected in hemoglobin. However, hemoglobin samples contained unlabeled p-NH_2-musk xylene (11.4-18.9 fmol/mg Hb), likely derived from chronic environmental exposures (Riedel and Dekant, 1999)

Remark: The estimates of dermal uptake percentages may be too low, because they were based on plasma peak levels and an estimate of the total body plasma volume. However, for a lipophylic substance like musk xylene the volume of distribution is likely to be higher than the volume of the plasma compartment.

Inhalation

No data available.

Special investigations

Several studies have identified the presence of musk xylene in human milk and human adipose tissue (see Section 4.1.1.4 for more details). Recent results on synthetic musk fragrances in human milk come from the study by Sönnichsen et al. (1999), who took milk samples from 108 women and analysed these for several polycyclic musks and nitromusks. The concentration of musk xylene in the milk showed a mean value of 7.43 µg/kg milk fat and a maximum value of 68.3 µg/kg milk fat. In earlier studies (early to mid nineties) somewhat higher values were found, with a highest mean and maximum concentration found of 100 and 1,220 µg musk xylene/kg milk fat, respectively.

In human adipose tissue, Rimkus et al. (1994) found levels of musk xylene varying from 0.02-0.22 mg/kg fat. Müller et al. (1996) found levels of 6.7-69 (2 outliers of 106 and 288) µg musk xylene/kg human fat.

Riedel et al. (1999) studied the occurrence of musk xylene-derived materials in hemoglobin samples taken from 10 human volunteers not "knowingly" (probably meaning "not occupationally") exposed to musk xylene. The metabolite p-NH_2-musk xylene appeared to be covalently bound to their hemoglobin in concentrations ranging from 13.3 to 45.9 fmol/mg hemoglobin.

4.1.2.1.3 Summary of toxicokinetics, metabolism and distribution

There are no data available on the toxicokinetics of musk xylene after inhalation exposure.

After oral administration with ^{3}H-musk xylene to rats, the major route of excretion was via the faeces via the bile. Within 7 days, excretion into urine and faeces was approximately 10.3% and 75.5%, respectively, while about 2% remained in the carcass. Based on plasma peak levels, the estimated systemic availability of an oral dose in humans was 0.6 to 3.8%.

It is difficult to accurately estimate oral uptake percentages for rat and humans based on the available data. For humans, the calculated percentages are probably underestimations of the totally absorbed quantity because they are based on plasma levels and an assumed volume of distribution equal to the plasma volume. This volume of distribution is too low because musk xylene will preferably distribute into the fatty compartment. For the rat, based on the amount excreted in the urine and carcass, an oral bioavailability of at least 12% can be derived. This percentage is also an underestimate of the actual intestinal uptake, because biliary excretion is not accounted for. If it is assumed, however, that the contribution of the biliary excretion is equal after oral and dermal exposure (which seems reasonable in view of the long plasma half-life time (40 hours in rat, 60-94 days in humans) of musk xylene), the ratio urinary/faecal excretion after dermal exposure (viz. 4% / 15%) can be used to estimate the total uptake after oral exposure, when the experimental data of two different studies (Minegishi et al., 1991, Hawkins et al., 1984) are combined. The resulting estimate of total uptake after oral dosing is 10% + (15/4) · 10% = *ca.* 50%. For both rats and humans a percentage for uptake after oral exposure of 50% will be taken forward to the risk characterisation.

After a 6 hour dermal application of ^{14}C-labelled musk xylene (under occlusion) to rats about 20% of the applied dose was absorbed in 48 hours, with 2% remaining in the skin. Between 6 and 48 hours, the skin acted as a reservoir from which musk xylene continued to be absorbed. Excretion via urine and faeces (predominantly via bile) was virtually complete within 48 hours, with only very small amounts additionally excreted between 48 and 120 hours. After 120 hours, about 4% of the applied dose was excreted in urine and 14-15.2% in faeces, with only 0.2% remaining in the carcass. Radioactivity was detected in nearly all the tissues with peak concentrations after 8 hours in gastrointestinal tract followed by adipose tissue, liver, adrenals, thyroid, pancreas and kidneys.

After dermal application, ^{14}C-musk xylene was very poorly absorbed from the human skin as only 0.26 and <0.1% of the applied dose, respectively, was excreted in urine and faeces within 120 hours, and about 90% of the applied dose was recovered from the site of application. Based on plasma peak levels, the estimated systemic availability of a dermal dose in humans was 0.03 to 0.06%. These percentages are probably underestimations of the totally absorbed quantity because they are based on plasma levels and an assumed volume of distribution equal to the plasma volume. This volume of distribution is too low because musk xylene will preferrably distribute into the fatty compartiment.

In vitro experiments with skin from rats and humans also indicate that the percutaneous absorption of musk xylene from both occluded and unoccluded skin is poor, and that after removal of the test substance, the skin acts as depot from which musk xylene continues to be systemically released. For dermal absorption of musk xylene in rats and humans, values of 20% and 10% respectively, are taken forward to the risk characterisation.

Metabolism of musk xylene in rats involves both reduction of a nitro group to an amine and hydroxylation of methyl groups, hydroxymethyl-musk xylene being the main metabolite in bile. Human urine contained a single metabolite which was chromatographically distinct from both musk xylene and hydroxymethyl-musk xylene. Other studies showed the presence of p-NH_2-musk xylene in human urine, but not N-acetyl-musk xylene.

From studies with rats and humans, in which musk xylene was administered intravenously or orally, respectively, it can be concluded that the plasma half life of musk xylene in rats is about 40 hours, while the plasma half life in humans is in the range of 60 to 94 days.

When administered orally to rats from 10 weeks before mating through to lactation, musk xylene levels in adults were highest in adipose tissue (in females 3.7-6.8 times higher than in males) and in milk. Transplacental passage occurred as in the offspring musk xylene accumulated in body fat. Musk xylene is also found in human milk fat and in adipose tissue.

4.1.2.2 Acute toxicity

4.1.2.2.1 Studies in animals

Oral

In an acute toxicity test musk xylene was suspended in 0.25% arabic gum solution and groups of 5 male and 5 female mice were given by tube single doses of 125, 250, 500, 1,000, 2,000, or 4,000 mg musk xylene/kg bw. The mice were observed for 14 days. Tremor was observed 3-18 hours after treatment in males and females given 4,000 mg/kg bw. No abnormal symptoms were found in any of the other groups. One female at 4,000 mg/kg bw died. Macroscopic examination revealed no clear toxic lesions in any of the mice at the end of the study. The acute oral LD_{50} for mice is >4,000 mg musk xylene/kg bw (Maekawa et al., 1990).

Three groups of 6 male rats (strain not specified) were orally treated with 2,500, 5,000 or 10,000 mg musk xylene/kg bw in corn oil. At 10,000 mg/kg bw 3 rats died, at 5,000 mg/kg bw 1 rat and at 2,500 mg/kg bw no mortality was seen up to 7 days post treatment. Unspecified depression was seen, but it was not specified at which dose levels (Bukva et al., 1970).

Dermal

In a limitedly reported study, groups of three albino rabbits received a dermal application of 10,000 or 15,000 mg musk xylene/kg bw as a suspension in mazola oil for 24 hours under occluded conditions on intact skin. No mortality occurred and no signs of irritation were seen. Haematology and clinical chemistry parameters were not affected at 5 days post treatment (Fogleman and Margolin, 1970).

Inhalation

No data available.

4.1.2.2.2 Studies in humans

No data available.

4.1.2.2.3 Summary of acute toxicity

The acute oral LD_{50} in mice and rats was established at >2,000 mg/kg bw. In a limited dermal study an application of 10,000 or 15,000 mg/kg bw caused no mortality in groups of three rabbits. The dermal test is not performed according to current standards. However, it is expected that the acute dermal toxicity is >2,000 mg/kg bw. According to the EC criteria musk xylene needs not be classified for its acute oral and dermal toxicity.

Data for acute inhalation toxicity were not available.

4.1.2.3 Irritation/Corrosivity

4.1.2.3.1 Studies in animals

Skin

In a dermal LD_{50} study, musk xylene applied as a suspension in mazola oil to rabbit skin for 24 hours under occlusion at levels of 10,000 or 15,000 mg/kg bw was not irritating (Fogleman and Margolin, 1970).

Eye

In a study performed according to OECD guideline 405, each of six NZW rabbits received a dose of 0.075 g (0.1 ml weight equivalent) musk xylene in the conjunctival sac of the right eye. At 30 seconds post instillation, both eyes of three rabbits were rinsed with physiological saline. The eyes of the three remaining animals were not rinsed. The contralateral eye of each animal served as a control. Test and control eyes were examined for signs of irritation for up to 14 days following dosing.

In the non-rinse group musk xylene caused iritis (score 1) in all three rabbits at 1 and 24 hours. The iridal irritation resolved completely by the 48 hour scoring interval. Conjunctivitis (redness, swelling and discharge) score 1 was also noted in all three rabbits. This irritation was completely resolved in all animals by study day 10. In the rinsed group iritis (score 1) was seen in 1/3 test eyes at the 1, 24 and 48 hour scoring interval. Conjunctivitis (redness, swelling and/or discharge) was noted in all three test eyes only at the 1 hour scoring interval. The conjunctival irritation resolved completely in all animals by study day 14. Although there is some slight irritation observed musk xylene is not considered an eye irritant (Merriman, 1997).

Respiratory tract

No data available.

4.1.2.3.2 Studies in humans

When tested as 5% in petrolatum, musk xylene produced mild irritation when studied in a pre-test for sensitisation in a maximisation test according to Kligman (1966). Further details on this dermal irritation test were not available (Kligman, 1970).

In a patch test, concentrations of 0.1% and 1% produced irritation responses after 2 days of contact in 1% and 1.6% of the dermatological patients tested, respectively (Frosch et al., 1995). Further details on this study can be found in Section 4.1.2.4.

4.1.2.3.3 Summary of irritation / corrosivity

Base set requirements have not been met for testing of skin irritation as adequate skin irritation studies are lacking. The limited data available indicate that musk xylene was not irritating when applied to intact rabbit skin at extremely high dose levels under occlusive conditions for 24 hours. In two sensitisation studies with guinea pigs no indications were obtained for dermal irritation when applied at concentrations up to 10%. Musk xylene was also not found to possess dermal irritating properties in a 90 days dermal toxicity study at dose levels up to 240 mg/kg bw (see Section 4.1.2.5). However, in human volunteers, 5% musk xylene in petrolatum was reported to be mildly irritating, while in a patch test, concentrations of 0.1% and 1% produced irritation responses after 2 days of contact in 1% and 1.6% of the dermatological patients tested, respectively (see Section 4.1.2.4). Thus it appears that in humans musk xylene can induce skin irritation, albeit (very) mild. Primary irritation scores and other study details are not available and it is not possible to classify musk xylene for this property. However, based on the available information in animals it is not considered appropriate to require additional testing according to current guidelines, as even extremely high or prolonged dermal exposure did not elicit significant dermal reactions in rabbits or rats, respectively.

From a well performed eye irritation study it can be concluded that musk xylene is not eye irritating. According to the EC criteria musk xylene needs not to be classified for eye irritating properties.

For respiratory tract irritation no data are available.

4.1.2.4 Sensitisation and photoallergy

4.1.2.4.1 Studies in animals

Klecak (1979) reported the results of an open epicutaneous test with groups of 6 to 8 guinea pigs. Animals were exposed to concentrations up to 5% musk xylene, according to the authors the maximum non-irritating concentration in this animal species. Up to this level no signs of sensibilisation were obtained. The Klecak study is insufficiently documented to allow proper evaluation of its results.

In order to detect the potential of musk xylene to cause phototoxic, photoallergic, and contact sensitivity responses at a concentration known to induce a photoallergic response to musk ambrette (10% w/v in acetone), groups of 10 guinea pigs were treated with 10% w/v musk xylene in acetone by occluded patch for 4 hours/day, 3 times weekly for 3 consecutive weeks in the induction phase. The patches were applied to the clipped and depilated dorsal midline area between the shoulders. Subsequent to removal of the patches at each treatment, the sites of the selected treatment groups were irradiated for 2 hours with 12 blacklite lamps (UVA, 320-400 nm) via light wheel. Special patches were used to avoid skin damage produced by the combination of depilation, tape stripping and irradiation. Ten to 14 days after the final induction treatment, the test and naive control groups were challenged with 10% musk xylene in acetone by a single 4-hour occluded patch applied to a naive site that had been depilated. Upon patch removal, the sites of the selected groups were irradiated for 2 hours using the light weel with 12 blacklite lamps. The challenge sites were depilated again 18-20 hours following light exposure to allow scoring. All challenge sites were scored for severity of response at 24 and 48 hours after challenge. Results are given in **Table 4.11**. The positive results for regimen 2 and 4 suggest that musk xylene is only weakly allergenic with regard to delayed contact hypersensitivity; the responses were not exacerbated by UVA exposure (regimen 1, 3). Hence, in contrast to musk ambrette, musk xylene does not have the potential to produce photoallergy (Parker et al., 1986).

Table 4.11 Results of photoallergy testing musk xylene.

Test regimen	Induction conditions	Challenge conditions	Incidence
1	MX+UVA*	MX+UVA	0/10
2	MX+UVA	MX	2/10
3	MX	MX+UVA	0/10
4	MX	MX	1/10
5	Naïve	MX+UVA	0/10
6	Naïve	MX	0/10

* MX=musk xylene, UVA= 2 h UVA exposure

Remark: The number of animals tested in this non-adjuvans study is too small (a minimum of 20 is required according to the guidelines). This makes it difficult to interpret the response of 1/10 after induction and challenge (according to the guidelines a score of 15% (3/20) in a non-adjuvans test should be considered as positive). Besides, no primary irritation was reported at the concentration used for induction. Hence, it must be assumed that musk xylene was not tested at a concentration causing mild irritation, which might be induced at concentrations higher than 10%. A test with adjuvans would have been more appropriate.

Groups of 12 female Dunkin-Hartley guinea pigs with clipped and shaven interscapular skin were used for photoallergy tests. Inductions were performed using 0.1 ml of 10% musk xylene in dimethylacetamide/acetone/ethanol 4:3:3 for 25 minutes on an area of 900 mm^2 that had been defined by 4·0.1 ml injections of FCA. After 25 minutes excess substance was removed and the guinea pigs were irradiated with 100 $kJ.m^{-2}$ UV. This procedure, excluding injection of adjuvant, was repeated 24 hours later. Ten to 14 days after induction the guinea pigs were challenged using clipped and shaved lumbar skin with 0.1, 1 or 10% musk xylene. Thirty minutes later the animals were irradiated with 100 $kJ.m^{-2}$ UV. After irradiation the test substance was applied to fresh skin sites to check for contact sensitivity. Reactions in skin were observed up to 72 hours. A second confirmatory challenge was performed one week later. Photo-crossreaction to the known photoallergen musk ambrette was studied at the third challenge stage, using 1% concentrations of each substance.

After the first and second challenge one animal at 1% and one animal at 10% showed positive reactions. At 0.1% no reactions were seen. There was no contact sensitivity and musk xylene only weakly photo-crossreacted with musk ambrette (Lovell and Sanders, 1988).

Remark: The maximum non-photo-irritant concentration from preliminary photo-irritation tests was chosen as the concentration for induction and the maximum concentration for challenge. As musk xylene was not (photo)-irritant at the concentrations tested (up to an arbitrary upper limit of 10%), 10% was taken. This means that musk xylene was not tested at a concentration causing mild irritation, which might be induced at concentrations higher than 10%. It was stated that contact sensitivity was not observed, but it should be noted that in the induction phases exposure to musk xylene was always followed by UV irradiation.

4.1.2.4.2 Studies in humans

To evaluate the contact-sensitising potential of musk xylene twenty-five healthy adult males received an application of 5% musk xylene in petrolatum on the skin for 5 alternate day 48-hour periods under occlusion. Pretreatment with sodium lauryl sulphate was omitted because the test solution caused mild irritation. Following a 10-day rest period, occlusive challenge patches were applied to fresh skin sites for 48 hours. The challenges were evaluated at 48 and 72 hours. No reactions were observed (Kligman, 1970).

Cronin (1984) found some evidence of cross-reactivity to musk xylene in patients photoallergic to musk ambrette with 3/19 patients showing evidence of cross-reactivity. One of these three patients showed a weak reaction.

A study was performed to determine the frequency of reactivity to a series of commonly used fragrances (amongst others musk xylene) in dermatological patients. In a pilot study on a total of 1,069 patients in 11 centres, the appropriate test concentration and vehicle were examined for each fragrance. In the main study, a set of 5 to 10 fragrances at 2 concentrations was patch tested in each centre on a minimum of 100 consecutive patients seen in the patch test clinic. With respect to musk xylene, 0.1% and 1% concentrations in petrolatum were patch tested on 192 patients (116 females, 76 males) in the test centre Menné, Copenhagen. Patches were applied to the back and kept in place for two days. Two to three reactions, rated as ‘irritant’ or ‘doubtful positive’, were observed for the 0.1 and 1% musk xylene concentrations, respectively, but no allergic reactions were observed (Frosch et al., 1995).

Bruze et al. (1985) have studied the sensitising properties of musk xylene in a photopatch test according to a protocol developed by the Scandinavian Photodermatitis Research Group (Jansén et al., 1982). 13 Patients, suspected to suffer from a photoallergic contact dermatitis, were screened by means of a questionaire and clinical tests. Dermal sensitivity to UV light was pre-tested after which nitromusks (amount and vehicle not specified) were applied on the skin under occlusive patches (two sites per substance). After 24 hours of contact the patches were removed under dim light and the skin examined for any signs of contact-dermatitis. Half of the contact sites was covered again and the other half was irradiated with ultra-violet light. All persons studied showed a photoallergic reaction to musk ambrette. In one person also a photoallergic reaction to musk xylene was obseved. No reaction was seen at the non-irradiated site. The study is inconclusive as to whether this patient shows either cross-photoallergy between musk ambrette and musk xylene or that this patient shows a concomittant independent photo-allergy to two nitromusks. The study is also inconclusive as to the prevalence of the condition.

4.1.2.4.3 Summary of sensitisation and photoallergy

Due to several shortcomings in the studies with guinea pigs it is not possible to conclude on the skin sensitising properties of musk xylene in animals. From studies with human volunteers, however, it can be concluded that musk xylene is not skin sensitising when tested at an irritating concentration. When patch tested to musk xylene, dermatological patients did not show allergic reactions either. It is concluded that musk xylene is not a skin sensitising substance in humans and does not need to be classified for this end point.

Data on respiratory tract sensitisation or occupational asthma are not available.

4.1.2.5 Repeated dose toxicity

4.1.2.5.1 Studies in animals

Oral

In a preliminary study to a 17 weeks study, groups of eight B6C3F1 mice/sex received a diet containing 0, 0.3, 0.6, 1.25, 2.5 or 5% musk xylene (equivalent to 0, 429, 857, 1,786, 3,571, or 7,143 mg/kg bw/day) for 14 days. Clinical signs and deaths were recorded daily. At the end of the study all survivors were killed for gross examination. All the mice fed ≥0.6% in the diet died after 2-4 days of treatment, except for one female at 0.6%. All mice at 0.3% survived to the end of the study. Tremor was evident in mice at the higher dose groups. Macroscopically, haemorrhages were found in the stomachs and small intestines of mice that died during the study. Histological examination of a few mice that died during the study revealed haemorrhagic erosions in the glandular stomach, but no toxic lesions which could be related to musk xylene were noted in the brain, spinal cord or other organs (Maekawa et al., 1990).

Remark: more details were not available.

In a preliminary test to a carcinogenicity study, groups of 10 male and 10 female mice (B6C3F1) received a diet containing 0, 0.0375, 0.075, 0.15, 0.3 or 0.6% musk xylene for 17 weeks (equivalent to 0, 54, 107, 214, 429 or 857 mg/kg bw/day). Animals were observed daily and at the end of the study all survivors were killed and major organs and or tissues were taken for gross and histological examination. All of the mice given 0.6% musk xylene, 8 males and all of the females at 0.3% died during the study. No mortalities occurred in any of the other groups. Throughout the experiment, no significant differences in body weight or food intake were seen between the treated groups of either sex given ≤0.15% musk xylene and the controls. Regarding organ weights, there were no significant differences between treated (≤0.15% musk xylene) and control groups although the absolute and relative liver weights were increased slightly in all treated groups except the 0.075% males. The increase was not dose related. Histologically, enlargement and irregularity of liver cells were observed in males and females at 0.15%. There were no remarkable or dose related toxic lesions in any other organs of any of the treated groups. From these results it was concluded that the maximum long-term tolerable dose of musk xylene administered in the diet would be 0.15% for both sexes. Therefore, 0.15 and 0.075% were selected as appropriate dose levels in the carcinogenicity study (Maekawa et al., 1990).

Remark: The test is as a preliminary study marginally described and has a limited study design (e.g. biochemical and heamatological parameters were not studied). Because more details are not available no NOAEL can be derived.

In a carcinogenicity study by Maekawa et al. (1990), no exposure-related non-neoplastic lesions (including effects on the liver) were observed in B6C3F1 mice exposed to 0, 0.075 or 0.15% musk xylene via the diet for 80 weeks after histological examination of all tissues / organs. In the males of the 0.15% group, a reduced body weight during weeks 4 to 80 was found. At the end of the study, after a 10 weeks recovery period, there were no body weight differences between the groups. For futher details on this study, reference is made to Section 4.1.2.7.

Dermal

Groups of 15 Sprague-Dawley rats/sex received dermal applications of 7.5, 24, 75 or 240 mg musk xylene (in phenylethyl alcohol)/kg bw/day for 90 days. The material was applied with a repeating syringe over approximately 25% of the body surface. The application was made to the clipped surface of the backs of rats under nonoccluded conditions. The rats were fitted with collars to prevent ingestion. The study design slightly deviated from OECD guideline 411, in that approximately 25% in stead of 10% of the body surface was used and that there was no occlusion. For comparative purposes (see remark) two positive control groups, treated with 240 mg musk ambrette (in phenylethyl alcohol)/kg bw/day, were used in this experiment; one group was fitted with collars, the other not. The vehicle control group (30 rats/sex) was treated with phenylethyl alcohol alone. Observations were made according to OECD guideline 411, and included dermal irritation and the reproductive organs. Neuropathological evaluations were performed at the end of the treatment period. From at least three rats/sex/dose the following areas of the nervous system were selected and prepared for microscopic examination. From CNS: lumbar spinal cord, mid-thoracic spinal cord, cervical spinal cord, medulla oblongata, cerebellar vermis, lateral geniculate, cerebral cortex and optic nerve. From peripheral nervous system: sciatic nerve from mid-thigh region and at sciatic notch, lumbar dorsal roots and dorsal root ganglia, lumbar ventral root, cervical roots, tibial branches to the calf musculature and the gastrocnemius muscle.

One male rat at 240 mg/kg bw/day died. Necropsy findings revealed renal disease, probably uraemia, which was judged a consequence of urinary calculi, and therefore likely to be unrelated to treatment. There were no treatment-related effects on clinical signs, body weight, haematological and clinical chemistry parameters. It was stated that apart from variable desquamation and occasionally atony of the skin[12] there were no significant treatment-related dermatological changes. Absolute liver weights were increased at 240 mg/kg bw/day with 16% in both males and females, and with 15% in females exposed to 75 mg/kg bw/day. Relative liver weights were increased at 240 mg/kg bw/day with 13% and 18% in males and females, respectively, and with 12% in females exposed to 75 mg/kg bw/day. No gross or microscopic changes were observed in any of the organs and tissues examined, including the reproductive organs, liver and skin. No effects were seen on neuropathological parameters (Ford et al., 1990). The NOEL in this study can be established at 24 mg/kg bw/day.

Remark: The structurally related musk ambrette was chosen as positive control in this study because musk ambrette is known to cause neurotoxicity and testicular atrophy in rats at high

[12] It is not clear from the report for which substance (musk xylene, musk ambrette or both) these effects were observed.

dietary and dermal doses. In this study, musk ambrette was clearly neurotoxic and caused testicular atrophy in rats, whether or not they had collars.

Inhalation

No data available.

4.1.2.5.2 Studies in humans

No data available.

4.1.2.5.3 Summary of repeated dose toxicity

In preliminary studies for a carcinogenicity study with mice, oral dose levels equivalent to 429 and 857 mg/kg/day for 17 weeks caused mortality in mice and dose levels equivalent to 214 mg/kg bw/day or higher caused a significantly decreased body weight (gain) and food consumption. At these dose levels an increased absolute and relative liver weight was seen as well as enlargement and irregularity of liver cells. As these studies were only dose range finding studies and very limited in design, no NOAELs are established. Moreover, the effects on the liver were not confirmed in an 80 weeks carcinogenicity study, while the only non-neoplastic effect in this study (decreased body weights) was reversible during the recovery period at the end of the study.

In a well performed dermal 90-days study with rats the two highest dose levels tested, 75 and 240 mg/kg bw/day, caused an increased absolute and relative liver weight of approximately 13-18%, not associated with any pathological finding. In this experiment no neuropathological effects and no effects on the reproductive organs were observed. No effects were observed at 24 mg/kg bw/day, which dose level can be established as the NOEL in this study. This NOEL can be considered as a NOAEL, although the extent of the liver weight changes at the next higher dose level was only marginal and of questionable biological significance. The value of 24 mg/kg bw/day is taken forward to the risk characterisation.

Inhalation repeated dose studies with musk xylene were not available.

4.1.2.6 Genotoxicity

The available *in vitro* and *in vivo* studies are summarised in **Table 4.12**. All tests were performed according to, or closely resembling, current guidelines.

Table 4.12 Genotoxicity studies with musk xylene.

Assay	Species	Protocol	Result	Reference
In vitro				
Bacterial gene mutation test	*S.typhimurium* (TA 98, 100, 1535, 1537, 1538)	Other; Ames et al., 1975	negative (-/+ S9)	Schüpbach, 1981
Bacterial gene mutation test	*S.typhimurium* (TA 98, 100)	Other; Ames et al., 1975	negative (-/+ S9)	Nair et al., 1986
Bacterial gene mutation test	*S.typhimurium* (TA 97, 98, 100, 102)	Other; OECD-like	negative (-/+ S9)	Mersch-Sundermann et al., 1996a; Emig et al., 1996
SOS chromotest	E.*coli* PQ37 *sfiA::lacZ*	Other	negative (-/+ S9)	Mersch-Sundermann et al., 1996a; Emig et al., 1996
SOS chromotest	E.*coli* PQ37 *sfiA::lacZ*	Other	negative (-/+ S9)	Kevekordes et al., 1996
Gene mutation test	mouse lymphoma L5178Y TK+/- cells	OECD 476	negative (-/+ S9)	Bigger and Clarke, 1992; Api et al., 1995
SCE test	human lymphocytes	OECD-like	negative (-/+ S9)	Kevekordes et al., 1996
Chromosome aberration test	CHO-cells	OECD 473	negative (-/+ S9)	Putman and Morris, 1992; Api et al., 1995
Micronucleus test	human lymphocytes; human hepatoma cell line Hep G2	Other	negative; negative	Kevekordes et al., 1997
Unscheduled DNA synthesis test	rat hepatocytes	OECD 482	negative	San and Raabe, 1992; Api et al., 1995
In vivo				
Unscheduled DNA synthesis test	rat liver	OECD 486	negative	San and Raabe, 1994; Api et al., 1995

4.1.2.6.1 *In vitro* studies

Musk xylene was tested for mutagenicity in an Ames/Salmonella test. Concentrations up to 200 µg/plate, which induced no toxicity, caused no positive reactions either without or with metabolic activation (Schüpbach, 1981). In another Ames test musk xylene was tested in Salmonella typhimurium strains TA 100 and TA 98 up to 500 µg/plate (the highest concentration tested due to poor solubility in aqueous medium). Negative results were obtained, both with and without metabolic activation (Nair et al., 1986).

Mersch-Sundermann et al. (1996a) and Emig et al. (1996) tested musk xylene (in DMSO) in 4 strains of *Salmonella typhimurium*, and in *Escherichia coli* PQ37 *sfiA::lacZ* in the SOS chromotest. Both tests were negative either with or without metabolic activation. Mersch-Sundermann et al. (1996a) reported dose levels up to 5 mg/plate in the *Salmonella* test and dose levels up to 1.6 mg/assay in the SOS chromotest. Slightly different dose levels were reported by Emig et al. (1996): musk xylene up to the limit of solubility in the *Salmonella* mutagenicity test, and up to 100 µg/assay in the SOS chromotest.

In addition an SOS chromotest with E. coli PQ37 strain was run by Kevekordes et al. (1996). Cells were exposed to musk xylene in a concentration of 0.033% (v/v) with and without

metabolic activation. No indication for enhanced reporter gene expression was obtained (Kevekordes et al., 1996).

In an L5178Y TK+/- mouse lymphoma assay dose levels of 20 to 400 μg/ml were used without metabolic activation and dose levels of 10 to 125 μg/ml were used with metabolic activation. None of the treated cultures exhibited a positive result (Bigger and Clarke, 1992; Api et al., 1995). Musk xylene was tested in a chromosome aberration assay using CHO-cells. Based on the preliminary cytotoxicity test high dose levels for the definitive assay were selected to give at least a 50% reduction in cloning efficacy. Dose levels of 2.5-40 μg/ml were tested in the presence of metabolic activation and dose levels of 1.9-30 μg/ml were tested without metabolic activation. Toxicity as measured by mitotic inhibition was observed at the highest concentrations after 24 and 48 hour harvests. No increase in chromosome aberrations was observed (Putman and Morris, 1992; Api et al., 1995).

In a UDS test using primary cultures of rat hepatocytes musk xylene was tested at eight dose levels ranging from 1 to 150 μg/ml. Dose levels >30 μg/ml were too toxic to be evaluated. At the other dose levels only negative results were obtained (San and Raabe, 1992; Api et al., 1995).

Musk xylene was also studied for its ability to induce sister chromatid exchanges in human lymphocytes with and without metabolic activation. Dose levels tested were in the range of 0.068 to 135 μM (cytotoxic). Positive and negative control cultures were included. No indications for induction of SCEs by musk xylene were obtained (Kevekordes et al., 1996).

In an *in vitro* micronucleus test, musk xylene (in DMSO) at doses up to 135 and 350 μM did not increase the frequency of micronuclei (scored in 1,000 binucleate cells with two nuclei of approximately equal size) in human lymphocytes and in the human hepatoma cell line Hep G2, respectively. Musk xylene was tested up to cytotoxic doses (270 and 500 μM, respectively) (Kevekordes et al., 1997).

4.1.2.6.2 *In vivo* studies

In an *in vivo* UDS assay musk xylene was administered via oral gavage at three dose levels of 500, 1,500 or 5,000 mg/kg bw in corn oil to groups of 5 male rats (Fischer 344). Hepatocytes were harvested 2-4 and 12-18 hours after administration after which DNA repair was assayed by means of ^{3}H-thymidine incorporation. No significant increase in the mean number of net nuclear grain counts in isolated hepatocytes were observed (San and Raabe, 1994; Api et al., 1995).

4.1.2.6.3 Cogenotoxic activity

Liver microsomes from male Wistar rats treated with 0.1 mmol/kg bw/day musk xylene (i.p. for 5 days) were used to activate the promutagens benzo[a]pyrene and Glu-P-1. The extent of proximate genotoxicant formation was studied in a *Salmonella typhimurium umu*-test. Treatment with musk xylene resulted in enhanced bioactivation of Glu-P-1, but failed to increase the activation of benzo[a]pyrene. Liver microsomes from animals treated with the positive controls 3-methyl cholanthrene (0.1 mmol/kg bw/day, same dosing protocol) and isosafrole (1 mmol/kg bw/day, same dosing protocol) stimulated bioactivation of both promutagens (Iwata et al., 1993b).

Musk xylene was examined for its potency to induce toxifying enzymes in an *in vivo/in vitro* induction assay. For the *in vivo* part of the study, male Sprague Dawley rats received i.p. administrations of 10, 20 or 40 mg musk xylene (in corn oil)/day over a period of 5 days. Following sacrifice on day 6 after the first dose, the livers were taken out, homogenised and centrifuged, after which the liver S9 fractions were used for the *in vitro* SOS chromotest with *Escherichia coli* PQ37 *sfiA::lacZ.* In this assay, musk xylene showed enzyme inducing effects in the rat liver, which led to an increase in the toxification of the pregenotoxicants 2-aminoanthracene and aflatoxin B_1, but not of the promutagen benzo[a]pyrene (Mersch-Sundermann et al., 1996a/b).

4.1.2.6.4 Summary of genotoxicity

Musk xylene was negative in several *in vitro* tests (bacterial gene mutation tests, SOS-chromotests, a mammalian gene mutation test, tests for chromosome aberrations and SCEs in mammalian cells, a micronucleus test in mammalian cells and an UDS test). In an *in vivo-in vitro* rat hepatocyte UDS test also negative results were obtained. It can be concluded that musk xylene is a non-genotoxic substance. Due to its enzyme-inducing properties, musk xylene can exhibit cogenotoxic activity.

4.1.2.7 Carcinogenicity

4.1.2.7.1 Studies in animals

Oral

Three groups of 50 male and 50 female mice (B6C3F1) received a diet containing 0, 0.075 or 0.15% musk xylene (i.e. 0, 750 or 1500 mg/kg) for 80 weeks. The dietary intakes were 70-125 and 141-228 mg musk xylene/kg bw/day for males and 80-143 and 166-259 mg musk xylene/kg bw/day for females in the low and high dose group, respectively. After 80 weeks the administration of musk xylene was stopped and the mice were then maintained on a basal diet without musk xylene until week 90 when all survivors were killed. All tissues, including reproductive organs were microscopically examined. Males fed 0.15% showed reduced body weights during weeks 4 to 80. At the end of the study there was no difference between the groups. There were no significant differences between controls and treated groups in mean survival time. Tumours developed in several organs and tissues in both sexes of all groups, including controls. Musk xylene at both dose levels tested statistically significantly increased the incidence of liver adenomas in both sexes and of liver carcinomas in males. In male mice, the incidence of Harderian gland adenomas was also statistically significantly increased in both treated groups. Positive trends (not statistically significant) were noted for the occurrence of lung tumours (adenomas) in both sexes and Harderian gland tumours (adenomas) and lymphomas in females. The incidences for the above mentioned tumours are given in **Table 4.13**. As can be seen from this table, these tumours also occurred in controls. For other tumours there was no difference in incidence between treated and control animals. The lowest dose tested (0.075%, equivalent to 70-125 mg/kg bw/day in male mice and 80-143 mg/kg bw/day in female mice) is an effect dose. In this study no effects were seen on the reproductive organs (Maekawa et al., 1990).

Table 4.13 Tumour incidences in mice treated orally with musk xylene for 80 weeks.

Site and type of tumour	No. of mice with tumours					
	Males			Females		
Concentration in diet (mg/kg)	0	750	1,500	0	750	1,500
Effective no. 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***
- haemangioma	0	0	0	2	0	0
- haemangioendothelioma	2	0	0	0	0	0
Lung						
- adenoma	3	5	6	0	2	2
- carcinoma	0	0	0	0	1	0
Haematopoietic organs						
- lymphomas (lymphocytic)	4	4	2	3	5	6
Harderian gland						
- adenoma	2	9*	10*	3	3	5
- carcinoma	1	1	0	0	0	0

* P<0.05;
** P<0.01;
*** P< 0.001

Dermal / Inhalation

No data available.

Special investigations on enzyme induction

Rats were treated i.p. for 5 consecutive days with 50, 100, or 200 mg musk xylene/kg bw. Both total cytochrome P450 and cytochrome b5 contents in rat liver microsomes were increased. Cytochrome P450-1A2 was strongly induced and cytochrome P450-1A1 slightly, as determined by SDS-PAGE (Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis) followed by immunochemical quantification (Iwata et al., 1992).

In order to characterise the inducing effects of musk xylene on phase I and phase II drug-metabolising enzymes, male Wistar rats (4 per group) were intraperitoneally dosed with 50, 100 or 200 mg musk xylene/kg bw for 5 consecutive days.

Treatment with musk xylene resulted in increased relative liver weights and increased microsomal protein levels. Dose related increases in benzo[a]pyrene hydroxylation and 7-ethoxycoumarin de-ethylation were observed from 50 mg/kg upwards. 7-pentoxyresorufin depentylation was increased at all dose levels as compared to control but the increase diminished at the higher dose levels. Other activities (aniline hydroxylase, aminopyrine demethylase, benzphetamine demethylase and erythromycin demethylase) were not affected. Dose-related increases at 50 mg/kg and above were also observed for glutathione S-transferase (GST; two

types), DT diaphorase (two types), and UDP-glucuronyl transferase but not for N-acetyl transferase. For GST type Ya and both DT diaphorase forms these increases were also reflected in cytosolic protein contents. Other enzymes mentioned were not quantified by immunochemical methods (Iwata et al., 1993a).

Male wistar rats (5 weeks old; 4 per group) were injected intraperitoneally with 0.1 mmol (30 mg)/kg bw musk xylene or with equimolar amounts of 3-methyl cholanthrene (3MC) or 2,3-tertbutylhydroxyanisole (BHA) or with 0.1 or 0.93 mmol/kg bw isosafrole. After 5 days of treatment livers were removed and studied for induction of phase I and phase II biotransformation enzymes. Results in treatment groups were compared to those of a vehicle treated control group.

At the dose levels mentioned only 3MC produced a profound increase in total cytochrome P450. Western blot analyses revealed that musk xylene, isosafrole and BHA induced more strongly cytochrome P450 1A2 (CYP1A2) in microsomes than CYP1A1, while 3MC induced CYP1A1 in preference to CYP1A2. Musk xylene induced ethoxyresorufin O-deethylase (EROD), equipotent to low dose isosafrole, erythromycin N-demethylation (ERD) and aniline hydroxylation, but did not affect benzphetamine demethylation. EROD was far more strongly induced by 3MC and high dose isosafrole. These two treatments also enhanced pentoxyresorufin-O-dependentylase (PROD) activity, but depressed benzphetamine demethylation. Treatment with musk xylene at this dose level did not affect phase II enzyme activities (DT-diaphorase, glutathione S-transferases or UDP-glucuronyl transferase). These enzymes were strongly induced by 3MC and to a lesser extend also by high dose isosafrole. According to the authors musk xylene is at these dose levels a more specific inducer for CYP1A2 than 3MC (Iwata et al., 1993b).

Remark: although the authors measured PROD activity, effects on CYP2B protein were not directly studied.

Groups of 10 male B6C3F1 mice received daily for 7 days musk xylene dissolved in trioctanoin by i.p. injections at doses of 0, 50, 100 or 200 mg/kg. On day 8 the animals were sacrificed. Sections of the liver were taken for histological examination and protein and enzyme activity was determined. After i.p. administration total microsomal protein content, EROD, and methoxy resorufin O-demethylase (MROD) activities were dose relatedly increased in all treated groups when compared to the control group. No changes were observed in PROD or ERD activities. Electron microscopic examination of the livers showed proliferation of smooth and especially rough endoplasmic reticulum at 50 mg musk xylene/kg bw as well as signs of mitochondrial damage. These microscopical changes became more pronounced at the higher dose levels.

Other groups of 20 male mice (same strain)/dose were given musk xylene in the diet at 0, 0.015, 0.045, or 0.15% (equivalent to 21.4, 64.3, 214.3 mg/kg bw) for 4 weeks. 10 Mice of each group were sacrificed and their kidneys and brains removed and weighed. The liver was examined histologically and microsomal protein and enzyme activities were determined. The remaining 10 mice in each group were withdrawn from musk xylene containing diets for 14 days. Then the mice were sacrificed and the livers were examined as above.

After 28 days feeding relative liver weight, total liver microsomal protein, EROD, and MROD but not PROD were dose relatedly and significantly increased at 0.045 and 0.150%, whether expressed as an activity per mg microsomal protein per minute or as substrate turnover (nmole of substrate per nmole P450 per minute). Immunoblotting showed that musk xylene induced cytochrome P450 1A2 enzyme. The cytochrome P450 1A1 was much less enhanced. After a

14-day recovery all increases were reversed and not significantly different when compared to controls. (Caldwell and Thatcher, 1994).

Male B6C3F1 mice were dosed orally with musk xylene dissolved in corn oil at dosages of 10 and 200 mg/kg bw for 7 days. At the highest dose, increases were found in relative liver weight (38%), microsomal protein yield (125%), total P450 content (62%) and cytochrome P450-2B (CYP2B) protein level (about 20-fold). At 10 mg/kg bw no general hepatic effects consistent with cytochrome P450 induction were seen. Although musk xylene increased CYP2B protein levels, it did not increase CYP2B enzyme activity (as determined by PROD activity). When given over a range of 1 to 200 mg/kg bw for 7 consecutive days, musk xylene dose-relatedly increased immunoreactive CYP2B protein levels (at the highest dose about 20-fold over control levels) but did not reveal any change in CYP2B enzyme activity from control levels at any dose (Stuard et al., 1996).

To characterise the effects of musk xylene on mouse hepatic microsomal enzyme activities groups of 5 male B6C3F1 mice were dosed by gavage for 7 days with 0 or 200 mg musk xylene/kg bw after which microsomes were prepared. A series of enzyme assays was used to determine total cytochrome P-450 content, NADPH cytochrome P-450 reductase, and the activities of cytochromes P-450 1A1, 1A2 and 2B. Immunoblotting procedures were also used to evaluate the changes in protein levels for CYP1A1, 1A2 and 2B. Liver weight was increased by 40%. The increased liver weight was reflected histologically as centrilobular hepatocellular hyperthrophy. Treatment increased the total microsomal cytochrome P450 content about 2-fold and NADPH cytochrome-c-reductase was increased about 4-fold over the control values. Induction of CYP1A1 and 1A2 protein levels was 2.50- and 2-fold, respectively. These results were consistent with increased 1A1 and 1A2 protein levels determined by immunoblotting (Lehman-McKeeman et al., 1995).

As a follow-up to Lehman-McKeeman et al. (1995), a dose-response study was conducted in which musk xylene was dosed by gavage to groups of 5 male B6C3F1 mice for 7 days at dosages of 1, 5, 10, 20, 50, 100 and 200 mg/kg bw. Treatment with musk xylene resulted in a dose related increase in absolute and relative liver weights at 20 mg/kg and 50 mg/kg and higher, respectively. Total microsomal protein and total cytochrome P450 were dose-relatedly increased at 50 and 20 mg/kg, respectively.

Induction of cytochrome P450 1A2 (about 3-fold) and 3A proteins was observed, together with a small but unquantified induction of cytochrome P4501A1 at 100 and 200 mg/kg bw. Enhanced protein levels were reflected in increased activities of EROD (4-fold), MROD (2-fold), and ERD (2.5-4 fold) at 200, 200, and 50-200 mg/kg, respectively. From 20 mg/kg bw and upwards, cytochrome P450 2B protein was also induced up to 25-fold at 200 mg/kg, but this was not reflected in increased PROD activity. Induction of cytochrome P450 2B was also confirmed by a 10-fold increase in P450 2B mRNA levels at 200 mg/kg within four h post dosing. The NOEL for effects on liver enzyme induction in this study is 10 mg/kg bw/day for 7 days (Lehman-McKeeman et al., 1997a).

In an additional experiment male B6C3F1 mice (5 per group) were exposed to the classical CYP2B inducer, phenobarbital (PB; 500 mg/l in drinking water) for 5 days and then given a single oral dosage of corn oil or 200 mg musk xylene (in corn oil)/kg bw at 2 or 18 hours prior to necropsy. By immunoblotting, there was no difference in the CYP2B protein levels in the PB treated animals dosed with either corn oil or musk xylene. CYP2B enzyme activity in the PB/corn oil treated mice was about 1,200 to 1,300 pmol/min/mg protein, representing about 20-fold induction over uninduced levels. When musk xylene was dosed 2 hours before necropsy,

CYP2B enzyme activity was similar to the PB/corn oil treated mice (1,221 ± 76 pmol/min/mg protein). However, when dosed 18 hours before necropsy, CYP2B enzyme activity in the musk xylene-treated mice (99 ± 12 pmol/min/mg protein) was decreased 90% relative to the PB/corn oil treated mice. According to the authors these results demonstrated that musk xylene is a potent inhibitor of the CYP2B enzymes, but since the inhibitory effect was seen only at 18 hours after dosing, the results suggested that the inhibition of the CYP2B enzymes *in vivo* required metabolism of musk xylene (Lehman-McKeeman et al., 1997a).

To determine whether biotransformation of musk xylene by intestinal flora contributed to the enzyme inhibition, an experiment was conducted in which 8 male B6C3F1 mice were dosed with PB (500 mg/l in drinking water for 5 days) to induce the CYP2B enzymes. One group of 4 mice was also dosed (by gavage) with a combination of neomycin (400 mg/kg/day), tetracycline (200 mg/kg/day) and bacitracin (200 mg/kg/day) during PB exposure period to reduce the intestinal microflora. Control animals received daily dosis of normal saline. After the 5 days PB and antibiotic treatment all mice were dosed with musk xylene (200 mg/kg by gavage) and microsomes were prepared 18 hours after musk xylene treatment. CYP2B protein levels were similar in the PB/saline/musk xylene and PB/antibiotic/musk xylene groups. However, antibiotic treatment prevented the inhibition of the CYP2B enzyme activity by musk xylene compared to the saline dosed mice. These results indicate that the biotransformation of musk xylene by intestinal microflora is involved in the inhibition of CYP2B enzymes seen with musk xylene treatment (Lehman-McKeeman, 1997a).

To study the mechanism of inhibition naive or phenobarbital (PB) treated mice were orally dosed with 200 mg [methyl-^{14}C]-musk xylene/kg bw (150 μCi) and the covalent binding to microsomal proteins was assessed. In naive mice about 3% of ^{14}C-musk xylene equivalents in the microsomal fraction bound covalently to protein. In PB-treated mice the covalent binding increased 7-fold and musk xylene decreased PB-induced enzyme activity (PROD) by 90%. When musk xylene was dosed to mice receiving antibiotics to eliminate intestinal flora, no covalent binding was detected in microsomes neither in naive nor in PB-induced mice, suggesting that amine metabolites of musk xylene were responsible for the covalent binding. Two amine metabolites of musk xylene, p-NH_2-musk xylene and o-NH_2-musk xylene were synthetised to study enzyme inhibition. Musk xylene and the amines were mixed inhibitors of CYP2B activity, with K_i values of 10^{-7} M, and 10^{-8} M for parent musk xylene and metabolites, respectively. Musk xylene and o-NH_2-musk xylene did not inactivate the enzyme, but p-NH_2-musk xylene produced a time- and NADPH-dependent loss of 85% of CYP2B enzyme activity (PROD) after 5 minutes of incubation. The concentration K_i at which the rate constant for inactivation (k_{inact}) was half-maximal was determined at 10.5 μM. The k_{inact} was 1.2 min^{-1}, corresponding to a maximum rate of inactivation with a half-life of residual enzyme activity of about 35 seconds. Dosing of PB-treated mice with p-NH_2-musk xylene (154 mg/kg bw) resulted in a complete loss of microsomal PROD activity within 2 hours after dosing, while an equimolar dose of musk xylene did only affect PROD activity at 18 hours post dosing, but not at 2 hours post dosing. Microsomal levels of CYP2B protein were not affected by either p-NH_2-musk xylene or by musk xylene. (Lehman-McKeeman et al., 1996; Lehman-McKeeman et al., 1997b).

In order to identify the substance responsible for induction of CYP2B in mouse liver, male B6C3F1 mice (5 per group) were treated with 0.67 mmol/kg of musk xylene, o-NH_2-musk xylene, or p-NH_2-musk xylene dissolved in corn oil (200 mg/kg bw for musk xylene or 180 mg/kg bw for both amino derivatives) by gavage. Control animals received only corn oil. At 4, 8, 16, 24, or 48 h post dosing livers were removed and studied for CYP2B and CYP1A2 mRNA, cytochrome P450 enzyme activities (PROD, EROD, MROD), CYP2B protein contents

and several more general parameters for xenometabolism induction (liver weight, microsomal protein, total cytochrome P450, and cytochrome c reductase). In separate studies the effect of pretreatment with broad-spectrum antibiotics on cytochrome P450 induction was also studied. The induction pattern by musk xylene and metabolites was compared to the induction pattern by PB.

PB and musk xylene and o-NH_2 musk xylene induced CYP2B mRNA by a factor of 5 over control levels with a maximum response at 6 to 18 h post dosing. With p-NH_2-musk xylene also a five fold induction of mRNA expression was observed, but this increase lasted for up to 48 hours post dosing. With PB, musk xylene and metabolites also an induction of CYP1A2 mRNA was observed, but this was only by a factor of 1.5 above control level.

When animals were pretreated with broad-spectrum antibiotics to eliminate metabolism by intestinal flora, no evidence of microsomal enzyme induction was obtained following dosing with musk xylene. Furthermore, while musk xylene when given alone induced CYP2B and CYP1A proteins it did not do so when given after a dose of antibiotics. It was therefore concluded that intestinal nitro-reduction products of musk xylene were responsible for induction of microsomal biotransformation enzymes (Lehman-McKeeman et al., 1997c).

Male F344 rats (6 per group) were orally dosed with 0, 10, 50 or 200 mg musk xylene /kg bw for 7 days. The substance was dissolved in corn oil. Following sacrifice of the animals, livers were assayed for induction of microsomal enzymes by determination of enzyme activities, for CYP1A1/2, CYP2B1/2 and CYP3A, and by determination of protein levels and mRNA expression for CYP2B1/2.

Up to 200 mg/kg bw, clinical signs of toxicity were not observed. Induction of microsomal enzymes was reflected in a dose-related increase in absolute liver weight (statistically significantly at 200 mg/kg bw), and dose-related increases in total microsomal cytochrome P450, cytochrome b5 and NADPH-cytochrome P450 reductase which were statistically significant at 50 and 200 mg/kg bw.

CYP2B activity measured as PROD was significantly increased at all dose levels up to 3-fold maximally at 200 mg/kg bw. However, the concentration of the corresponding CYP2B enzyme was increased by about 50-fold at this dose level and the increase in steady-state CYP2B mRNA level amounted to 10-fold.

Exposure to musk xylene resulted also in increases in CYP1A1/2 and CYP3A1 enzyme activities which were reflected in dose related increases in EROD and MROD activities (statistically significant at all dose levels) and testosterone 6β-hydroxylation, which was statistically significant at 50 and 200 mg/kg bw. The induction profile of the biotransformation enzymes was similar to that observed with phenobarbital at 500 mg/l in the drinking water for 7 days. This study provided a LOEL of 10 mg/kg bw /day for microsomal liver enzyme induction in the rat after 7 days of dosing (Lehman-McKeeman et al., 1999).

Female and male Long Evans rats (5-6 weeks old) were fed musk xylene through their diet at dose levels of 0 10, 100 or 1,000 mg musk xylene/kg feed (corresponding to 0, 0.7 to 0.8, 7-8 or 70 to 80 mg/kg bw/day). The four different treatment groups consisted of 6 to 8 animals per group, half of which were females and half of which were males. After 10 to 11 weeks, animals were weighed, killed, livers collected and weighed analysed for PROD, MROD and EROD activities, while microsomal proteins were submitted to gel-electroforesis followed by immunoblotting for CYP1A, 2B and 3A proteins, which were quantified by densitometry.

No treatment related effects on absolute or relative liver weight or crude microsomal protein contents was observed. In the mid dose group MROD and EROD activities were enhanced by 2.5- to 4-fold in both sexes, while PROD activities were only enhanced by 1.7 fold. Immunoblot densitometry showed a 2- and 4-fold increase in CYP1A proteins at 10 and 100 mg/kg feed while at 1,000 mg/kg feed these proteins were about 7 times as high as in the controls. CYP3A was induced 1.1- to 1.6-fold in the exposed groups, while CYP2B proteins were enhanced *ca.* 10- and 6-fold in the 10 and 100 mg/kg feed groups, respectively. In the 1,000 mg/kg feed group CYP2B proteins were increased by a factor of about 180. Statistical significance for increased protein levels was only reached for CYP1A at 100 and 1,000 mg/kg feed and for CYP2B at 1,000 mg/kg feed (Suter-Eichenberger et al., 2000).

Female and male Long Evans rats (5-6 weeks old) were fed musk xylene through their diet at dose levels of 0 or 100 mg musk xylene/kg feed (corresponding to 7 to 8 mg/kg bw/day) for 18 weeks or 100 mg/kg feed for 16 weeks followed by 2 weeks of control diet or 100 mg/kg feed for 10 weeks followed by 8 weeks of control diet. The four different treatment groups consisted of 2 to 4 animals.

ELISA-determined protein levels of liver microsomal cytochrome P450 1A1 and 1A2 were enhanced about 1.5 to 2 times in males and about 2 times in females. Enzyme activities as expressed by EROD and PROD were increased about 3 times in both males and females. After 16 or 10 weeks of exposure to musk xylene diet followed by either 2 or 8 weeks of control diet no appreciable differences with the 18 weeks control groups could be found. Other cytochrome P450 forms were not studied (Suter-Eichenberger et al., 2000).

4.1.2.7.2 Studies in humans

No data available.

4.1.2.7.3 Summary of carcinogenicity

Musk xylene has been tested for carcinogenicity in mice by dietary administration in one experiment with duration of 80 weeks. Both dose levels tested (0.075 and 0.15%) resulted in statistically significantly increased incidences of hepatocellular adenomas in both sexes and of hepatocellular carcinomas in males. The incidence of Harderian gland adenomas was also statistically significantly increased in males at both dose levels. Some other tumours, like lung adenomas in both sexes and lymphomas and Harderian gland adenomas in females, occurred in greater number in the treated groups but the differences with control incidences were not statistically significant. The lowest dose tested, 0.075%, equivalent to 70-125 mg/kg bw/day in male mice and 80-143 mg/kg bw/day in female mice, is an effect dose. In this study no effects were seen on the reproductive organs.

Special investigations into the mechanism behind the mouse liver tumours indicated that musk xylene treatment caused a very significant induction of liver enzymes, including cytochromes P450 1A1, 1A2 and 2B and cytochrome b5. Levels of CYP2B protein in liver are as high as those seen with the classical CYP2B inducer phenobarbital. However, the metabolite p-NH2-musk xylene selectively inactivates the enzyme CYP2B. The toxicological significance of this induction/inhibition phenomenon is unclear. In a 7 days study in the mouse the NOEL for effects on liver enzymes was 10 mg/kg bw/day. Similar induction phenomena have been observed in rat liver and for this species a LOEL of 10 mg/kg bw/day after 7 days of exposure could be derived.

Even at dietary levels as low as 10 mg/kg feed, corresponding to 0.7 to 0.8 mg/kg bw/day, a slight inducing effect on CYP2B protein could be observed after *ca.* 75 days, while for CYP1A and 3A a ten times higher dose level appeared to be a LOEL. The induction phenomena were reversible and occured without simultaneous changes in liver weights. In absence of any other indication of liver toxicity the slight changes in levels of biotransformation enzyme activities are considered to be of an adaptive nature rather than adverse. Therefore this effect as such and the NOEL/LOEL for it will not be taken forward to the risk characterisation.

The mechanism behind the carcinogenic activity of musk xylene is not entirely understood. Statistically significantly increased incidences of malignancies were only observed in the livers of male B6C3F1 mice, a strain which is particularly prone to develop liver tumours. Other spontaneous tumours developed in the Harderian gland (adenomas), lungs (adenomas) and haematopoietic system (lymphomas). The treated groups showed somewhat higher numbers for these tumours (not statistically significantly different from controls, with the exception of Harderian gland adenomas in males). The carcinogenicity of musk xylene has not been studied in a second species, e.g. the rat.

It has been clearly demonstrated that musk xylene is not genotoxic. In addition, the carcinogenic properties of the substance seen in mouse liver seem to be related to induction of microsomal liver enzymes, notably cytochrome P450 1A1, 1A2 but most of all cytochrome P450 2B in a pattern which closely resembles the pattern of induction seen after administration of phenobarbital. The induction of these enzymes is observed both in rats and mice, and in both species the induced CYP2B enzyme is rapidly inactivated by p-NH_2-musk xylene, which is probably formed from musk xylene after nitro-reduction by intraintestinal micro-organisms. In contrast, the induced CYP1A1 and 1A2 enzymes are metabolically active and it has been demonstrated that exposure to musk xylene can result in enhanced bioactivation of several promutagens. Induction of microsomal liver enzymes is a threshold phenomenon with for musk xylene a NOEL of 10 mg/kg bw/day in the mouse and a LOEL of 10 mg/kg bw in the rat. It is conceivable that below a certain threshold the risk for promutagen bioactivation and carcinogenicity will be negligible.

As to the Harderian gland tumours, only benign malformations developed. This gland and tissue type does not occur in humans and therefore these benign tumours are difficult to interpret with respect to their relevance to humans. Like the liver and Harderian gland tumours, the tumours in the lung and haematopoietic system occurred spontaneously in the B6C3F1 mouse strain, with only slightly higher incidences in the treated animals.

Conclusion

It is difficult to deduce the carcinogenic risk of musk xylene to humans from the available data. This because:

- only one species has been tested, i.e. the B6C3F1 mouse;
- this strain of mice is particularly prone to develop certain types of tumours, especially liver tumours;
- the mechanism behind the tumour development is not entirely understood, although it is clear that musk xylene has no genotoxic potential and that enzyme induction plays an important role in the development of the liver tumours observed.

Although musk xylene has not been tested for carcinogenicity in rats, there is a concern that it might be carcinogenic in rats as well, given the comparable enzyme induction properties of musk

xylene in mice and rats. Further testing in e.g. rats or in mice to further elucidate the mechanism is, however, not considered to contribute much to the risk assessment of the carcinogenic risk of musk xylene to humans. This because the available data do allow the conclusions that musk xylene is a carcinogen in mice, that it acts by a non-genotoxic mode of action, and that the most serious type of tumour for which the incidence was statistically significantly increased (i.e. liver carcinomas in male mice) is mechanistically related to microsomal enzyme induction. Hence, for risk characterisation a threshold approach is considered justified, given that musk xylene is non-genotoxic and that enzyme induction is a threshold phenomenon. By taking the oral LOAEL of 70 mg/kg bw/day for tumour development (liver tumours in particular) as basis for the risk characterisation and by taking the mouse NOEL for enzyme induction into account in interpretation of the margin of safety (MOS), this will already result in a rather conservative approach when realising that the B6C3F1 mouse is especially prone to develop liver tumours.

As to classification, IARC concluded in 1996 that there is limited evidence for the carcinogenicity of musk xylene in animals, but that the substance is not classifiable as to its carcinogenicity to humans (group 3) (IARC, 1996). However, the effects on the liver observed with musk xylene resemble those that can be seen after dosing rats and mice with phenobarbital. Phenobarbital is clearly a (liver) carcinogenic substance in rodents and often used to promote the development of tumours that were initiated by preceding treatment with genotoxic substances. Although the relevance of the carcinogenicity of phenobarbital for humans has been questioned (e.g. Williams and Whysner, 1996; IARC, 2001), IARC (2001) nevertheless just recently classified phenobarbital as a group 2B substance ("possibly carcinogenic to humans"). Hence, given the resemblance to phenobarbital, it is now concluded that the non-genotoxic compound musk xylene is to be classified as a carcinogen category 3 (R40), although it is realised that it is a borderline case. The CMR Working Group of May 2002 decided positive on classification as carc. cat. 3 (R40), and confirmed this at their September 2002 meeting.

4.1.2.8 Toxicity to reproduction

4.1.2.8.1 Effects on fertility

Studies in animals

No multi-generation reproductive toxicity study was available. In the 90-dermal toxicity study with rats and also in an oral carcinogenicity study with mice, musk xylene caused no effects on the reproductive organs. This in contrast to the positive control in the 90-day dermal toxicity study, the structurally related compound musk ambrette. Musk ambrette is known to cause testicular atrophy, and indeed caused this effect in the 90-day dermal toxicity study. In addition, in a peri/postnatal toxicity study in which pups were exposed *in utero* and during lactation and were allowed to mate later on (see Section 4.1.2.8.2), no effect on reproductive performance was observed.

Studies in humans

No studies available.

From 1994 to 1996, 152 women (age 35 ± 7 years) consulting a clinic in Heidelberg (Germany) because of gyneacological problems were examined for the presence of synthetic fragrances,

amongst which musk xylene, in their blood. Additionally, various pituitary, adrenal and ovarian hormones were measured, a gyneacological examination was performed and a comprehensive history was taken of their use of cosmetics and detergents and the type and frequency of fish consumption. Of the 152 women, 106 had fertility problems. Among the remaining 46 patients, 28 had cycle disorders, 7 alopecia and hirsutism, and 11 diseases of the uterus, tubes or ovaries. Musk xylene was detected in 144/152 blood samples (detection limit 20 ng/l), with a median, mean and maximum concentration of 65.5, 114.6 and 1,183 ng/l, respectively. The authors reported that no significant correlations were found between the level of musk xylene in blood and age, body mass index, occupation, nationality, fish consumption, use of detergents and follicular and luteal phase hormones (although for the latter there seemed to be a trend). Significant associations were reported between musk xylene levels in blood and the frequency of cosmetics use (especially with perfumes), the level of androstanediol-glucuronide (but not with other adrenal hormones) and obstetric history (primary infertility as compared to previous pregnancies and previous births, nulliparae as compared to having given birth once or more, one or more miscarriages as compared to no miscarriage). A marked, but not significant association was found for certain disorders (anovulation, luteal insufficiency) (Eisenhardt et al., 2001).

Remark: No causal relationships between the level of musk xylene in blood and a reproductive or endocrine effect can be established from this study, a.o. because no proper control group (i.e. women with no gyneacological disorders) was used and confounding factors were not studied.

4.1.2.8.2 Developmental toxicity

Studies in animals

Oral

In a dosage range-finding study groups of 8 pregnant Sprague-Dawley rats received by gavage 0, 60, 200, 600 or 2,000 mg musk xylene/kg bw/day on days 7 through 17 of gestation. Musk xylene was administered in corn oil. On day 20 of gestation the rats were sacrificed. Tremors occurred in all treatment groups while urine stained abdominal fur occurred in groups treated with 60, 600 and 2,000 mg/kg bw. Dried red or red perioral substance occurred at 200 mg/kg bw. Chromodacryorrhea, dried red or red peioral substance and red substance on forepaws occurred at 600 and 2,000 mg/kg bw. Rats at 200, 600 and 2,000 mg/kg bw showed dose relatedly reduced body weight gains and food consumption. No caesarian sectioning or litter parameters were affected by administration up to 2,000 mg/kg bw. No gross external fetal alterations, malformations or variations were observed (Parker, 1997).

In an oral developmental toxicity study groups of 25 Sprague-Dawley rats received by gavage 0, 20, 60 or 200 mg musk xylene/kg bw/day during day 7 through 17 of gestation. Musk xylene was administered in corn oil. On day 20 of gestation the rats were sacrificed. A significant number of rats (12-25) at 200 mg/kg bw and a few (1-3) at 60 mg/kg bw had tremors, chromorhinorrhea and urine stained abdominal fur. These signs were first observed after the initial dose and were not observed after the fourth dosage. Body weight gain was significantly and dose relatedly decreased during the treatment period in rats at 60 and 200 mg/kg bw. Absolute and relative feed consumption values were significantly reduced for the entire dosage period in the 60 and 200 mg/kg bw body weight groups. Reproduction and litter parameters were unaffected by musk xylene administration. Extra thoracic ribs and increased ossification of hyoid sites and forepaw phalanges (both significant) were observed in the 200 mg/kg group. The

NOAEL for maternal toxicity in this study can be established at 20 mg/kg bw/day and the NOAEL for developmental toxicity at 60 mg/kg bw/day (Christian et al., 1997; Christian et al., 1999).

Female and male Long Evans rats (5-6 weeks old) were fed musk xylene through their diet at dose levels of 0, 1, 10, 33, 100 or 1,000 mg musk xylene/kg feed (corresponding to 0, 0.07-0.08, 0.7-0.8, 2-3, 7-8 or 70-80 mg/kg bw/day) for at least 10 weeks before pregnancy. Females were mated with males exposed to matching musk xylene diets. Young animals were examined one day before birth (GD22), at postnatal day 1 within 12 hours after birth (PN1) or at postnatal day 14 (PN14). For GD22 and PN1 offspring liver samples of males and females were pooled within one litter. Administration of musk xylene continued throughout pregnancy up to postnatal day 14, where applicable. F1 offspring was examined for effects on liver weights, liver crude microsomal protein levels, MROD and EROD activities and liver microsomal contents of CYP1A, 2B and 3A proteins.

In PN14 offspring, liver weights in the offspring of dams exposed to musk xylene tended to be lower than liver weights of control offspring, but effects were neither sex- nor dose-related and did not reach statistical significance. No effects were seen on liver crude microsomal protein levels. In PN14 offspring, EROD and MROD activities were enhanced by 1.6 and 1.8 fold, respectively, at a maternal exposure level of 33 mg/kg feed and by 3 and 3.7-fold at 100 mg/kg feed (1,000 mg/kg feed not studied). No induction of EROD was seen at 10 mg/kg feed (MROD not studied). There were no differences in sensitivity with respect to sex. Immunoblotting and densitometry confirmed induction of CYP1A, CYP2B and CYP3A proteins in the 100 and 1,000 mg/kg feed dose groups.

In PN1-offspring born to dams exposed to 100 mg/kg feed before and during pregnancy EROD activity was enhanced by 3 to 7 fold, as compared to PN1 offspring from control dams. MROD activity was also increased. It was stated that in GD22-offspring of dams exposd to 100 mg/kg feed, neither EROD nor MROD activities could be detected, but data on these pups or on comparable control animals were not provided (Suter-Eichenberger et al., 2000).

Cross-fostering study

Female and male Long Evans rats (5-6 weeks old) were fed musk xylene through their diet at dose levels of 0 and 100 mg musk xylene/kg feed (corresponding to 0 and 7-8 mg musk xylene/kg bw/day) for at least 10 weeks after which females were mated with males exposed to matching musk xylene diets. Offspring was exchanged between the various maternal exposure groups within 12 hours after birth, to reach the following study design:

	Pre-natally control	Pre-natally exposed
Post-natally control	group 1	group 3
Post-natally exposed	group 2	group 4

F1 offspring was examined at PN14 for effects on MROD and EROD activities. As compared to group 1 offsping, in the pups of both group 2 and 4, but not in those of group 3, the enzyme activities were enhanced to a similar degree (*ca*. 2.5-fold for MROD and *ca*. 2-fold for EROD). In combination with the observation that no EROD or MROD activities could be detected in GD22 offspring, the results of the cross-fostering study indicate that the observed difference in enzymatic activities was largely due to postnatal exposure (Suter-Eichenberger et al., 2000).

Peri/postnatal toxicity study

Musk xylene (in corn oil) was administered by gavage at dosages of 0, 2.5, 7.5 or 25 mg/kg bw/day to groups of 28 time-mated Charles River CD rats from day 14 of pregnancy (end of organogenesis) through to weaning on day 21 *post partum* (Brooker et al., 1998). The females were allowed to litter and rear their young to weaning. From all offspring the age at which certain developmental stages were attained was determined by examining surface righting reflex, startle reflex, air righting reflex and pupil reflex. From the litters, selected offspring were retained (24 males and 24 females per group) to maturity and assessed for behavioural changes (in motor-coordination and balance, activity and avoidance) and reproductive capability (by mating on a one male to one female basis, and following the pregnant animals through gestation, parturition and allowing the pups to grow to weaning). The only exposure the F_1 generation had to the test substance was *in utero* during the peri-natal phase or through any transfer in the milk of the lactating dams. Additional groups of 15 pregnant females were treated at 0, 2.5 or 25 mg/kg bw/day for the purpose of obtaining toxicokinetic samples for analysis of musk xylene in milk (see Section 4.1.2.1.1 – Special investigation).

A slight (not statistically significant) and transient reduction in body weight gain was noted for dams at 25 mg/kg bw during the first few days of treatment. In this group, reduced body weight gain was also seen during lactation (statistically significant in mid-lactation). During lactation food intake was also slightly lower at 25 mg/kg bw (95% of controls; not statistically significantly). In this group mean pup weight was slightly lower (not statistically significant) from day 4 through to weaning (4.4-7.6%). Linked with this lower pup weight was a slightly later day of attainment for air righting in these pups compared with controls. There were no effects on sexual maturition and reproductive performance. Lower body weight gains (6%; not statistically significant) during the pre-mating and mating phases were seen in F_1 males from F_0 dams receiving 25 mg/kg bw.

F_1 pups were exposed at levels in the mothers milk of up to 84,940 µg musk xylene/l (see Section 4.1.2.1.1 – Special investigation). These exposures caused no direct effect on performance in specific behavioural tests or on reproductive capacity in maturity.

Concentrations of musk xylene measured in adipose tissue (see **Table 4.14**) of F_1 pups killed on day 15 (additional groups) or day 22 *post partum* (excess pups from main groups) showed a sex-related difference: female pups had higher concentrations in fat than male pups. Concentrations in fat increased approximately proportionally with dose. At day 15 *post partum*, fat concentrations were higher than at day 22 *post partum* (Brooker et al., 1998).

Table 4.14 Concentrations in fat (in µg/ml).

Dose level (mg/kg bw)	Day 15 (mean of n=14-15)		Day 22 (mean of n= 26-28)	
	Male	Female	Male	Female
2.5	1.02	1.10	0.39	0.42
7.5	-	-	1.15	1.28
25	8.92	9.44	4.36	5.75

The NOAELs for maternal toxicity and peri/postnatal toxicity in this study can be established at 7.5 mg/kg bw/day. It is recognised that the effects seen at 25 mg/kg bw in both dams and pups were only marginal and, in general, not statistically significant. As to the effect seen in pups: the

same effects were seen for the related substance musk ketone, hence it cannot be excluded that the effects are biologically relevant and related to musk xylene treatment of the F_0 dams. Therefore, the NOAELs are (conservatively) set at 7.5 mg/kg bw/day.

Dermal / Inhalation

No data available.

Studies in humans

No data available.

4.1.2.8.3 Endocrine interactions

Receptor binding

Chou and Dietrich (1999) investigated the competitive binding capability of musk xylene and musk xylene metabolites to the estrogen receptor in trout (*Oncorhynchus mykiss*) and clawed frog (*Xenopus laevis*). No binding of the parent compound musk xylene was observed. Binding to the ER was noticed for 2-amino musk xylene and 4- amino musk xylene in both species (IC50 for 2-amino-musk xylene 1.3 ± 1.1 mM and 12.9±10.3 µM and for 4-amino-musk xylene > 1mM and 30.8 ±28.5 µM for trout and Xenopus, respectively).

E-screen

In a non-GLP study, musk xylene (purity 99.5%), 4-amino-musk xylene (purity >99.9%) and ortho-amino-musk xylene (purity 95%) in ethanol were added to estrogen receptor-positive human mammary carcinoma cells (MCF-7) and incubated for 6 days according to the E-screen method of Soto et al. (1995). They were tested at 5 different concentrations, up to 5 µmol/L (4-amino musk xylene) or 10 µmol/L (the other two substances) with a maximum solvent concentration of 0.1%. The rate of proliferation of the cells was determined by photometric analysis of the total protein content of the fixed cells and compared to that of a hormone-free control sample. The relative rate of proliferation (test substance relative to control) was then compared to that of 17β-estradiol. Musk xylene and 4-amino-musk xylene showed a, slightly higher rate of proliferation relative to the hormone-free control, whereas ortho-amino-musk xylene showed no increase. The potency of musk xylene and 4-amino musk xylene however was 10^{-5} less than that of 17β-estradiol (Bitsch et al., 2002). Although this result was statistically significant, it should be noted that the results of the tests were highly variable, whereas the results of the control sample (0.1% ethanol) were not shown.

4.1.2.8.4 Summary of toxicity to reproduction

With respect to fertility no multi-generation reproductive toxicity study was available for either route. In a 90-day dermal toxicity study with rats and also in an oral carcinogenicity study with mice, musk xylene caused no effects on the reproductive organs, whereas the structurally related compound musk ambrette caused testicular atrophy in the 90-day dermal toxicity study. In a peri/postnatal toxicity study no effects on sexual maturition and reproductive performance were reported in pups which were exposed to musk xylene *in utero* and during lactation.

In an oral developmental study with rats maternal toxicity, expressed as decreased body weight gain and food consumption, was seen in the mid and high dose level of 60 and 200 mg musk xylene/kg bw/day. Embryo toxicity (extra thoracic ribs and increased ossification) was seen at the highest dose level tested. The NOAEL for maternal toxicity in this study can be established at 20 mg/kg bw/day and the NOAEL for developmental toxicity at 60 mg/kg bw/day. There is no indication for teratogenicity.

In a limited one-generation study with special attention to induction of cytochrome P450 enzymes in the offspring, enhanced levels of CYP1A and 2B proteins and CYP1A-related enzyme activities were observed in pups at 14 days of age, which were born to dams exposed to 2 to 3 mg musk xylene/kg bw/day and above for at least 10 weeks before mating and through gestation and lactation. The NOEL for this effect was 0.7-0.8 mg/kg bw/day. From a cross-fostering study it appeared that the induction of cytochrome P450 enzymes in the pups may be attributed entirely to the postnatal exposure via the milk. However, in absence of any other indication of liver toxicity the slight changes in levels of biotransformation enzyme activities are considered to be of an adaptive nature rather than adverse.

An oral peri/postnatal toxicity study was performed, in which the F_1-generation was exposed to musk xylene *in utero* or through any transfer in the milk of the lactating dams. At the highest dose level of 25 mg/kg bw/day only very slight maternal toxicity (decreased body weight gain and food consumption) was seen. Slight pup toxicity, reflected in a slightly later day of attainment of air righting and slightly reduced body weight gain, was observed at the highest dose level. Dosing up to 25 mg/kg bw did not result in behavioural changes or in reduced reproductive capacity. The mid dose tested in this study, 7.5 mg/kg bw/day, can be considered the NOAEL for both maternal toxicity and peri/postnatal toxicity although it is recognised that the effects seen at 25 mg/kg bw in both dams and pups were only marginal and, in general, not statistically significant. Realising that this is a conservative approach, the fact that the effects at the next higher dose are very small and that the effect in pups is of uncertain biological significance has to be taken into account in the interpretation of the MOS values.

The available data obtained from the peri/postnatal toxicity study indicate that musk xylene needs not to be classified for reproductive toxicity. Given the marginal effects elicited in the offspring in that study and the fact that these effects are of uncertain biological significance, there is also no need to label musk xylene with R64 (“May cause harm to breast fed babies”). This was confirmed by the May 2002 meeting of the CMR Working Group.

Musk xylene and not 2-amino- and 4-amino-musk xylene, was demonstrated to be a very weak agonist in the E-screen assay. Binding to the estrogen receptor from trout or clawed frog showed binding of 2-amino- and 4-amino- musk xylene, and not for musk xylene itself.

These results are in conflict with each other. Furthermore, these weak estrogenicity has only been demonstrated *in vitro*, and no effects were found in the 90-day dermal repeated dose assays on reproductive organs, and in a peri/postnatal toxicity study on reproductive performance of the in utero exposed off-spring.

4.1.3 Risk characterisation (with regard to the effects listed in Annex 1A of Regulation 1488/94)

4.1.3.1 General aspects

There are no data available on the toxicokinetics of musk xylene after inhalation exposure.
After oral administration with ^{3}H-musk xylene to rats, the major route of excretion was via the faeces via the bile. Within 7 days, excretion into urine and faeces was approximately 10.3% and 75.5%, respectively, while about 2% remained in the carcass. Based on plasma peak levels, the estimated systemic availability of an oral dose in humans was 0.6 to 3.8%.

It is difficult to accurately estimate oral uptake percentages for rat and humans based on the available data. For humans, the calculated percentages are probably underestimations of the totally absorbed quantity because they are based on plasma levels and an assumed volume of distribution equal to the plasma volume. This volume of distribution is too low because musk xylene will preferably distribute into the fatty compartment. For the rat, based on the amount excreted in the urine and carcass, an oral bioavailability of at least 12% can be derived. This percentage is also an underestimate of the actual intestinal uptake, because biliary excretion is not accounted for. If it is assumed, however, that the contribution of the biliary excretion is equal after oral and dermal exposure (which seems reasonable in view of the long plasma half-life time (40 hours in rat, 60-94 days in humans) of musk xylene), the ratio urinary/faecal excretion after dermal exposure (viz. 4% / 15%) can be used to estimate the total uptake after oral exposure, when the experimental data of two different studies (Minegishi et al., 1991, Hawkins et al., 1984) are combined. The resulting estimate of total uptake after oral dosing is 10% + (15/4) · 10% = *ca.* 50%. For both rats and humans a percentage for uptake after oral exposure of 50% will be taken forward to the risk characterisation.

After a 6 hour dermal application of ^{14}C-labelled musk xylene (under occlusion) to rats about 20% of the applied dose was absorbed in 48 hours, with 2% remaining in the skin. Between 6 and 48 hours, the skin acted as a reservoir from which musk xylene continued to be absorbed. Excretion via urine and faeces (predominantly via bile) was virtually complete within 48 hours, with only very small amounts additionally excreted between 48 and 120 hours. After 120 hours, about 4% of the applied dose was excreted in urine and 14-15.2% in faeces, with only 0.2% remaining in the carcass. Radioactivity was detected in nearly all the tissues with peak concentrations after 8 hours in gastrointestinal tract followed by adipose tissue, liver, adrenals, thyroid, pancreas and kidneys.

After dermal application, ^{14}C-musk xylene was very poorly absorbed from the human skin as only 0.26 and <0.1% of the applied dose, respectively, was excreted in urine and faeces within 120 hours, and about 90% of the applied dose was recovered from the site of application. Based on plasma peak levels, the estimated systemic availability of a dermal dose in humans was 0.03 to 0.06%. These percentages are probably underestimations of the totally absorbed quantity because they are based on plasma levels and an assumed volume of distribution equal to the plasma volume. This volume of distribution is too low because musk xylene will preferrably distribute into the fatty compartiment.

In vitro experiments with skin from rats and humans also indicate that the percutaneous absorption of musk xylene from both occluded and unoccluded skin is poor, and that after removal of the test substance, the skin acts as depot from which musk xylene continues to be

systemically released. For dermal absorption of musk xylene in rats and humans, values of 20% and 10% respectively, are taken forward to the risk characterisation.

Metabolism of musk xylene in rats involves both reduction of a nitro group to an amine and hydroxylation of methyl groups, hydroxymethyl-musk xylene being the main metabolite in bile. Human urine contained a single metabolite which was chromatographically distinct from both musk xylene and hydroxymethyl-musk xylene. Other studies showed the presence of p-NH_2-musk xylene in human urine, but not N-acetyl-musk xylene.

From studies with rats and humans, in which musk xylene was administered intravenously or orally, respectively, it can be concluded that the plasma half life of musk xylene in rats is about 40 hours, while the plasma half life in humans is in the range of 60 to 94 days.

When administered orally to rats from 10 weeks before mating through to lactation, musk xylene levels in adults were highest in adipose tissue (in females 3.7-6.8 times higher than in males) and in milk. Transplacental passage occurred as in the offspring musk xylene accumulated in body fat. Musk xylene is also found in human milk fat and in adipose tissue.

The acute oral LD_{50} in mice and rats was established at >2,000 mg/kg bw. In a limited dermal study an application of 10,000 or 15,000 mg/kg bw caused no mortality in groups of three rabbits. The dermal test is not performed according to current standards. However, it is expected that the acute dermal toxicity is >2,000 mg/kg bw. According to the EC criteria musk xylene needs not be classified for its acute oral and dermal toxicity.

Data for acute inhalation toxicity were not available.

Base set requirements have not been met for testing of skin irritation as adequate skin irritation studies are lacking. The limited data available indicate that musk xylene was not irritating when applied to intact rabbit skin at extremely high dose levels under occlusive conditions for 24 hours. In two sensitisation studies with guinea pigs no indications were obtained for dermal irritation when applied at concentrations up to 10%. Musk xylene was also not found to possess dermal irritating properties in a 90 days dermal toxicity study at dose levels up to 240 mg/kg bw. However, in human volunteers, 5% musk xylene in petrolatum was reported to be mildly irritating, while in a patch test, concentrations of 0.1% and 1% produced irritation responses after 2 days of contact in 1% and 1.6% of the dermatological patients tested, respectively. Thus it appears that in humans musk xylene can induce skin irritation, albeit (very) mild. Primary irritation scores and other study details are not available and it is not possible to classify musk xylene for this property. However, based on the available information in animals it is not considered appropriate to require additional testing according to current guidelines, as even extremely high or prolonged dermal exposure did not elicit significant dermal reactions in rabbits or rats, respectively.

From a well performed eye irritation study it can be concluded that musk xylene is not eye irritating. According to the EC criteria musk xylene needs not to be classified for eye irritating properties.

For respiratory tract irritation no data are available.

Due to several shortcomings in the studies with guinea pigs it is not possible to conclude on the skin sensitising properties of musk xylene in animals. From studies with human volunteers, however, it can be concluded that musk xylene is not skin sensitising when tested at an irritating concentration. When patch tested to musk xylene, dermatological patients did not show allergic

reactions either. It is concluded that musk xylene is not a skin sensitising substance in humans and does not need to be classified for this end point.

Data on respiratory tract sensitisation or occupational asthma are not available.

In preliminary studies for a carcinogenicity study with mice, oral dose levels equivalent to 429 and 857 mg/kg/day for 17 weeks caused mortality in mice and dose levels equivalent to 214 mg/kg bw/day or higher caused a significantly decreased body weight (gain) and food consumption. At these dose levels an increased absolute and relative liver weight was seen as well as enlargement and irregularity of liver cells. As these studies were only dose range finding studies and very limited in design, no NOAELs are established. Moreover, the effects on the liver were not confirmed in a 80 weeks carcinogenicity study, while the only non-neoplastic effect in this study (decreased body weights) was reversible during the recovery period at the end of the study.

In a well performed dermal 90-days study with rats the two highest dose levels tested, 75 and 240 mg/kg bw/day, caused an increased absolute and relative liver weight of approximately 13-18%, not associated with any pathological finding. In this experiment no neuropathological effects and no effects on the reproductive organs were observed. No effects were observed at 24 mg/kg bw/day, which dose level can be established as the NOEL in this study. This NOEL can be considered as a NOAEL, although the extent of the liver weight changes at the next higher dose level was only marginal and of questionable biological significance. The value of 24 mg/kg bw/day is taken forward to the risk characterisation.

Inhalation repeated dose studies with musk xylene were not available.

Musk xylene was negative in several *in vitro* tests (bacterial gene mutation tests, SOS-chromotests, a mammalian gene mutation test, tests for chromosome aberrations and SCEs in mammalian cells, a micronucleus test in mammalian cells and an UDS test). In an *in vivo/in vitro* rat hepatocyte UDS test also negative results were obtained. It can be concluded that musk xylene is a non-genotoxic substance. Due to its enzyme-inducing properties, musk xylene can exhibit cogenotoxic activity.

Musk xylene has been tested for carcinogenicity in mice by dietary administration in one experiment with a duration of 80 weeks. Both dose levels tested (0.075 and 0.15%) resulted in statistically significantly increased incidences of hepatocellular adenomas in both sexes and of hepatocellular carcinomas in males. The incidence of Harderian gland adenomas was also statistically significantly increased in males at both dose levels. Some other tumours, like lung adenomas in both sexes and lymphomas and Harderian gland adenomas in females, occurred in greater number in the treated groups but the differences with control incidences were not statistically significant. The lowest dose tested, 0.075%, equivalent to 70-125 mg/kg bw/day in male mice and 80-143 mg/kg bw/day in female mice, is an effect dose. In this study no effects were seen on the reproductive organs.

Special investigations into the mechanism behind the mouse liver tumours indicated that musk xylene treatment caused a very significant induction of liver enzymes, including cytochromes P450 1A1, 1A2 and 2B and cytochrome b5. Levels of CYP2B protein in liver are as high as those seen with the classical CYP2B inducer phenobarbital. However, the metabolite p-NH2-musk xylene selectively inactivates the enzyme CYP2B. The toxicological significance of this induction/inhibition phenomenon is unclear. In a 7 days study in the mouse the NOEL for effects on liver enzymes was 10 mg/kg bw/day. Similar induction phenomena have been observed in rat liver and for this species a LOEL of 10 mg/kg bw/day after 7 days of exposure could be derived.

Even at dietary levels as low as 10 mg/kg feed, corresponding to 0.7 to 0.8 mg/kg bw/day, a slight inducing effect on CYP2B protein could be observed after *ca.* 75 days, while for CYP1A and 3A a ten times higher dose level appeared to be a LOEL. The induction phenomena were reversible and occured without simultaneous changes in liver weights. In absence of any other indication of liver toxicity the slight changes in levels of biotransformation enzyme activities are considered to be of an adaptive nature rather than adverse. Therefore this effect as such and the NOEL/LOEL for it will not be taken forward to the risk characterisation.

The mechanism behind the carcinogenic activity of musk xylene is not entirely understood. Statistically significantly increased incidences of malignancies were only observed in the livers of male B6C3F1 mice, a strain which is particularly prone to develop liver tumours. Other spontaneous tumours developed in the Harderian gland (adenomas), lungs (adenomas) and haematopoietic system (lymphomas). The treated groups showed somewhat higher numbers for these tumours (not statistically significantly different from controls, with the exception of Harderian gland adenomas in males). The carcinogenicity of musk xylene has not been studied in a second species, e.g. the rat.

It has been clearly demonstrated that musk xylene is not genotoxic. In addition, the carcinogenic properties of the substance seen in mouse liver seem to be related to induction of microsomal liver enzymes, notably cytochrome P450 1A1, 1A2 but most of all cytochrome P450 2B in a pattern which closely resembles the pattern of induction seen after administration of phenobarbital. The induction of these enzymes is observed both in rats and mice, and in both species the induced CYP2B enzyme is rapidly inactivated by p-NH_2-musk xylene, which is probably formed from musk xylene after nitro-reduction by intraintestinal micro-organisms. In contrast, the induced CYP1A1 and 1A2 enzymes are metabolically active and it has been demonstrated that exposure to musk xylene can result in enhanced bioactivation of several promutagens. Induction of microsomal liver enzymes is a threshold phenomenon with for musk xylene a NOEL of 10 mg/kg bw/day in the mouse and a LOEL of 10 mg/kg bw in the rat. It is conceivable that below a certain threshold the risk for promutagen bioactivation and carcinogenicity will be negligible. As to the Harderian gland tumours, only benign malformations developed. This gland and tissue type do not occur in humans and therefore these benign tumours are difficult to interpret with respect to their relevance to humans. Like the liver and Harderian gland tumours, the tumours in the lung and haematopoietic system occurred spontaneously in the B6C3F1 mouse strain, with only slightly higher incidences in the treated animals.

It is difficult to deduce the carcinogenic risk of musk xylene to humans from the available data, given that:

- only one species has been tested, i.e. the B6C3F1 mouse;
- this strain of mice is particularly prone to develop certain types of tumours, especially liver tumours;
- the mechanism behind the tumour development is not entirely understood, although it is clear that musk xylene has no genotoxic potential and that enzyme induction plays an important role in the development of the liver tumours observed.

Although musk xylene has not been tested for carcinogenicity in rats, there is a concern that it might be carcinogenic in rats as well, given the comparable enzyme induction properties of musk xylene in mice and rats. Further testing in e.g. rats or in mice to further elucidate the mechanism is, however, not considered to contribute much to the risk assessment of the carcinogenic risk of musk xylene to humans. This is because the available data does allow the conclusions that musk

xylene is a carcinogen in mice, that it acts by a non-genotoxic mode of action, and that the most serious type of tumour for which the incidence was statistically significantly increased (i.e. liver carcinomas in male mice) is mechanistically related to microsomal enzyme induction. Hence, for risk characterisation a threshold approach is considered justified, given that musk xylene is non-genotoxic and that enzyme induction is a threshold phenomenon. By taking the LOAEL of 70 mg/kg bw/day for tumour development (liver tumours in particular) as basis for the risk characterisation and by taking the mouse NOEL for enzyme induction into account in the interpretation of the MOS, this will already result in a rather conservative approach when realising that the B6C3F1 mouse is especially prone to develop liver tumours.

As to classification, IARC concluded in 1996 that there is limited evidence for the carcinogenicity of musk xylene in animals, but that the substance is not classifiable as to its carcinogenicity to humans (group 3). However, the effects on the liver observed with musk xylene resemble those that can be seen after dosing rats and mice with phenobarbital. Phenobarbital is clearly a (liver) carcinogenic substance in rodents and often used to promote the development of tumours that were initiated by preceding treatment with genotoxic substances. Although the relevance of the carcinogenicity of phenobarbital for humans has been questioned, IARC nevertheless in 2001 classified phenobarbital as a group 2B substance ("possibly carcinogenic to humans"). Hence, given the resemblance to phenobarbital, it is now concluded that the non-genotoxic compound musk xylene is to be classified as a carcinogen category 3 (R40), although it is realised that it is a borderline case.

With respect to fertility no multi-generation reproductive toxicity study was available for either route. In a 90-day dermal toxicity study with rats and also in an oral carcinogenicity study with mice, musk xylene caused no effects on the reproductive organs, whereas the structurally related compound musk ambrette caused testicular atrophy in the 90-day dermal toxicity study. In a peri/postnatal toxicity study no effects on sexual maturition and reproductive performance were reported in pups which were exposed to musk xylene *in utero* and during lactation.

In an oral developmental study with rats maternal toxicity, expressed as decreased body weight gain and food consumption, was seen in the mid and high dose level of 60 and 200 mg musk xylene/kg bw/day. Embryo toxicity (extra thoracic ribs and increased ossification) was seen at the highest dose level tested. The NOAEL for maternal toxicity in this study can be established at 20 mg/kg bw/day and the NOAEL for developmental toxicity at 60 mg/kg bw/day. There is no indication for teratogenicity.

In a limited one-generation study with special attention to induction of cytochrome P450 enzymes in the offspring, enhanced levels of CYP1A and 2B proteins and CYP1A-related enzyme activities were observed in pups at 14 days of age, which were born to dams exposed to 2 to 3 mg musk xylene/kg bw/day and above for at least 10 weeks before mating and through gestation and lactation. The NOEL for this effect was 0.7-0.8 mg/kg bw/day. From a cross-fostering study it appeared that the induction of cytochrome P450 enzymes in the pups may be attributed entirely to the postnatal exposure via the milk. However, in absence of any other indication of liver toxicity the slight changes in levels of biotransformation enzyme activities are considered to be of an adaptive nature rather than adverse.

An oral peri/postnatal toxicity study was performed, in which the F_1-generation was exposed to musk xylene *in utero* or through any transfer in the milk of the lactating dams. At the highest dose level of 25 mg/kg bw/day only very slight maternal toxicity (decreased body weight gain and food consumption) was seen. Slight pup toxicity, reflected in a slightly later day of attainment of air righting and slightly reduced body weight gain, was observed at the highest

dose level. Dosing up to 25 mg/kg bw did not result in behavioural changes or in reduced reproductive capacity. The mid dose tested in this study, 7.5 mg/kg bw/day, can be considered the NOAEL for both maternal toxicity and peri/postnatal toxicity although it is recognised that the effects seen at 25 mg/kg bw in both dams and pups were only marginal and, in general, not statistically significant. Realising that this is a conservative approach, the fact that the effects at the next higher dose are very small and that the effect in pups is of uncertain biological significance has to be taken into account in the interpretation of the MOS values.

The available data obtained from the peri/postnatal toxicity study indicate that musk xylene needs not to be classified for reproductive toxicity. Given the marginal effects elicited in the offspring in that study and the fact that these effects are of uncertain biological significance, there is also no need to label musk xylene with R64 ("May cause harm to breast fed babies").

In a 90-day dermal toxicity study with rats no indications for a neurotoxic potential was found for musk xylene, in contrast to the structurally related compound musk ambrette.

4.1.3.2 Workers

4.1.3.2.1 Introduction

Assuming that oral exposure is prevented by personal hygienic measures, the risk characterisation for workers is limited to the dermal and inhalation routes of exposure.

4.1.3.2.2 Comparison of exposure and effects

Acute toxicity

From a limited dermal toxicity study it can be concluded that musk xylene needs not to be classified for dermal toxicity. Given the absence of lethality or other systemic effects in the acute dermal study, and the anticipated occupational dermal exposure levels (2.5-42 mg/dag), it is concluded that musk xylene is of no concern for workers with regard to acute dermal effects: **conclusion (ii)**. There are no data on the acute inhalation toxicity of musk xylene. However given the estimated inhalation exposure levels (0.1-10 mg/m^3) and the low acute toxicity after oral and dermal administration, there are no indications for concern with respect to acute toxicity by inhalation exposure: **conclusion (ii)**.

Irritation and corrosivity

Acute dermal irritation
Base set requirements have not been met for testing of skin irritation as adequate skin irritation studies are lacking. Based on the available data it is not possible to classify musk xylene for skin irritation properties. However, it is not considered appropriate to require additional testing according to current guidelines, as even extremely high or prolonged dermal exposure did not elicit significant dermal reactions in rabbits or rats, respectively: **conclusion (ii)**.

Dermal irritation after repeated exposure

Repeated dermal exposure may induce local skin effects. Starting-points for the risk characterisation after repeated dermal exposure with respect to these effects are (a) the results from the dermal repeated-dose toxicity studies (see Section 4.1.2.5.1) and (b) the dermal occupational exposure estimates (see Section 4.1.1.1 and **Table 4.3**). In the 90-day dermal toxicity study with rats no skin effects were reported up to a dose of 240 mg/kg bw/day. This NOAEL is equivalent to 1.7 mg/cm^2 (based on a body weight of the rat (0.3 kg) and the exposed dermal area of the rat (42.5 cm^2, which is 10% of the estimated total body surface area). Given the estimated frequency of exposure (225d/year) chronic exposure is assumed for risk characterisation. The MOSs between the NOAEL and the dermal exposure levels are mentioned in **Table 4.15**. The MOSs are evaluated by comparison with the minimal MOS (9). In annex 1 to this RAR the assessment factors used to establish the minimal MOS are given (**Table A.1**). There is concern when the MOS is significantly lower than the minimal MOS.

Table 4.15 Occupational risk assessment of musk xylene for repeated dose toxicity after dermal exposure (local effects).

Scenario/subscenario	Risk characterisation for dermal exposure		
	Estimated dermal exposure in mg/day (mg/cm^2)[A]	MOS[B]	Conclusion[C]
1. The production of fragrance compounds	42 (0.1)	17	ii
2. The use of liquid fragrance compounds:			
- addition	4 (0.01)	170	ii
- cleaning	6.5 (0.005)	340	ii
3. The use of cleaning agents by professional cleaners	2.5 (0.003)	567	ii

A Between brackets the estimated dermal exposure in mg/cm^2 used for calculating MOSs; assuming an exposed dermal area of 420 cm^2 for Scenario 1 and 2 (except cleaning), an area of 1,300 cm^2 for Scenario 2 'cleaning', and and area of 840 cm^2 for Scenario 3;
B Based on a dermal NOAEL in rats of 1.7 mg/cm^2;
C Based on a comparison of the MOS with a minimal MOS of 9.

Given the MOSs for dermal exposure as mentioned in **Table 4.15**, it is concluded that, based upon the present information, there is no concern for local effects due to repeated dermal exposure: **conclusion (ii)**.

Eye irritation

Exposure to the eyes is possible via vapours or accidentally by splashing. Given the effects observed in the acute eye irritation study in rabbits, it is concluded that musk xylene is of no concern for workers with regard to acute eye irritation: **conclusion (ii)**.

Respiratory irritation

No data are available on the local effects in the respiratory tract after acute or repeated respiratory exposure. The risk for local effects after respiratory exposure cannot be derived from oral or dermal toxicity studies, so a quantitative risk characterisation is not possible. However, given the low or negligible estimated inhalation exposure there are no indications for concern for respiratory irritation: **conclusion (ii)**.

Sensitisation

Based on the available data on sensitisation it is concluded that musk xylene is not a skin sensitising substance in humans and does not need to be classified for this end point: **conclusion (ii)**.

Repeated-dose toxicity

Dermal exposure

Risk characterisation for local skin effects after repeated exposure to musk xylene is described in the paragraph 'Irritation and corrosivity=. This paragraph is limited to the systemic effects due to repeated exposure to musk xylene.

Starting-points for the risk characterisation for workers exposed by skin contact for systemic effects (excluding carcinogenicity) are (a) the NOAEL of 24 mg/kg bw/day from the 90-day dermal toxicity study with rats, and (b) the estimated dermal exposure levels for the different occupational scenarios (see Section 4.1.1.1 and **Table 4.3**). Given the estimated frequency of exposure (225 days/year), chronic exposure is assumed for risk characterisation. It is noted that the frequency of exposure during cleaning in Scenario 2 'use of liquid fragrance compounds/cleaning' is only 20-50 days/year. However, because of the long half life in blood and the accumulation potential of musk xylene it is justifiable to base the risk assessment on chronic exposure. The MOSs between the NOAEL and the dermal exposure levels are mentioned in **Table 4.16**. The MOSs are evaluated by comparison with the minimal MOS (180). In Annex 1 to this RAR the assessment factors used to establish the minimal MOS are given (**Table A.2**). There is concern when the MOS is significantly lower than the minimal MOS.

Table 4.16 Occupational risk assessment of musk xylene for repeated dose toxicity after dermal exposure (systemic effects).

Scenario/subscenario	Risk characterisation for dermal exposure		
	Estimated dermal exposure in mg/day (mg/kg bw/day)[A]	MOS[B]	Conclusion[c]
1. The production of fragrance compounds	42 (0.6)	40	ii [D]
The use of liquid fragrance compounds:			
- addition	4 (0.06)	400	ii
- cleaning	6.5 (0.09)	267	ii
3. The use of cleaning agents by professional cleaners	2.5 (0.04)	600	ii

A Between brackets the estimated dermal exposure in mg/kg bw/day (assuming a worker body weight of 70 kg) used for calculating MOSs;
B Based on a dermal NOAEL in rats of 24 mg/kg bw/day;
C Based on a comparison of the MOS with the minimal MOS (180);
D In view of the significantly overestimated exposure conclusion (ii) is drawn although the MOS is lower than the minimal MOS (see text for details).

Given the MOSs for dermal exposure as mentioned in **Table 4.16**, it is concluded that, based upon the present information, there is no reason for concern for systemic effects due to repeated dermal exposure in scenarios 2 and 3: **conclusion (ii)**. Comparison of the calculated MOS value for Scenario 1 (40) with the minimal MOS (180), indicates a concern for Scenario 1. However,

due to the crystalline nature of the substance, the exposure for Scenario 1 is substantially overestimated. Moreover, the strong odour of the substance will urge workers to wear protective clothing, thus further reducing the exposure. Based on these considerations **conclusion (ii)** is drawn for Scenario 1 as well.

Inhalation exposure

Starting-points for the risk characterisation for workers exposed by inhalation are (a) the NOAEL of 24 mg/kg bw/day from the dermal toxicity study with rats, and (b) the estimated inhalation exposure levels for the different occupational scenarios (see Section 4.1.2.5 and **Table** 4.3). Given the estimated frequency of exposure (225 days/year) chronic exposure is assumed for risk characterisation. The MOSs between the NOAEL and the inhalation exposure levels are mentioned in **Table 4.17**. The MOSs are evaluated by comparison with the minimal MOS (1,800). In Annex 1 to this RAR the assessment factors used to establish the minimal MOS are given (**Table A.3**). There is concern when the MOS is significantly lower than the minimal MOS.

Table 4.17 Risk assessment for musk xylene for repeated-dose toxicity after respiratory exposure.

Scenario/subscenario	Risk characterisation for respiratory exposure		
	Estimated respiratory exposure in mg/m^3 (mg/kg/bw/day)[A]	MOS[B]	Conclusion[C]
1. The production of fragrance compounds	0.3 (0.04)	600	ii
The use of liquid fragrance compounds:			
- addition	Negligible	High	ii
- cleaning	Negligible	high	ii
3. The use of cleaning agents by professional cleaners	Negligible	high	ii

A Between brackets the exposure in mg/kg bw/day, based on a respiratory volume of 10 m^3/workday and 70 kg worker;
B Based on a dermal NOAEL of 24 mg/kg bw/day in rats and assuming a worker body weight of 70 kg and a respiratory volume of 10 m^3 for a working day;
C Based on a comparison of the MOS with the minimal MOS (1,800).

Given the MOSs for inhalation exposure as mentioned in **Table 4.17**, it is concluded that, based upon the present information, there is no reason for concern for systemic effects due to repeated inhalation exposure in scenario 2 and 3: **conclusion (ii)**.. Comparison of the calculated MOS for Scenario 1 (600) with the minimal MOS (1,800) indicates a concern for this scenario. However, in view of the worst-case chracter of the minimal MOS because of the multiplication of the different assessment factors, a **conclusion (ii)** is considered justified.

Combined exposure

The total body burden (systemic dose) is determined by uptake after dermal as well as inhalation exposure to musk xylene. In general, a risk characterisation for systemic effects for combined exposure introduces a lot of uncertainties, e.g. due to differences in build up of the internal exposure after both exposure routes and due to difficulties in the choice of the most appropriate toxicity study as starting point. In case of musk xylene, the starting-point for both the risk characterisation after dermal and inhalation exposure is the dermal toxicity study with rats.

Therefore, it is justifiable to estimate the risk for combined exposure, starting with the NOAEL of 24 mg/kg bw/day. The MOSs between the NOAEL and the calculated systemic dose are mentioned in **Table 4.18**. The MOSs are evaluated by comparison with the minimal MOS (360). In Annex 1 to this RAR the assessment factors used to establish the minimal MOS are given (**Table A.4**). There is concern when the MOS is significantly lower than the minimal MOS.

Table 4.18 Risk assessment for combined exposure to musk xylene based on the NOAEL from the dermal toxicity study.

Scenario/subscenario	Risk characterisation for combined exposure				
	Estimated dermal exposure in mg/day (systemic dose in mg/kg bw/day)[A]	Estimated respiratory exposure in mg/m³ (systemic dose in mg/kg bw/day)[B]	Total systemic dose as result from dermal and inhalation exposure in mg/kg bw/day [C]	MOS [D]	Conclusion[E]
1. The production of fragrance compounds	42 (0.06)	0.3 (0.04)	0.1	48	ii[F]
2. The use of liquid fragrance compounds					
-addition	4 (0.006)	negligible	0.006	800	ii
Cleaning	6.5 (0.009)	negligible	0.009	533	ii
3. The use of cleaning agents by professional cleaners	2.5 (0.004)	negligible	0.004	1,200	Ii

A Between brackets the systemic dose due to dermal exposure in mg/kg bw/day, assuming a worker body weight of 70 kg and a dermal absorption of 10%;
B The systemic dose due to respiratory exposure in mg/kg bw/day, assuming a worker body weight of 70 kg, a respiratory volume of 10 m³ per workday, and 100% inhalation absorption;
C Total systemic dose, i.e., the sum of the systemic dose due to dermal exposure and the systemic dose due to respiratory exposure;
D Based on an internal dermal NOAEL of 4.8 mg/kg bw/day with rats (based on an external NOAEL of 24 mg/kg bw/day and a dermal absorption of 20%);
E Based on a comparison of the MOS with the minimal MOS (360);
F In view of the significantly overestimated dermal exposure, conclusion (ii) is drawn although the MOS is lower than the minimal MOS (see text for details).

Given the MOSs for combined exposure as mentioned in **Table 4.18**, it is concluded that, based upon the present information, there is no reason for concern for systemic effects due to repeated combined exposure in scenarios 2 and 3: **conclusion (ii)**. Comparison of the calculated MOS value for Scenario 1 (48) with the minimal MOS (360) indicates a concern for Scenario 1. However, due to the crystalline nature of the substance the exposure for Scenario 1 is substantially overestimated. Moreover, the strong odour of the substance will urge workers to wear protective clothing, thus further reducing the exposure. Based on these considerations **conclusion (ii)** is drawn for scearnio 1 as well.

Mutagenicity

Given the results from the mutagenicity studies, it is concluded that musk xylene is of no concern for workers with regard to mutagenicity: **conclusion (ii)**.

Carcinogenicity

Musk xylene is considered to be a carcinogen acting by a non-genotoxic mode of action. Therefore, a threshold approach is appropriate. Carcinogenicity studies performed by the dermal

and inhalation route were not available. In an oral study with mice a LOAEL of 70 mg/kg bw/day was observed based on carcinogenicity (tumours in the liver). This LOAEL can be used as starting-point for the risk characterisation for carcinogenicity for workers exposed by the skin or by inhalation, or by both routes (combined exposure). The estimated dermal and inhalation exposure levels for the different occupational scenarios (see Section 4.1.2.5 and **Table 4.3**) are compared with the oral LOAEL. The MOSs between the LOAEL and the dermal, inhalation and combined exposure levels are mentioned in **Table 4.19-4.21**. The MOSs are evaluated by comparison with the minimal MOSs. In annex 1 to this RAR the assessment factors used to establish the minimal MOSs are given (**Table A.5-A.7**). There is concern when the MOS is significantly lower than the minimal MOS.

Table 4.19 Risk assessment for musk xylene for carcinogenicity after dermal repeated exposure.

Scenario/subscenario	Risk characterisation for carcinogenicity		
	Estimated dermal exposure in mg/day (mg/kg/bw/day)[A]	MOS[B]	Conclusion[C]
1. The production of fragrance compounds	42 (0.6)	116	ii
2. The use of liquid fragrance compounds			
- addition	4 (0.06)	1,167	ii
- cleaning	6.5 (0.09)	778	ii
3. The use of cleaning agents by professional cleaners	2.5 (0.04)	1,750	ii

A Estimated dermal exposure in mg/kg bw/day assuming a worker body weight of 70 kg used for calculating MOSs;
B Based on an oral LOAEL of 70 mg/kg bw/day ;
C Based on a comparison of the MOS with the minimal MOS (126).

Table 4.20 Risk assessment for musk xylene for carcinogenicity after inhalation repeated exposure.

Scenario/subscenario	Risk characterisation for carcinogenicity		
	Estimated respiratory exposure in mg/m^3 (mg/kg/bw/day)[A]	MOS[B]	Conclusion
1. The production of fragrance compounds	0.3 (0.04)	1,750	ii
2. The use of liquid fragrance compounds:			
- addition	Negligible	High	ii
- cleaning	negligible	High	ii
3. The use of cleaning agents by professional cleaners	Negligible	High	ii

A Between brackets the exposure in mg/kg bw/day, based on a respiratory volume of 10 m^3/workday and 70 kg worker;
B Based on a LOAEL of 70 mg/kg bw/day in rats and assuming a worker body weight of 70 kg and a respiratory volume of 10 m^3 for a working day;
C Based on a comparison of the MOS with the minimal MOS (1,260).

Table 4.21 Risk assessment for carcinogenicity after combined exposure to musk xylene.

Scenario/subscenario	Risk characterisation for combined exposure				
	Estimated dermal exposure in mg/day (systemic dose in mg/kg bw/day)[A]	Estimated respiratory exposure in mg/m³ (systemic dose in mg/kg bw/day)[B]	Total systemic dose as result from dermal and inhalation exposure in mg/kg bw/day [C]	MOS [D]	Conclusion[E]
1. The production of fragrance compounds	42 (0.06)	0.3 (0.04)	0.1	350	ii
2. The use of liquid fragrance compounds					
- addition	4 (0.006)	Negligible	0.006	5,833	ii
- cleaning	6.5 (0.009)	negligible	0.009	3,889	ii
3. The use of cleaning agents by professional cleaners	2.5 (0.004)	negligible	0.004	8,750	ii

A Between brackets the systemic dose due to dermal exposure in mg/kg bw/day, assuming a worker body weight of 70 kg and a dermal absorption of 10%;
B The systemic dose due to respiratory exposure in mg/kg bw/day, assuming a worker body weight of 70 kg, a respiratory volume of 10 m³ per workday, and 100% inhalation absorption;
C Total systemic dose, i.e., the sum of the systemic dose due to dermal exposure and the systemic dose due to respiratory exposure;
D Based on a systemic oral LOAEL of 35 mg/kg bw/day with rats (based on an oral LOAEL of 70 mg/kg bw and an oral absorption of 50%); E Based on a comparison of the MOS with the minimal MOS (630).

Given the MOSs for dermal, inhalation, and combined exposure as mentioned in **Table 4.19-4.21**, it is concluded that, based upon the present information, there is no reason for concern for systemic effects due to repeated dermal, inhalation, or combined exposure in Scenario 2 and 3: **conclusion (ii)**. Comparison of the calculated MOS value for Scenario 1 (350) with the minimal MOS (630) indicates a concern for Scenario 1. However, due to the crystalline nature of the substance the exposure for Scenario 1 is substantially overestimated. Moreover, the strong odour of the substance will urge workers to wear protective clothing, thus further reducing the exposure. Based on these considerations **conclusion (ii)** is drawn for scearnio 1 as well.

The risk for local carcinogenicity after repeated dermal and inhalation exposure cannot be derived from the oral carcinogenicity study.

Reproductive toxicity

No information on reproduction toxicity of musk xylene is available. There are no indications for effects on reproductive organs based on a dermal 90-day toxicity study with rats, although in this study investigations were limited to histological examination of the reproductive organs: **conclusion (ii)**. Developmental studies performed by inhalation or dermal exposure were not available. In an oral developmental toxicity study in rats a NOAEL of 20 mg/kg bw/day is established for maternal toxicity and a NOAEL of 60 mg/kg bw/day for developmental toxicity. Musk xylene is not teratogenic.

In an oral peri/postnatal toxicity study in rats a NOAEL of 7.5 mg/kg bw/day was observed based on a slightly but significantly decreased body weight gain in pups at the next higher dose level (25 mg/kg bw/day). This NOAEL is used for risk characterisation by route-to-route extrapolation in order to get insight in the effects of peri/postnatal exposure to musk xylene on

the offspring. By use of this NOAEL as starting point for the risk assessment, it is assumed that the pre-natal effects as observed in the developmental toxicity study (NOAEL 60 mg/kg bw/day) are covered. The MOSs between the oral NOAEL and the dermal, respiratory, and combined exposure levels are shown in **Table 4.22-4.24**. The MOSs are evaluated by comparison with the minimal MOSs. In annex 1 to this RAR the assessment factors used to establish the minimal MOSs are given (**Table A.8-A.10**). There is concern when the MOS is significantly lower than the minimal MOS.

Table 4.22 Risk assessment for the offspring after dermal exposure to musk xylene.

Scenario/subscenario	Risk characterisation for dermal exposure		
	Estimated dermal exposure in mg/day (mg/kg bw/day)[A]	MOS[B]	Conclusion[C]
1. The production of fragrance compounds	42 (0.6)	13	ii
2. The use of liquid fragrance compounds			
- addition	4 (0.06)	125	ii
- cleaning	6.5 (0.09)	83	ii
3. The use of cleaning agents by professional cleaners	2.5 (0.04)	188	ii

A Estimated dermal exposure in mg/kg bw/day assuming a worker body weight of 70 kg used for calculating MOSs;
B Based on an oral peri/postnatal NOAEL in rats of 7.5 mg/kg bw/day;
C Based on a comparison of the MOS with the minimal MOS (7.2).

Table 4.23 Risk assessment for the offspring after respiratory exposure to musk xylene.

Scenario/subscenario	Risk characterisation for respiratory exposure		
	Estimated respiratory exposure in mg/m^3 (mg/kg/bw/day)[A]	MOS[B]	Conclusion[C]
1. The production of fragrance compounds	0.3 (0.04)	188	ii
2. The use of liquid fragrance compounds:			
- addition	Negligible	High	ii
- cleaning	negligible	High	ii
3. The use of cleaning agents by professional cleaners	Negligible	High	ii

A In brackets the exposure in mg/kg bw/day, based on a respiratory volume of 10 m^3/workday and 70 kg worker;
B Based on an oral peri/postnatal NOAEL of 7.5 mg/kg bw/day in rats and assuming a worker body weight of 70 kg;
C Based on a comparison of the MOS with the minimal MOS (72).

Table 4.24 Risk assessment for the offspring after combined exposure to musk xylene.

Scenario/subscenario	Risk characterisation for combined exposure				
	Estimated dermal exposure in mg/day (systemic dose, mg/kg bw/day)[A]	Estimated respiratory exposure in mg/m³ (systemic dose, mg/kg bw/day)[B]	Total systemic dose as result from dermal and inhalation exposure in mg/kg bw/day [C]	MOS [D]	Conclusion[E]
1. The production of fragrance compounds	42 (0.06)	0.3 (0.04)	0.1	38	ii
2. The use of liquid fragrance compounds:					
- addition	4 (0.006)	Negligible	0.006	625	ii
- cleaning	6.5 (0.009)	Negligible	0.009	417	ii
3. The use of cleaning agents by professional cleaners	2.5 (0.004)	Negligible	0.004	938	ii

A Between brackets the systemic dose due to dermal exposure in mg/kg bw/day, assuming a worker body weight of 70 kg and a dermal absorption of 10%;
B The systemic dose due to respiratory exposure in mg/kg bw/day, assuming a worker body weight of 70 kg, a respiratory volume of 10 m³ per workday, and 100% inhalation absorption;
C Total systemic dose, i.e., the sum of the systemic dose due to dermal exposure and the systemic dose due to respiratory exposure;
D Based on an internal peri/postnatal NOAEL of 3.75 mg/kg bw/day (based on an oral NOAEL of 7.5 mg/kg bw/day with rats and an oral absorption of 50%);
E Based on a comparison of the MOS with the minimal MOS (36).

Given the MOSs for dermal, inhalation, and combined exposure as mentioned in **Table 4.22-4.24**, it is concluded that, there is no reason for concern for effects on the offspring due to occupational exposure by inhalation, skin or combined exposure for all scenarios with regard to offspring: **conclusion (ii)**.

Occupational limit values

At the moment, occupational limit values for musk xylene have not been established.

4.1.3.2.3 Summary of risk characterisation for workers

For workers, for all relevant endpoints a **conclusion (ii)** was reached.

4.1.3.3 Consumers

4.1.3.3.1 Introduction

For consumers, the main exposure to musk xylene results from its use as a fragrance in body care products. Exposure to these products occurs frequently. The main exposure route for consumers is considered to be the dermal route.

Starting point for the risk characterisation is the external exposure level of 210 μg/kg bw/day (see Section 4.1.1.3). Because the absorption of musk xylene through human skin is at maximum 10%, this external exposure level results in an internal exposure level of 21 μg/kg bw/day.

4.1.3.3.2 Comparison of exposure and effects

Irritation

As musk xylene has only (very) mild dermal irritation properties in humans, merely at concentrations of musk xylene that do not occur in consumer cosmetic articles (in which the maximum fraction is 0.568% according to SCCNFP, 1999), there is no concern for consumers for skin irritation: **conclusion (ii)**.

There is no concern for consumers for eye irritation, because musk xylene is not an eye irritating substance: **conclusion (ii)**.

Sensitisation

There are no indications that musk xylene is a skin sensitiser in humans. Consumers are therefore not at risk after repeated dermal exposure: **conclusion (ii)**.

Repeated dose toxicity

Starting point for the risk assessment is the dermal NOAEL of 24 mg/kg bw/day from the 90-day toxicity study with rats. Assuming a dermal absorption value of 20% for rats, this NOAEL corresponds to an internal no-effect dose of 4.8 mg/kg bw/day.

Comparing this internal no-effect dose with the calculated human systemic exposure level of 21 μg/kg bw/day, a MOS of 229 can be calculated.

Taking into account intra- and interspecies differences, the use of a NOAEL from a semi-chronic study but also the worst-case character of the exposure estimate and the marginal effects observed at the LOAEL, this MOS indicates no concern for consumers following repeated dermal exposure: **conclusion (ii)**.

Genotoxicity

Musk xylene is a non-genotoxic substance: **conclusion (ii)**.

Carcinogenicity

Musk xylene is a carcinogen in mice (a second species, e.g. the rat was not tested). It acts by a non-genotoxic mode of action. Although the mechanism behind the carcinogenic activity of musk xylene is not entirely understood, at least for the observed liver tumours microsomal enzyme induction is involved. Therefore, for risk characterisation a threshold approach is considered justified, given that musk xylene is non-genotoxic and that enzyme induction is a threshold phenomenon. No dermal carcinogenicity studies were available. In an oral carcinogenicity study with B6C3F1 mice the LOAEL was 70 mg/kg bw/day for tumour development (liver tumours in particular).

To characterise the risk for consumers for carcinogenicity following dermal exposure to musk xylene, the oral LOAEL of 70 mg/kg bw/day is used as starting point. Assuming 50% oral absorption, this LOAEL corresponds to an internal low-effect dose of 35 mg/kg bw/day.

Comparing this internal low-effect dose with the calculated human systemic exposure level of 21 µg/kg bw/day, a MOS of 1,667 can be calculated. Taking into account intra- and interspecies differences (while realising that the B6C3F1 mouse is particularly prone to develop certain types of tumours, especially liver tumours) and the use of a LOAEL in stead of a NOAEL, the MOS of 1,667 indicates no concern for consumers for carcinogenicity after dermal exposurev: **conclusion (ii)**.

Reproductive toxicity

There are no indications for effects on fertility in the dermal 90-day toxicity study with rats and in the oral carcinogenicity study with mice, although in these studies investigations were limited to histological examination of the reproductive organs. Dermal developmental studies are lacking. In an oral developmental toxicity study with rats developmental toxicity only occurred at maternal toxic dose levels ($NOAEL_{developmental\ toxicity}$ 60 mg/kg bw/day and $NOAEL_{maternal\ toxicity}$ 20 mg/kg bw/day). In an oral peri/postnatal study in which rats were exposed to musk xylene from day 14 of gestation through weaning, the NOAEL for effects on the pups was 7.5 mg/kg bw/day.

In order to get insight in the risk for the progeny of pregnant consumers, the oral NOAEL of 7.5 mg/kg bw/day for peri/postnatal effects and the oral NOAEL of 60 mg/kg bw/day for developmental effects are used for risk characterisation. Assuming 50% oral absorption, these NOAELs correspond to internal no-effect doses of 3.75 and 30 mg/kg bw/day, respectively.

Comparing these internal no-effect doses with the calculated human systemic exposure level of 21 µg/kg bw/day, the MOSs are 179 and 1,429, respectively. Taking into account intra- and interspecies differences and the fact that the effect seen at the LOAEL in the peri/postnatal study was marginal in nature and of uncertain biological significance, these MOSs indicate no concern for peri/postnatal and developmental effects to the progeny of consumers: **conclusion (ii)**.

4.1.3.3.3 Summary of risk characterisation for consumers

For consumers, for all relevant endpoints a **conclusion (ii)** was reached.

It should be noted that the SCCNFP (1999) has recommended that the exposure of consumers due to the cosmetic use of musk xylene should be reduced by 50%. If this advice is implemented in EU-law the external exposure would then become 206.3/2 = 103.2 µg/kg bw/day giving an internal exposure of approximately 10.5 µg/kg bw/day (see introduction of this paragraph). This reduction of the exposure with 50% would only strengthen the **conclusion (ii)** already drawn for the current consumer exposure.

4.1.3.4 Indirect exposure via the environment

4.1.3.4.1 Introduction

For man exposed via the environment the inhalation route and oral route are applicable. Starting point for the risk characterisation for the local scale is private use, which shows the highest total daily intake. The regional scale takes into account all relevant life cycle steps mentioned in Section 3.1.2.1. In the EUSES calculations the total daily intake (external exposure) is 0.0136 and 3.55e-3 mg/kg bw/day for private use and the regional scale, respectively. Assuming an oral absorption of 50% for humans, these external exposures correspond to internal exposures of 6.8e-3 and 1.78e-3 mg/kg bw/day, respectively. Only for repeated dose toxicity the internal exposure is necessary for route-to-route extrapolation.

Because of the occurrence of musk xylene in mother's milk, a separate risk characterisation is necessary for breast-fed babies (highest exposure value 5.12 μg/kg bw/day).

4.1.3.4.2 Comparison of exposure and effects

Inhalation exposure

No inhalation toxicity data are available for long-term effects (repeated dose and reproductive toxicity, carcinogenicity). A direct comparison with the inhalation toxicity data and local and regional air concentrations can therefore not be carried out. However, from **Table 4.6** it can be seen that the contribution of the inhalation of musk xylene via air is negligible compared to other uptake routes. Hence, for man indirectly exposed via the environment, **conclusion (ii)** can be derived for inhalation exposure for both the local and regional scale.

Total daily intake

The total daily intake covers exposure via food and air, but as can be seen from **Table 4.6** the contribution of the latter is negligible. Hence, the main exposure route is oral.

Repeated dose toxicity

From the oral dose-range finding studies no NOAELs could be established. Starting point for the risk assessment is therefore the dermal NOAEL of 24 mg/kg bw/day from the 90-day toxicity study with rats. Assuming a dermal absorption value of 20% for rats, this NOAEL corresponds to an internal no-effect dose of 4.8 mg/kg bw/day.

Comparing this internal no-effect dose with the estimated internal total human daily intake levels, the MOSs for both local and regional scale are >700 (see **Table 4.25**).

Taking into account intra- and interspecies differences, the use of a NOAEL from a semi-chronic study but also the marginal effects observed at the LOAEL, these MOSs indicate no concern for man repeatedly exposed indirectly via the environment: **conclusion (ii)**.

Genotoxicity

Musk xylene is a non-genotoxic substance: **conclusion (ii)**.

Carcinogenicity

Musk xylene is a carcinogen in mice (a second species, e.g. the rat was not tested). It acts by a non-genotoxic mode of action. Although the mechanism behind the carcinogenic activity of musk xylene is not entirely understood, at least for the observed liver tumours microsomal enzyme induction is involved. Therefore, for risk characterisation a threshold approach is considered justified, given that musk xylene is non-genotoxic and that enzyme induction is a threshold phenomenon. No dermal carcinogenicity studies were available. In an oral carcinogenicity study with B6C3F1 mice the LOAEL was 70 mg/kg bw/day for tumour development (liver tumours in particular).

To characterise the risk for carcinogenicity for man indirectly exposed via the environment to musk xylene, the oral LOAEL of 70 mg/kg bw/day is used as starting point. Comparing this low-effect dose with the estimated total human daily intake levels, the MOSs for both local and regional scale are >>1,000 (see **Table 4.25**). Taking into account intra- and interspecies differences (while realising that the B6C3F1 mouse is particularly prone to develop certain types of tumours, especially liver tumours) and the use of a LOAEL in stead of a NOAEL, these MOSs indicate no concern for carcinogenicity for man exposed indirectly via the environment: **conclusion (ii)**.

Reproductive toxicity

There are no indications for effects on fertility in the dermal 90-day toxicity study with rats and in the oral carcinogenicity study with mice, although in these studies investigations were limited to histological examination of the reproductive organs. In an oral developmental toxicity study with rats developmental toxicity only occurred at maternal toxic dose levels ($NOAEL_{developmental\ toxicity}$ 60 mg/kg bw/day and $NOAEL_{maternal\ toxicity}$ 20 mg/kg bw/day). In an oral peri/postnatal study in which rats were exposed to musk xylene from day 14 of gestation through weaning, the NOAEL for effects on the pups was 7.5 mg/kg bw/day.

In order to get insight in the risk for the progeny of pregnant women indirectly exposed via the environment, the oral NOAEL of 7.5 mg/kg bw/day for peri/postnatal effects and the oral NOAEL of 60 mg/kg bw/day for developmental effects are used for risk characterisation.

Comparing these no-effect doses with the estimated total human daily intake levels, the MOSs for both local and regional scale are >500 (see **Table 4.25**).

Taking into account intra- and interspecies differences and the fact that the effect seen at the LOAEL in the peri/postnatal study was marginal in nature and of uncertain biological significance, the MOSs indicate no concern for peri/postnatal and developmental effects to the progeny of women exposed indirectly via the environment: **conclusion (ii)**.

Table 4.25 Margins of safety for local and regional scale for musk xylene.

		Total daily intake (internal / external exposure) in mg/kg bw/day	MOS Repeated dose toxicity	MOS Carcinogenicity	MOS Reproductive toxicity	
					peri/postnatal	developmental
Local	Private use	6.8e-3 / 0.0136	706	5,147	551	4,412
Regional	All life cycle steps	1.78e-3 / 3.55e-3	2697	1,9718	2,213	16,901

Exposure via mother's milk

The highest exposure of musk xylene via mother's milk was calculated to be 5.12 µg/kg bw/day. Data from a peri/postnatal toxicity study would be the most suitable to characterise the risk for babies exposed via mother's milk. For musk xylene, the NOAEL for peri/postnatal effects is 7.5 mg/kg bw. Comparing this no-effect dose with the maximum exposure level via mother's milk, a MOS of 1,465 is derived. Taking into account intra- and interspecies differences and the fact that the effect seen at the LOAEL in the peri/postnatal study was marginal in nature and of uncertain biological significance, this MOS indicates no concern for breast-fed babies: **conclusion (ii)**.

4.1.3.4.3 Summary of risk characterisation for exposure via the environment

A **conclusion (ii)** was reached for man exposed indirectly via the environment at the local scale and at the regional scale, and also for breast-fed babies.

4.1.3.5 Combined exposure

As indicated in Section 4.1.1.5, a worst-case estimate for the combined (external) exposure to musk xylene would be the sum of the worst-case estimates for the three individual populations, i.e. 0.6 mg/kg bw/day (dermal, workplace) + 0.043 mg/kg bw/day (inhalation, workplace) + 0.21 mg/kg bw/day (dermal, consumers) + 0.0136 mg/kg bw/day (oral, locally via the environment). Assuming figures of 10%, 100% and 50% for dermal, inhalation and oral absorption, respectively, an internal exposure of 0.13 mg/kg bw/day (i.e. 0.06 mg/kg bw/day (dermal, workplace) + 0.043 mg/kg bw/day (inhalation, workplace) + 0.021 mg/kg bw/day (dermal, consumers) + 0.0068 mg/kg bw/day (oral, locally via the environment)) can be calculated. Note that approximately 79% of the combined internal exposure estimate originates from occupational sources.

Acute toxicity / Irritation / Sensitisation / Genotoxicity

Given that musk xylene is not acutely toxic, eye irritating, skin sensitising and genotoxic, and musk xylene has only weak, if any, skin irritating potential, there is no concern for these endpoints after combined exposure to musk xylene: **conclusion (ii)**.

Repeated dose toxicity

Starting point for the risk assessment is the dermal NOAEL of 24 mg/kg bw/day from the 90-day toxicity study with rats. Assuming a dermal absorption value of 20% for rats, this NOAEL corresponds to an internal no-effect dose of 4.8 mg/kg bw/day.

Comparing this internal no-effect dose with the calculated combined human systemic exposure level of 0.13 mg/kg bw/day, a MOS of 37 can be calculated.

Taking into account intra- and interspecies differences, the use of a NOAEL from a semi-chronic study but also the worst-case character of the combined exposure estimate and the marginal effects observed at the LOAEL, this MOS indicates no concern for repeated combined exposure: **conclusion (ii)**.

Carcinogenicity

Musk xylene is a carcinogen in mice (a second species, e.g. the rat was not tested). It acts by a non-genotoxic mode of action. Although the mechanism behind the carcinogenic activity of musk xylene is not entirely understood, at least for the observed liver tumours microsomal enzyme induction is involved. Therefore, for risk characterisation a threshold approach is considered justified, given that musk xylene is non-genotoxic and that enzyme induction is a threshold phenomenon. No dermal carcinogenicity studies were available. In an oral carcinogenicity study with B6C3F1 mice the LOAEL was 70 mg/kg bw/day for tumour development (liver tumours in particular).

To characterise the risk for carcinogenicity following combined exposure to musk xylene, the oral LOAEL of 70 mg/kg bw/day is used as starting point. Assuming 50% oral absorption, this LOAEL corresponds to an internal low-effect dose of 35 mg/kg bw/day.

Comparing this internal low-effect dose with the calculated combined human systemic exposure level of 0.13 mg/kg bw/day, a MOS of 269 can be calculated. Taking into account intra- and interspecies differences (while realising that the B6C3F1 mouse is particularly prone to develop certain types of tumours, especially liver tumours), the use of a LOAEL in stead of a NOAEL and the worst-case character of the combined exposure estimate, this MOS indicates no concern for carcinogenicity after combined exposure: **conclusion (ii)**.

Reproductive toxicity

There are no indications for effects on fertility in the dermal 90-day toxicity study with rats and in the oral carcinogenicity study with mice, although in these studies investigations were limited to histological examination of the reproductive organs. In an oral developmental toxicity study with rats developmental toxicity only occurred at maternally toxic dose levels ($NOAEL_{developmental\ toxicity}$ 60 mg/kg bw/day and $NOAEL_{maternal\ toxicity}$ 20 mg/kg bw/day). In an oral peri/postnatal study in which rats were exposed to musk xylene from day 14 of gestation through weaning, the NOAEL for effects on the pups was 7.5 mg/kg bw/day.

In order to get insight in the risk for the progeny of pregnant women, the oral NOAEL of 7.5 mg/kg bw/day for peri/postnatal effects and the oral NOAEL of 60 mg/kg bw/day for developmental effects are used for risk characterisation. Assuming 50% oral absorption, these NOAELs correspond to internal no-effect doses of 3.75 and 30 mg/kg bw/day, respectively. Comparing these internal no-effect doses with the calculated combined human systemic exposure level of 0.13 mg/kg bw/day, the MOSs are 29 and 231, respectively.

Taking into account intra- and interspecies differences and the worst-case character of the combined exposure estimate, the MOS of 231 indicates no concern for developmental effects to the progeny of pregnant women after combined exposure: **conclusion (ii)**. As to peri/postnatal effects, a MOS of 29 also indicates no concern for the progeny of pregnant women after combined exposure: **conclusion (ii)**. This is because the peri/postnatal study was directed towards this specific subpopulation, and that for any subpopulation the intraspecies differences in sensitivity will be smaller than for the population in total. Hence, it is reasonable to apply a smaller intraspecies factor for the progeny than 10, which is in concurrence with the risk characterisation for the progeny of workers. A MOS of 29 would then lead to a **conclusion (ii)**, also because the effect seen at the LOAEL in the peri/postnatal study was marginal in nature and of uncertain biological significance and because of the worst-case character of the combined exposure estimate.

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

4.2.1 Effects assessment: Hazard identification and Dose (concentration) - response (effect) assessment

4.2.1.1 Explosivity

Explosivity of musk xylene is indicated by shock and heat. Propagation in allowed packaging is limited.

4.2.1.2 Flammability

Musk xylene is flammable.

4.2.1.3 Oxidising potential

Musk xylene is not oxidising.

4.2.2 Risk characterisation

Given the physico-chemical data, musk xylene is considered not to form a risk with respect to oxidising properties: **conclusion (ii)**.

It is noted that musk xylene is flammable and explosive by shock and heat, and should be labelled with respect to these aspects. Therefore, measures to avoid flammability and explosion are indicated. If the appropriate conditions of handling and storage are adhered to, there are no concerns for risks to human health arising from the physicochemical properties of musk xylene and **conclusion (ii)** applies.

5 RESULTS

5.1 ENVIRONMENT

Conclusion (i) There is a need for further information and/or testing.

This conclusion is reached, because the substance is considered to be a PBT candidate chemical. A further PBT- testing strategy is proposed.

5.2 HUMAN HEALTH

5.2.1.1 Workers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those, which are being applied already.

Table 5.1 Overview of conclusions with respect to occupational risk characterisation of musk xylene.

End point	Conclusions valid for the occupational scenario's					
	Scenario 1		Scenario 2		Scenario 3	
	MOS	Conclusion	MOS	conclusion	MOS	conclusion
acute toxicity						
- dermal	n.a.	ii	n.a.	ii	n.a.	ii
- inhalation	n.a.	ii	n.a.	ii	n.a.	ii
irritation and corrosivity, single exposure						
- dermal	n.a.	ii	n.a.	ii	n.a.	ii
- inhalation	n.a.	ii	n.a.	ii	n.a.	ii
- eyes	n.a.	ii	n.a.	ii	n.a.	ii
Sensitisation						
- dermal	n.a.	ii	n.a.	ii	n.a.	ii
repeated dose toxicity, local toxicity						
- dermal						
(dermal NOAEL 240 mg/kg bw/day)	17	ii	170/340	ii	567	ii
repeated dose, systemic toxicity						
- dermal	40	ii	400/267	ii	600	ii
(dermal NOAEL 24 mg/kg bw/day)						
- inhalation	600	ii	high	ii	high	ii
(dermal NOAEL 24 mg/kg bw/day)						
- combined	48	ii	800/533	ii	1,200	ii
(dermal NOAEL 24 mg/kg bw/day)						
Mutagenicity	n.a	Ii	n.a.	ii	n.a.	ii

Table 5.1 continued overleaf

Table 5.1 continued Overview of conclusions with respect to occupational risk characterisation of musk xylene

End point	Conclusions valid for the occupational scenario's					
	Scenario 1		Scenario 2		Scenario 3	
	MOS	Conclusion	MOS	conclusion	MOS	conclusion
carcinogenicity						
- dermal	116	ii	1,167/778	ii	1,750	ii
(oral LOAEL 70 mg/kg bw/day)						
- inhalation	1,750	ii	high	ii	high	ii
(oral LOAEL 70 mg/kg bw/day)						
- combined	350	ii	5,833/3,889	ii	8,750	ii
(oral LOAEL 70 mg/kg bw/day)						
reproductive toxicity, fertility						
- dermal	n.a.	ii	n.a.	ii	n.a.	ii
- inhalation	n.a.	ii	n.a.	ii	n.a.	ii
reproductive toxicity, developmental effects (peri/postnatal exposure)						
- dermal	13	ii	125/83	ii	188	ii
(oral NOAEL of 7.5 mg/kg bw/day)						
- inhalation	188	ii	high	ii	high	ii
(oral NOAEL of 7.5 mg/kg bw/day)						
- combined	38	ii	625/417	ii	938	ii
(oral NOAEL of 7.5 mg/kg bw/day)						
Flammability	n.a.	ii	n.a.	ii	n.a.	ii
explosive properties	n.a.	ii	n.a.	ii	n.a.	ii
oxidising properties	n.a.	ii	n.a.	ii	n.a.	ii

n.a. not applicable

5.2.1.2 Consumers

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those, which are being applied already.

5.2.1.3 Humans exposed indirectly via the environment

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those, which are being applied already.

5.3 COMBINED EXPOSURE

Conclusion (ii) There is at present no need for further information and/or testing and no need for risk reduction measures beyond those, which are being applied already.

5.4 RISKS FROM PHYSICO-CHEMICAL PROPERTIES

Given the physico-chemical data, musk xylene is considered not to form a risk with respect to oxidising properties: **conclusion (ii)**.

It is noted that musk xylene is flammable and explosive by shock and heat, and should be labelled with respect to these aspects. Therefore, measures to avoid flammability and explosion are indicated. If the appropriate conditions of handling and storage are adhered to, there are no concerns for risks to human health arising from the physico-chemical properties of musk xylene and **conclusion (ii)** applies.

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ABBREVIATIONS

Standard term / Abbreviation	Explanation/Remarks and Alternative Abbreviation(s)
ADI	Acceptable Daily Intake
AF	Assessment Factor
Ann	Annex
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
BOD	Biochemical Oxygen Demand
bw	body weight / Bw, bw
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
°C	degrees Celsius (centigrade)
C_{50}	median immobilisation concentration or median inhibitory concentration 1 / explained by a footnote if necessary
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CEPE	European Committee for Paints and Inks
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT50	Clearance Time, elimination or depuration expressed as half-life
d	Day(s)
d.wt	dry weight / dw
dfi	daily food intake
DG	Directorate General

DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT_{50}	Degradation half-life or period required for 50 percent dissipation / degradation
DT_{50lab}	Period required for 50 percent dissipation under laboratory conditions (define method of estimation)
DT_{90}	Period required for 90 percent dissipation / degradation
$DT_{90field}$	Period required for 90 percent dissipation under field conditions (define method of estimation)
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]
EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests
EC	European Communities
EC	European Commission
EC_{10}	Effect Concentration measured as 10% effect
EC_{50}	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
f_{oc}	Organic carbon factor (compartment depending)
G	Gram(s)

GLP	Good Laboratory Practice
h	hour(s)
ha	Hectares/h
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 tonnes/annum)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database (existing substances)
IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues
kg	kilogram(s)
K_{oc}	organic carbon normalised distribution coefficient
K_{ow}	octanol/water partition coefficient
Kp	solids-water partition coefficient
kPa	kilo Pascals
l	litre(s)
$L(E)C_{50}$	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
log	logarithm to the basis 10
m	Meter
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration

MC	Main Category
mg	Milligram(s)
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidising (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
OC	Organic Carbon content
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
Pa	Pascal unit(s)
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling
PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration
pH	logarithm (to the base 10) of the hydrogen ion concentration {H+}
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC(s)	Predicted No Effect Concentration(s)
$PNEC_{water}$	Predicted No Effect Concentration in Water
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
(Q)SAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation

RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst-Case
S phrases	Safety phrases according to Annex IV of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCHER	Scientific Committee on Health and Environmental Risks
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
ThOD	Theoritical Oxygen Demand
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations
UNEP	United Nations Environment Programme
US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material
µg	microgram(s)
vB	very Bioaccumulative
VOC	Volatile Organic Compound
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w	gram weight

w/w	weight per weight ratio
WHO	World Health Organisation
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)

Annex A Establishment of the minimal MOSs used for the risk characterisation by the Netherlands

Note**:**This annex represents the view of the Netherlands. In particular, it presents the approach used by the Netherlands to determine, in a transparant way, which conclusion is to be drawn for worker risk characterisation based on the magnitude of the MOS. The (default) assessment factors used below are derived from Hakkert et al. (1996).

Table A.1 Assessment factors applied for the calculation of the minimal MOS for local toxicity after dermal repeated exposure.

Aspect	Assessment factors
Interspecies differences[a]	3
Intraspecies differences	3
Differences between experimental conditions and exposure[b]	1
Type of critical effect	1
Dose response	1
Confidence of the database	1
Overall	9

a Extrapolation based on differences in sensitivity, for local effects adjustment for differences in metabolic size is inappropriate.
b For local skin effects it is assumed that the exposure duration can influence the severity of the effects but will not influence the height of the NOAEL. Therefore, for local skin effects no assessment factor for the duration of exposure is applied.

Table A.2 Assessment factors applied for the calculation of the minimal MOS for systemic toxicity after dermal repeated exposure.

Aspect	Assessment factors
Interspecies differences[a]	4 x 3
Differences in dermal absorption between animal and human[b]	0.5
Intraspecies differences	3
Differences between experimental conditions and exposure[c]	10
Type of critical effect	1
Dose response	1
Confidence of the database	1
Overall	180

a Extrapolation based on differences in caloric demands, together with a factor 3 for differences in sensitivity.
b A factor 0.5 for differences in dermal absorption (20% dermal absorption in animals and 10% dermal absorption in humans).
c A factor 10 is applied as default for the extrapolation of subchronic to chronic exposure.

Table A.3 Assessment factors applied for the calculation of the minimal MOS for systemic effects after repeated inhalation exposure.

Aspect	Assessment factors
Interspecies differences[a]	4 x 3
Intraspecies differences	3
Differences between experimental conditions and exposure[b]	10
Type of critical effect	1
Dose response	1
Route-to-route extrapolation[c]	5
Confidence of the database	1
Overall	1,800

a Extrapolation based on differences in caloric demands, together with a factor 3 for remaining uncertainties.
b A factor 10 is applied as default for the extrapolation of subchronic to chronic exposure.
c For route-to-route extrapolation correction is made by differences between dermal and inhalation absorption. A default value for inhalation absorption is used, because data are lacking, i.e. 100%. Based on experimental data a dermal absorption percentage of 20% is used.

Table A.4 Assessment factors applied for the calculation of the minimal MOS for combined repeated exposure.

Aspect	Assessment factors
Interspecies differences[a]	4 x 3
Intraspecies differences	3
Differences between experimental conditions and exposure[b]	10
Type of critical effect	1
Dose response	1
Confidence of the database	1
Overall	360

a Extrapolation based on differences in caloric demands, together with a factor 3 for remaining uncertainties.
b A factor 10 is applied as default for the extrapolation of subchronic to chronic exposure.

Table A.5 Assessment factors applied for the calculation of the minimal MOS for carcinogenic effects after dermal exposure.

Aspect	Assessment factors
Interspecies differences[a]	7 x 3
Intraspecies differences	3
Differences between experimental conditions and exposure	1
Type of critical effect	1
Dose response[b]	10
Route-to-route extrapolation[c]	0.2
Confidence of the database	1
Overall	126

a Extrapolation based on differences in caloric demands, together with a factor 3 for remaining uncertainties.
b A factor 10 is applied as default for the extrapolation of LOAEL to NAEL. Despite the observed dose-response relationship this is justifiable because the calculated NAEL (7 mg/kg bw/day) lies in the same order of magnitude as the NOEL (10 mg/kg bw/day) based on the 7 day toxicity study with mice (special investigation on enzyme activity).
c For route-to-route extrapolation correction is made by differences between oral and dermal absorption. Based on experimental data 50% oral absorption and 10% dermal absorption is used.

Table A.6 Assessment factors applied for the calculation of the minimal MOS for carcinogenic effects after inhalation exposure.

Aspect	Assessment factors
Interspecies differences[a]	7 x 3
Intraspecies differences	3
Differences between experimental conditions and exposure	1
Type of critical effect	1
Dose response[b]	10
Route-to-route extrapolation[c]	2
Confidence of the database	1
Overall	1,260

a Extrapolation based on differences in caloric demands, together with a factor 3 for remaining uncertainties.
b A factor 10 is applied as default for the extrapolation of LOAEL to NAEL. Despite the observed dose-response relationship this is justifiable because the calculated NAEL (7 mg/kg bw/day) lies in the same order of magnitude as the NOEL (10 mg/kg bw/day) based on the 7 day toxicity study with mice (special investigation on enzyme activity).
c For route-to-route extrapolation correction is made by differences between oral and inhalation absorption. Based on experimental data 50% oral absorption is used. A default value for inhalation absorption is used, because data are lacking, i.e. 100%.

Table A.7 Assessment factors applied for the calculation of the minimal MOS for carcinogenic effects after combined exposure.

Aspect	Assessment factors
Interspecies differences[a]	7 x 3
Intraspecies differences	3
Differences between experimental conditions and exposure	1
Type of critical effect	1
Dose response[b]	10
Confidence of the database	1
Overall	630

a Extrapolation based on differences in caloric demands, together with a factor 3 for remaining uncertainties.
b A factor 10 is applied as default for the extrapolation of LOAEL to NAEL. Despite the observed dose-response relationship this is justifiable because the calculated NAEL (7 mg/kg bw/day) lies in the same order of magnitude as the NOEL (10 mg/kg bw/day) based on the 7 day toxicity study
with mice (special investigation on enzyme activity).

Table A.8 Assessment factors applied for the calculation of the minimal MOS for offspring effects after dermal exposure.

Aspect	Assessment factors
Interspecies differences[a]	4 x 3
Intraspecies differences[b]	3
Differences between experimental conditions and exposure[c]	1
Type of critical effect	1
Dose response	1
Route-to-route extrapolation[d]	0.2
Confidence of the database	1
Overall	7.2

a Extrapolation based on differences in caloric demands, together with a factor 3 for differences in sensitivity.
b Because the progeny comprises only a subpopulation of the general population, a factor 3 is considered to be sufficient to compensate for differences within this subpopulation.
c A factor for exposure duration is not required since it concerns peri/postnatal exposure to pups.
d For route-to-route extrapolation correction is made by differences between oral and dermal absorption. Based on experimental data 50% oral absorption and 10% dermal absorption is used.

Table A.9 Assessment factors applied for the calculation of the minimal MOS for offspring effects after inhalation exposure.

Aspect	Assessment factors
Interspecies differences[a]	4 x 3
Intraspecies differences[b]	3
Differences between experimental conditions and exposure[c]	1
Type of critical effect	1
Dose response	1
Route-to-route extrapolation[d]	2
Confidence of the database	1
Overall	72

a Extrapolation based on differences in caloric demands, together with a factor 3 for differences in sensitivity.
b Because the progeny comprises only a subpopulation of the general population, a factor 3 is considered to be sufficient to compensate for differences within this subpopulation.
c A factor for exposure duration is not required since it concerns peri/postnatal exposure to pups.
d For route-to-route extrapolation correction is made by differences between oral and inhalation absorption. Based on experimental data 50% oral absorption is used. A default value for inhalation absorption is used, because data are lacking, i.e. 100%.

Table A.10 Assessment factors applied for the calculation of the minimal MOS for offspring effects after combined exposure.

Aspect	Assessment factors
Interspecies differences[a]	4 x 3
Intraspecies differences[b]	3
Differences between experimental conditions and exposure[c]	1
Type of critical effect	1
Dose response	1
Confidence of the database	1
Overall	36

a Extrapolation based on differences in caloric demands, together with a factor 3 for differences in sensitivity.
b Because the progeny comprises only a subpopulation of the general population, a factor 3 is considered to be sufficient to compensate for differences within this subpopulation.
c A factor for exposure duration is not required since it concerns peri/postnatal exposure to pups.

European Commission

EUR 21506 EN **European Union Risk Assessment Report**
5-tert-butyl-2,4,6-trinitro-m-xylene (musk xylene), Volume 55

Editors: S.J. Munn, R. Allanou, K. Aschberger, F. Berthault, O. Cosgrove, M. Luotamo S. O'Connor, S. Pakalin, A. Paya-Perez, G. Pellegrini, S. Scheer, B. Schwarz-Schulz, S. Vegro

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Environment and quality of life series

The report provides the comprehensive risk assessment of the substance 5-tert-butyl-2,4,6-trinitro-m-xylene (musk xylene). It has been prepared by The Netherlands in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to humans and the environment, laid down in Commission Regulation (EC) No. 1488/94.

The evaluation considers the emissions and the resulting exposure to the environment and the human populations in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined. For human health the scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.

The human health risk assessment for musk xylene concludes that risks are not expected.

The environmental risk assessment for musk xylene concludes that there is a need for further information and/or testing because the substance is considered to be a PBT candidate chemical.

A further PBT – testing strategy is proposed

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European Commission – Joint Research Centre
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European Chemicals Bureau (ECB)

European Union Risk Assessment Report

5-tert-butyl-2,4,6-trinitro-m-xylene (musk xylene)

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