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Institute for Health and Consumer Protection
European Chemicals Bureau
I-21020 Ispra (VA) Italy

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

CAS No: 81-15-2

EINECS No: 201-329-4

Summary Risk Assessment Report

2005 **Special Publication I.05.15**

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(MUSK XYLENE)

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SUMMARY RISK ASSESSMENT REPORT

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.

Contact point:
Chemical Substances Bureau
P.O. Box 1
3720 BA Bilthoven
The Netherlands

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PREFACE

This report provides a summary, with conclusions, of the risk assessment report of the substance 5-tert-butyl-2,4,6-trinitro-m-xylene (musk xylene) that has been prepared by The Netherlands in the context of Council Regulation (EEC) No. 793/93 on the evaluation and control of existing substances.

For detailed information on the risk assessment principles and procedures followed, the underlying data and the literature references the reader is referred to the comprehensive Final Risk Assessment Report (Final RAR) that can be obtained from the European Chemicals Bureau[1]. The Final RAR should be used for citation purposes rather than this present Summary Report.

[1] European Chemicals Bureau – Existing Chemicals – http://ecb.jrc.it

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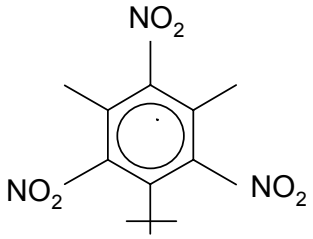
1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS Number: 81-15-2
EINECS Number: 201-329-4
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 weight: 297.3
Molecular formula: $C_{12}H_{15}N_3O_6$
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
Physical state	solid, powder	
Melting point	112-114°C	#, *
Boiling point	not applicable	**
Relative density	0.77, 0.85 g/cm^3 Recommended: 0.77 g/cm^3	* $
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	*
Surface tension	not applicable	&
Water solubility	0.15 mg/l (measured) 0.49 mg/l (calculated) Recommended: 0.15 mg/l	*
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	* $
Flash point	168°C	*
Flammability	flammable	*
Autoflammability temperature	305-341°C	*
Explosive properties	initiated by shock and heat, propagation depends on packaging size and characteristics, and is limited in typical transport packaging	*
Oxidising properties	not oxidising	***
Granulometry	100% v/v < 100 μm 21.8% v/v < 10 μm 14.4% v/v < 4 μm	*

\# 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 determination 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.

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[2]:

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.

[2] 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

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.

Uses

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.

The EU import volume for musk xylene amounts to 67 tonnes/year (year 2000).

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General

Musk xylene may be released into the environment during its compounding into the fragrance, the formulating of the fragrance into end products and the use of those end products (private use).

General characteristics of musk xylene which are relevant for the exposure assessment are given below.

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.

Photolysis of musk xylene was studied under experimental conditions. Based on these data and on structural grounds it can be concluded 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. 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

Based on the experimental test results a biodegradation rate constant of 0 hr^{-1} could be assumed as musk xylene is not readily biodegradable. The use of the BIOWIN model 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. 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}.

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 $Pa.m^3/mol$ is 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 TGD equation for predominantly hydrophobics. 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%).

EUSES (SimpleTreat) estimates the following default distribution for musk xylene in a STP: air: 0 %, water: 43 % and sludge: 57 %.

Bioaccumulation

The BCF fish can be calculated using the QSAR mentioned in the TGD. Using a log K_{ow} of 4.9 a BCF of 2,900 L/kg is obtained.

In addition to the calculated BCF a number of experimental data are available for musk xylene. These bioaccumulation studies showed a number of uncertainties. However, based on a weight of evidence approach, with a number of studies 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 a value of 4,400 l/kg in the current risk assessment on musk xylene.

No experimental data are available on accumulation in earthworms. Therefore, the BCF earthworm is estimated according to the TGD QSAR: 4.6 kg/kg.

3.1.2 PECs at processing and private use

The environmental exposure assessment of musk xylene will be based on the expected releases of the substance during the following life cycle stages:

I Fragrance compounding (six compounding sites)
II Formulation into end products
III Private use

Local PECs for the above-mentioned scenarios were calculated based on the TGD principles using both default information and site-specific data.

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.

In addition to these estimated PECs also a number of local and regional monitoring data are available for musk xylene in various environmental compartments (mainly water and fish). The monitoring data set comprises various EU regions (esp. musk xylene levels in biota) and 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.

3.2 EFFECTS ASSESSMENT

3.2.1 Aquatic compartment (incl. sediment)

For the determination of the PNEC water both short and long-term toxicity test results studies are available for musk xylene. The 5d-growth test with algae and the 2-day-reproduction test with *Daphnia magna* are considered long term tests. The 14-day-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* (56 µg/l) giving a $PNEC_{water}$ of 1.1 µg/l.

The 14d-fish toxicity data may be used to support this PNEC. That is, if the LOEC of the 14-day-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.

No experimental data are available for sediment organisms. Applying the equilibrium partitioning theory, a PNEC of 0.3mg/kg ww is calculated.

3.2.2 Terrestrial compartment

The toxicity of musk xylene to earthworms was studied in a 14-day test. No effects were observed on survival up to the highest test concentration of 50 mg/kg dw. Because no effects were found up to the highest concentration the $PNEC_{soil}$ was derived from the $PNEC_{water}$ using the equilibrium partitioning theory, leading to a value of 0.26 mg/kg dw.

3.2.3 Atmosphere

No data available.

3.2.4 Non compartment specific effects relevant to the food chain

No toxicological data are available for (top-) predators. 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. An AF of 150 is subsequently used as a reasonable 'compromise' between 90 and 300. The $PNEC_{oral}$ then becomes: $7.5 \cdot 20/150 = 1$ mg/kg food.

3.3 RISK CHARACTERISATION

3.3.1 General

Table 3.1 presents the local PEC/PNEC ratios for the various relevant life cycle stages of musk xylene. Details will be discussed in sections 3.3.2 through 3.3.4.

Table 3.1 Local PEC/PNEC ratios.

	STP	Surface water	Soil	Soil alternative*	Fish	Worm	Worm alternative*
Site 1	< 0.01	0.2	0.02	0.01	1.6	0.3	<0.1
Site 2	< 0.01	0.2	< 0.01	< 0.01	1.6	0.3	<0.1
Site 3	n.r.	0.2	0.02	0.02	1.6	0.3	0.1
Site 4	< 0.01	0.2	0.19	0.2	1.6	0.4	<0.1
Site 5	< 0.01	0.2	0.02	0.01	1.6	0.3	<0.1
Site 6	< 0.01	0.2	< 0.01	< 0.01	1.6	0.3	<0.1
end product formulation	<0.01-<0.01	0.2-0.4	0.1-0.5	0.08 - 0.4	1.7 - 2.2	0.4-0.6	< 0.1- 0.1
private use	<0.01	0.9	1.7	0.06	5	1.1	<0.1

* Based on maximum sludge concentration of 1 mg/kg dwt

3.3.2 Aquatic compartment (incl. sediment)

From **Table 3.1** it can be seen that all aquatic 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. This conclusion holds also for the regional assessment.

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 aquatic monitoring data were available.

3.3.3 Terrestrial compartment

For compounding 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. For this reason **conclusion (ii)** is considered as most relevant for the private use scenario.

3.3.4 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.

3.3.5 Non compartment specific effects relevant to the food chain

All PEC/PNEC ratios for fish eating predators are found to be above 1 (**Table 3.1** of the RAR). 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. Therefore a **conclusion (ii)** is considered 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 (**Table 3.1** of the RAR). 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. For this reason **conclusion (ii)** is considered as most relevant for the private use scenario.

3.3.6 Metabolites of musk xylene

A limited risk assessment has been carried out for the major metabolites of musk xylene. Overall, a **conclusion (ii)** is therefore considered most relevant for musk xylene metabolites in the environment.

3.3.7 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

Occupational exposure

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 largely and may be up to 1%. The substance is not produced within the EU, but imported from China. Inside the EU the pure substance is used in fragrance compounding.

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. 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.

Scenario 1 The production of fragrance compounds

Musk xylene is imported as a crystalline powder. At room temperature the substance has a very low vapour pressure, so inhalation exposure to the vapour is probably negligible, but exposure to dust may be possible. The fragrance compounds are probably mixed on costumers 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%.

Fragrance compounding

For risk assessment, the results of the estimation with the EASE model and the analogue substances were used. 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 seemed 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.

Dermal exposure was estimated with the EASE model for dumping only one or two bags per day.

Drumming of liquid fragrance

For inhalation exposure the estimates of the USEPA model was used for the risk evaluation.

For dermal exposure the result of the EASE model was used.

Scenario 2 The use of liquid fragrance compounds

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 of no exposure to musks.

For the risk characterisation, the values estimated with the EASE model were used.

Scenario 3 The use of cleaning agents by professional cleaners

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 no special high pressure spraying equipment is used, so that no aerosol formation takes place, and that the products are diluted before use.

The values, estimated with the EASE model were taken forward to the risk characterisation.

Results of the estimates are presented in **Table 4.1**.

Table 4.1 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
2. The use of liquid fragrance compounds:									
- addition	0-1	225	negl.	EASE	negl.	EASE	negl.	Expert	4
- cleaning and maintenance	0-1	20-50	negl.	EASE	negl.	EASE	negl.	Expert	6.5
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

Consumer exposure

Consumer exposure occurs from consumer products to which musk xylene is added intentionally. Musk xylene is used as fragrance and fragrance enhancer in cosmetics, detergents and air fresheners. The main exposure of consumers is via cosmetics via the dermal route. According to the EU Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCCNFP) this dermal exposure amounts to 210 μg/kg bw/day. As the SCCNFP based their calculation on a range of cosmetic products likely to be used in any one weekly period, and all products were assumed to be perfumed with the upper 97.5th percentile level of the fragrance ingredient, this value must be regarded as conservative. Compared to cosmetics, the exposure of consumers to musk xylene in detergents and air fresheners is negligible. Therefore only the figure of 210 μg/kg bw/day is taken forward to the risk characterisation. It should be noted that in 1999 the SCCNFP recommended that the exposure of consumers due to the cosmetic use of musk xylene should be reduced by 50%. This because musk xylene is retained in human fat and is excreted in human milk (see Section 4.1.2 below). If this measure comes into effect, the exposure would drop to 105 μg/kg bw/day.

Humans exposed via the environment

Local emissions of musk xylene to the environment may occur at formulation (fragrance compounding and end product formulation) and from private use. For both the local and regional scale, human intake via air is negligible compared to other uptake routes (especially root crops and fish). Hence, the main exposure route is oral. On a local scale, private use showed the highest total daily intake of all life cycle steps (0.0136 mg/kg bw/day). For the regional scale, the total daily intake was calculated to be 3.55e-3 mg/kg bw/day.

Musk xylene is retained in human adipose tissue and is excreted in human breast milk. The levels of musk xylene in human breast milk seem to have declined in the last decade, given that the mean and maximum levels found in 1999/2000 were considerably lower than those found in the early nineties. The exposure (worst case estimate) via mother's milk for infants varies between 0.42 and 5.12 μg musk xylene/kg bw/day.

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

The human population may be exposed by the oral, dermal and inhalatory route.

In the data set for musk xylene animal studies as well as human studies are available.

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 the oral and dermal studies 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 preferably distribute into the fatty compartment.

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 an 80-week 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 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-day 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 occurred 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. These gland and tissue types 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 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 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 maturation 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 Risk characterisation

4.1.3.1 Workers

For the purpose of risk characterisation, it is assumed that inhalation of dust and skin contacts are the main routes of exposure. Oral exposure is not considered to be a significant route of exposure under normal working practices. If applicable, quantitative risk assessment is performed by calculation of the MOS (the ratio between NOAEL/LOAEL and exposure levels) and comparison of this value with the minimal MOS. This minimal MOS is established via assessment factors, taking into account inter- and intraspecies differences, differences between experimental conditions and the exposure pattern of the worker, type of critical effects, dose-response relationship, confidence in the database, and correction for route-to-route extrapolation. A risk is indicated when the MOS is lower than the minimal MOS. In case of combined exposure the calculations are based on internal NOAELs and systemic exposure levels.

Acute 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/day), 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

The occupational risks for local effects are characterised for exposure via the skin, the respiratory tract and the eyes.

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. The NOAEL for local effects of the 90-day dermal toxicity study with rats (1.7 mg/cm^2)[3] is used as starting point for the risk characterisation. Comparison of the calculated MOSs (17-567) between this NOAEL and the dermal exposure levels (0.003-0.1 mg/cm^2) with the minimal MOS (9)[4], indicates that 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

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.

The NOAEL for systemic effects from the dermal 90-day rat study (24 mg/kg bw/day) is used as starting point. Assuming a dermal absorption value of 20% for rats, this NOAEL corresponds to an internal level of 4.8 mg/kg bw/day. The minimal MOSs required for chronic occupational exposure using this NOAEL, the actual exposure levels (see also **Table 4.1**) and the MOSs calculated between the NOAEL and the exposure levels are given in **Table 4.2**.

[3] Based on a NOAEL of 240 mg/kg bw/day assuming a body weight of the rat of 0.3 kg and an exposed dermal area of the rat of 42.5 cm^2 (which is 10% of the total body surface area)

[4] Minimal MOS local effects dermal (9): 3 (interspecies) x 3 (intraspecies)

Table 4.2 Occupational risk assessment for repeated-dose toxicity of musk xylene

	Scenario 1			Scenario 2			Scenario 3		
	Derm	**Resp**	**Comb**	**Derm**	**Resp**	**Comb**	**Derm**	**Resp**	**Comb**
NOAEL (in mg/kg bw/day)	24	24	4.8	24	24	4.8	24	24	4.8
Exposure (in mg/kg bw/day)	0.6	0.04	0.10#	0.06-0.09	negl	0.006-0.009#	0.04	negl	0.004#
calculated MOS	40	600	48	267-400	high	533-800	600	high	1,200
minimal MOS	180[a]	1,800[b]	360[c]	180[a]	1,800[b]	360[c]	180[a]	,1800[b]	360[c]

Derm: Dermal exposure;
Resp: Respiratory exposure; comb: combined exposure; negl: negligible exposure
The total systemic exposure, based on 10% dermal human absorption and 100% inhalatory absorption
a 180 = 12 (interspecies) · 3 (intraspecies) · 10 (exposure duration) · 0.5 (absorption differences; 10% human/ 20% animal)
b 1,800 = 12 (interspecies) · 3 (intraspecies) · 10 (exposure duration) · 5 (absorption differences; 100% inhalatory/20% dermal)
c 360 = 12 (interspecies) · 3 (intraspecies) · 10 (exposure duration)

Comparison of the minimal MOSs and the calculated MOSs indicates no concern for systemic effects due to repeated dermal, inhalatory, or combined exposure in scenarios 2 and 3 **conclusion (ii)** and a concern for all routes in scenario 1. However, due to the crystalline nature of the substance, the repeated dermal and combined 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 systemic effects due to dermal and combined exposure in scenario 1 as well. Furthermore, in view of the worst case character of the minimal MOS for inhalation exposure (caused by multiplication of the different assessment factors) **conclusion (ii)** is also considered justified for systemic effects due to inhalatory exposure in Scenario 1.

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 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. Assuming 50% oral absorption this LOAEL corresponds to an internal low-effect dose of 35 mg/kg bw/day. The minimal MOSs required for chronic occupational exposure using this NOAEL, the actual exposure levels and the MOSs calculated between the NOAEL and the exposure levels are given in **Table 4.3**.

Table 4.3 Occupational risk assessment for carcinogenic effects of musk xylene

	Scenario 1			Scenario 2			Scenario 3		
	derm	**resp**	**comb**	**derm**	**resp**	**comb**	**Derm**	**resp**	**comb**
LOAEL (in mg/kg bw/day)	70	70	35	70	70	35	70	70	35
Exposure (in mg/kg bw/day)	0.6	0.04	0.10#	0.06-0.09	negl	0.006-0.009#	0.04	negl	0.004#
calculated MOS	116	1,750	350	778-1167	high	3889-5833	1750	high	8,750
minimal MOS	126[a]	1,260[b]	630[c]	126[a]	1,260[b]	630[c]	126[a]	1,260[b]	630[c]

derm: dermal exposure; resp: respiratory exposure; comb: combined exposure; negl: negligible exposure

\# the total systemic exposure, based on 10% dermal human absorption and 100% inhalatory absorption

a 126 = 21 (interspecies) · 3 (intraspecies) · 10 (LOAEL to NAEL) · 0.2 (absorption differences; 10% dermal/ 50% oral)

b 1260 = 21 (interspecies) · 3 (intraspecies) · 10 (LOAEL to NAEL) · 2 (absorption differences; 100% inhalatory/50% oral)

c 630 = 21 (interspecies) · 3 (intraspecies) · 10 (LOAEL to NAEL)

Comparison of the minimal MOSs and the calculated MOSs indicates no concern for carcinogenic effects due to repeated exposure in scenarios 2 and 3 and respiratory exposure in Scenario 1: **conclusion (ii)**. The comparison indicates a concern for carcinogenic effects due to dermal and combined exposure in Scenario 1. However, due to the crystalline nature of the substance, the dermal and combined 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 systemic effects due to dermal and combined exposure in scenario 1 as well.

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, developmental toxicity only occurred at maternal toxic dose levels ($NOAEL_{developmental\ toxicity}$ 60 mg/kg bw/day, $NOAEL_{maternal\ toxicity}$ 20 mg/kg bw/day). 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. Assuming 50% oral absorption, the NOAEL of 7.5 corresponds to an internal no-effect dose of 3.75 mg/kg bw/day.

The minimal MOSs required for chronic occupational exposure using this NOAEL, the actual exposure levels and the MOSs calculated between the NOAEL and the exposure levels are given in **Table 4.4**.

Table 4.4 Occupational risk assessment for reproductive effects of musk xylene

	Scenario 1			Scenario 2			Scenario 3		
	Derm	**Resp**	**Comb**	**Derm**	**Resp**	**Comb**	**Derm**	**Resp**	**Comb**
NOAEL (in mg/kg bw/day)	7.5	7.5	3.75	7.5	7.5	3.75	7.5	7.5	3.75
Exposure (in mg/kg bw/day)	0.6	0.04	0.10#	0.06-0.09	negl	0.006-0.009#	0.04	negl	0.004#
calculated MOS	13	188	38	83-125	high	417-625	188	high	938
minimal MOS	7.2[a]	72[b]	36[c]	7.2[a]	72[b]	36[c]	7.2[a]	72[b]	36[c]

derm: dermal exposure; resp: respiratory exposure; comb: combined exposure; negl: negligible exposure
\# the total systemic exposure, based on 10% dermal human absorption and 100% inhalatory absorption
a 7.2 = 12 (interspecies) · 3 (intraspecies) · 0.2 (absorption differences; 10% dermal/ 50% oral)
b 72 = 12 (interspecies) · 3 (intraspecies) · 2 (absorption differences; 100% inhalatory/50% oral)
c 36 = 12 (interspecies) · 3 (intraspecies)

Comparison of the minimal MOSs and the calculated MOSs indicates no concern for effects on the offspring due to repeated dermal, inhalatory, or combined exposure in all occupational scenarios: **conclusion (ii)**.

Occupational limit values

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

4.1.3.2 Consumers

Starting point for the risk characterisation is the (frequent) dermal exposure of consumers to musk xylene in cosmetic products, for which an external exposure level of 210 μg/kg bw/day was calculated. 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.

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, there is no concern for consumers for skin irritation: **conclusion (ii)**. There is also no concern for consumers for eye irritation en skin sensitisation: **conclusion (ii)**.

Starting point for the risk assessment for repeated dose toxicity 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 the latter with the calculated human systemic exposure level of 21 μg/kg bw/day results in a MOS of 229. This MOS indicates no concern for consumers, 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: **conclusion (ii)**.

Musk xylene is a carcinogen in mice (a second species, e.g. the rat was not tested). 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. For risk characterisation a threshold approach is considered justified, given that musk xylene is non-genotoxic **conclusion (ii)** and that enzyme induction is a threshold phenomenon. Because no dermal carcinogenicity studies were available, the oral LOAEL of 70 mg/kg bw/day from the

carcinogenicity study with B6C3F1 mice is used as starting point for the risk characterisation. 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 exposure: **conclusion (ii)**.

There are no indications for effects on fertility in the dermal 90-day toxicity study with rats, in the oral carcinogenicity study with mice and in the oral peri/postnatal study in which rats were exposed to musk xylene *in utero* and during lactation. Developmental effects have been observed in an oral developmental toxicity study with rats (but only at maternal toxic dose levels; NOAEL for developmental toxicity 60 mg/kg bw/day) and in the oral peri/postnatal study with rats (NOAEL for pup toxicity 7.5 mg/kg bw/day). In the absence of dermal developmental toxicity studies, these oral NOAELs are used as starting point for the risk characterisation for the progeny of pregnant consumers. Assuming 50% oral absorption, these NOAELs correspond to internal no-effect doses of 30 and 3.75 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 1,429 and 179, 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 Humans exposed via the environment

For man exposed via the environment inhalation exposure is negligible (**conclusion (ii)** for all relevant endpoints). The main exposure route for man indirectly exposed is oral. Starting point for the risk characterisation for the local scale is private use, which shows the highest total daily intake of 0.0136 mg/kg bw/day. For the regional scale the total daily intake is 3.55e-3 mg/kg bw/day. 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).

Total daily intake

In the absence of oral repeated dose toxicity studies, the dermal NOAEL of 24 mg/kg bw/day from the 90-day toxicity study with rats is used as starting point for the risk assessment. 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 the latter with the estimated internal total human daily intake levels, the MOSs for both local and regional scale are >700. These MOSs indicate no concern for man repeatedly exposed indirectly via the environment, 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: **conclusion (ii)**.

Musk xylene is a carcinogen in mice (a second species, e.g. the rat was not tested). 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. For risk characterisation a threshold approach is considered justified, given that musk xylene is non-genotoxic **conclusion (ii)** and that enzyme induction is a threshold phenomenon. The oral LOAEL of 70 mg/kg bw/day from the carcinogenicity study with B6C3F1 mice is used as starting point for the risk characterisation. Comparing this low-effect dose with the estimated total human daily intake levels, the MOSs for both local and regional scale are >>1,000. 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)**.

There are no indications for effects on fertility in the dermal 90-day toxicity study with rats, in the oral carcinogenicity study with mice and in the oral peri/postnatal study in which rats were exposed to musk xylene *in utero* and during lactation. Developmental effects have been observed in an oral developmental toxicity study with rats (but only at maternal toxic dose levels; NOAEL for developmental toxicity 60 mg/kg bw/day) and in the oral peri/postnatal study with rats (NOAEL for pup toxicity 7.5 mg/kg bw/day). These oral NOAELs are used as starting point for the risk characterisation for the progeny of pregnant women indirectly exposed via the environment. Comparing these no-effect doses with the estimated total human daily intake levels, the MOSs for both local and regional scale are >500. 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)**.

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 Combined exposure

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 for repeated dose toxicity 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 the latter with the calculated combined human systemic exposure level of 0.13 mg/kg bw/day results in a MOS of 37. This MOS indicates no concern for repeated combined exposure, 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: **conclusion (ii)**.

Carcinogenicity

Musk xylene is a carcinogen in mice (a second species, e.g. the rat was not tested). 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. For risk characterisation a threshold approach is considered justified, given that musk xylene is non-genotoxic **conclusion (ii)** and that enzyme induction is a threshold phenomenon. The oral LOAEL of 70 mg/kg bw/day from the carcinogenicity study with B6C3F1 mice is used as starting point for the risk characterisation. 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, in the oral carcinogenicity study with mice and in the oral peri/postnatal study in which rats were exposed to musk xylene *in utero* and during lactation. Developmental effects have been observed in an oral developmental toxicity study with rats (but only at maternal toxic dose levels; NOAEL for developmental toxicity 60 mg/kg bw/day) and in the oral peri/postnatal study with rats (NOAEL for pup toxicity 7.5 mg/kg bw/day). These oral NOAELs are used as starting point for the risk characterisation for the progeny of pregnant women. Assuming 50% oral absorption, these NOAELs correspond to internal no-effect doses of 30 and 3.75 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 231 and 29, 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)

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 applies because the substance is considered a PBT candidate chemical. A further PBT- testing strategy is proposed.

5.2 HUMAN HEALTH

5.2.1 Human health (toxicity)

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 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.

5.2.2 Human health (risks from physico-chemical properties)

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.

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.