European Chemicals Bureau

Institute for Health and Consumer Protection

European Chemicals Bureau

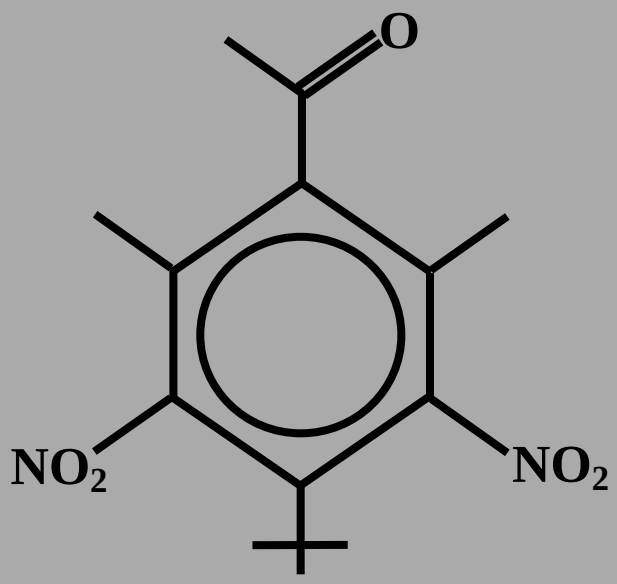
Existing Substances

European Union Risk Assessment Report

CAS No: 81-14-1

EINECS No: 201-328-9

4’-tert-butyl-2’,6’-dimethyl-3’,5’-dinitroacetophenone (musk ketone)



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European Union Risk Assessment Report
4’-tert-butyl-2’,6’-dimethyl-3’,5’-dinitroacetophenone (musk ketone)
CAS: 81-41-1
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European Union Risk Assessment Report

4'-TERT-BUTYL-2',6'-DIMETHYL-3',5'-DINITROACETOPHENONE (MUSK KETONE)

CAS No: 81-14-1

EINECS No: 201-328-9

RISK ASSESSMENT

Cataloguing data can be found at the end of this publication
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4'-TERT-BUTYL-2',6'-DIMETHYL-3',5'-DINITROACETOPHENONE (MUSK KETONE)

CAS No: 81-14-1

EINECS No: 201-328-9

RISK ASSESSMENT

Final Report, 2005

The Netherlands

Rapporteur for the risk evaluation of 4'-tert-butyl-2',6'-dimethyl-3',5'-dinitroacetophenone (musk ketone) 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 Organization for Applied Scientific Research (TNO) and the National Institute of Public Health and Environment (RIVM), by order of the rapporteur.

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

Date of Last Literature Search:	**2003**
Review of report by MS Technical Experts finalised:	**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
DG Joint Research Centre

Catherine Day
Director-General
DG Environment

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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:	81-14-1
EINECS No:	201-328-9
IUPAC Name:	4’-tert-butyl-2’,6’-dimethyl-3’,5’-dinitroacetophenone

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.

Human Health

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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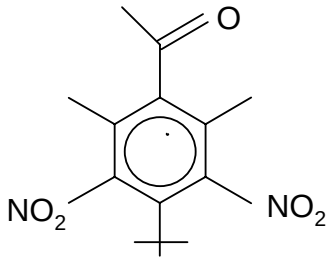
1 GENERAL SUBSTANCE INFORMATION

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS No: 81-14-1
EINECS No: 201-328-9
IUPAC Name: 4'-tert-butyl-2',6'-dimethyl-3',5'-dinitroacetophenone
Synonyms: [4-(1,1-dimethylethyl)-2,6-dimethyl-3,5-dinitrophenyl]-ethanone, musk ketone
Molecular formula: $C_{14}H_{18}N_2O_5$
Molecular weight: 294.3

Structural formula:



1.2 PURITY/IMPURITIES, ADDITIVES

Purity: >98.5%
Impurities: musk xylene, <1.5%
Additives: none

1.3 PHYSICO-CHEMICAL PROPERTIES

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

Table 1.1 Physico-chemical properties of musk ketone.

Property	Result	Comment	References
Physical state	solid, powder		
Melting point	135-137°C	*	Ph. Eur., 1990; B. Ph., 1993; Bauer *et al.*, 1990
Boiling point	not applicable	**	Givaudan, 1990[A]
Relative density	0.73 g/cm^3	*	RVO-TNO, 1974
Vapour pressure	0.27 Pa at 50°C, 7.6 Pa at 80°C 0.00004 Pa (calculated) at 20°C Recommended: 0.00004 Pa at 20°C	*	Grain, 1990; Tas and Van de Plassche., 1996
Surface tension	not applicable	&	-
Water solubility	0.46 mg/l (measured) 1.9 mg/l (calculated) Recommended: 0.46 mg/l	*	Schramm *et al.,* 1996; Tas and Van de Plassche., 1996
Solubility in other solvents	-		-
Partition coefficient n-octanol/water (log value)	4.3, 3.8, 3.2 (measured) 3.78, 4.3 (calculated) Recommended: 4.3	* # 	Rudio, 1996; Schramm *et al.*, 1996; Tas and Van de Plassche., 1996; Johnson *et al.*, 1984
Flash point	>168°C	*	Aroma Chemicals
Flammability	not flammable	*	RVO-TNO, 1974
Autoflammability temperature	not applicable	***	-
Explosive properties	not explosive	*	RVO-TNO, 1974; Givaudan, 1990[B]
Oxidizing properties	not oxidising	***	-
Granulometry	100% v/v < 200 μm 22.1% v/v < 10 μm 13.5% v/v < 4 μm	*	Rodriguez, 1998

* One or several values found in literature, all in the same range, not all methods are specified.

** Not applicable, decomposition starts at 250°C.

*** Conclusion based on theoretical, and/or structural considerations.

& The low water solubility renders further determinations as superfluous.

Recommended value based on test report.

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, the relative density and one measured value for 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:

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 0.46 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. Tas and Van de Plassche (1996) report that a log K_{ow} of 3.2 has been measured using the shake-flask procedure. However, the original report was not available. Rudio (1996) applied the HPLC method according to OECD Test-Guideline 117, resulting in a log K_{ow} of 4.3. Schramm *et al.* (1996) measured a value of 3.8 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 3.78 and 4.31, 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 4.3 described in detail by Rudio (1996) is preferred.

Summary of physico-chemical properties

All data are considered as sufficiently reliable to fulfill the Annex VIIA requirements. The substance propagates burning but may be considered not flammable on account of the high flashpoint and the limited effect in the explosive burning test.

1.4 CLASSIFICATION AND LABELLING

Current classification according to Annex 1

No classification.

Decision of the TC C&L

The Meeting of the Technical Committee C&L on the Classification and Labelling of Dangerous Substances in June 2002 (environment) and January 2003 (human health) recommended the following classification and labelling:

Classification

Carc. Cat. 3, R40
N;R50-53

Labelling

Xn; N
S(2)-36/37-46-60-61
R40-50/53

Explanation:

Carc. Cat. 3, R40	Limited evidence of carcinogenic effect.
N;R50-53	Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment.
Xn; N	Harmful; Very toxic to aquatic organisms.
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 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION

There is no production of musk ketone 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 ketone is manufactured in a batch process not exceeding 500 kg by Friedel-Crafts acylation of 5-tert.butyl-1,3-dimethylbenzene with acetyl chloride using aluminium chloride as a catalyst. The resulting 4-tert.butyl-2,6-dimethylacetophenone is subsequently nitrated to yield musk ketone, 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 ketone has to be dissolved. Musk ketone 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 and/or;
  category 15: cosmetics;
  category 36: odour agents.

Musk ketone is received by fragrance companies from suppliers in and outside the EU or through the intervention of brokers importing the substance. Subsequently, fragrance compounds or preparations 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 2.2**) have been taken from the IUCLID data sheets and additional, more recent sources (industry information).

Table 2.2 Import volumes per major site (> 10 tonnes in 1990-1994 period).

Site	Import volume (tonnes)	Year
site 1	13	1996
site 2	4	1996
site 3	13	1996
site 4	4	1997
site 5	2	1997

The Research Institute of Fragrance Materials (RIFM) has carried out surveys on the usage of fragrances by fragrance compounding facilities in Europe. For the survey carried out in 1996 32 companies involved in fragrance compounding responded to the survey, which included all of the major fragrance producers world wide. According to RIFM the 1995 volumes of use for musk ketone account for approximately 90% of the total use. For musk ketone the results are shown in **Table 2.3.**

Table 2.3 Results of RIFM surveys: import of musk ketone for Europe in tonnes.

1992 (survey carried out in 1993)	124 tonnes
1995 (survey carried out in 1996)	61 tonnes
1996 (survey carried out in 1997)	54 tonnes
1998	40 tonnes
2000	35 tonnes

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 ketone in amounts well below the reporting level of 10 tonnes/year. Also, the RIFM data are more recent than the information from the IUCLID data sheets. It should be noted that the RIFM reported the import volumes for Europe and not for the EU.

For the exposure calculations for the life-cycle part “end product formulation” and “private use” the volume reported by RIFM for 2000 of 35 tonnes in **Table 2.3** 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 ketone.

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 General

3.1.1.1 Degradation

Abiotic degradation

Studies on hydrolysis of musk ketone 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 ketone was studied by Butte et al. (1999). Under laboratory conditions using an UV immersion lamp, photolysis of musk ketone was observed in which an initial phase where the reaction followed first order kinetics (k: 0.171 min^{-1} and $t_{1/2}$: 4.1 min). 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 12.5 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 ketone 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

The inherent biodegradability was tested by the MITI II test (OECD Guideline 302C). The Biological Oxygen Demand (BOD) was measured during a 28-day test with 100 mg/l activated sludge and a concentration of 39 mg/l musk ketone. Throughout the test the level of BOD in the sample with musk ketone was identical to the sample without test compound. It was therefore concluded that musk ketone was not biodegradable under the test conditions (Calame and Ronchi, 1989).

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 82.5% for musk ketone. Simonich et al. (2000) and Sabaliunas et al. (2001) confirmed that the removal for musk ketone within a STP is high i.e. 80-92%. 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), Rimkus et al. (1999) and Herren and Berset (2000) presented measurements in influent and effluent of STPs, surface waters and biota for metabolites of musk ketone assuming that nitro musks will be transformed to the corresponding amino compounds. They analysed and detected the metabolite 3-nitro-5-amino-2, 6-dimethyl-4-tert-butylacetophenone referred to in their publications as "2-amino-MK". They did not analyse for the "diamino-MK" metabolite. 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), that white rot fungi or ectomycorrhizal basidiomycetes can degrade TNT (Gorontzy et al., 1994; Meharg et al., 1997). For musk ketone no such experimental data are available. However, the measurements described above show that reduction of nitro groups occurs for musk ketone in sewage treatment plants and fish.

Subsequently, for the environmental risk assessment:

- the results of these measurements of MK 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 characterization for MK metabolites;
- based on the test results from Calame and Ronchi (1989) a biodegradation rate constant of 0 hr^{-1} could be assumed as musk ketone is not inherently biodegradable. The use of the BIOWIN model (TGD, 2002) for estimating aerobic biodegradability also points to the lack of biodegradation of musk ketone. However, the amino reaction product has been measured in substantial amounts in effluents - where the concentration in influent was below the detection limit - showing that primary degradation of musk ketone occurs in an STP (see Section 3.1.2.4). In principle the measured metabolite MK in effluent can also be a degradation product of another substance, 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 ketone will be calculated in the present RAR 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 $0.04 \cdot 10^{-3}$ Pa and a water solubility of 0.46 mg/l a Henry's law constant of 0.026 $Pa.m^3/mol$ is calculated.

From the measured log K_{ow} of 4.3 a log Koc of 3.58 l/kg can be estimated using the equation for predominantly hydrophobics[4] from the TGD:

$$log\ Koc = 0.81\ log K_{ow} + 0.10 \quad (1)$$

[4] 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 ketone this QSAR may be more accurate than the general one for hydrophobics. The Koc would become 7261 l/kg in stead of 3800 l/kg. The rapporteur has used this alternative value in the current risk assessment settings and no significant difference was found between the two options.

This results in the following partition coefficients:

- $K_{soil\text{-}water}$: 115 m^3/m^3;
- $K_{susp\text{-}water}$: 96 m^3/m^3;
- $K_{sed\text{-}water}$: 96.6 m^3/m^3.

In addition to these theoretical values experimental data are available on the partitioning of musk ketone between water and suspended matter. Winkler et al. (1998) determined partition coefficients between water and suspended matter collected from the river Elbe in Germany. Both field and laboratory experiments were carried out for determining partition coefficients. The Kp susp from the laboratory experiment (distilled water and spiked suspended matter) was found to be 17,700 l/kg. Field data were found to be lower: 440 l/kg (min), 1,700 l/kg (mean) and 3,750 l/kg (max). The TGD default value of 96 m^3/m^3 correlates with a value of 383 l/kg. This default value is nearly the same as the minimum field value of 440 l/kg. The TGD value of 383 l/kg is used in the current RAR. The rapporteur has verified that the use of field values did not affect the general conclusions of the risk assessment.

No experimental data are available on the partitioning of musk ketone between water and soil, sediment or sludge

EUSES (SimpleTreat) estimates the following default distribution for musk ketone in a STP: air: 0%, water: 68% and sludge: 32%.The results of Simonich et al. (1998 and 2000) and Sabaliunas et al. (2001) indicated that the musk ketone removal within a STP can be very high i.e. 80-92%. As these data do not allow to make 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 with the current default setting the aquatic emission load of musk ketone may be overestimated, whereas the load to sludge may be underestimated.

3.1.1.3 Accumulation

Accumulation in fish

Bioconcentration of ^{14}C-musk ketone has been tested using two different concentrations in a flow-through system according to OECD Guideline 305E (Van Dijk and Burri, 1986). The uptake and depuration in rainbow trout (*Onchorynchus mykiss*) was followed in edible and non-edible parts and a whole body concentration was calculated (see **Table 3.1**). The bioconcentration factor is derived by C_{fish} /C_{water} from extrapolation of the uptake curve to an expected steady state. Plateau levels of radiolabel were reached in edible tissue in 5 days and in non-edible tissue and in the whole fish in 8 days. The elimination curve is very steep, slightly bent and is consistent with first order kinetics. Depuration half-lives were recalculated to be approximately 2.5 days indicating that the uptake of musk ketone was highly reversible. During the 8d-accumulation period, besides musk ketone, three polar degradation products were detected by TLC and LSC in water forming up to 14.8% of the total recovered radioactivity. From the mass balance for the system fish plus water, the amount of degradation products in the water is related to the amount of parent substance in the fish and it is concluded that the major part (between 70 and 100%) of the excreted radioactivity consist of polar metabolites of musk ketone. The BCF of 1,380 is based on radiolabelled material, including parent material and metabolites. The BCF based on parent material will only be lower.

In the test a solubiliser (DMF, Tween) was used to dissolve musk ketone. The test was carried out using a radiolabel, without identification of the parent compound in the fish. In water the parent compound was identified by HPLC. For rapidly metabolising compounds this is not a reliable method to determine the bioaccumulation potential. For musk ketone it is not clear whether biotransformation in fish is an important factor. In mammals metabolites have been identified (Ford et al., 1990). However in fish no detailed information on metabolism is available, although amino metabolites were identified in fish by Gatermann et al. (1998).

Table 3.1 Bioconcentration of musk ketone (low and high refer to high and low dose of 5 and 47 μg/l, respectively) (Van Dijk and Burri, 1996).

^{14}C-radiolabel identified species	No Rainbow trout
Low dose [μg/l] High dose [μg/l]	5 ± 0.5 47 ± 5
Period of exposure [day] Period of elimination [day]	8 21
r.a. day 8 parent material in water r.a. day 8 polar degradation products in water	85.2% (low); 91.3% (high) 14.8% (low); 8.7% (high)
Uptake rate constant [l/kg/day] Elimination rate constant [d^{-1}] $t_{1/2}$ elimination [day] bioconcentration factor (whole fish, wet weight) [l/kg]	140[a] (low), r^2=0.95[a]; 943 (low) 120[a] (high), r^2=0.92[a]; 631 (high) 0.27[a] (low), r^2=0.90[a]; 0.355 (low); r^2=0.99 0.32[a] (high), r^2=0.93[a]; 0.474 (high); r^2=0.98 2.6[a] (low); 2.2[a] (high) 1,380[b]

a) Recalculated from the original data;
b) Based on radiolabelled residue in fish.

The BCF can also be calculated using the QSAR mentioned in the TGD: log BCF (wet weight) = 0.85. log K_{ow} - 0.70. Using log K_{ow} (HPLC) a BCF of 920 l/kg is obtained. This value corresponds to the measured BCF value, although it should be stated that the experimentally determined BCF is based on radioactivity and not on identification of musk ketone.

In Yamagishi et al. (1983) a mean concentration ratio between fish-muscle and water of 1,100 l/kg for musk ketone is reported. These values are obtained by dividing the concentration in fish by the concentration in water in the environment. The reliability of bioconcentration factors obtained in the environment is questionable, since it is unknown whether a steady state has occurred. Therefore, these values for the BCF will not be used for the risk assessment.

In the environmental risk assessment and the calculations with EUSES the experimental BCF value of 1380 l/kg, derived from the study from Van Dijk and Burri (1995) will be used.

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 revised 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:

$(0.84 + 0.012\ K_{ow}.\ RHO_{soil})/(K_{soil\text{-}water} \cdot CONV_{water})$

Using a log K_{ow} of 4.3 this leads to a BCF_{worm} of 3.6 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 ketone in the EU.

Fragrance compounding

Emissions of musk ketone 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 ketone. Several emission routes 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 or recycled. 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 ketone present in the remaining fragrance oils are discharged with the waste water.

Local PECs are calculated for the 5 compounding sites, as presented in **Table 2.2**. Site-specific emission data are submitted for these sites. As they cover approximately 90% of the total EU compounding tonnage of musk ketone, the exposure assessment for this life cycle stage is assumed to be represented sufficiently. Therefore no additional generic scenario is carried out for compounding. (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 13 tonnes/year. The estimated weight percent loss to waste water 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 ketone the tonnage has to be corrected by multiplying the tonnage with the tonnage divided by the percentage of musk ketone in a formulation. According to EFFA (1997) this is 3.5%, being equal to the 50th percentile of thousands of fragrance formulations. The corrected tonnage is: $100 / 3.5 \cdot 13 = 371$ ton/yr. The number of emission days is: $0.6^{5} \cdot 371 = 223$ days (see

[5] Although the fraction of the main source should in fact be 1, the value of 0.6 is used for the calculation of the number of emission days (default). The formula $f \cdot T$ 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 (371).

Table B2.1 of Appendix I of the TGD). The release for the emission episode is: 0.0001 · 13 / 223 = 5.8 g/day. A pre-treatment facility, consisting of sedimentation, oil-skimming and pH control by neutralization, treats the on-site waste water. 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.

The effluent from the pre-treatment facility goes to a municipal STP in which a removal rate of 32 % (TGD) is assumed. The remaining load of musk ketone after these two steps is (0.27 to 0.51) · 0.68 = 18 to 34 % of the amount originally emitted. This means that 18 to 34% of 5.8 g/day is 1.0 to 1.9 g/d 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}: 0.02-0.03 µg/l

As the dilution factor for this site is 1 the C_{local} in water, calculated according to the TGD, becomes 0.02 to 0.03 µg/l.

$PEClocal_{water}$ (dissolved) = 0.02/0.03 + 0.11 (PEC regional) = 0.13/0.14 µg/l.

Subsequently, based on the equilibrium partitioning theory, the $PEClocal_{sed}$ = 0.03mg/kg dw.

Site 2

The tonnage per year for fragrance compounding is 3 tonnes/year. The weight percent loss to waste water 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 · 3 = 86 tonnes/year. The number of emission days is: 2 · 86 = 171 days (see Table B2.1 of Appendix I of the TGD). The release for the emission episode is: 0.0001 · 3 /171 = 1.8 g/day.

A pre-treatment facility, consisting of flocculation, coagulation, filtration and pH control by neutralisation, treats the on-site waste water. Based on the measured removal percentage of oil and assuming that all musk substances are in the oil, the removal percentage for musk ketone for that particular treatment facility is > 99%. This site does not (yet) have an STP which means that <1% of musk ketone is emitted to water, that is $<0.01 \cdot 1.8 = < 0.018$ 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 0.004 ng/l.

Adding the $PECregional_{water}$ calculated in Section 3.1.2.3 this gives:

$PEClocal_{water}$ (dissolved) = 0.000004 + 0.11 = 0.11 µg/l

Subsequently, based on the equilibrium partitioning theory, the $PEClocal_{sed}$ = 0.025 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 off by an authorised waste disposal company.

Site 4

The tonnage per year for fragrance compounding is 4 tonnes/year. The use of musk ketone is evenly distributed over all working days (250, actual figure) and the weight percent loss to waste water 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: $0.002 \cdot 4 / 250 = 32$ g/day. A pre-treatment facility, consisting of sedimentation, oil-skimming, pH-neutralisation, coagulation, flocculation and filtration, treats the waste water. On average the percentage of oil separation is roughly 90% which is assumed to be equal to the removal percentage of musk ketone. 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 32% (default) which means that the total load passing both treatment steps is $0.1 \cdot 0.68 \cdot 32$ g/day = 2.2 g/day. The effluent concentration of the STP thus becomes 1.4/2700 = 0.8 µg/l.

The effluent is discharged into a river with a flow of $9.5 \cdot 10^9$ l/day. The resulting dilution factor is $(9.5 \cdot 10^9 + 2.7 \cdot 10^6) / 2.7 \cdot 10^6 = 3{,}520$, leading to a $Clocal_{water}$ for the emission episode of: 0.2 ng/l. Adding the $PECregional_{water}$ calculated in Section 3.1.2.3 this gives:

$PEClocal_{water}$ (dissolved) = 0.0002 + 0.11 = 0.11 µg/l.

Subsequently, based on the equilibrium partitioning theory, the $PEClocal_{sed}$ = 0.025 mg/kg dw.

Site 5

The tonnage per year for fragrance compounding is 2 tonnes/year. The use of musk ketone is evenly distributed over all working days (250, actual figure) and the weight percent loss to waste water 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: $0.002 \cdot 2/250 = 16$ g/day. The daily waste water flow of the STP is $1.4 \cdot 10^9$ litre, resulting in a concentration of $16/1.4 \cdot 10^9 = 11.4$ ng/l. Removal is assumed to be 32% (default). The resulting PEC_{STP} is $0.68 \cdot 11.4 = 7.8$ ng/l. The effluent is discharged into a river with a flow of $7.8 \cdot 10^9$ l/day. The resulting dilution factor is $(7.8 \cdot 10^9 + 1.4 \cdot 10^9) / 1.4 \cdot 10^9 = 6.6$, leading to a $Clocal_{water}$ for the emission episode of: 1.2 ng/l (dissolved concentration). Adding the $PECregional_{water}$ calculated in Section 3.1.2.3 this gives:

$PEClocal_{water}$ (dissolved) = 0.001 + 0.11 = 0.11 µg/l.

Subsequently, based on the equilibrium partitioning theory, the $PEClocal_{sed}$ = 0.025 mg/kg dw.

End product formulation

Fragrance compounding (first formulation step) is followed by formulation of musk ketone in end products (cosmetics, detergents, fabric softeners etc). Industry submitted some general statements that major detergents companies are not using nitromusks any longer. Limited 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[6] 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"[7] for waste water is 0.0009 and 0.00002 for air (Table 2). The site-specific emission factor to waste water 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 are 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 is 300[8]. 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 ketone in compounded fragrances and end product is needed. In the first formulation step, 3.5% musk ketone is used in fragrance compounds (EFA, 1997). The second formulation step, 0.02% (see Section 4.1.1.3) musk ketone was selected for the formulated end products (household detergents). It should be noted that a higher percentage of musk ketone in cosmetics (0.02-0.5%) 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 formulating company. The fraction of main source of 0.4 will be used, although industry submitted information about the number of formulating sites (soaps, detergents, cosmetics) in Europe. There may be a total of a few thousand of those sites in the Europe. It is not known; however, which part of those sites is actually using nitromusks. Using this unbalanced information would therefore be highly speculative. Moreover, according to industry the formulating company, for which the site-specific data on water emissions and emission days were generated, is "in its home country one of the larger formulators among the small enterprises". For this reason the default fraction of main source of 0.4, literally meaning that 40% of the formulation in the region may take place at one site, therefore doesn't seem to be an unrealistic value.

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6 The emission scenario document does not include air fresheners and/or odour agents.

7 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).

8 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 ketone is present at 3.5% in fragrance compounds and 0.02% in formulated end products (households detergents). 35 tonnes in the EU gives 1,142 tonnes of fragrance compounds (100/3.5 · 35 = 1,000 tonnes) and 517,000,0 tonnes of formulated end product (100/0.02 · 1,000 = 5,000,000 tonnes). Applying the 10% rule this leads to a tonnage of 500,000 tonnes at regional scale. This tonnage of 500,000 is used as input for the B-tables (B2.1).

Note: According to very recent industry information (2003) on the number of nitromusk end product formulators in the EU there are reasons to assume that a fraction of main source of 0.4 is too high. Values of 0.04 and 0.2 may seem more appropriate. These alternative values for the fraction of main source have been used now in the RAR on musk xylene. As the conclusions for the RAR on musk ketone would not be influenced by these alternative fraction of main source values, only the value of 0.4 is used here as a conservative estimate.

The total volume of musk ketone used end product formulation in Europe for 2000 is assumed to be 35 tonnes (see Section 2.2). Applying the 10% rule leads to a local emission to waste water of $(0.4 \cdot 0.002 \cdot 0.10 \cdot 35$ tonnes/year)/250 days = 0.011 kg/day. Using a default sewage flow of 200 l/eq/d gives a concentration in untreated wastewater of $5.5 \cdot 10^{-3}$ mg/l. The fraction (default) of musk ketone in the STP directed to air, water and sludge is 0.00034, 0.68 and 0.32, respectively. These defaults will be used for the assessment (see Section 3.1.1.2). The resulting effluent concentration is $0.68 \cdot 5.5 \cdot 10^{-3} = 3.74$ μg/l. Using a dilution factor of 10 the $Clocal_{water}$ is 0.37 μg/l (dissolved). Adding the $PECregional_{water}$ (dissolved) = 0.37 + 0.11 = 0.49 μg/l.

Subsequently, based on the equilibrium partitioning theory, the $PEClocal_{sed}$ = 0.041 mg/kg dw.

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 ketone will be emitted with the waste water of households. It is assumed that the total volume of musk ketone use in compounding fragrances in Europe for 2000, i.e. 35 tonnes, is released to waste water going to STPs. In reality the release can also be lower for reasons given already in Section 2.2 (a.o. export outside EU) and because some musk ketone will probably remain on the fabric. For the latter factor no quantitative data are available. Musk ketone used in cosmetics will generally have a lower emission factor than 1 that holds for detergents (see TGD A-tables). According to the OSPAR report (p.m. reference) on musks, the principal use of musk ketone is in cosmetics. However, no quantitative figures are available and as a worst case scenario 100% detergent usage is assumed in the current risk assessment.

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 35/10 = 3.5 tones/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 $35/370 \cdot 10^6$ (number of EU citizens) = 0.09 g/year can be calculated. For a theoretical EU region with 20 million inhabitants this would lead to a regional use volume of 1.9 tonnes/year ($20 \cdot 10^6 \cdot$ 0.09 g/year). Multiplying this average EU region with a factor of 1.9 (see above) results in a volume of 3.6 tonnes/year for a Southern European region. As this volume of 3.6 tonnes/year equals the volume calculated with the 10% rule (3.5 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, this leads to a local emission to waste water of (0.002 · 35 · 0.10 tonnes/year) / 365 days = 0.019kg/day. Using a default sewage flow of 2,000 m^3/day gives a concentration in untreated waste water of 0.0095 mg/l. The fraction (default) of musk ketone in the STP directed to air, water and sludge is 0.00034, 0.68 and 0.32, respectively (see Section 3.1.1.2). This leads to an effluent concentration of 0.0065 mg/l which is used as the PEC_{stp}. Using a dilution factor of 10 the $Clocal_{water}$ is 0.65 µg/l (dissolved). Adding the $PECregional_{water}$ calculated in Section 3.1.2.3 this gives:

$PEClocal_{water}$ (dissolved) = 0.65 + 0.11 = 0.76 µg/l.

Subsequently, based on the equilibrium partitioning theory, the $PEClocal_{sed}$ = 0.16 mg/kg dw.

The rapporteur is aware that the current private use scenario for musk ketone is a rather worst-case scenario. This is because of the reasons mentioned above already, but also due to the fact that a relatively high fraction of the emissions to waste water 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 are negligible compared to those from private use. Assuming the whole EU tonnage of 35 tonnes/year to be released to water (rather worst case scenario; see also private use scenario) results in a continental aquatic emission of 35/365= 95 kg/day. Applying the 10% rule the regional emission becomes 9.5 kg/day. In the EUSES the input for regional emissions is 9.5 kg/day and for continental emissions 85.5 kg/day (0.9 · 95). Assuming a split up of 30% discharge directly to surface water and 70% to an STP, results in emission values of 6.7 (indirect) and 2.9 kg/day (direct) for the regional scale and 60.5 (indirect) and 25.9 kg/day (direct) for continental emissions. EUSES finally calculates a PEC regional of 0.11 µg/l for surface water and 0.04 mg/kg for sediment.

3.1.2.4 Monitoring data

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

In a study by Eschke et al. (1994) influent and effluent concentrations of musk ketone were measured in 25 community sewage treatment plants along the River Ruhr in Germany. Influent concentrations were at the level of 0.57 to 2.4 µg/l and the median effluent level was 0.8 µg/l. From these data a percentage removal of 50% can be estimated. Surface water concentrations in the Ruhr were generally at a level of 0.03 µg/l. Higher levels, up to 0.23 µg/l, were found where tributaries entered the main stream. Under dry weather conditions these tributaries mainly consisted of treated waste water (Eschke et al., 1994). Based on these figures, the mean dilution factor for effluents in the Ruhr seems to be approximately 10.

In Japan (1981) mean concentrations in effluent from three treatment plants along the river Tama were 0.26 µg/l with a median level in the River Tama of 0.01 µg/l (Yamagishi et al., 1983). 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 the same as in the main river.

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 ketone (UK and USA) showed concentrations between 0.04-0.099 µg/l. The river water concentrations for musk ketone immediately downstream of the effluent discharge were 0.024 µg/l. The river water concentration for musk ketone upstream of the effluent discharge was 0.002 µg/l.

In a Swiss river, which is influenced by the outlet of an STP, the concentration of musk ketone was 0.0083 µg/l (Müller et al., 1996).

In a study on the main rivers in the Netherlands musk ketone 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). As in The Netherlands, the concentration in sediment is taken to be half of the concentration in suspended solids, sediment concentrations are estimated to be below 0.025 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 metres 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 below the detection limit (<0.02 ng/l). Relatively high levels of 4.6 ng/l were found near the River Elbe (Gatermann et al., 1995).

Gatermann et al. (1998) measured musk ketone and the possible transformation product 2-amino-MK at one location in the river Elbe (Hamburg). Concentrations of musk ketone and 2-amino-MK were <1-4 ng/l (n = 3) and 7 ng/l (n = 1), respectively. From the STP Hamburg also a 24 hour composite influent sample and effluent sample was taken. Concentrations of musk ketone were 550 ng/l and 6 ng/l for influent and effluent, while for 2-amino-MK these concentrations were <0.5 ng/l and 250 ng/l.

Herren and Berset (2000) reported musk ketone and musk ketone metabolite levels in sewage sludge samples from different catchment areas in Switzerland. Musk ketone was detected in 7 out of 12 sludges with a mean concentration of 5 µg/kg dwt. The maximum level was 7 µg/kg dwt. The metabolite amino musk ketone was only found in a few sludges with a maximum of 13.1 µg/kg dwt.

Sludge was sampled in six 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 70%. In almost all 31 samples of primary, secondary or digested sludge musk ketone concentrations were lower than the reporting level of 1 mg/kg. Two samples resulted

in a concentration of 1.1 mg/kg, while one sample resulted in a concentration of 2.0 mg/kg. These high levels refer to activated sludge. Digested sludge data were all below 1 mg/kg. The authors considered concentrations in sludge below 1 mg/kg dw irrelevant and did not determine detection levels.

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

Location	Concentration (µg/l)	N	Reference
Influent, effluent and surface water			
Effluent, Japan	0.26 median (0.14-0.41)	18	Yamagishi et al. (1983)
Tama River, Japan	0.01 median (nd-0.028)	18	Yamagishi et al. (1983)
Waste water influent Effluent	1.5 median (0.57-2.4) 0.75 median (0.22-1.3)	19 36	Escke et al. (1994)
River Ruhr Germany	0.03 mean (0.02-0.23)	34	Escke et al. (1994)
River Elbe, Germany	<0.002-0.010	31	Winkler et al. (1998)
Surface waters, Berlin	0.08 mean (n.d.-0.390)	30	Heberer et al. (1999)
River Glatt, Switzerland	0.0083	1	Muller et al. (1996)
River Rhine, Netherlands	<0.01	31	Breukel and Balk, 1996
River Meuse, Netherlands	<0.01	34	Breukel and Balk, 1996
North Sea	<0.00002 median; max 0.00008	30	Gatermann et al., 1995
STP (communal), USA		-[d]	Simonich et al., 2000
Influent (AS)*	0.569		
Effluent	0.099		
STP (communal), USA		-[e]	
Influent (TF)*	0.488		
Effluent	0.096		
STP (communal), UK		-[d]	Sabaliunus et al., 2001
Influent (AS)*	0.37		
Effluent	0.04-0.06		
STP (communal), UK		-[e]	
Influent (TF)*	0.75		
Effluent	0.04-0.06		
Rivers in Yorkshire, UK	0.002 (upstream)	-[f]	
	0.024 (downstream)		
Suspended matter concentration (mg/kg dwt)			
River Rhine, the Netherlands	<0.05	14	Breukel and Balk, 1996
River Meuse, the Netherlands	<0.05	14	Breukel and Balk, 1996
River Elbe, Germany	0.009 mean (0.004-0.022)	31	Winkler et al. (1998)
Various rivers, Germany	0.106 (median) (0.024-0.408)	13	

Table 3.2 continued overleaf

Table 3.2 continued Monitoring results of musk ketone in the aquatic environment.

Location	Concentration (µg/l)	N	Reference
Influent, effluent and surface water			
Sediment concentration (µg/kg dwt)			
Elbe river, Hamburg	0.135-0.189		Rimkus et al. , 1999
Elbe river, Wedel-Schulau	0.162		Rimkus et al. , 1999
Various German rivers	0.2-3.8		Lach and Steffen (1997)
STP sludge (communal) concentration (mg/kg)			
Sludge, Switzerland	0.005 (mean; dwt) 0.007 (max.; dwt)	12	Herren and Berset (2000)
Sludge, Germany	0.030 (mean; dwt) < 0.010-0.060 (min-max)	2	Sauer et al. (1997)
Sludge, the Netherlands	n.d. – 2 (dwt) < 1 (median value)	31	Blok (1998)

d) Samples were collected hourly over 3-day period from an Activated Sludge Wastewater Treatment. The samples were composite 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 composite 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 can be made when comparing calculated with measured concentrations:

- measured as well as calculated concentrations are a direct reflection of the use volume of musk ketone: however, these volumes vary from country to country and, more important, do vary in time;
- measured concentrations in
- Table**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 of STPs along the river Ruhr. The median value of 0.75 µg/l is a factor 10 lower than the calculated local effluent concentration for private use of 6.7 µg/l. The maximum measured value of 1.3 µg/l in the Eschke study differs a factor 5 from the calculated one. The measured concentration by Gatermann et al. (1998) is much lower than the value from Eschke et al. (1994): 0.006 µg/l, resulting in a larger difference with the calculated PEC. 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 ketone between 0.04-0.099 µg/l. These data also confirm that measured concentrations are lower than the calculated local effluent concentration for private use (6.7 µg/l).

Surface water: recent (mean or median) measured surface water concentrations vary from 0.08 to 0.0083 µg/l, being at least a factor 10 and 1.4 lower than the calculated $PEClocal_{water}$ of 0.76µg/l for private use and the $PECreg_{water}$ of 0.11 µg/l, respectively. The maximum value of 0.39 µg/l as reported in the Heberer et al. (1999) study is a factor 2 lower than the calculated PEC for private use and a factor 3 higher than the PEC regional of 0.11 µg/l. The concentration of 0.39 µg/l (Heberer et al., 1999) should be considered as an extreme value as it refers to water highly influenced by several STP effluents. The representativity of this sampling point is discutable. Recently measured river water concentrations of musk ketone in the UK (Sabaliunas et al., 2001) vary from 0.002 to 0.024 µg/l, being respectively a factor > 440 and 40 lower than the calculated $PEClocal_{water}$ for private use (0.76 µg/l) and a factor > 65 and 5 lower than the $PECreg_{water}$ (0.11 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 more or less equal to the calculated PEC reg_{sed}. Sediment data from the Elbe river (Rimkus et al., 1999) of 0.135-0.189 µg/kg WWT are about two orders of magnitude lower than the calculated PECs for sediment.

STP sludge: a sludge concentration of 7.6 mg/kg is calculated for the private use scenario. Measured data from communal (private use) STPs in Germany, Switzerland and The Netherlands were all (much) lower. The maximum measured concentration (Blok, 1998) of 2 mg/kg is a factor 5 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, monitoring data will be taken into account as well. Available monitoring data generally point to 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 (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 2 mg/kg (see Sections 3.1.4 and 3.1.5.1). The rapporteur is aware that this value of 2 mg/kg refers to an activated sludge value, which is in most cases of lesser relevance than digested sludge levels (< 1mg/kg). The latter is the form of sludge that is (mostly) applied on agricultural soils. The currently used value of 2 mg/kg should thus be considered as a worst-case value.

3.1.3 Atmosphere

No site specific data are available on the emission of musk ketone to the atmosphere. In Section 3.1.2 it is mentioned that for sites 4 and 5 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 formulation using a regional volume of 3.5 (= 10% of 35 tonnes) tonnes/year (see Section 3.1.2.1). Emission factor (ESD in TGD) is 0.00002;
- private use using a regional volume of 3.5 (= 10% of 35 tonnes) tonnes/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 3.5 tonnes/year (10% of 35 tonnes).

Results are presented in **Table 3.3**.

Table 3.3 Local PECs for musk ketone in air.

	PEC (ng/m^3)
Site 1	25
Site 2	5.7
Site 3	25
Site 4	7.6
Site 5	3.8
End product formulation	$3.02.10^{-2}$
private use	0.01
Regional	0.01

A measured value in air of 4-45 pg/m^3 has been reported in Norway (Kallenborn *et al.,* 1999). In indoor air, 120 pg/m^3 were detected. 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 ketone 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 3.5 tonnes/year (see Section 3.1.2.1);
- private use using a regional volume of 3.5 tonnes/year (see Section 3.1.2.2).

Results are presented in **Table 3.4**

Table**.** 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 2 mg/kg[9]. 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 2 mg/kg$_{dwt}$. 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 2 mg/kg$_{dwt}$ can directly be entered in EUSES. For the regional scenario this is not possible. Indirectly the

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

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 waste water 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 Local PECs for the terrestrial compartment.

	PEC in soil (µg/kg ww) Default scenario	PEC in soil (µg/kg ww) Alternative scenario (highest measured sludge value of 2 mg/kg)
Site 1	2.0-2.3	1.9
Site 2	0.51	0.4
Site 3	1.7	1.6
Site 4	16	15.7
Site 5	0.53	0.4
End product formulation	63	71.8
Private use	122	32

Measured concentrations in soils are not available.

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 1,380 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 3.6 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 3.5 tonnes/year (see Section 3.1.2.1);
- private use using a regional volume of 3.5 tonnes/year (see Section 3.1.2.2).

Results are presented in **Table 3.5**. The revised TGD prescribes the use of a biomagnifications factor (BMF) for the aquatic route. Musk ketone falls in the category ($\log K_{ow} < 4.5$ and BCF (fish) < 2000) where a BMF of 1 is applicable.

Table 3.5 PECs in fish and worm.

	$PEC_{oral, fish}$ (mg/kg ww)	$PEC_{oral, worm}$ (mg/kg ww) Default scenario	$PEC_{oral, worm}$ (mg/kg ww) Alternative scenario (highest measured sludge value of 2 mg/kg)
Site 1	0.17	0.03	0.005
Site 2	0.16	0.02	0.003
Site 3	0.16	0.03	0.004
Site 4	0.16	0.05	0.03
Site 5	0.16	0.02	0.003
End product formulation	0.34	0.14	0.12
Private use	0.6	0.22	0.05

3.1.5.2 Monitoring data

Concentrations found in fish and shellfish are summarised in **Table 3.6**. Concentrations are given in μg/kg wet weight and/or in μg/kg fat. Some additional information on the test results is given below.

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 1.5 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 0.07 mg/kg fat. Concentrations were lower in lakes than in rivers like Rhine and Meuse, e.g. 0.07 mg/kg fat at two sample sites in the IJssel Lake. The highest concentration was measured in the river Rhine near Lobith: 0.29 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 0.053 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 ketone levels in fish ranging from < 4 to 27 μg/kg (fresh weight). Fish samples were from major Dutch rivers and other large fresh water bodies.

Rimkus et al. (sub.) measured musk ketone and 2-amino-MK in fish in the rivers Elbe and Stör, in aquacultures in Denmark, Spain and Austria and in ponds of an STP. Mean concentrations of 2-amino-MK were 12 (<3-17), 6.2 (<1-23), 21 (6-34) μg/kg fat in fish. 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. Of the STP also influent and effluent concentrations were measured. Concentrations of musk ketone in influent and effluent were 72 and 22 ng/l, respectively. For 2-amino-MK these were 17 and 14 ng/l, respectively.

Fromme et al. (1999) measured levels of nitromusks in eel in the Berlin area. The mean values of musk ketone during the measurement periods 1995 and 1996 were, respectively, 41 and 39 µg/kg fresh wt. Maximum levels were 260 µg/kg fresh wt (1995) and 380 µg/kg fresh wt (1996).

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 Oslo fjord and Greenland fjord areas. The measured MK concentrations in 25 different fish samples ranged form < 5 to 42 µg/kg lipids. The highest MK level (42 µg/kg lipid) was found in the liver of Atlantic cod near Oslo. Cod fish is a rather big, predator fish, so these data are representative for musk ketone levels in animals that are 'high' in the marine food chain.

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 ketone was present in two hand lotions. The authors did not identify the source of musk ketone detected in their analysis of fish samples, and therefore did not publish the concentrations.

Table 3.6 Monitoring data for musk ketone in aquatic biota.

Sample	Concentration [µg/kg]	N	Reference
Carp (Japan)	Median: 2 (n.d.-27 muscle ww Median: 2 (n.d.-70 viscera ww	31	Yamagishi et al., 1983
Shellfish (Japan)	Median: 2 (0.9-26) ww	9	Yamagishi et al., 1983
Freshwater fish (Germany)	Median: 70 (10-380) fat	26	Rimkus and Wolf, 1993
Mussels (Germany)	10-30 fat	9	Rimkus and Wolf, 1993
Trout (fish farm) (Germany)	Mean: 140 (20-330) fat	46	Rimkus and Wolf, 1993
Fish River Ruhr (Germany)	Median: 7 (3-66) muscle ww Median: 560 (500-1,500) fat	9	Eschke et al., 1994
Fish effluent pond (Germany)	Median: 128 (74-1,605) muscle, ww Median: 5,500 (4,300-17,000) fat	13	Eschke et al., 1994
Fish farm (Germany)	Mean: 30 (10-110) fat	36	Rimkus and Wolf, 1995
Fish German rivers	Mean: 90 (<10-380) fat	22	Rimkus and Wolf, 1995
Shrimps (Germany)	30-50 fat	3	Rimkus and Wolf, 1995
Fish (German rivers)	Mean: 92 (23-235) fat	7	Rimkus et al. (sub)
Fish (effluent pond) (Germany)	Mean: 1,095 (350-1,560) fat	11	Rimkus et al. (sub)
Mussels (effluent pond) (Germany)	1,280 fat	1	Rimkus et al. (sub)
Trout (fish farms 3 countries)	Mean: 61 (22-117) fat	7	Rimkus et al. (sub)
Eel, the Netherlands	Median: 20 (<4-60) ww Median: 70 (<20-290) fat	13	Wiertz, 1995
Eel, the Netherlands	Median 11 (<5-33) ww	9	De Boer and Wester, 1996

Table 3.6 continued overleaf

Table 3.6 continued Monitoring data for musk ketone in aquatic biota.

Sample	Concentration [µg/kg]	N	Reference
Other fish, the Netherlands	n.d.	7	De Boer and Wester, 1996
Shellfish, the Netherlands	n.d.	4	De Boer and Wester, 1996
Fish, the Netherlands (large surface waters)	<4-27 wwt		RIVO, 1997
Fish (landlocked shad), Italy (Lago Verbano)	0.5 (med.) ww range 0.3-0.7 ww	102	Ceschi et al. 1996
Fish, Norway; harbour and fjord areas	< 5 – 42 (lipid)	25	Kallenborn et al, 2001
Fish, Germany (various rivers)	n.d-<0.005	5	Janda et al. (2000)
Eel, Germany	41 mean (1- 260) fwt (1995) 39 mean (1 - 380) fwt (1996)	84 122	Fromme et al., 1999

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 0.6 mg/kg for private use. Assuming a fat content of 5% this is equal to 12 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 mostly around a factor 10 or more lower than the calculated one. The highest maximum reported levels (e.g. 380 µg/kg WWT) in Germany, however, only differs a factor 2 from the estimated value.

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

3.2.1 Aquatic compartment

3.2.1.1 Toxicity data

Schramm et al. (1996) tested the acute toxicity of musk ketone 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 0.46 mg/l for bacteria and the water solubility for algae and daphnids.

From the test on inherent biodegradability (see Section 3.1.1.1) it can be concluded that musk ketone was not toxic to the inoculums used at a concentration far above the water solubility (NOEC > 39 mg/l).

For musk ketone three toxicity tests by Grützner et al. (1995) were available: one with algae, one with *Daphnia magna* and one with fish (**Table 3.7**). The tests were performed with a

combination of DMF and Tween 80 (0.005% each) to dissolve the musk compound. The concentrations of musk ketone in water are analyzed using HPLC. The measured concentrations range between 50-110% of nominal. The test results are expressed as measured concentrations.

The 72-h green algae growth inhibition test was carried out according to OECD Guideline 201, Section 2, with *Selenastrum capricornutum* (in the study referred to by its new name *Pseudokirchneriella subcapitata*). The chronic NOEC is based on growth rate (g) and biomass (b). For these parameters also an EC50 was determined.

For *Daphnia* a 21-d reproduction test was carried out according to OECD Guideline 202, part II. The reproduction was significantly reduced at 0.338 mg/l, while at 0.675 mg/l reproduction was absent. In the highest concentration after 15 days parent mortality was 100%, and reproduction did not take place. In the other concentrations no mortality occurred. In 0.084 mg/l reproduction was 90% compared to control, while in 0.338 mg/l this was reduced to 45%. 0% reproduction could not be determined due to 100% mortality in the highest concentration, therefore the EC50 is given as a range.

A 21-d prolonged toxicity test with young rainbow trout (*Onchorhynchus mykiss*) was carried out according to OECD Guideline 204. The mean fish weight was 2.3 g at the start of the experiment. A total of three fish died (30% mortality) in the highest concentration (0.5 mg/l), hence the LC50 could not be determined. Fish weight and size were significantly reduced at 0.25 mg/l, and weight was significantly reduced for 0.5 mg/l. Clinical signs, such as irregular respiratory rate and reduced food uptake, occurred at 0.125 mg/l and higher.

An embryo larvae (Zebra fish) toxicity test according to Swedish Standard (SS 02 81 93) was performed with musk ketone (Carlsson and Norrgren 2002 *in press*). Seven concentrations between 1 and 1,000 µg/l of musk ketone 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 100 µg/l and a NOEC of 33 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 fertilized Zebrafish eggs were exposed in 96-well microtitre plates to a series of musk ketone 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 33 µg/l and 100 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.

Very recently some (sub)chronic crustacean toxicity tests investigated the effects of one nitromusk (musk ketone) as well as three polycyclic musks (Tonalide™, Galaxolide™ and Celestolide™) on the larval development rate of the marine copepod *Acartia tonsa* (Wollenberger et al. 2001; in prep.) and several life cycle parameters of the brackish water copepod Nitocra spinipes (Breitholtz et al. 2003).

The inhibition of larval development of Acartia was shown to be a very sensitive endpoint, with 5-day-EC_{50}-values ranging from 0.03 to 0.16 mg/l (nominal values). These values were generally more than one order of magnitude below the 48-hour-LC_{50}-values found for adults, which ranged from 0.32 to 2.5 mg/l. The larval development 5-day-EC_{50} and 5-day-EC_{10} values for musk ketone amounted to, respectively, 0.066 mg/l and 0.010 mg/l. The Wollenberger et al. (2001) paper is a draft version and is still subject to alterations (the Rapporteur has actually asked the authors for clarification on some issues). For this reason the data will not (yet) be used in the current ecotoxicological hazard identification for musk ketone. The preliminary results, however, indicate that this test species, especially the larval stage, may be sensitive to musk ketone and polycyclic musks.

The NOEC and LOEC for the larval development rate of Nitocra were found to be 10 and 30 µg/l, respectively (Breitholtz et al., 2003). The 22 days exposure resulted in decreased population growth with a LOEC of 100 µg/l and a NOEC of 30 µg/l. Further details on the Nitocra study are not (yet) available.

(Please note that if the Acartia or Nitocra data would be used in the current RAR the PNEC would become 0.010/10= 0.001 mg/l (1 µg/l) which is lower than the current PNEC of 6.3 µg/l. However, this lower PNEC would not alter the final conclusions of the report).

Table 3.7 Toxicity data for aquatic organisms.

Species	Test	Result (mg/l)	Remark[a]	Reference
Vibrio fischeri	30 minute	EC50 = >0.37	DIN 38412, part 34	Schramm et al., 1996
Selenastrum capricornutum[b]	72-hour static	NOEC = 0.088 EbC50 = 0.12 (0.11-0.13) EgC50 = 0.24 (0.21-0.30)	OECD TG 201; carrier: DMF, Tween 80 (each 0.005%); 0.044-0.84 mg/l (n=5); HPLC identification; actual concentrations 54-92% of nominal; results as measured concentrations	Grützner et al., 1995
Scenedesmus subspicatus	72-hou static	EbC50 = >0.46	OECD TG 201	Schramm et al., 1996
Daphnia magna	48-hour static	EC50 = >0.46	OECD TG 202	Schramm et al., 1996
Daphnia magna	21-day semi-static	EC50 = 0.34-0.68 (imm) EC50 = 0.17-0.34 (rep) NOEC = 0.17 (rep) LOEC = 0.34 (rep)	OECD TG 202 part II; carrier: DMF, Tween 80 (each 0.005%); 0.042-0.68 mg/l (n= 5); HPLC identification; actual concentrations 61-97% of nominal; results as measured concentrations	Grützner et al., 1995
Rainbow trout *O. mykiss*	21-day flow-through	LC50 = > 0.50 NOEC = 0.063 LOEC = 0.13 (clinical signs)	OECD TG 204; carrier: DMF, Tween 80 (each 0.005%); 0.031-0.50 mg/l (n=5); HPLC identification; actual concentrations 82-110% of nominal; results as measured concentrations	Grützner et al., 1995

a) the number of concentrations tested (n) is without control and solvent control
b) in the study referred to by its new name *Pseudokirchneriella subcapitata*

In the TGD several QSARs for non-polar narcosis are given for calculating toxicity data for aquatic organisms. Results for musk ketone are presented in **Table 3.8**. Comparing the experimental results as presented in **Table 3.8** with the QSAR estimates shows that there are only minor differences between both values. This might indicate that musk ketone acts by non-polar narcosis.

Table 3.8 Toxicity data using QSARs for non-polar narcosis (TGD Chapter 4) using a log K_{ow} of 4.3 and a MW of 294.3g/mol.

Species	Endpoint	Result (mg/l)
Pimephales promelas	96-hour LC50	2.7 (1.2-6.1)
Brachydanio rerio/Pimephales promelas	28-32-day NOEC (ELS)	0.20 (0.093-0.43)
Daphnia magna	48-hour EC50	1.2 (0.53-2.5)
	16-day NOEC	0.13 (0.052-0.31)
Selenastrum capricornutum	72-96-hour EC50	0.87 (0.59-1.3)

Results of an *in vitro* competitive estrogen receptor binding study (Chou and Dietrich, 1999) with musk ketone and musk ketone 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. The 72h-growth test with algae and the 21d-reproduction test for *Daphnia magna* are considered long term tests. The 21d-fish growth test for musk ketone is considered to be a test on chronic effects as well. An assessment factor of 10 is applied to the lowest of three NOECs leading to a $PNEC_{water}$ of 6.3 µg/l. (A tentative PNEC of 1 ug/l could be derived on the basis of the Acartia and Nitocra studies).

Subsequently, via the equilibrium partitioning theory, a $PNEC_{sed}$ of 0.5 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 (96.7 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 0.37 mg/l. However, according to the TGD these tests with photoluminescent bacteria can not be used for deriving a $PNEC_{STP}$. From the test on inherent biodegradability a NOEC of > 39 mg/l could be derived. Applying an assessment factor of 10 leads to a $PNEC_{STP}$ of > 3.9 mg/l. It is realised that this PNEC is higher than the water solubility of musk ketone of 0.46 mg/l.

3.2.1.3 Atmosphere

No data are available on exposure of organisms via the air. Therefore, no $PNEC_{air}$ can be derived.

3.2.2 Terrestrial compartment

3.2.2.1 Toxicity data

Toxicity tests were carried out with earthworms and springtails (see **Table 3.9**). In the test with earthworms no mortality of the adults was observed after 4 weeks at any of the test concentrations. At the NOEC for growth (100 mg/kg), the body weight increase was 47% of the control (40 ± 33 compared to 85 ± 32), but this difference was not statistically significant. The inhibition of reproduction was measured as young worms per container after 8 weeks. Inhibition was 15% at the LOEC of 100 mg/kg (235 ± 24 compared to 275 ± 26). The amount of food was visually approximated at each feeding. Food consumption was measured as total of food added over 5 weeks per test containers. Inhibition of food consumption occurred at 32 mg/kg and higher test concentrations. The method applied does not measure the "real" food consumption. Therefore the result is not used for derivation of the PNEC.

The toxicity to springtails was tested according to the ISO/CD 11267 draft guideline (Klepka and Petto, 1997). The mortality in the test concentrations varied between 4 and 20% but a concentration-response relation could not be established. Survival after 4 weeks in 1,000 mg/kg was 90%. Reproduction, measured as produced juveniles per test unit, in 3, 32 and 100 mg/kg was increased as compared to the control, whereas it was inhibited by 65% in 316 mg/kg (LOEC) and by 87% in 1,000 mg/kg.

Table 3.9 Toxicity data for soil organisms.

Species	Result [mg/kg dw]	Remark	reference
Springtail Folsomia candida	28-d LC50 = >1,000 NOEC = 100 and LOEC = 316 (reproduction)	ISO/CD 11267 draft 1996, 10-12day-old juveniles; artificial soil; pH 6.0 ± 0.5, 6% o.c.; range 3 – 1,000 mg/kg (n=5)	Klepka and Petto, 1997
Earthworm Eisenia foetida	28/56-d LC50 = >1,000 NOEC = 100 and LOEC = 316 (28 day-growth) NOEC = 32 and LOEC = 100 (8w-reproduction)	ISO/DIS 11268-2, initial weight 370-480 mg, artificial soil, pH 6.0 ± 0.5, 6% o.c.; range 3-1,000 mg/kg (n=5); no mortality at 1,000 mg/kg	Goβman and Petto, 1997

3.2.2.2 PNEC for the terrestrial compartment

For musk ketone two long-term toxicity tests are available: for a shredder (4 weeks, springtail) and a detritivorous species (8 weeks, earthworm), allowing an assessment factor of 50 to be applied to the lowest NOEC. This lowest NOEC should first be normalised to the standard soil defined in the TGD of 3.4% organic matter. This leads to a value of 11 mg/kg dw (i.e. 32 divided by 10/3.4). Subsequently, applying the assessment factor of 50 gives a $PNEC_{soil}$ of 0.22 mg/kg dw.

3.2.3 Non compartment specific effects relevant to the food chain

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 ketone. The oral NOAEL of 2.5 mg/kg bw/d for postnatal toxicity in rats is used for this purpose (see Chapter 4). As toxicity is based 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, 2002). However, a number of arguments can be adduced why the use of such factor may be over-protective in this case. One reason is that even at the next concentration in the test, i.e. 7.5 mg/kg bw/d, only marginal (7%) effects were seen on the body weight gain of the pups. This makes this LOAEL, and, implicitly, the selected NOAEL, rather conservative. In addition, a semi-chronic dermal rat study is available (Ford *et al.*, 1990) from which an oral NOAEL of 19 mg/kg bw/day can be calculated (route-to-route). This value is higher than the value of 2.5 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 2.5 mg/kg bw/day as a starting point for the $PNEC_{oral}$ derivation of musk ketone it is therefore suggested to use an assessment factor of 150 as a reasonable 'compromise' between 90 and 300. The $PNEC_{oral}$ then becomes: $2.5 \cdot 20/150 = 0.3$ mg/kg food.

$PNEC_{oral} = 0.3$ mg/kg food

3.3 RISK CHARACTERISATION

In Chapter 2 some uncertainties were mentioned about the total volume of musk ketone being used in the EU. This a.o. 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 that the available monitoring data 'implicitly' comprise the overall emissions from the use of musk ketone 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 (esp. musk ketone 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 nitro musks have been taken.

3.3.1 Aquatic compartment

The PEC/PNEC ratios for the aquatic compartment (STP and surface water) are presented in **Table 3.10**. The PNECs used are < 3.9 mg/l (STP) and 6.3 µg/l (water).

Table 3.10 PEC/PNEC ratios for STP and surface water.

	STP	Surface water
Site 1	< 0.01	0.02-0.03
Site 2	< 0.01	0.02
Site 3	n.r	0.02
Site 4	< 0.01	0.02
Site 5	< 0.01	0.02
End product formulation	<0.01	0.08
Private use	< 0.01	0.1
Regional	n.r	0.02

From **Table 3.10** Tableit can be seen that all PEC/PNEC ratios are below 1: **conclusion (ii)**. This conclusion is supported by the available monitoring data. The same conclusion would be true if the tentative PNEC water of 1 µg/l would be used.

The 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, also lead to a PEC/PNEC of less than one: **conclusion (ii)**.

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 the soil compartment are presented in **Table 3.11**. The $PNEC_{soil}$ of 0.22 mg/kg dwt is used for this comparison.

Table 3.11 PEC/PNEC ratios for the terrestrial environment.

Scenario	Default scenario	Alternative scenario (based on maximum measured sludge value of 2 mg/kg)
Site 1	<0.01-0.01	<0.01
Site 2	<0.01	<0.01
Site 3	<0.01	<0.01
Site 4	0.06	0.06
Site 5	<0.01	<0.01
End product formulation	0.29	0.29
Private use	0.5	0.1

Table 3.11 indicates that all PEC/PNEC ratios for the terrestrial compartment, both in the default and alternative scenario, are below 1: **conclusion (ii)**.

3.3.4 Non compartment specific effects relevant to the food chain

The PNEC oral is 0.3 mg/kg bw/day. This PNEC is compared with the PECs (**Table 3.12**). All PEC/PNEC ratios are found to be at or below 1: **conclusion (ii)**, except for the private use scenario. The default PEC/PNEC ratio of 1.8 for the private use scenario (fish-route) can be overruled, however, by the rather large monitoring data set for fish from a number of different EU regions. All measured values are lower than the calculated value of 600 µg/kg WWT. 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. Only the highest maximum value in fat (17,000 µg/kg WWT) would yield a PEC/PNEC significantly above 1 (2.5). This value comes from the data from Escke et al (1994) with fish from effluent ponds. Both the sampling year (before 1994) and the location (effluent pond) reflect a worst case situation. From the mean value of 5,500, with a minimum of 4,300 and a maximum of 17,000, a 90 P-value of around 8,000 µg/kg fat can be estimated (log normal (skewed) distribution). This 90 P-value corresponds with a value of 400 µg/kg WWT based on a fat percentage of 5%, which is below the calculated PEC of 600 µg/kg, and this results in a PEC/PNEC of 1.2. The 90 P value is preferred above the maximum value according to the TGD. Due to the still very conservative character of this 90P value (sampling point, year) in comparison with the other available fish monitoring data, a **conclusion (ii)** is considered most appropriate for the private use scenario (fish route)

For worm predators all PEC/PNEC ratios are calculated to be below 1 for both the default and alternative scenario: **conclusion (ii)**.

Table 3.12 PEC/PNEC ratios for fish-eating and worm-eating predators.

	PEC/PNEC fish	PEC/PNEC worm Default scenario	PEC/PNEC worm Alternative scenario (based on maximum measured sludge value of 2 mg/kg)
Site 1	0.5	<0.1	<0.1
Site 2	0.5	<0.1	<0.1
Site 3	0.5	<0.1	<0.1
Site 4	0.5	0.15	<0.1
Site 5	0.5	<0.1	<0.1
End product formulation scenario	1	0.41	0.35
Private use	1.8	0.65	0.16

3.3.5 Metabolites of musk ketone

2-amino-MK has been measured in water and fish in Germany (Gatermann et al., 1998). Concentrations of musk ketone and 2-amino-MK were <1-4 ng/l (n = 3) and 7 ng/l (n = 1), respectively at one location in the river Elbe (Hamburg). In influent the concentration of musk ketone was much higher than 2-amino-MK, 550 versus <0.5 ng/l, while in effluent the concentration of 2-amino-MK was the highest, 250 versus 6 ng/l. In fish from two German rivers the concentration of 2-amino-MK was almost an order of magnitude lower than the concentration of musk ketone. Herren and Berset (2000) found the metabolite amino musk

ketone in only one sludge sample. The value (13 µg/kg dwt) was slightly higher than the maximum musk ketone level (7 µg/kg dwt).

The amount of ecotoxicity data for musk ketone metabolites is very limited. Chou and Dietrich (1999) investigated the competitive binding capability of musk ketone and musk ketone metabolites to the estrogen receptor in trout (*Oncorhynchus mykiss*) and clawed frog (*Xenopus laevis*). No binding of the parent compound musk ketone was observed. In contrast, however, binding to the ER was noticed for 2-amino-musk ketone in both species, Xenopus being the most sensitive species. The IC50 (competitive binding at the ER) of 2K in Xenopus was found to be 70.1 ± 88.3 µM (= 20.5 mg/l). Although competitive binding was demonstrated for the metabolites of MK, the relevance of such *in vitro* assays for the environmental situation is still unclear. IC-50 values for 2K are many orders of magnitude higher than those levels found at present in the environment.

No further ecotoxicity data are available for the MK metabolites (pers. com. IFF). This in contrast to musk xylene for which some recent ecotoxicity data (short term Daphnia test) have become available (see RAR on musk xylene). No obvious difference was noticed between the toxicity of the parent compound and the musk xylene metabolites. It should be emphasised, however, that for bioaccumulative compounds long term test results outweigh short term data.

Conclusion metabolites

On the one hand there are no indications that ketone metabolites may be more toxic than the parent compound (parallel with musk xylene, lesser hydrophobicity of MK metabolite compared to parent compound), but on the other hand the biological activity is different as concluded in the estrogen receptor binding study. However, as 1) the ecological relevance of the *in vitro* ER binding study is unknown, 2) effects in the *in vitro* study occurred at much higher levels than those currently measured in the environment, 3) current environmental levels of metabolites are low compared to parent compound (and related compounds) toxicity and 4) EU nitromusk usage is expected to further decrease due to political decisions, for musk ketone metabolites a **conclusion (ii)** is drawn for the environment.

3.3.6 PBT assessment

Musk ketone is considered not to be a PBT candidate substance. Although the Persistence criterion seems to be fulfilled (one experimental biodegradation test clearly showing no (ready) biodegradability, accompanied by some inconclusive influent/effluent studies and the BIOWIN model results), the Bioaccumulation criterion is not met as the experimental BCF is below 2,000. The Toxicity-criterion would be a borderline case for ecotoxicity with the tentative NOECs of 10 µg/l for Acartia and Nitocra. The T-criterion in the TGD is that long-term NOECs should be less than 10 µg/l. For human health toxicity, the situation around musk ketone fulfilling the T-criterion is not clear yet. This is because the CMR group has decided that more information should/could be provided about the potential carcinogenicity (R40?). The outcome of this discussion on carcinogenicity has no influence on the final PBT assessment for musk ketone, as the B-criterion is not met.

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 ketone.

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 ketone 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).

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

Data were received from six European facilities and are considered as representative for Europe.

Table 4.1 Use of musk ketone.

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 ketone 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 ketone (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 ketone 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 ketone is imported as a crystalline powder. Determination of the particle size showed that all material grains were smaller than 200 μm. The respirable fraction (particle size lower than 4 μm) was up to 13.5% (Rodriguez B, 1998).

At room temperature the substance has a very low vapour pressure: 4.10^{-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 ketone 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 ketone 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/m³)
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 analogous 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.

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 the 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 the 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 ketone 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 ketone and the descriptions of the study are such, that the relevance of the results for this assessment is 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 US-EPA transfer model (US-EPA, 1991).

The US-EPA 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 – US-EPA 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 = 294
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	5,100	850
k	0.5	0.1

The calculated range for filling of cans of 50 L is 0.04 and 1.4 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 estimated values <0.01-0.14 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 analogue substances are used for estimating exposure due to compounding. The quantities of musk ketone 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 0.1 mg/m^3 (rounded), 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 value of the EASE model is taken, 42 mg/day. This value corresponds well with the mentioned lower range of the analogue substance: 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.

Drumming of liquid fragrance

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 estimates of the US-EPA model, including the effect of LEV, are considered most relevant for drumming: may be used for the risk evaluation, <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. Dermal exposure is estimated to be equal. For the risk characterisation the 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 of 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 1,950 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 musk ketone 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 ketone) 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 $15 \cdot 840 \cdot 0.01 \cdot 1/50$).

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 ketone; mg/m3)						Estimated skin exposure level (musk ketone; 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 ketone is added intentionally.

Musk ketone was assessed by the EU Scientific Committee on Cosmetic Products and Non-Food Products intended for Consumers (SCC, 1997; SCCNFP, 1999): musk ketone 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 ketone may also be used in household detergents (HEDSET; Tas *et al.*, 1997). In the HEDSET the amount of musk ketone in detergents is stated to be <0.02%. Müller (1997) reports the detection of musk ketone in laundry detergents (maximum value is 0.011% in several products, based on data from 1993 for Switzerland). According to the Consumentengids (1995) musk ketone is not used in detergents any more nor in 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 ketone 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 ketone 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 ketone concentration in product (in %)[6]	Dermal exposure to musk ketone in µg/kg bw/day during normal use[7]
Body lotion[2]	8	0.71	100%	5.68	0.0276 (0.013)	26.1
Face cream[3]	0.8	2	100%	1.6	0.0207	5.5
Fragrance cream[2]	5	0.29	100%	1.45	0.276	66.7
Eau de toilette[4]	0.75	1	100%	0.75	0.552 (0.9)	69
Other[5]						33.1
					TOTAL	200.4

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-perspirent/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 ketone can be estimated at 200 µg/kg bw/day. 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 ketone (vapour pressure 0.00004 Pa and Henry coefficient 0.0256 Pa.m^3/mol). In a draft report by Hellewegen and van Bergen (draft 5, 2000) it was in fact shown that for 2-benzylidene octanal, a substance with a vapour pressure and log K_{ow} similar to musk ketone, evaporation from the skin was only 7%.

It should be noted that the SCCNFP (1999), based on the retention of musk ketone in human fat and its excretion in human milk (see Section 4.1.1.2.2 below), recommended that the exposure of consumers due to the cosmetic use of musk ketone should be reduced by 50%. If this measure comes into effect, the exposure would drop to 200.4/2 = 100.2 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 ketone is used in fragrances the upper level of use (97.5 percentile) is reported as 6.9% (data from industry). This corresponds to 207 mg musk ketone/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 0.00207 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 0.0000207 mg/cm^2. The exposed area is 840 cm^2 (hands both sides), therefore the total exposure will be 0.0174 mg/event. This latter value corresponds to 0.29 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 ketone is used as a fragrance in air fresheners. Air freshener aerosol may contain up to 1% of fragrance. The amount of musk ketone in the fragrance is 6.9%. The estimated worst case exposure to musk ketone in air freshener is 6.4 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 Summary

The dermal exposure of consumers to musk ketone via cosmetics amounts to 200 µg/kg bw/day. Compared to this value, the exposure of consumers to musk ketone in

detergents and air fresheners is negligible. Therefore only the figure of 200 μg/kg bw/day is taken forward to the risk characterisation.

4.1.1.4 Indirect exposure via the environment

EUSES calculations

In the EUSES model, a log K_{ow} value of 4.3 has been used as being representative for distribution in the environment. A measured fish bioconcentration factor of 1,380 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 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 ketone 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 1	0.175	4.68e-3	0.125	6.34e-5	4.25e-3	1.34e-3	2.48e-5
Private use		1.05	0.277	1.27e-3	1.58e-3	7.1e-5	2.25e-5	1.08e-8
Regional		0.16	0.0334	1.92e-4	1.91e-4	1.48e-5	4.68e-6	9.05e-9

Table 4.6 Estimated human daily intake of musk ketone 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 1	2.88e-4	2.57e-5	2.15e-3	1.81e-6	1.83e-5	1.08e-5	5.31e-6	2.5e-3
Private use		1.73e-3	1.52e-3	2.18e-5	4.53e-5	3.05e-7	1.8e-7	2.32e-9	3.31e-3
Regional		2.63e-4	1.83e-4	3.3e-6	5.46e-6	6.36e-8	3.75e-8	1.94e-9	4.55e-4

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 all fragrance compounding steps the estimated human 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 1 (2.5e-3 mg/kg bw/day).

Private use gives a total daily intake of 3.31e-3 mg/kg bw; intake is mainly via root crops and fish. Musk ketone was not coming from air but from pore 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 3.31e-3 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 98% to the total daily intake of 4.55e-4 mg/kg bw. The musk ketone 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 ketone, Sönnichsen et al. (1999) did not find a correlation of fish consumption with levels of musk ketone in human milk (see below).

Drinking water concentrations

No data on musk ketone in drinking water were available. The amount of musk ketone in surface water from literature data is usually at or below the detection limit around 0.01 μg/L (see **Table 3.2** for details).

Literature data on food concentrations

Monitoring data for musk ketone in aquatic biota are summarised in **Table 3.6**. 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 1999. 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 ketone in the milk showed a mean value of 3.49 μg/kg milk fat with a standard deviation of 9.24. The minimum and maximum value found were close to zero (detection limit <2 μg/kg milk fat) and 82.9 μg/kg milk fat, respectively. There was no convincing correlation of musk ketone 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 a significant correlation with the use of skin products, but not 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 ketone concentration was 9.6 μg/kg milk fat (range 2.1-82.9 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 ketone in 1991 (48 samples) and 1992 (343 samples). Levels of musk

ketone varied from <10 to 240 µg/kg milk fat, with a mean of 40 µg/kg milk fat (90- and 95-percentile 80 and 110 µg/kg milk fat, respectively) (Liebl and Ehrenstorfer, 1993). In another study in 23 human milk samples from 1992/3 in Northern Germany, musk ketone levels up to 90 (average 31) µg/kg milk fats were found in by Rimkus et al. (1994). These milk samples showed a fat content of 0.1-5.1%. Ott et al. (1999) found mean levels of 10 µg musk ketone/kg milk fat in human milk samples (n=55) from women in Middle Hesse, Germany in 1995 (maximum level approximately 100 µg/kg milk fat). In a 1993/5 survey of human milk (n=73 samples from different European countries), musk ketone levels varied from approximately 20-130 µg /kg milk fat, with a mean of approximately 25 µg/kg milk fat. The mean fat content of the milk was 3.1% (Ramseier et al., 1998).

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 is 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 ketone 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 milk by a factor of 0.12, the approximate daily intake of the substance in mg/kg bw/day can be estimated. If the 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 ketone in mother's milk is based on the highest mean and maximum concentrations found for musk ketone in the early to mid ninety studies (40 and 240 µg/kg milk fat, respectively) and the consumption of 0.120 kg milk/day per kg bw containing 3.5% fat:

Mean: 40 µg musk ketone/kg milk fat = 40 · 0.120 · 0.035 = 0.17 µg musk ketone/kg bw/day.

Maximum: 240 µg musk ketone/kg milk fat = 240 · 0.120 · 0.035 = 1 µg musk ketone/kg bw/day.

The exposure (worst case estimate) via mother's milk for infants thus varies between 0.17 and 1 µg musk ketone/kg bw/day.

Human adipose tissue

Musk ketone was determined in 32 human adipose tissue samples (13 from females and 19 from males) from Northern Germany. All samples were from 1992/3. Levels of musk ketone in male and female adipose tissue varied from 0.01 to 0.05 mg/kg fat, with the exception of one female (0.22 mg/kg fat) (Rimkus et al., 1994).

Müller et al. (1996) found levels of <1-11 (2 outliers of 173 and 40) µg musk ketone/kg fat in 15 human adipose tissue samples (age group 3-100 years, 5 from males, 10 from females) collected in Switzerland in 1983/4 and 1994.

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 ketone 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) + 0.20 mg/kg bw/day (dermal, consumers) + 3.31e-3 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 / Inhalation

No data available.

Dermal

The absorption, distribution and excretion of radioactivity have been determined after a 6-hour topical application with 0.5 mg/kg bw of ring-labelled ^{14}C-musk ketone (in a mixture of phenylethyl alcohol and ethanol) to the shaven backs of 21 male rats (16 CD Sprague-Dawley and 5 Long-Evans). 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 with aluminium foil and a waterproof dressing. After 6 h application the dressing and foil were removed and the remaining dose at the treated area was wiped off. Urine, faeces and expired air were collected

for rats killed at 6 hours after start of dosing or later and were analysed for radioactivity and metabolite identification. 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 for analysis. At sacrifice organs and tissues were (including untreated skin and treated skin area) removed for analysis of radioactivity. From two additional male CD rats (one with its bile duct cannulated) treated topically on the shaved back (area of 16 cm^2) with 2.5 mg ^{14}C-musk ketone (in phenylethyl alcohol/ethanol)/kg bw under occlusion for 48 hours, urine and bile were collected at 24 and 48 hours.

Result: In CD rats and Long-Evans rats 14.6-26.3% and 13.3%, respectively, of the radiolabelled dose was absorbed from the shaven backs during 6 hours. After removal of the ^{14}C-musk ketone at 6 hours of application ca. 16% of the dose remained on the skin which continued to be absorbed. This is supported by data obtained on animals killed at later times which show a steady decrease in the amount of material found on the treated skin from 7.2-9.85% of the dose at 8 hours to 3.1-4.1% after 24 h and 2.0-3.4% after 48 hours. The actual absorption at 8 hours was approximately 19%, at 24 hours 25.1-28.3%, and at 48 hours 26.2-32.7%. After 48 hours, absorption essentially ceased while approximately 3% of the dose remained unabsorbed on the skin. In Long-Evans rats the disposition of radioactivity was similar with absorption of ^{14}C-musk ketone rising to 29.3-40.2% of the dose between 48 and 120 hours. The mean proportion of the dose remaining on the treated skin after 24 hours was approximately 3%.

In CD rats means of 7.3% and 17.2% of the applied dose had been excreted in the urine and faeces, respectively, after 120 hours. In Long-Evans rats the rate of elimination was comparable with 10.8% and 27.2% of the dose excreted during 5 days in the urine and faeces, respectively. Most radioactivity was eliminated in the first 48 h after start of dosing. No radioactivity was detected in expired air.

In the bile duct cannulated CD rats only 1.8% of the dose was eliminated in the urine in 48 hours, while 25.3% was collected in the bile (of which 15.8% within 24 hours). The non-cannulated rat excreted 8% of the dose in the urine in 48 hours. These results indicate that the predominant route of excretion for ^{14}C-musk ketone is via the bile, and therefore that most of the radioactivity in the urine of cannulated animals is due to material that had been reabsorbed from the gastro-intestinal tract. In bile, at least six drug-related components were present as β-glucuronic acid conjugates which were apparently deconjugated and further metabolised in the gastro-intestinal tract to other more polar components, some of which were at least partially reabsorbed giving rise to a complex profile of urinary metabolites.

Radioactivity was detected in nearly all the tissues of animals killed at 1-120 hours after start of dose application. Concentrations were highest at about 6 hours after start of dosing in all tissues. Between 8 and 120 h the concentration of radioactivity declined steadily in all tissues so that at 120 hours after start of dosing the concentration of radioactivity in each tissue was in general less than 20% of its peak value. Throughout the study the highest concentrations of radioactivity were found in the gastro-intestinal tract, liver, adipose tissue, adrenals, thyroid and kidneys, which at 6 hours after start of dosing contained means of 0.645 µg musk ketone equivalents/g, 0.32 µg/g, 0.19 µg/g, 0.12 µg/g, 0.10 µg/g and 0.08 µg/g, respectively, in CD rats. The distribution of radioactivity in the tissues of Long-Evans rats was similar with the highest peak concentrations of radioactivity at 6 h after start of dosing in the gastro-intestinal tract (0.47 µg musk ketone equivalents/g), liver (0.26 µg/g), adrenals (0.1 µg/g), thyroid (0.18 µg/g) and fat (0.16 µg/g) (Hawkins et al., 1984; Hawkins and Ford, 1999).

Ring-labelled ^{14}C-musk ketone (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 caecal contents, large intestine contents, and bile ducts. Lower levels were present in the small intestine contents and liver. Tissues of the rat killed at 24 hours after the 14th dose generally contained more radioactivity, although the highest concentrations were still associated with the site of application and the gastro-intestinal tract and lower levels were present in liver, blood, and thyroid. Hence, the absorption of radioactivity was incomplete, given the large amounts of the applied radioactivity remaining at the site of application.

Means of 1.48 and 2.34 μg musk ketone equivalents were excreted in urine and faeces, respectively, in the 24 hours following the application of dose 1. The mean rate of excretion in the urine increased to 6.54 μg/day during the 24 hours following the application of dose 14. The mean rate of excretion in faeces increased to maxima of about 14.8 μg/day in the 24 hours following application of both dose 12 and dose 14.

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 (0.22-0.37% in liver, and even less in fat, blood, kidneys, brain, and thyroid) (Hawkins et al., 1989; Hawkins and Ford, 1999).

A bile duct cannulated rat received a single dose of 4.63 mg ring-labelled ^{14}C-musk ketone (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-lucuronidase treated rat bile showed a complex pattern of (unidentified) metabolites (Hawkins et al., 1989).

Intravenous

A group of four male Sprague-Dawley CD rats received a single intravenous administration of ring-labelled ^{14}C-musk ketone (0.5 mg/kg bw in polyethylene glycol, aqueous sodium chloride and ethanol). Blood samples were taken at 5, 30, and 90 minutes and at 3, 6, 24, 48, 72, 96, 120, 168, and 240 hours after dosing. The concentration of radioactivity in plasma showed a single peak of approximately 0.57 μg musk ketone equivalents/ml between 1.5 and 6 hours after injection, the time varying between individual animals. Concentrations declined with a mean terminal elimination half-life of 60 hours. The mean area under the curve was 45 μg.h/ml (Hawkins et al., 1989).

Special investigation

Musk ketone was administered by gavage to pregnant CD rats (n=18/group) at 2.5 and 25 mg/kg bw as a solution in corn oil, daily from day 14 of gestation up to 7 days post-parturition. Milk samples of ca. 0.5 ml were obtained manually from 3 dams per dose level per time point (after administration of oxytocin) at 4, 8 and 24 hours after dosing on days 3 and 7 post-parturition. Highest mean concentrations of musk ketone were found in the 4 hour

samples, declining more than 20-fold by 24 hours after dosing (see **Table 4.7**) Assuming that the musk ketone was completely associated with fat and total fats accounted for 134 g/l rat milk, mean concentrations of musk ketone in milk fat were calculated as in **Table 4.8** (Ford and Hawkins, 1996; Hawkins et al., 1996).

Table 4.7 Mean (n=3) concentrations in milk (in μg/ml).

Sample time	Day 3 - Dose level (mg/kg bw)		Day 7 - Dose level (mg/kg bw)	
(hour)	2.5	25	2.5	25
4	0.72	4.21	0.98	20.90
8	0.07	1.84	0.34	9.29
24	<0.05	0.16	<0.05	0.71

Table 4.8 Corresponding mean (n=3) concentrations in milk fat (in μg/g).

Sample time	Day 3 - Dose level (mg/kg bw)		Day 7 - Dose level (mg/kg bw)	
(hour)	2.5	25	2.5	25
4	5.37	31.4	7.31	156
8	0.52	13.7	2.54	69.3
24	<0.37	1.19	<0.37	5.30

In vitro studies

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 ketone (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 >80%. After 24 hours, musk ketone was poorly absorbed through unoccluded skin, given that on average 3.28 ± 2.35% was found in the receptor fluid. Occluding of the skin did not really affect this absorption at 24 hours (5.68 ± 6.47% on average). Significant amounts of radioactivity were recovered from within the skin (at 24 hours, 47% in both unoccluded and occluded skin). Over 48 hours, musk ketone 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.

To determine the penetration rate into and through skin, 3% and 10% solutions of ^{14}C-musk ketone in ethanol/acetone (1:1) (corresponding to doses of 180 and 600 μg/cm^2) and a 10% solution of ^{14}C-musk ketone in benzoeacid benzyl ester (corresponding dose 600 μg/cm^2) were applied to intact explanted mini pig skin (area 5 cm^2) for a maximum of 16 hours. The total penetration rate, as calculated separately from the total amounts in stratum corneum and

living skin layers of epidermis, corium and subcutis, was very low for all tested solutions. The stratum corneum acts as a very effective penetration rate limiting membrane. 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**) (Klecak, 1982).

Table 4.9 Penetration rate into and through 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 h	3.1%	0.6%	1.2%	0.3%	2.3%	0.2%
6 h	3.7%	0.7%	1.6%	0.4%	2.9%	0.3%
16 h	3.8%	0.8%	2.1%	0.5%	3.1%	0.5%

Percentages in this table refer to the applied dose.

4.1.2.1.2 Studies in humans

Two healthy male volunteers received an application of 2.2 mg ring-labelled ^{14}C-musk ketone (in a mixture of phenylethyl alcohol and ethanol) on the unshaven skin of the upper left quadrant of the chest for 6 hours. The dose was applied evenly over an area of 100 cm^2. The application rate was 0.02 mg/cm^2. The treated area was covered with protective gauze held in position with adhesive tape. After 6 hours, the dressing was removed from the test area and the treated skin was wiped. Blood, urine and faeces were collected up to 120 hours after start of dosing. After 120 hours the treated area of the skin was stripped with adhesive tape. All samples (including strips, dressing and swabs) were analysed for radioactivity.

The ^{14}C-musk ketone was poorly absorbed from the skin during the 6-h application since only 0.5% of the dose was excreted in urine and faeces during 120 hours, and approximately 86% of the dose was recovered from the site of application. Moreover, 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 12%). When urine samples were treated with β-glucuronidase and extracted with ethyl acetate the recovery was about 5-fold larger, indicating that a large proportion of the metabolites of musk ketone in human urine were present as glucuronide conjugates. Extracts of β-glucuronidase treated human urine contained a single major (unidentified) metabolite which was probably also present as a minor constituent of rat bile extract (Hawkins et al., 1989; Hawkins et al., 2002).

Several studies have identified the presence of musk ketone in human milk and human adipose tissue (see Section 4.1.1.42.2 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 ketone in the milk showed a mean value of 3.49 μg/kg milk fat and a maximum value of 82.9 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 40 and 240 μg musk ketone/kg milk fat, respectively.

In human adipose tissue, Rimkus et al. (1994) found levels of musk ketone varying from 0.01 to 0.05 mg/kg fat, with the exception of one female (0.22 mg/kg fat). Müller et al. (1996) found levels of <1-11 (2 outliers of 173 and 40) µg musk ketone/kg human fat.

4.1.2.1.3 Summary of toxicokinetics, metabolism and distribution

There are no data available on the toxicokinetics of musk ketone after oral and inhalation exposure. For the related compound musk xylene some toxicokinetic data were available after oral exposure, on the basis of which for both rats and humans a percentage of 50% for oral absorption is taken forward to the risk characterisation of musk xylene. Musk ketone is quite comparable to musk xylene with respect to physico-chemical properties. Dermal uptake and penetration rates do not indicate a major difference for the two substances either (see **Table 4.10**). Based on these similarities between musk ketone and musk xylene, for musk ketone a percentage of 50% for oral absorption will be taken forward to the risk characterisation in concordance with musk xylene.

Table 4.10 Comparison of characteristics of musk ketone versus musk xylene.

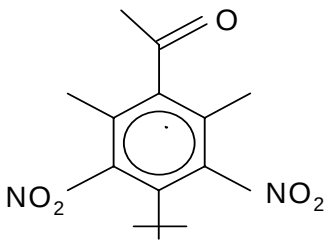
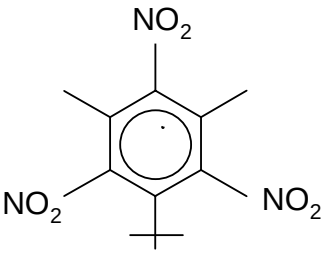
	Musk ketone[1]	**Musk xylene[1,2]**
Molecular properties		
IUPAC name:	4'-tert-butyl-2', 6'-dimethyl-3',5'-dinitroacetophenone	5-tert-butyl-2,4,6-trinitro-mxylene
Structural formula:		
Physical state	solid, powder	solid, powder
Melting point	135-137 °C	112-114°C
Molecular weight:	294.3 D	297.3 D
Relative density	0.73 g/cm^3	0.77 g/cm^3
Vapour pressure	0.00004 Pa	0.00003 Pa
Water solubility	0.46 mg/l	0.15 mg/l
Partition coefficient n-octanol/water (log value)	4.3	4.9

Table 4.10 continued overleaf

Table 4.10 continued Comparison of characteristics of musk ketone versus musk xylene.

	Musk ketone[1]	**Musk xylene**[1,2]
Molecular properties		
In vitro dermal studies		
Ashcroft and Hotchkiss, 1996	< 6% absorption after 24hours in rat skin disks, but depot in skin (47% at 24 hours).	< 2% absorption after 24 hours in rat skin disks, but depot in skin (30-43% at 24 hours).
Klecak, 1982	2.6-4.6% penetration in intact mini-pig skin explants after 16 hours	4.4-4.5% penetration in intact mini-pig skin explants after 16 hours
In vivo dermal studies		
Hawkins et al, 1984, Hawkins and Ford, 1999	40% absorption in rats and 14% in humans	20% absorption in rats and 10% in humans

1) Physico-chemical data in this table have been taken from Section 1, Table 1.1.
2) Data from the Risk Assessment Report on Musk Xylene (version of 2005).

After a 6 hour dermal application of ^{14}C-labelled musk ketone (under occlusion) to rats, a total of about 40% of the applied dose was absorbed within 48 hours, with 2-3.5% remaining in the skin. Between 6 and 48 hours, the skin acted as a reservoir from which musk ketone continued to be absorbed. Excretion via urine and faeces (predominantly via bile) was highest during the first 48 hours, with small amounts additionally excreted between 48 and 120 hours. After 120 hours, about 7-11% of the applied dose was excreted in urine, and 17-27% in faeces. Radioactivity was detected in nearly all tissues, with highest levels at 6 hours in gastrointestinal tract, followed by liver, adipose tissue, adrenals, thyroid, fat, and kidneys.

^{14}C-labelled musk ketone was poorly absorbed from human skin during a 6 hour application, as only 0.5% of the applied dose was excreted in urine and faeces within 120 hours, and >86% of the applied dose was recovered from the site of application.

In vitro experiments with unoccluded rat skin indicate that the percutaneus absorption of musk ketone is poor, and that the skin acts as a depot from which musk ketone can be absorbed.

Metabolism of musk ketone in rats and humans involves glucuronide conjugation.

For dermal absorption of musk ketone in rats and humans, values of 40% and 14%, respectively, are taken forward to the risk characterisation.

The plasma elimination half-life in rats after intravenous administration of musk ketone is approximately 60 hours. No data on plasma half-life in humans are available for musk ketone.

When administered orally to rats from day 14 of gestation up to 7 days post-parturition, musk ketone appeared in milk and milk fat. Musk ketone 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 a limitedly reported oral gavage study, musk ketone (in corn oil) was administered to 3 groups of 6 male rats (strain not specified) at doses of 2,500, 5,000 or 10,000 mg/kg bw. At 2,500 and 5,000 mg/kg bw no rats died, while at 10,000 mg/kg bw 3/6 rats died. Effects observed were bloody discharge around the nose, hyperexcitability, depression, coma and death. From the study description it was not clear at which dose level the non-lethal effects were observed (Bukva et al., 1970; Opdyke, 1975).

Remark: in contrast to these findings, in a repeated dose toxicity study (Lehmann-McKeeman et al., 1999, see Section 4.1.2.5), signs of severe intoxication and death (no further details) were observed within 2 days of dosing, when male F344 rats were dosed orally at a much lower level (500 mg musk ketone /kg bw in corn oil). There is no explanation for the discrepancy between the acute and the repeated dose study.

Dermal

A limitedly reported dermal acute toxicity study describes the application of musk ketone to the clipped skin of groups of 3 albino rabbits at dosages of 2,000 or 10,000 mg/kg bw. Musk ketone was applied as 40% suspension in corn oil under occlusion for 24 hours. During the 7-day observation period no mortality occurred and no signs of dermal irritation or systemic toxicity were observed (Fogleman and Margolin, 1970; Opdyke, 1975).

Inhalation

No data available.

4.1.2.2.2 Studies in humans

No data available.

4.1.2.2.3 Summary of acute toxicity

Although the available studies for acute toxicity testing of musk ketone have not been performed according to OECD guidelines, it can be concluded that the oral LD_{50} for rats and the dermal LD_{50} for rabbits are both greater than 2,000 mg/kg bw. Data for acute inhalation toxicity are not available.

According to EC criteria musk ketone needs not to be classified for acute oral and dermal toxicity based on the reported LD_{50} values.

4.1.2.3 Irritation/Corrosivity

4.1.2.3.1 Studies in animals

Skin

In a dermal LD_{50} study with rabbits musk ketone, in a 40% suspension in corn oil under occlusion for 24 hours, showed no signs of dermal irritation (Fogleman and Margolin, 1970; Opdyke, 1975).

Eye

In an OECD 405 guideline study six New Zealand White rabbits received an instillation of 0.1 ml musk ketone (0.07 g) into one eye. At 30 seconds post-instillation both eyes of three rabbits were rinsed with physiological saline. The eyes of the remaining three rabbits remained unrinsed. The contralateral eye of each animal served as a control. Examinations were performed up to 72 hours post-installation.

No effects were seen on the cornea and iris. In the unrinsed animals slight to moderate redness (score 1-2) and slight swelling (score 1) were observed 1-24 hours after instillation. One animal with redness score 2 developed discharge score 1 at 1 h post-instillation. All signs had disappeared at the 48 hours observation. In the rinsed animals slight redness (score 1) and swelling (score 1) were observed at 1 hour post-instillation. In two animals these signs were completely resolved at 24 hours post-instillation, while in the third animal slight redness (score 1) lasted up to 48 hours post-instillation (Merriman, 1997).

Respiratory tract

No data available.

4.1.2.3.2 Studies in humans

No data available.

4.1.2.3.3 Summary of irritation / corrosivity

Base set requirements for testing of skin irritation have not been met as adequate skin irritation studies are lacking. However, a request for a skin irritation study performed according to current guidelines is not deemed appropriate because:

- the available data on musk ketone do not point to a skin irritating potential: upon single treatment very high doses of musk ketone (as a 40% suspension in corn oil) are not irritating to the rabbit skin when applied for 24 hours under occlusion. After repeated dosing (see 4.1.2.5) no signs of skin irritation were seen in rats, while in rabbits slight irritation was observed at high doses which partly could be attributed to the vehicle used. In sensitisation studies (see 4.1.2.4) no irritation was observed in guinea pigs and in humans when applied at concentrations up to 75% and 5%, respectively.

- the test conditions used in a guideline study on rabbits (0.5 g for 4 hours under occlusion) are not expected to result in skin irritation given the results of the acute dermal study at much higher doses and longer duration.

From a well performed experiment it can be concluded that musk ketone is not eye irritating. According to EC criteria musk ketone needs not to be classified for skin and eye irritating properties.

No data on respiratory tract irritation are available.

4.1.2.4 Sensitisation and photoallergy

4.1.2.4.1 Studies in animals

Klecak (1979) performed an Open Epicutaneous Test in guinea pigs. Musk ketone did not show sensitising properties in this system, but the test report is unclear with respect to numbers of animals, number of dose groups and dose levels and vehicle used. Therefore the relevance of this finding cannot be established.

In order to detect the potential of musk ketone 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 ketone 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 backlight 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 ketone in acetone by a single 4 hours 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 backlight 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**. Comparisons to the naive control data (regimens 5, 6) suggest that musk ketone may be weakly phototoxic (regimen 5), while positive responses with regimens 1, 2, and 4 suggest that musk ketone may also be weakly allergenic. The contact hypersensitivity response was not obviously exacerbated by UVA light (regimens 1, 3). Hence, in contrast to musk ambrette, musk ketone does not have the potential to produce photoallergy (Parker et al., 1986).

Table 4.11 Results of photoallergy testing with musk ketone.

Test regimen	Induction conditions	Challenge conditions	Incidence
1	MK+UVA*	MK+UVA	2/10
2	MK+UVA	MK	2/10
3	MK	MK+UVA	0/9
4	MK	MK	2/10
5	naïve	MK+UVA	1/10
6	naïve	MK	0/10

* MK=musk ketone, 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 2/10 in group 4 (induction and challenge) as compared to that in group 6 (0/10; challenge only), because according to the guidelines a score of at least 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 ketone 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 ketone 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 ketone. 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-cross-reaction to the known photoallergen musk ambrette was studied at the third challenge stage, using 1% concentrations of each substance.

There were no reactions after the first challenge at concentrations up to 10%. After the second challenge only one animal in both the 10 and 1% dose groups, but none at 0.1%, showed a photoallergic reaction. There was no contact sensitivity, and musk ketone did not photo-cross-react 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 ketone was not (photo)-irritant at the concentrations tested (up to an arbitrary upper limit of 10%), 10% was taken. This means that musk ketone 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 ketone was always followed by UV irradiation, meaning that non-irradiated musk ketone has not been tested as such.

In a recent dermal sensitisation test according to GLP and OECD guideline 406 (GPMT), 20 female guinea pigs (strain Albino Dunkin Hartley, weight 280 to 350 g) per group were treated with musk ketone dissolved in 1:9 acetone/olive oil (purity minimum 98% RPA). The control group with 10 animals was dosed with the vehicle. In the induction phase, the animals were treated with 3% w/v musk ketone intradermally with/without Freund's Complete Adjuvant. This was followed at day 7 with a topical application of 75% w/v musk ketone. Challenge doses of 7.5, 25 and 75 % w/v were applied at day 21. Scattered mild redness (score: grade 1) was seen in some animals at all challenge doses (at 75% 5/20 compared to 1/10 controls, at 25% and 7.5% 3/20 compared to 0/10 controls) at 24 h but not at 48 hours after challenge (Johnson, 2001). In this test musk ketone had only weak sensitising properties.

Remark: The dose levels used for induction and challenge were determined in a preliminary study, in which the intradermal 3% preparation was the highest dose to cause mild irritation but the topical 75% preparation did not cause irritation. Although no sodium lauryl sulphate was used at topical induction, it is expected that this would not have contributed much given the high dose tested.

4.1.2.4.2 Studies in humans

A maximisation test was carried out with 25 healthy adult volunteers. Musk ketone was applied to the fore-arms at a concentration of 5% in petrolatum under occlusion for a total of five alternate-day 48-hour periods. Each application was preceded by a 24-hour occlusive treatment of the patch site with 5% aqueous sodium lauryl sulfate. Following a 10-day rest period, a challenge application (5% musk ketone in petrolatum) was applied to fresh sites on the scapular back of each subject under occlusion for 48 hours. The challenge site was pretreated for 1 hour with 10% aqueous sodium lauryl sulfate. Under these conditions, musk ketone failed to elicit a sensitisation reaction after 48 and 72 hours (Kligman, 1970; Opdyke, 1975). In another maximisation test carried out with 25 volunteers, following the same procedure, musk ketone at a concentration of 3.2% also produced no sensitisation reaction (Greif, 1967; Opdyke, 1975).

Remark: Both studies mentioned above were stated to be performed according to the principles laid down in Kligman (1966). However, they deviate from the principles because these require that a non-irritating solid (like musk ketone) should be tested up to concentrations of 25% in combination with sodium lauryl sulphate. Therefore these two human studies are inconclusive with respect to exposure to concentrations higher than 5%. More recent information indicates that musk ketone cannot be fully dissolved in petrolatum at concentrations of 5% and above (Rudio, 2000).

A study was performed to determine the responsivity to a series of commonly used fragrances (amongst others musk ketone) 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 ketone, 1% and 5% concentrations in petrolatum were patch tested on 100 patients (48 females, 52 males) in the test centre Wahlberg, Stockholm. The patch was applied for 2 days to the back. No irritant or allergic reactions were observed for both concentrations of musk ketone (Frosch et al., 1995).

Bruze et al. (1985) have studied the sensitising properties of musk ketone 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 questionnaire 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 were 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 ketone was observed. 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 ketone 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

Based on the results of a recently performed guinea pig maximisation study it can be concluded that musk ketone has weak sensitising properties. From two maximisation studies

with human volunteers it can only be concluded that musk ketone up to a concentration of 5% is not skin sensitising in humans. There is no need for classification according to EU guidelines.

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

The available oral toxicity data are limited to observations obtained in special studies on enzyme induction (see at the end of this section) of rather short duration. For completeness sake, the observations of more general nature are given below.

Male B6C3F1 mice were orally dosed with musk ketone 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 (14%). At 10 mg/kg bw no general hepatic effects were seen (Stuard et al., 1996).

Male B6C3F1 mice were dosed daily by gavage for 7 consecutive days with 0, 5, 10, 20, 50, 100, 200, or 500 mg/kg bw musk ketone in corn oil. Musk ketone treatment resulted in dose-related increases in relative liver weight at dose levels of 50 mg/kg bw, up to 50% at 500 mg/kg bw. Musk ketone also caused histological changes in the liver, primarily centrilobular hepatocellular hypertrophy, and at the highest dose panlobular hepatocellular hypertrophy (Stuard et al., 1997).

Remark: more details as to the dose-response relationship for the histological effects on the liver were not available.

Male F344 rats were dosed orally with 0, 20, 100, or 200 mg musk ketone (in corn oil)/kg bw for 7 consecutive days. Treatment with musk ketone resulted in a dose-related increase in absolute liver weight, which reached statistical significance at 100 and 200 mg/kg bw (Lehmann-McKeeman et al.,1999). At the highest dose level, the liver weight was increased with 42% over the control group.

Remark: Although mice tolerated dosages of musk ketone up to 500 mg/kg bw, rats exhibited signs of severe intoxication and death within 2 days of dosing at 500 mg/kg bw. Therefore, 200 mg/kg bw was chosen as the highest dose.

Dermal

Groups of 15 Sprague-Dawley rats/sex received dermal applications of 7.5, 24, 75 or 240 mg musk ketone (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.

There were no treatment-related deaths or clinical signs observed. It was stated that apart from variable desquamation and occasional atony of the skin[10] there were no significant treatment-related dermatological changes. Both sexes at 240 mg/kg bw/day and females at 75 mg/kg bw/day showed a significantly lower body weight gain during the study, resulting in a *ca.* 10% or 20% smaller body weight in males and females, respectively, at 240 mg/kg bw and a 10% smaller body weight in the females of the 75 mg/kg bw group at the end of the study, despite a similar to or greater food consumption than that of the controls. Both sexes at 240 mg/kg bw/day showed variations in haematological parameters, notably decreases in haemoglobin, haematocrit, RBC counts and MCV. The study authors did not consider the changes, which were claimed to be within historical control ranges, of biological significance but numerical information was not provided. Absolute liver weights were increased in rats at 240 mg/kg bw/day with 19% (not statistically significant) in males and with 20% (statistically significant) in females. Relative liver weights were increased at 240 mg/kg bw/day with 37% (not statistically significant) in males and with 50% (statistically significant) in females. 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. Based on the body weight changes at 75 mg/kg bw/day, the NOEL in this study can be established at 24 mg/kg bw/day (Ford et al., 1990).

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

Musk ketone was tested on the abdomen of New Zealand White rabbits, 3/group with intact skin and 3/group with abraded skin, at dose levels of 0 (vehicle control), 175 or 750 mg/kg bw/day, 5 days/week for 3 weeks. Musk ketone was dissolved in dimethyl phthalate (DMP). To preclude ingestion of the dose, each animal was provided with a plastic collar and the trunk was wrapped lightly with a gauze.

During week 3 symptoms of disease (including bloating of the abdomen, diarrhoea, anorexia, raspy repiration and in some cases hindlimb weakness) began to appear in all groups, including controls. Deaths, not considered treatment-related, occurred in all groups, including controls. In the control and low dose groups, slight to moderate erythema followed by slight desquamation was noted in the abraded skin, but only during the first week. In the high dose group, these effects were seen in both abraded and intact skin, and persisted until week 2/3. No consistent changes in clinical values, gross pathology or histopathology (only liver, kidneys and bone marrow were examined) were observed, with the exception of a variable decrease in bone marrow haematogenic activity in 3 high dose animals and a slight increase in

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

hepatocellular vacuolisation, mainly in the high dose group (Rutter and Ferrell, 1971; Opdyke, 1975).

Remark: As skin irritation was also observed in the controls, the vehicle DMP may have contributed to the dermal effects seen in the treated animals. The other treatment-related effects observed in the low and high dose group animals were not very eminent. As the animals in the high dose group received 3 ml DMP, while those in the control and low dose groups received only 2 ml of this carrier substance the relevance of the findings with respect to the toxicity of musk ketone is questionable.

Almost the same experiment was performed by Powers and Ferrell (1972), however with 4 rabbits/group with abraded skin and 2 rabbits/group with intact skin, and for a total of 20 successive applications. Musk ketone was again applied as a suspension in DMP. There was no treatment-related mortality. Terminal body weight loss, general appearance and behaviour were comparable between the three groups. Signs of irritation were generally slight in all groups (slight transient erythema and/or desquamation in the control and low dose groups, slight to moderate erythema and slight desquamation in the high dose group). Clinical chemistry reflected at termination a treatment-related increase in ALAT in 5 rabbits at 750 mg/kg bw and in 1 rabbit at 175 mg/kg bw. Gross pathology showed discolouration of the liver ('nutmeg' appearance or 'white' lobes) at 750 mg/kg bw. Microscopic pathology (only performed on liver, kidneys and bone marrow) revealed hepatocyte vacuolisation in 5/6 rabbits at 750 mg/kg bw and necrosis in the 6th. Bone marrow haematogenic activity was slightly decreased in 2 rabbits at 750 mg/kg bw and in one control animal. According to the study authors, the bone marrow effects cannot be attributed unequivocally to the treatment because it occurred also in a control animal, although to a lesser degree (Powers and Ferrel, 1972).

Remark: In this study the animals of the high dose group received 1.5 ml DMP, while control and low dose animals received 1.5 ml and 0.35 ml, respectively. Thus at a lower total DMP dose with no difference in DMP dose between the high dose and control animals, the same effects were observed on the bone marrow and liver cells as in the above study by Rutter and Ferrel (1971). This may indicate that at least part of the effect on the bone marrow and hepatocytes might be attributable to musk ketone.

Inhalation

A group of 20 female CD rats was exposed by inhalation (whole body) to fragrance mixtures at a nominal concentration of 5 mg/m^3 for 4 hours per day, 5 days per week for 6 weeks (mixture A) and groups of 12 female CD rats and 12 female Syrian golden hamsters were exposed to 50 mg/m^3 for 4 hours per day, 5 days per week for 13 weeks (mixture D). Musk ketone was part of these fragrance mixtures, and the level of musk ketone to which the animals were exposed was 7.2 μg/m^3 for mixture A and 170.5 μg/m^3 for mixture D. The results were compared to those obtained in appropriate control groups. Exposure to either mixture A or mixture D did not result in mortality, skin reactions or effects on body weight, behaviour or physical appearance, heamatology and clinical chemistry, organ weights and gross pathology (including uterus and ovaries), or histopathology (uterus but not ovaries examined) (Fukayama et al., 1999).

Remark: the study is of limited value because the animals were not exposed to musk ketone alone, but to mixtures of fragrances. In these mixtures musk ketone was only present at rather low levels.

Special investigations on enzyme induction

Male B6C3F1 mice were orally dosed with musk ketone 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 (14%), microsomal protein yield (49%), total P450 content (64%) and cytochrome P450-2B (CYP2B) protein level (about 7-fold). Musk ketone also increased CYP2B enzyme activity (determined by pentoxyresorufin-O-dealkylase (PROD) activity) about 6-fold, consistent with the increase in CYP2B protein level. At 10 mg/kg bw no general hepatic effects consistent with cytochrome P450 induction were seen. When given over a range of 5 to 500 mg/kg bw for 7 consecutive days, musk ketone above 20 mg/kg bw dose-relatedly increased immunoreactive CYP2B protein levels (at the highest dose about 10-fold over control levels) as well as CYP2B enzyme (PROD) activity (Stuard et al., 1996).

To study the induction of cytochromes P450 by musk ketone, male B6C3F1 mice were dosed daily by gavage for 7 consecutive days with 0, 5, 10, 20, 50, 100, 200, or 500 mg/kg bw musk ketone in corn oil. Musk ketone treatment resulted in dose-related increases in relative liver weight and microsomal protein (at 50 mg/kg bw and higher, reaching 50% and 75% increases, respectively, relative to control at 500 mg/kg bw) and in total cytochrome P450 content (at 10 mg/kg bw and higher; 75% increase at the highest dose). Musk ketone also caused histological changes in the liver, primarily centrilobular hepatocellular hypertrophy, and at the highest dose panlobular hepatocellular hypertrophy. Musk ketone induced dose-related increases in enzyme activities of CYP2B (determined by PROD; 28-fold increase at the highest dose; NOEL 20 mg/kg bw), CYP1A (determined by ethoxyresorufin-O-dealkylation (EROD) and methoxyresorufin-O-dealkylation (MROD); 2- and 4-fold increases at 500 mg/kg bw, respectively; NOELs of 100 and 20 mg/kg bw, respectively) and CYP3A (determined by erythromycin-n-demethylation (ERND); 2-fold increase at the highest dose; NOEL 10 mg/kg bw). Blotting indicated a dose-related increase in immunoreactive CYP2B, CYP1A and CYP3A protein levels (with the largest overall increase for CYP2B), and consistent increases in CYP2B10, CYP1A2 and CYP3A11/13 mRNA levels (Stuard et al., 1997).

In a second experiment, the inhibition of phenobarbital (PB)-induced CYP2B activity by musk ketone, in comparison with musk xylene, was studied. Male B6C3F1 mice received 500 ppm PB in the drinking water *ad libitum* for 4 days to induce CYP2B isoenzymes. On the fourth day, the mice were gavaged with either corn oil (control) or musk xylene or musk ketone (0.67 mmol/kg bw each, corresponding to 200 or 198 mg/kg bw, respectively). Musk xylene reduced PB-induced CYP2B enzyme activity by more than 90%, while musk ketone reduced it by only 20%. With both musk xylene and musk ketone, no decrease in CYP2B immunoreactive protein levels was observed, despite the decreased CYP2B enzyme activity (Stuard et al., 1997).

The above mentioned experiment was more or less repeated with male F344 rats, which were dosed orally with 0, 20, 100, or 200 mg musk ketone (in corn oil)/kg bw for 7 consecutive days. An additional group of rats received 500 ppm PB in their drinking water for 7 days. Following sacrifice of the animals, livers were assayed for induction of microsomal enzymes by determination of enzyme activities (CYP1A1/2, CYP2B1/2 and CYP3A) and by determination of protein levels and mRNA expression for CYP2B1/2.

Musk ketone at all doses induced general hepatic effects very similar to those observed with PB, but to a somewhat lower extent. The effects consisted of a dose related increases in absolute liver weight (up to 42% over the control group; statistically significant at 100 and 200 mg/kg bw), NADPH-cytochrome P450 reductase activity, total microsomal cytochrome

P450 content and cytochrome b5 levels (all three statistically significant at all doses, but the latter two not dose-related). CYP2B activity measured as PROD was significantly increased at all dose levels up to 8-fold maximally at 100 and 200 mg/kg bw (PB 50-fold). Consistent with this, CYP2B1/2 protein levels were increased about 10-fold (PB 20-fold) while the increase in steady-state CYP2B1 mRNA level amounted to 3-fold (PB 15-fold). Exposure to musk ketone also resulted at all doses in increases in CYP1A enzyme activity measured as EROD (10-fold at 20 mg/kg bw and 30-fold at 100 and 200 mg/kg bw) and MROD (7-fold at 20 mg/kg bw and 20-fold at 100 and 200 mg/kg bw). This induction was much greater than for PB (5-fold and 2-fold, respectively). Musk ketone treatment decreased CYP3A enzyme activity (as determined by testosterone 6β-hydroxylation) at 100 and 200 mg/kg bw to approximately 70 and 50% of control levels, respectively. This is in contrast with PB, that resulted in a 2-fold increase in CYP3A enzyme activity. On a protein level, musk ketone induced CYP1A2 and especially CYP1A1 (both at least 10-fold), with no change in CYP3A (Lehman-McKeeman et al., 1999).

Remark: Although mice tolerated dosages of musk ketone up to 500 mg/kg bw, rats exhibited signs of severe intoxication and death within 2 days of dosing at 500 mg/kg bw. Therefore, 200 mg/kg bw as chosen as the highest dose.

Musk ketone was tested in female A/J mice at a gavage dose of 20 mg once every 2 days for a total of 3 doses for its ability to induce glutathione S-transferase (GST) activity in liver, lung, forestomach, and small and large intestinal mucosa. Compared with controls, that were given the vehicle cottonseed oil alone, musk ketone increased GST enzyme activity in liver, small intestinal mucosa and colon (not statistically significant in the latter), but not in lung and forestomach. Musk ketone also elevated the glutathione level (as measured by acid-soluble sulfhydryl) in the small intestine mucosa, but decreased it in the other tissues (Zheng et al., 1992).

Musk ketone was examined for its potency to induce biotransformation 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 ketone (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 ketone showed enzyme inducing effects in the rat liver, which led to an increase in the toxification of the pregenotoxicants benzo[a]pyrene (B(a)P), 2-aminoanthracene and aflatoxin B1 at all doses (Mersch-Sundermann et al., 1996a/b).

Enzyme measurements (EROD) in microsomal preparations of human hepatoma (Hep G2) cells (i.e. cells that have retained the activities of phase I and phase II enzymes) indicated that treatment with 500, 1,000 or 5,000 ng/ml musk ketone results in increased CYP1A isoenzyme activities (Mersch-Sundermann et al., 2001).

4.1.2.5.2 Studies in humans

No data available.

4.1.2.5.3 Summary of repeated dose toxicity

The available oral repeated dose toxicity studies with musk ketone are limited to special studies into biotransformation enzyme induction of comparatively short duration. In these studies (apart from enzyme induction) the only toxic responses reported were liver enlargement at dose levels greater than 20 mg/kg bw/day for 7 days in mice (NOEL) and at 20 mg/kg bw/day and above in the rat (LOEL). In mice, musk ketone also caused histological changes in the liver (primarily centrilobular hepatocellular hypertrophy and at 200 mg/kg bw/day panlobular hepatocellular hypertrophy). Details as to the dose-response relationship for the histological effects on the liver were not availabe. In rats liver histology was not studied. These studies are too limited to derive an NOAEL for oral repeated dose toxicity.

In a well performed dermal 90-days toxicity study with rats effects at the highest dose of 240 mg musk ketone/kg bw included a decreased body weight gain without a concommittant decrease in food consumption, decreases in red blood cell parameters and an increase in absolute and relative liver weight without a histopathological correlation. The decrease in body weight gain was also seen in females at the lower dose of 75 mg/kg bw. In the experiment no neuropathological effects and no effects on the reproductive organs were seen. The NOEL of 24 mg/kg bw/day in this study can be considered as a NOAEL, although the extent of the body 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.

In dermal experiments with rabbits doses up to 750 mg musk ketone/kg bw for 20 days, microscopic pathology revealed hepatocyte vacuolisation and decreased bone marrow haematogenic activity, but these may have been caused, at least in part, by the vehicle (dimethyl phthalate).

When administered as part of a fragrance mixture, inhalatory exposure to musk ketone up to a maximum tested dose of 170.5 $\mu g/m^3$ for 4 hours per day, 5 days per week for 13 weeks did not result in any toxicity. This study is of limited value because the animals were not exposed to musk ketone alone, and musk ketone was only present at rather low levels in the mixtures.

In special studies for enzyme induction, 7 consecutive daily oral musk ketone doses of up to 500 mg/kg bw for mice and up to 200 mg/kg bw for rats resulted in general hepatic effects consistent with those associated with PB-like microsomal enzyme inducers. In mice, musk ketone treatment resulted in a markedly increased CYP2B enzyme activity, together with increases in CYP2B protein and mRNA levels. Small changes in CYP1A and CYP3A enzyme activities were also observed, with concomitant increases in protein and mRNA levels. Although to a smaller degree, musk ketone treatment in rats resulted in identical effects on CYP2B. In contrast to mice, however, musk ketone induced CYP1A enzyme activities even more than CYP2B enzyme activity, while it reduced CYP3A enzyme activity. In both mice and rats, 20 mg/kg bw was the LOEL for enzyme induction.

In mice, three oral doses of 20 mg musk ketone have also been shown to induce GST enzyme activity in liver, small intestinal mucosa and colon. Intraperitoneal doses of 10 to 40 mg musk ketone/kg bw proved to be strong inducers of biotransformation enzymes in rat liver.

From information on the related compound musk xylene (see RAR musk xylene, 2003) it is clear that musk ketone is quite similar to musk xylene with respect to enzyme induction properties, musk xylene being the more potent one, with a NOEL of 10 mg/kg bw in mice and a LOEL of 10 mg/kg bw in rats. However, although musk xylene CYP2B enzyme induction is

characterised by large increases in mRNA and immunoreactive protein for the CYP2B enzymes, in contrast to musk ketone there is no commensurate increase in CYP2B enzyme activity with musk xylene. This inhibition of induced CYP2B enzyme activity is caused by the *p*-NH_2-metabolite of musk xylene which is formed by nitroreduction. Musk ketone possesses an acetyl rather than a nitro group para to the t-butyl substitution, and therefore musk ketone lacks the appropriate nitro reduction needed to inactivate the CYP2B enzymes.

In the 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 LOEL for it will not be taken forward to the risk characterisation.

4.1.2.6 Genotoxicity

The available *in vitro* and *in vivo* studies are summarized in **Table 4.12**. These studies are well performed, according to, or closely resembling, current guidelines.

Table 4.12 Genotoxicity studies with musk ketone.

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)	McConville, 1980
Bacterial gene mutation test	*S.typhimurium* (TA 97, 98, 100, 1535)	Other; Ames et al., 1975	negative (-/+ S9)	Zeiger et al., 1988
Bacterial gene mutation test	*S.typhimurium* (TA 97, 98, 100, 102)	Other; modified Ames-test	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; Kevekordes et al., 1996
Gene mutation test	mouse lymphoma L5178Y TK+/- cells	OECD 476	negative (-/+ S9)	Bigger and Clarke, 1993; Api et al., 1996
SCE test	human lymphocytes	OECD-like	negative (-/+ S9)	Kevekordes *et al.*, 1996
Chromosome aberration test	CHO-cells	OECD 473	equivocal (-/+ S9)	Putman et al., 1994; Api et al., 1996
Micronucleus test	human lymphocytes; human hepatoma cell line Hep G2	Other	negative; negative	Kevekordes et al., 1997
Micronucleus test	human hepatoma cell line Hep G2	Other	negative	Mersch-Sundermann et al., 2001
Unscheduled DNA synthesis test	rat hepatocytes	OECD 482	negative	San, 1994; Api et al., 1996
In vivo				
Micronucleus test	ICR mice	Other; OECD-like	negative	Gudi, 1996; Api and Gudi, 2000

4.1.2.6.1 *In vitro* studies

Several bacterial assays were carried out, all of which were negative without and with metabolic activation (McConville, 1980; Zeiger et al., 1988; Mersch-Sundermann et al., 1996a; Emig et al., 1996; Kevordes et al., 1996). In the study by McConville (1980) dose levels up to 10 mg musk ketone/plate in DMSO were tested in 5 strains of *Salmonella typhimurium*. At concentrations ≥1.0 mg/plate precipitation occurred. Zeiger *et al*. (1988) also tested up to 10 mg musk ketone/plate, but used acetone as solvent and 4 strains of *Salmonella typhimurium*. Mersch-Sundermann et al. (1996a) tested musk ketone (in DMSO) at dose levels up to 5 mg/plate in 4 strains of *Salmonella typhimurium*, and at dose levels up to 1.6 mg/assay in *Escherichia coli* PQ37 *sfiA::lacZ* in the SOS chromotest. Slightly different dose levels were reported for the latter tests by Emig et al. (1996): musk ketone up to the limit of solubility in DMSO for the *Salmonella* mutagenicity test, and up to 100 μg/assay in the SOS chromotest. In the SOS chromotest by Kevekordes et al.(1996) musk ketone was tested up to the limit of solubility in the aqueous medium of the assay.

Bigger and Clarke (1993) studied the effects of musk ketone (in acetone) in the L5178Y TK+/- mouse lymphoma mutagenesis assay in the absence (dose levels up to 4 mg/ml) and presence of S9 (dose levels op to 35 μg/ml). The test method used was according to OECD 476. No positive reactions were obtained either without or with metabolic activation.

Musk ketone at doses of 0.068 to 68 μM in DMSO did not induce SCEs in human lymphocytes in the absence or presence of S9. Higher doses were 100% cytotoxic (Kevekordes et al., 1996).

In a chromosome aberration test performed according to OECD 473 musk ketone (in acetone) was tested in CHO-cells at concentrations up to 34 μg/ml without metabolic activation and up to 10 μg/ml with metabolic activation. Toxicity occurred at the highest dose levels. At 10 μg/ml with metabolic activation, a significant increase in structural aberrations but no numerical aberrations was observed at 24 hours harvest. In a confirmatory test at concentrations up to 14 μg/ml, again with metabolic activation no structural aberrations were found, but at 14 μg/ml an increase in numerical aberrations was found. In this confirmatory test cells were only evaluated after 24 hours. Cytotoxicity occurred at the highest dose level, but not at lower dose levels. The study is considered to be equivocal (Putman et al., 1994).

Musk ketone (in acetone) was negative for unscheduled DNA synthesis (test performed according to OECD method 482) in primary cultures of rat hepatocytes up to 50 μg/ml. Toxicity occurred at the highest dose levels (San, 1994).

In an *in vitro* micronucleus test, musk ketone (in DMSO) at doses up to 136 and 250 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 ketone was tested up to cytotoxic doses (272 and 340 μM, respectively) (Kevekordes et al., 1997). [Remark: the frequency of micronuclei was expressed as total number of micronuclei per 1,000 cells, rather than frequency of micronucleated cells. However, as no effect was observed on the total number of micronuclei, this aberrant methodological procedure did not affect the study result.]

In another *in vitro* micronucleus test with human Hep G2 cells, musk ketone (in DMSO; tested concentrations 5-5,000 ng/ml) did also not affect the micronuclei frequency (Mersch-Sundermann et al., 2001).

4.1.2.6.2 *In vivo* studies

In a micronucleus assay, male and female IRC mice received a single i.p. injection with 250, 500, or 1,000 mg musk ketone/kg bw in corn oil. Mortality was observed in 2/20 male and 1/20 female mice receiving 1,000 mg/kg bw. Clinical signs following administration included lethargy at all dose levels and tremors and diarrhoea in male and female mice at 1,000 mg/kg bw. The number of micronucleated polychromatic erythrocytes per 1,000 polychromatic erythrocytes in treated animals was not significantly increased when compared to controls after 24, 48 or 72 hours. Slight reductions (up to 28%) in the ratio of polychromatic erythrocytes to total erythrocytes were observed in all groups at all time points (Gudi, 1996; Api and Gudi, 2000).

4.1.2.6.3 Cogenotoxic activity

Compared to liver S9 fractions from untreated rats, liver S9 fractions from rats treated with musk ketone showed an increased potency to toxify the pregenotoxicants B(a)P, 2-aminoanthracene and aflatoxin B_1 in the *in vitro* SOS chromotest with *Escherichia coli* PQ37 *sfiA::lacZ*. Hence, musk ketone is a cogenotoxicant by inducing toxifying enzymes (see also Section 4.1.2.5.1 – Special investigations on enzyme induction) (Mersch-Sundermann et al., 1996a/b).

When tested in an *in vitro* micronucleus test with metabolically competent human hepatoma cells (Hep G2 line), a cogenotoxic effect of musk ketone was observed when the cells were pre-treated with musk ketone for 28 hours and subsequently exposed to B(a)P, but not when the cells were simultaneously treated with musk ketone and B(a)P. Pretreatment with musk ketone resulted in a significant increase in B(a)P-induced micronuclei. This amplification of B(a)P genotoxicity was seen with 500-5,000 ng/ml musk ketone, concentrations that were effectively inducing CYP1A-activities (as was shown by EROD measurements in the Hep G2 cells), i.e. enzymes playing a key role in the activation of B(a)P (Mersch-Sundermann et al., 2001).

4.1.2.6.4 Summary of genotoxicity

Musk ketone was negative in several *in vitro* tests (bacterial gene mutation tests, SOS chromotests, a mammalian gene mutation test, tests for micronuclei induction and SCEs in mammalian cells, and an UDS test). A chromosome aberration test in mammalian cells *in vitro* provided an equivocal result, but as an *in vivo* mouse micronucleus test was negative, it can be concluded that musk ketone is a non-genotoxic substance. Due to its enzyme-inducing properties, musk ketone can exhibit cogenotoxic activity.

4.1.2.7 Carcinogenicity

There are no carcinogenicity data available for musk ketone. However, the related compound musk xylene was tested for carcinogenicity in mice. It was concluded (see RAR musk xylene, 2005) that musk xylene is carcinogenic 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. Therefore, for the characterisation of the carcinogenic risk of musk xylene to humans a threshold approach was taken, in which the (oral) LOAEL of

70 mg/kg bw/day for tumour development (liver tumours in particular) served as starting point and in which the NOEL for enzyme induction was taken into account in the interpretation of the margin of safety (MOS).

Given that:

- musk ketone is quite comparable to musk xylene with respect to physico-chemical and toxicokinetic properties, and in particular
- both musk ketone and musk xylene are phenobarbital-like inducers of liver enzymes in both rats and mice (with a LOEL of 20 mg/kg bw for musk ketone in both species, while for musk xylene 10 mg/kg bw is a NOEL in mice and a LOEL in rats),

there is a concern that musk ketone may be hepatocarcinogenic in mice as well. Just like musk xylene, musk ketone is a phenobarbital-like inducer of liver enzymes, but as such it is somewhat less potent than musk xylene. In concurrence with the risk characterisation for musk xylene, for the characterisation of the carcinogenic risk of musk ketone to humans a threshold approach would thus seem justified, also because musk ketone is a non-genotoxic substance. Given all this, it is concluded that, despite the lack of data on the carcinogenicity of musk ketone itself, there is no need for further testing because from the information above it is felt that the data available on musk xylene can be safely used for the risk characterisation of musk ketone.

As to classification: realising that it is a borderline case (musk xylene was not tested for carcinogenicity in rats, and the strain of mice used in the carcinogenicity study is particularly prone to develop certain types of tumours, especially liver tumours), it was nevertheless concluded (see RAR musk xylene, 2005) that the non-genotoxic compound musk xylene should be classified as a carcinogen category 3 (R40). In addition, the liver effects induced by musk xylene resemble those that can be seen after dosing rats and mice with phenobarbital, a (liver) carcinogenic substance in rodents and recently classified by IARC (IARC, 2001) as a group 2B substance ("possibly carcinogenic to humans"). For musk ketone, the case is even more borderline as no carcinogenicity data on musk ketone are available. However, on the basis of its similarity to musk xylene, musk ketone should be classified as a carcinogen category 3 (R40). This was agreed upon by the CMR Working Group at their January 2003 meeting.

4.1.2.8 Toxicity for reproduction

4.1.2.8.1 Effects on fertility

Studies in animals

No multi-generation reproductive toxicity study was available. In the 90-day dermal toxicity study with rats, musk ketone caused no effects on the reproductive organs. This in contrast to the positive control in that 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 4.1.2.9), 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 ketone, 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 ketone was detected in 130/152 blood samples (detection limit 20 ng/l), with a median, mean and maximum concentration of 55.5, 79.4 and 518 ng/l, respectively. The authors reported that no significant correlations were found between the level of musk ketone in blood and age, body mass index, occupation, nationality, fish consumption, use of detergents, follicular and luteal phase 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). Significant associations were reported between musk ketone levels in blood and the frequency of cosmetics use (especially with perfumes), the levels of androstenedione and androstanediol-glucuronide (but not with other adrenal hormones) and certain disorders (premenstrual syndrome, hirsutism) (Eisenhardt et al., 2001).

Remark: No causal relationships between the level of musk ketone 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.

4.1.2.9 Developmental toxicity

4.1.2.9.1 Studies in animals

Oral

In a range-finding study groups of 8 pregnant Sprague-Dawley rats received by gavage 0, 60, 200, 600 or 2,000 mg musk ketone (in corn oil)/kg bw/day during days 7-17 of gestation. At day 20 of gestation the animals were sacrificed. Two rats at 2,000 mg/kg bw and 3 rats at 600 mg/kg bw were found dead during the study, due to maternal toxicity. Examination of the uterus of these animals showed conceptuses that appeared normal for the developmental age. Treatment-related clinical signs (including urine-stained abdominal fur, excessive salivation, alopecia, ungroomed coat, cold to touch, emaciation, red perioral and perivaginal substance, chromodacryorrhea, chromorhinorrhea and/or decreased motor activity) were observed at 200 mg/kg bw and higher. At 200 mg/kg bw a treatment related increase in the occurrence of tremors was seen, but only at gestation days 7 through 9. In all treatment groups reduced body weight gain and food consumption occurred. Observations after caesarean sectioning showed decreases in fetal body weights, litter sizes and live fetuses and increases in early and late resorptions and percent resorbed conceptuses at 200 mg/kg bw and higher. No gross external fetal alterations were observed (Parker, 1997).

Based on the results of the range-finding study dosages of 0, 15, 45 and 150 mg musk ketone (in corn oil)/kg bw/day were selected for the main developmental toxicity study, which was performed according to current guidelines. These dosages were administered to groups of 25 pregnant Sprague-Dawley rats by gavage during days 7 through 17 of gestation. At day 20 of gestation the animals were sacrificed. No abortions, premature deliveries or deaths occurred during the study. Dose-related increased incidences of dried faeces and perioral substance occurred in the 45 and 150 mg/kg bw groups. In the highest dose group also urine-stained abdominal fur, excessive salivation, dehydration, red substance on forepaws and tremors occurred in significantly increased numbers. One or two rats in the highest dose group showed also chromorhinorrhea or chromodacryorrhea. Effects were first observed on gestation days 13 and 7 in the 45 and 150 mg/kg bw groups, respectively. Body weight gain and food consumption were dose-relatedly and statistically significantly reduced in the 45 and 150 mg/kg groups for the entire dosage period, with a rebound in these parameters on gestation days 18-20. Significant weight loss occurred at 150 mg/kg during days 7-10 of gestatation. Pregnancy incidences were comparable in all four groups. Increased post-implantation loss (evident as significant increases in litter averages for total and early resorptions, a slight tendency for increased late resorptions and percentage of resorbed conceptuses per litter, and increased numbers of dams with any resorptions or with all conceptuses dead or resorbed) were observed at 150 mg/kg bw. Two dams from this group showed litters consisting of only resorbed conceptuses. There were no dead fetuses. Significantly reduced fetal body weight was seen at 150 mg/kg bw. There was no indication for teratogenicity. The NOAEL for maternal toxicity can be established at 15 mg/kg bw/day, while the NOAEL for developmental toxicity can be established at 45 mg/kg bw/day (Christian et al., 1997; Christian et al., 1999).

Peri/postnatal toxicity study

Musk ketone (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* (Makin and Bottomley, 1997). The females were allowed to litter and rear their young to weaning (litters were standardised to 8 pups on day 6 *post partum*). 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.

A statistically significantly lower body weight gain was noted for dams at 25 mg/kg bw during the first two days of treatment (74.6% of that of controls). Due to this, lower absolute body weights were apparent in these dams from mid-pregnancy onwards and became slightly more pronounced during lactation. During lactation food intake was slightly but statistically significantly lower at 25 mg/kg bw (90% of controls). In this group mean pup weight was lower at birth (4.8%) and through to weaning (11.8%). Linked with this lower pup weight was a slightly later day of attainment for surface and air righting in these pups compared with controls and a later day of attainment of sexual maturation, although there was no effect on reproductive performance. Lower body weight gains during the pre-mating and mating phases

were seen in F_1 males from F_0 dams receiving 7.5 and 25 mg/kg bw (6.5-7.6% and 11.5-12.9%, respectively).

F_1 pups were exposed at levels in the mothers milk of up to 20,900 µg musk ketone/l throughout the entire nursing period (3 weeks) (data from Hawkins et al., 1996; 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 ketone measured in adipose tissue of excess F_1 pups killed on day 6 or day 22 *post partum* showed no sex-related differences. For the samples on day 6, although the fat concentration increased with dose, there was evidence that the concentration of musk ketone in the fat was not proportional to the dose. For the samples on day 22 a proportionality of the musk ketone concentration in adipose tissue to the dose level could not be excluded (Makin and Bottomley, 1997).

The NOAEL for maternal toxicity is 7.5 mg/kg bw/day. The NOAEL for peri/postnatal toxicity in this study can be established at 2.5 mg/kg bw/day. It is recognised that the biological significance of the only effect seen at 7.5 mg/kg bw (a marginal, but statistically significant decrease in body weight gain in F_1 males during a period in which the F_1 males no longer were exposed to musk ketone via mothers milk) is unclear. However, as the cause cannot be deducted from the parameters investigated (possibilities are exposure via the milk, reduced milk production by the dams, reduced maternal care), and the same, but even stronger, effect is seen at the next higher dose of 25 mg/kg bw, it cannot be excluded that the effect is biologically relevant and related to musk ketone treatment of the F_0 dams. Therefore, the NOAEL is (conservatively) set at 2.5 mg/kg bw/day.

Dermal / Inhalation

No data available.

4.1.2.9.2 Studies in humans

No data available.

4.1.2.9.3 Endocrine interactions

Receptor binding

Chou and Dietrich (1999) investigated the competitive binding capability of musk ketone and musk ketone metabolites to the estrogen receptor in trout (*Oncorhynchus mykiss*) and clawed frog (*Xenopus laevis*). No binding of the parent compound musk ketone was observed. Binding to the ER was noticed for 2-amino-musk ketone in both species (IC50 > 1 mM and 70.1 ± 88.3 µM for trout and Xenopus, respectively).

E-Screen

In a non-GLP study, musk ketone (purity 99.5%) and 2-amino musk ketone (purity 99.9%) 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 10 µmol/L 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 17 β-estradiol. Musk ketone showed a slightly higher rate of proliferation relative to the hormone-free control. The potency however was 10^{-5} lower than that of 17 β-estradiol. The 2-amino-musk ketone showed no increase at all. (Bitsch et al., 2002). Although the result with musk ketone 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.

4.1.2.9.4 Summary of toxicity to reproduction

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

In a well performed oral developmental toxicity study with rats, maternal toxicity occurred in a dose-related way at 45 and 150 mg/kg bw/day. This toxicity included reduced body weight gain and reduced food consumption. Developmental toxicity, including increased post implantation loss and reduced fetal body weight, was only seen at 150 mg/kg bw/day. There was no indication for teratogenicity. The NOAEL for maternal toxicity can be established at 15 mg/kg bw, and the NOAEL for developmental toxicity can be established at 45 mg/kg bw. No developmental toxicity studies are available for the dermal and inhalatory route.

In an oral peri/postnatal toxicity study slight toxicity (decreased body weight gain and food consumption) was seen at the highest dose level of 25 mg/kg bw in the dams (NOAEL for maternal toxicity is 7.5 mg/kg bw/day). Pup toxicity at this dose included a lower weight (at birth and through to weaning) and a later day of attainment for surface and air righting and fluxual maturation. Lower body weight gains up to post-natal week 20 were seen in F_1 males from F_0 dams receiving 7.5 and 25 mg/kg b/w/day. It is to be noted that exposure of the F_1-generation to musk ketone was only *in utero* during the peri-natal phase or through any transfer in the milk of the lactating dams. Next to a direct effect of the substance, reduced milk production or wasting cannot be excluded as (alternative) causes of the effect on body weight gain. Dosing up to 25 mg/kg bw did not result in behavioural changes or in reduced reproductive capacity. The lowest dose tested, 2.5 mg/kg bw/day, can be considered the NOAEL in this study. Realising that this is a conservative approach, the fact that the effect at the next higher dose is very small and that it is limited to males and of uncertain biological significance has to be taken into account in the interpretation of the MOS values for this endpoint.

The available data obtained from the peri/postnatal toxicity study indicate that musk ketone 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 ketone with R64 ("May cause harm to breast fed babies"). This was confirmed by the May 2002 meeting of the CMR Working Group.

Musk ketone and not 2-amino musk ketone 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-musk ketone, and not for musk ketone 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 ketone after oral and inhalation exposure. For the related compound musk xylene some toxicokinetic data were available after oral exposure, on the basis of which for both rats and humans a percentage of 50% for oral absorption is taken forward to the risk characterisation of musk xylene. Musk ketone is quite comparable to musk xylene with respect to physico-chemical properties. Dermal uptake and penetration rates do not indicate a major difference for the two substances either. Based on these similarities between musk ketone and musk xylene, for musk ketone a percentage of 50% for oral absorption will be taken forward to the risk characterisation in concordance with musk xylene.

After a 6 hour dermal application of ^{14}C-labelled musk ketone (under occlusion) to rats, a total of about 40% of the applied dose was absorbed within 48 hours, with 2-3.5% remaining in the skin. Between 6 and 48 hours, the skin acted as a reservoir from which musk ketone continued to be absorbed. Excretion via urine and faeces (predominantly via bile) was highest during the first 48 hours, with small amounts additionally excreted between 48 and 120 hours. After 120 hours, about 7-11% of the applied dose was excreted in urine, and 17-27% in faeces. Radioactivity was detected in nearly all tissues, with highest levels at 6 hours in gastrointestinal tract, followed by liver, adipose tissue, adrenals, thyroid, fat, and kidneys.

^{14}C-labelled musk ketone was poorly absorbed from human skin during a 6 hour application, as only 0.5% of the applied dose was excreted in urine and faeces within 120 hours, and >86% of the applied dose was recovered from the site of application.

In vitro experiments with unoccluded rat skin indicate that the percutaneus absorption of musk ketone is poor, and that the skin acts as a depot from which musk ketone can be absorbed.

Metabolism of musk ketone in rats and humans involves glucuronide conjugation.

For dermal absorption of musk ketone in rats and humans, values of 40% and 14%, respectively, are taken forward to the risk characterisation.

The plasma elimination half-life in rats after intravenous administration of musk ketone is approximately 60 hours. No data on plasma half-life in humans are available for musk ketone.

When administered orally to rats from day 14 of gestation up to 7 days post-parturition, musk ketone appeared in milk and milk fat. Musk ketone is also found in human milk fat and in adipose tissue.

Although the available studies for acute toxicity testing of musk ketone have not been performed according to OECD guidelines, it can be concluded that the oral LD_{50} for rats and

the dermal LD_{50} for rabbits are both greater than 2,000 mg/kg bw. Data for acute inhalation toxicity are not available.

According to EC criteria musk ketone needs not to be classified for acute oral and dermal toxicity based on the reported LD_{50} values.

Base set requirements for testing of skin irritation have not been met as adequate skin irritation studies are lacking. However, a request for a skin irritation study performed according to current guidelines is not deemed appropriate because:

- the available data on musk ketone do not point to a skin irritating potential: upon single treatment very high doses of musk ketone (as a 40% suspension in corn oil) are not irritating to the rabbit skin when applied for 24 hours under occlusion. After repeated dosing no signs of skin irritation were seen in rats, while in rabbits slight irritation was observed at high doses which partly could be attributed to the vehicle used. In sensitisation studies no irritation was observed in guinea pigs and in humans when applied at concentrations up to 75% and 5%, respectively.
- the test conditions used in a guideline study on rabbits (0.5 g for 4 hours under occlusion) are not expected to result in skin irritation given the results of the acute dermal study at much higher doses and longer duration.

From a well performed experiment it can be concluded that musk ketone is not eye irritating. According to EC criteria musk ketone needs not to be classified for skin and eye irritating properties.

No data on respiratory tract irritation are available.

Based on the results of a recently performed guinea pig maximisation study it can be concluded that musk ketone has weak sensitising properties. From two maximisation studies with human volunteers it can only be concluded that musk ketone up to a concentration of 5% is not skin sensitising in humans. There is no need for classification according to EU guidelines.

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

The available oral repeated dose toxicity studies with musk ketone are limited to special studies into biotransformation enzyme induction of comparatively short duration. In these studies (apart from enzyme induction) the only toxic responses reported were liver enlargement at dose levels greater than 20 mg/kg bw/day for 7 days in mice (NOEL) and at 20 mg/kg bw/day and above in the rat (LOEL). In mice, musk ketone also caused histological changes in the liver (primarily centrilobular hepatocellular hypertrophy and at 200 mg/kg bw/day panlobular hepatocellular hypertrophy). Details as to the dose-response relationship for the histological effects on the liver were not availabe. In rats liver histology was not studied. These studies are too limited to derive an NOAEL for oral repeated dose toxicity.

In a well performed dermal 90-days toxicity study with rats effects at the highest dose of 240 mg musk ketone/kg bw included a decreased body weight gain without a concommittant decrease in food consumption, decreases in red blood cell parameters and an increase in absolute and relative liver weight without a histopathological correlation. The decrease in body weight gain was also seen in females at the lower dose of 75 mg/kg bw. In the experiment no neuropathological effects and no effects on the reproductive organs were seen. The NOEL of 24 mg/kg bw/day in this study can be considered as a NOAEL, although the

extent of the body 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.

In dermal experiments with rabbits doses up to 750 mg musk ketone/kg bw for 20 days, microscopic pathology revealed hepatocyte vacuolisation and decreased bone marrow haematogenic activity, but these may have been caused, at least in part, by the vehicle (dimethyl phthalate).

When administered as part of a fragrance mixture, inhalatory exposure to musk ketone up to a maximum tested dose of 170.5 $\mu g/m^3$ for 4 h per day, 5 days per week for 13 weeks did not result in any toxicity. This study is of limited value because the animals were not exposed to musk ketone alone, and musk ketone was only present at rather low levels in the mixtures.

In special studies for enzyme induction, 7 consecutive daily oral musk ketone doses of up to 500 mg/kg bw for mice and up to 200 mg/kg bw for rats resulted in general hepatic effects consistent with those associated with PB-like microsomal enzyme inducers. In mice, musk ketone treatment resulted in a markedly increased CYP2B enzyme activity, together with increases in CYP2B protein and mRNA levels. Small changes in CYP1A and CYP3A enzyme activities were also observed, with concomitant increases in protein and mRNA levels. Although to a smaller degree, musk ketone treatment in rats resulted in identical effects on CYP2B. In contrast to mice, however, musk ketone induced CYP1A enzyme activities even more than CYP2B enzyme activity, while it reduced CYP3A enzyme activity. In both mice and rats, 20 mg/kg bw was the LOEL for enzyme induction.

In mice, three oral doses of 20 mg musk ketone have also been shown to induce GST enzyme activity in liver, small intestinal mucosa and colon. Intraperitoneal doses of 10 to 40 mg musk ketone/kg bw proved to be strong inducers of biotransformation enzymes in rat liver.

From information on the related compound musk xylene (see RAR musk xylene, 2005) it is clear that musk ketone is quite similar to musk xylene with respect to enzyme induction properties, musk xylene being the more potent one, with a NOEL of 10 mg/kg bw in mice and a LOEL of 10 mg/kg bw in rats. However, although musk xylene CYP2B enzyme induction is characterized by large increases in mRNA and immunoreactive protein for the CYP2B enzymes, in contrast to musk ketone there is no commensurate increase in CYP2B enzyme activity with musk xylene. This inhibition of induced CYP2B enzyme activity is caused by the *p*-NH_2-metabolite of musk xylene which is formed by nitroreduction. Musk ketone possesses an acetyl rather than a nitro group para to the t-butyl substitution, and therefore musk ketone lacks the appropriate nitro reduction needed to inactivate the CYP2B enzymes.

In the 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 LOEL for it will not be taken forward to the risk characterisation.

Musk ketone was negative in several *in vitro* tests (bacterial gene mutation tests, SOS chromotests, a mammalian gene mutation test, tests for micronuclei induction and SCEs in mammalian cells, and an UDS test). A chromosome aberration test in mammalian cells *in vitro* provided an equivocal result, but as an *in vivo* mouse micronucleus test was negative, it can be concluded that musk ketone is a non-genotoxic substance. Due to its enzyme-inducing properties, musk ketone can exhibit cogenotoxic activity.

There are no carcinogenicity data for musk ketone. However, the related compound musk xylene was tested for carcinogenicity in mice. It was concluded (see RAR musk xylene, 2005) that musk xylene is carcinogenic 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. Therefore, for the characterisation of the carcinogenic risk of musk xylene to humans a threshold approach was taken, in which the (oral) LOAEL of 70 mg/kg bw/day for tumour development (liver tumours in particular) served as starting point and in which the NOEL for enzyme induction was taken into account in the interpretation of the MOS.

Given that:

- musk ketone is quite comparable to musk xylene with respect to physico-chemical and toxicokinetic properties, and in particular,
- both musk ketone and musk xylene are phenobarbital-like inducers of liver enzymes in both rats and mice (with a LOEL of 20 mg/kg bw for musk ketone in both species, while for musk xylene 10 mg/kg bw is a NOEL in mice and a LOEL in rats),

there is a concern that musk ketone may be hepatocarcinogenic in mice as well. Just like musk xylene, musk ketone is a phenobarbital-like inducer of liver enzymes, but as such it is somewhat less potent than musk xylene. In concurrence with the risk characterisation for musk xylene, for the characterisation of the carcinogenic risk of musk ketone to humans a threshold approach would thus seem justified, also because musk ketone is a non-genotoxic substance. Given all this, it is concluded that, despite the lack of data on the carcinogenicity of musk ketone itself, there is no need for further testing because from the information above it is felt that the data available on musk xylene can be safely used for the risk characterisation of musk ketone.

As to classification, realising that it is a borderline case (musk xylene was not tested for carcinogenicity in rats, and the strain of mice used in the carcinogenicity study is particularly prone to develop certain types of tumours, especially liver tumours), it was nevertheless concluded (see RAR musk xylene, 2005) that the non-genotoxic compound musk xylene should be classified as a carcinogen category 3 (R40). In addition, the liver effects induced by musk xylene resemble those that can be seen after dosing rats and mice with phenobarbital, a (liver) carcinogenic substance in rodents and recently classified by IARC (IARC, 2001) as a group 2B substance ("possibly carcinogenic to humans"). For musk ketone, the case is even more borderline as no carcinogenicity data on musk ketone are available. However, on the basis of its similarity to musk xylene, musk ketone should be classified as a carcinogen category 3 (R40).

With respect to fertility no multi-generation reproductive toxicity study was available for either route. In the 90-day dermal toxicity study with rats, musk ketone caused no effects on the reproductive organs, whereas the structurally related compound musk ambrette caused testicular atrophy in the same study.

In an oral peri/postnatal toxicity study slight toxicity (decreased body weight gain and food consumption) was seen at the highest dose level of 25 mg/kg bw in the dams (NOAEL for maternal toxicity is 7.5 mg/kg bw/day). Pup toxicity at this dose included a lower weight (at birth and through to weaning) and a later day of attainment for surface and air righting and fluxual maturation. Lower body weight gains up to post-natal week 20 were seen in F_1 males from F_0 dams receiving 7.5 and 25 mg/kg b/w/day. It is to be noted that exposure of the F_1-generation to musk ketone was only *in utero* during the peri-natal phase or through any

transfer in the milk of the lactating dams. Next to a direct effect of the substance, reduced milk production or wasting cannot be excluded as (alternative) causes of the effect on body weight gain. Dosing up to 25 mg/kg bw did not result in behavioural changes or in reduced reproductive capacity. The lowest dose tested, 2.5 mg/kg bw/day, can be considered the NOAEL in this study. Realising that this is a conservative approach, the fact that the effect at the next higher dose is very small and that it is limited to males and of uncertain biological significance, has to be taken into account in the interpretation of the MOS values for this endpoint.

In a well performed oral developmental toxicity study with rats, maternal toxicity occurred in a dose-related way at 45 and 150 mg/kg bw/day. This toxicity included reduced body weight gain and reduced food consumption. Developmental toxicity, including increased post implantation loss and reduced fetal body weight, was only seen at 150 mg/kg bw/day. There was no indication for teratogenicity. The NOAEL for maternal toxicity can be established at 15 mg/kg bw, and the NOAEL for developmental toxicity can be established at 45 mg/kg bw. No developmental toxicity studies are available for the dermal and inhalatory route.

The available data obtained from the peri/postnatal toxicity study indicate that musk ketone 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 ketone 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 ketone, 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 dermal toxicity study in secondary literature it can be concluded that musk ketone 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 exposure levels, it is concluded that musk ketone is of no concern for workers with regard to acute dermal effects: **conclusion (ii)**. There are no data on acute inhalation toxicity, however given the estimated inhalation exposure levels 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

Adequate skin irritation studies are lacking. Based on the available data it is not possible to classify musk ketone for skin irritation properties. However, it is not considered appropriate to require additional testing according to current guidelines. The test conditions used in a guideline study on rabbits (0.5 g for 4 hours under occlusion) are not expected to result in skin irritation since musk ketone is not irritating to rabbit skin after single exposure, musk ketone is not irritating to rat skin after repeated exposure and only slight irritation was observed at rabbit skin after exposure to high doses which partly could be attributed to the vehicle used: **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 4.1.1.2 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 total body surface area). Given the estimated frequency of exposure (225days/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 20-52 days/year. The MOSs between the NOAEL and the dermal exposure levels are mentioned in **Table 4.13**. The MOSs are evaluated by comparison with the minimal MOS (9). In Annex A 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 4.13 Occupational risk assessment of musk ketone for repeated dose toxicity after dermal exposure (local effects).

Scenario/subscenario	Risk characterisation for dermal exposure		
	Estimated dermal exposure in mg/day (mg/cm²)[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/cm2 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 cm2 for Scenario 2 'cleaning', and an 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 4.13**, 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 ketone is of no concern for workers with regard to acute eye irritation: **conclusion (ii)**.

Respiratory irritation

No data are available on the local respiratory tract effects of musk ketone 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. Musk ketone administered for 13 weeks (4 hours per day, 5 days per week) by the inhalatory route as part of fragrance mixture did not result in any toxicity up to a dose of 0.17 mg/m^3. Based on this inhalation study and given the low or negligible estimated inhalation exposure there are no indications for concern for respiratory irritation: **conclusion (ii)**.

Sensitisation

Based on the dermal sensitisation study in guinea pigs it can be concluded that musk ketone only has weak sensitising properties. In humans, musk ketone is not a skin sensitiser in concentrations up to 5%. Therefore musk ketone is of no concern for workers with regard to skin sensitisation: **conclusion (ii)**.

Repeated-dose toxicity

Dermal exposure

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

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' is only 20-50 days/year. However, because of the high half life in blood and the accumulation potential of musk ketone, 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 4.14**. The MOSs are evaluated by comparison with the minimal MOS (126). In Annex A 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 4.14 Occupational risk assessment of musk ketone for repeated dose toxicity after dermal exposure (systemiceffects).

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]
2. 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 exposure mg/kg bw/d 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/d;
C) Comparison of the MOS with the minimal MOS (126);
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 4.14**, 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 (126), 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. The MOSs are evaluated by comparison with the minimal MOS (900). In Annex A 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 4.15 Risk assessment for musk ketone for repeated-dose toxicity after respiratory exposure**.**

Scenario/subscenario	Risk characterisation for respiratory exposure		
	Estimated respiratory exposure in mg/m³ (mg/kg/bw/day)[A]	MOS[B]	Conclusion
1. The production of fragrance compounds	0.3 (0.04)	600	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/d, based on a respiratory volume of 10 m³/workday and 70 kg worker;
B) Based on a dermal NOAEL of 24 mg/kg bw/d in rats and assuming a worker body weight of 70 kg and a respiratory volume of 10 m³ for a working day;
C) Based on a comparison of the MOS with the minimal MOS (900).

Given the MOSs for inhalation exposure as mentioned in **Table 4.15**, 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 (900) indicates a concern for this scenario. However, in view of the worst case character 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 ketone. 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 ketone, 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 4.16**. The MOSs are evaluated by comparison with the minimal MOS (360). In Annex A 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 4.16 Risk assessment for combined exposure to musk ketone 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/d)[A]	Estimated respiratory exposure in mg/m³ (systemic dose in mg/kg bw/d)[B]	Total systemic dose as result from dermal and inhalation exposure in mg/kg bw/d [C]	MOS [D]	Conclusion[E]
1. The production of fragrance compounds	42 (0.08)	0.3 (0.04)	0.12	80	ii [F]
2. The use of liquid fragrance compounds					
- addition	4 (0.008)	Negligible	0.008	1200	ii
- cleaning	6.5 (0.013)	negligible	0.013	739	ii
3. The use of cleaning agents by professional cleaners	2.5 (0.005)	Negligible	0.005	1920	ii

A) Between brackets the systemic dose due to dermal exposure in mg/kg bw/d, assuming a worker body weight of 70 kg and a dermal absorption of 14%;
B) The systemic dose due to respiratory exposure in mg/kg bw/d, 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 9.6 mg/kg bw/d with rats (based on an external NOAEL of 24 mg/kg bw/day and a dermal absorption of 40%);
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 4.16**, it is concluded that, based upon the present information, there is no reason for concern for systemic effects due to combined exposure in scenarios 2 and 3: **conclusion (ii)**. Comparison of the calculated MOS value for Scenario 1 (80) 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 ketone is of no concern for workers with regard to mutagenicity: **conclusion (ii)**.

Carcinogenicity

There are no carcinogenicity studies available. However, in the dossier of musk xylene (RAR musk xylene, 2005) there was information available on the carcinogenic properties of the substance (the observed LOAEL for carcinogenic effects in mice was 70 mg/kg bw/day). Since musk ketone and musk xylene are comparable with regard to their toxicokinetic and toxicodynamic properties, and given the comparable results observed in the enzyme induction

studies it is assumed that the information available on musk xylene can be used for the risk characterization of musk ketone. For musk xylene it was concluded that there is no concern for workers with regard to systemic carcinogenicity after dermal, inhalation, and combined exposure for all scenarios: **conclusion (ii)**. Because the exposure levels for both substances are the same, it is assumed that the same conclusions are applicable for musk ketone.

Reproductive toxicity

Reproduction toxicity studies by inhalation or dermal exposure are lacking. There are no indications for effects on the 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 a NOAEL of 15 mg/kg bw/day is established for maternal toxicity and a NOAEL of 45 mg/kg bw/day for developmental toxicity. The substance is not teratogenic.

In an oral peri/postnatal toxicity study in rats a NOAEL of 2.5 mg/kg bw/day was observed based on a slightly but significantly and dose-related decreased growth of male pups from the next higher dose level (7.5 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 ketone on the offspring although the biological significance of this effect is uncertain. 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 45 mg/kg bw/day) are covered. The MOSs between the oral NOAEL and the dermal, respiratory, and combined exposure levels are shown in **Table 4.17-4.19**. The MOSs are evaluated by comparison with the minimal MOSs. In Annex A 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 4.17 Risk assessment for the offspring after dermal exposure to musk ketone.

Scenario/subscenario	Risk characterisation for dermal exposure		
	Estimated dermal exposure in mg/day (mg/kg bw/d)[A]	MOS[B]	Conclusion[C]
The production of Fragrance compounds	42 (0.6)	4	ii
2. The use of liquid fragrance compounds:			
- addition	4 (0.06)	42	ii
- cleaning	6.5 (0.09)	28	ii
3. The use of cleaning agents by professional cleaners	2.5 (0.04)	63	ii

A) Estimated dermal exposure in mg/kg bw/d assuming a worker body weight of 70 kg used for calculating MOSs;
B) Based on an oral peri/postnatal NOAEL in rats of 2.5 mg/kg bw/d;
C) Based on a comparison of the MOS with the minimal MOS (10).

Table 4.18 Risk assessment for the offspring after respiratory exposure to musk ketone.

Scenario/subscenario	Risk characterisation for respiratory exposure		
	Estimated respiratory exposure in mg/m³ (mg/kg/bw/d)[A]	MOS[B]	Conclusion[C]
1. The production of fragrance compounds	0.3 (0.04)	63	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/d, based on a respiratory volume of 10 m³/workday and 70 kg worker;
B) Based on an oral peri/post natal NOAEL of 2.5 mg/kg bw/d 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 4.19 Risk assessment for the offspring after combined exposure to musk ketone.

Scenario/subscenario	Risk characterisation for combined exposure				
	Estimated dermal exposure in mg/day (systemic dose in mg/kg bw/d)[A]	Estimated respiratory exposure in mg/m³ (systemic dose in mg/kg bw/d)[B]	Total systemic dose as result from dermal and inhalation exposure in mg/kg bw/d [C]	MOS [D]	Conclusion[C]
1. The production of fragrance compounds	42 (0.08)	0.3 (0.04)	0.12	10	ii
2. The use of liquid fragrance compounds:					
- addition	4 (0.008)	Negligible	0.008	156	ii
- cleaning	6.5 (0.013)	Negligible	0.013	96	ii
3. The use of cleaning agents by professional cleaners	2.5 (0.005)	Negligible	0.005	250	ii

A) Between brackets the systemic dose due to dermal exposure in mg/kg bw/d, assuming a worker body weight of 70 kg and a dermal absorption of 14%;
B) The systemic dose due to respiratory exposure in mg/kg bw/d, 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/post natal NOAEL of 1.25 mg/kg bw/d (based on an oral peri/post natal NOAEL of 2.5 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 **Table 4.17-4.19**, and taking into account the small effect limited to males (at the LOAEL) and the considerations for dermal exposure in Scenario 1 put forward in the risk characterisation for repeated-dose toxicity, it is concluded that based upon the present

information there seems to be no reason for concern with regard to effects on the offspring: **conclusion (ii)**.

Occupational limit values

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

4.1.3.2.3 Summary of risk characterisation for workers

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

4.1.3.3 Consumers

4.1.3.3.1 Introduction

For consumers, the main exposure to musk ketone 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 200 μg/kg bw/day (see Section 4.1.1.32.1). Because the absorption of musk ketone through human skin is at maximum 14%, this external exposure level results in an internal exposure level of 28 μg/kg bw/day.

4.1.3.3.2 Comparison of exposure and effects

Irritation

The available data on musk ketone do not point to a skin irritating potential. Hence, there is no concern for consumers for skin irritation: **conclusion (ii)**.

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

Sensitisation

In animals, musk ketone only has weak sensitising properties. In humans, musk ketone is not a skin sensitiser in concentrations up to 5%. Higher concentrations of musk ketone do not occur in consumer cosmetic articles (in which the maximum fraction is 0.552% according to SCCNFP, 1999), hence consumers are 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 40% for rats, this NOAEL corresponds to an internal no-effect dose of 9.6 mg/kg bw/day.

Comparing this internal no-effect dose with the calculated human systemic exposure level of 28 μg/kg bw/day, a MOS of 343 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 ketone is a non-genotoxic substance: **conclusion (ii)**.

Carcinogenicity

There are no data available on the carcinogenic potential of musk ketone. However, the related compound musk xylene appeared to be carcinogenic in mice, acting by a non-genotoxic mode of action that, at least for the observed liver tumours, involved microsomal enzyme induction (see RAR musk xylene, 2005). Therefore, for the characterisation of the carcinogenic risk of musk xylene to humans a threshold approach was taken, in which the (oral) LOAEL of 70 mg/kg bw/day for tumour development (liver tumours in particular) served as starting point and in which the NOEL for enzyme induction was taken into account in the interpretation of the MOS. It is assumed that the information available on musk xylene can be used for the risk characterisation of musk ketone, because:

- musk ketone is quite comparable to musk xylene with respect to physico-chemical and toxicokinetic properties, and
- both musk ketone and musk xylene are non-genotoxic substances, and
- both musk ketone and musk xylene are phenobarbital-like inducers of liver enzymes in both rats and mice, with musk ketone being somewhat less potent than musk xylene.

For musk xylene, the risk characterisation did not indicate concern for consumers for carcinogenicity after dermal exposure: **conclusion (ii)**. It is assumed that the same conclusion for carcinogenicity - **conclusion (ii)** - is applicable for consumers after dermal exposure to musk ketone, because the human systemic exposure level for musk ketone and musk xylene is comparable.

Reproductive toxicity

There are no indications for effects on fertility in the dermal 90-day toxicity study with rats, although in this study 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 ($NOAEL_{developmental\ toxicity}$ 45 mg/kg bw/day, $NOAEL_{maternal\ toxicity}$ 15 mg/kg bw/day). In an oral peri/postnatal study in which rats were exposed to musk ketone from day 14 of gestation through weaning, the NOAEL for effects on the pups was 2.5 mg/kg bw/day.

In order to get insight in the risk for the progeny of pregnant consumers, the oral NOAEL of 2.5 mg/kg bw/day for peri/postnatal effects and the oral NOAEL of 45 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 1.25 and 22.5 mg/kg bw/day, respectively. Comparing these internal no-effect doses with the calculated human systemic exposure level of 28 μg/kg bw/day, the MOSs are 45 and 804, respectively.

Taking into account intra- and interspecies differences the MOS of 804 indicates no concern for developmental effects to the progeny of consumers: **conclusion (ii)**. As to peri/postnatal effects, a MOS of 45 also indicates no concern for the progeny of consumers: **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 45 would then lead to a **conclusion (ii)**, also because the effect seen at the LOAEL in the peri/postnatal study only occurred in males and was marginal in nature and of uncertain biological significance.

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 ketone should be reduced by 50%. If this advice is implemented in EU-law the external exposure would then become 200/2 = 100 μg/kg bw/day giving an internal exposure of 14 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 3.31e-3 and 4.55e-4 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 1.66e-3 and 2.28e-4 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 ketone in mother's milk, a separate risk characterisation is necessary for breast-fed babies (highest exposure value 1 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 ketone 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

Oral repeated dose toxicity studies are lacking. 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 40% for rats, this NOAEL corresponds to an internal no-effect dose of 9.6 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 >>1,000 (see **Table 4.20**).

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

Carcinogenicity

There are no data available on the carcinogenic potential of musk ketone. However, the related compound musk xylene appeared to be carcinogenic in mice, acting by a non-genotoxic mode of action that, at least for the observed liver tumours, involved microsomal enzyme induction (see RAR musk xylene, 2003). Therefore, for the characterisation of the carcinogenic risk of musk xylene to humans a threshold approach was taken, in which the (oral) LOAEL of 70 mg/kg bw/day for tumour development (liver tumours in particular) served as starting point and in which the NOEL for enzyme induction was taken into account in the interpretation of the MOS. It is assumed that the information available on musk xylene can be used for the risk characterisation of musk ketone, because:

- musk ketone is quite comparable to musk xylene with respect to physico-chemical and toxicokinetic properties, and
- both musk ketone and musk xylene are non-genotoxic substances, and
- both musk ketone and musk xylene are phenobarbital-like inducers of liver enzymes in both rats and mice, with musk ketone being somewhat less potent than musk xylene.

For musk xylene, the risk characterisation did not indicate concern for carcinogenicity for man exposed indirectly via the environment: **conclusion (ii)**. It is assumed that the same conclusion for carcinogenicity - **conclusion (ii)** - is applicable for man exposed indirectly via the environment to musk ketone, because the human total daily intake levels for musk ketone and musk xylene are comparable.

Reproductive toxicity

There are no indications for effects on fertility in the dermal 90-day study with rats, although in this study 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 ($NOAEL_{developmental\ toxicity}$ 45 mg/kg bw/day,

$NOAEL_{maternal\ toxicity}$ 15 mg/kg bw/day). In an oral peri/postnatal study in which rats were exposed to musk ketone from day 14 of gestation through weaning, the NOAEL for effects on the pups was 2.5 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 2.5 mg/kg bw/day for peri/postnatal effects and the oral NOAEL of 45 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 >700 (see **Table 4.20**).

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

Table 4.20 Margins of safety for local and regional scale for musk ketone.

		Total daily intake (internal / external exposure) in mg/kg bw/day	MOS Repeated dose toxicity	MOS Reproductive toxicity	
				Peri/postnatal	Developmental
Local	Private use	1.66e-3 / 3.31e-3	5,783	755	13,595
Regional	All life cycle steps	2.28e-4 / 4.55e-4	42,105	5,495	98,901

Exposure via mother's milk

The highest exposure of musk ketone via mother's milk was calculated to be 1 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 ketone, the NOAEL for peri/postnatal effects is 2.5 mg/kg bw/day. Comparing this no-effect dose with the maximum exposure level via mother's milk, a MOS of 2,500 is derived. Taking into account intra- and interspecies differences, and the fact that the effect seen at the LOAEL in the peri/postnatal study only occurred in males and 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 4.1.1.52.3, a worst case estimate for the combined (external) exposure to musk ketone 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) + 0.20 mg/kg bw/day (dermal, consumers) + 3.31e-3 mg/kg bw/day (oral, locally via the environment). Assuming figures of 14%, 100% and 50% for dermal, inhalation and oral absorption, respectively, an internal exposure of 0.15 mg/kg bw/day (i.e. 0.08 mg/kg bw/day (dermal, workplace) + 0.043 mg/kg bw/day (inhalation, workplace) + 0.028 mg/kg bw/day (dermal, consumers) + 1.66e-3 mg/kg bw/day (oral, locally via the

environment)) can be calculated. Note that approximately 80% of the combined internal exposure estimate originates from occupational sources.

Acute toxicity / Irritation / Sensitisation / Genotoxicity

Given that musk ketone is not acutely toxic, eye irritating and genotoxic, and musk ketone has no skin irritating and only weak, if any, skin sensitising potential, there is no concern for these endpoints after combined exposure to musk ketone: **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 40% for rats, this NOAEL corresponds to an internal no-effect dose of 9.6 mg/kg bw/day.

Comparing this internal no-effect dose with the calculated combined human systemic exposure level of 0.15 mg/kg bw/day, a MOS of 64 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

There are no data available on the carcinogenic potential of musk ketone. However, the related compound musk xylene appeared to be carcinogenic in mice, acting by a non-genotoxic mode of action that, at least for the observed liver tumours, involved microsomal enzyme induction (see RAR musk xylene, 2005). Therefore, for the characterisation of the carcinogenic risk of musk xylene to humans a threshold approach was taken, in which the (oral) LOAEL of 70 mg/kg bw/day for tumour development (liver tumours in particular) served as starting point and in which the NOEL for enzyme induction was taken into account in the interpretation of the MOS. It is assumed that the information available on musk xylene can be used for the risk characterisation of musk ketone, because:

- musk ketone is quite comparable to musk xylene with respect to physico-chemical and toxicokinetic properties, and
- both musk ketone and musk xylene are non-genotoxic substances, and
- both musk ketone and musk xylene are phenobarbital-like inducers of liver enzymes in both rats and mice, with musk ketone being somewhat less potent than musk xylene.

For musk xylene, the risk characterisation indicated no concern for carcinogenicity after combined exposure: **conclusion (ii)**. It is assumed that the same conclusion for carcinogenicity - **conclusion (ii)** - is applicable for combined exposure to musk ketone, because the combined human systemic exposure level for musk ketone and musk xylene is comparable.

Reproductive toxicity

There are no indications for effects on fertility in the dermal 90-day toxicity study with rats, although in this study 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 ($NOAEL_{developmental\ toxicity}$ 45 mg/kg bw/day,

$NOAEL_{maternal\ toxicity}$ 15 mg/kg bw/day). In an oral peri/ postnatal study in which rats were exposed to musk ketone from day 14 of gestation through weaning, the NOAEL for effects on the pups was 2.5 mg/kg bw/day.

In order to get insight in the risk for the progeny of pregnant women, the oral NOAEL of 2.5 mg/kg bw/day for peri/postnatal effects and the oral NOAEL of 45 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 1.25 and 22.5 mg/kg bw/day, respectively. Comparing these internal no-effect doses with the calculated combined human systemic exposure level of 0.15 mg/kg bw/day, the MOSs are 8 and 150, respectively.

Taking into account intra- and interspecies differences and the worst case character of the combined exposure estimate, the MOS of 150 indicates no concern for developmental effects to the progeny of pregnant women after combined exposure: **conclusion (ii)**. As 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, 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). Given also the worst case character of the combined exposure estimate and the fact that the effect seen at the LOAEL in the peri/postnatal study only occurred in males and was marginal in nature and of uncertain biological significance, it is concluded that also for peri/postnatal effects the MOS of 8 represents no concern - **conclusion (ii)** - for the progeny of pregnant women after combined exposure.

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

Musk ketone is not explosive.

4.2.1.2 Flammability

Musk ketone may be considered not flammable on account of the high flashpoint and the limited effect in the explosive burning test.

4.2.1.3 Oxidising potential

Musk ketone is not oxidising.

4.2.2 Risk characterisation

Given the physico-chemical data, musk ketone is considered not to form a risk with respect to flammability, and explosive and oxidizing properties: **conclusion (ii)**.

5 RESULTS

5.1 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.

5.2 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 ketone.

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						

Table 5.1 continued overleaf

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

End point	Conclusions valid for the occupational scenario's					
	Scenario 1		Scenario 2		Scenario 3	
	MOS	Conclusion	MOS	Conclusion	MOS	Conclusion
- 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	80	ii	1,200/739	ii	1,920	ii
(dermal NOAEL 24 mg/kg bw/day)						
mutagenicity	n.a	ii	n.a.	ii	n.a.	ii
carcinogenicity (based on musk xylene)						
- dermal	n.a.	ii	n.a.	ii	n.a.	ii
- inhalation	n.a.	ii	n.a.	ii	n.a.	ii
- combined	n.a.	ii	n.a.	ii	n.a.	ii
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	4	ii	42/28	ii	63	ii
(oral NOAEL of 2.5 mg/kg bw/day)						
- inhalation	63	ii	high	ii	high	ii
(oral NOAEL of 2.5 mg/kg bw/day)						
- combined	10	ii	156/96	ii	250	ii
(oral NOAEL of 2.5 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
oxidizing 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 PHYSICOCHEMICAL PROPERTIES

Given the physico-chemical data, musk ketone is considered not to form a risk with respect to flammability, and explosive and oxidizing properties: **conclusion (ii)**.

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ABBREVIATIONS

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)
CT_{50}	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

DT50	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)
DT90	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
EC10	Effect Concentration measured as 10% effect
EC50	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
foc	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 [software tool]
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)
Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
kPa	kilo Pascals
l	litre(s)
L(E)C50	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
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	Predicted No Effect Concentration
$PNEC_{water}$	Predicted No Effect Concentration in Water
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(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
SCE	Sister Chromatic Exchange
SCHER	Scientific Committee on Health and Environmental Risks
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 · 3
Differences in dermal absorption between animal and human[b]	0.35
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	**126**

A) Extrapolation based on differences in caloric demands, together with a factor 3 for differences in sensitivity.
B) A factor 0.35 for differences in dermal absorption (40% dermal absorption in animals and 14% 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 · 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]	2.5
Confidence of the database	1
Overall	**900**

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 40% 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 · 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 offspring effects after dermal exposure.

Aspect	Assessment factors
Interspecies differences[a]	4 · 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]	0.28
Confidence of the database	1
Overall	**10**

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 a dermal absorption of 14% is used. In line with musk xylene an oral absorption of 50% is used.

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

Aspect	Assessment factors
Interspecies differences[a]	4 · 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 dermal absorption. Based on experimental data a dermal absorption of 14% is used. In line with musk xylene an oral absorption of 50% is used.

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

Aspect	Assessment factors
Interspecies differences[a]	4 · 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 21507 EN **European Union Risk Assessment Report 4'-tert-butyl-2',6'-dimethyl-3',5'-dinitroacetophenone (musk ketone), Volume 62**

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Environment and quality of life series

The report provides the comprehensive risk assessment of human health part of the substance Musk ketone. 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 environmental and human health risk assessment for 4'-tert-butyl-2',6'-dimethyl-3',5'-dinitroacetophenone (musk ketone) concludes that risks are not expected.

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European Union Risk Assessment Report

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