

European Union Risk Assessment Report

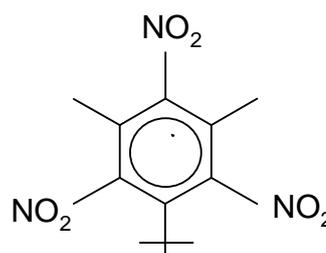
CAS: 81-15-2

EINECS No: 201-329-4

PBT ASSESSMENT OF MUSK XYLENE

5-TERT-BUTYL-2,4,6-TRINITRO-M-XYLENE

Addendum to the final report (2005)
of the risk assessment



The mission of the JRC-IHCP is to protect the interests and health of the consumer in the framework of EU legislation on chemicals, food, and consumer products by providing scientific and technical support including risk-benefit assessment and analysis of traceability.

European Commission
Joint Research Centre
Institute for Health and Consumer Protection

Address: Via E. Fermi 2749 - 21027 Ispra (VARESE) - Italy
E-mail: jrc-ihcp-communication@ec.europa.eu
Tel.: +39 0332 785959
Fax: +39 0332 785730

<http://ihcp.jrc.ec.europa.eu/>
<http://www.jrc.ec.europa.eu/>
<http://ecb.jrc.ec.europa.eu/>

Legal Notice

Neither the European Commission nor any person acting on behalf of the Commission is responsible for the use which might be made of this publication.

***Europe Direct is a service to help you find answers
to your questions about the European Union***

Freephone number (*):

00 800 6 7 8 9 10 11

(*) Certain mobile telephone operators do not allow access to 00 800 numbers or these calls may be billed.

A great deal of additional information on the European Union is available on the Internet. It can be accessed through the Europa server <http://europa.eu/>

JRC 61687

EUR 24607 EN
ISBN 978-92-79-18438-3
ISSN 1831-9424
DOI 10.2788/45153

Luxembourg: Publications Office of the European Union

© European Union, 2010

Reproduction is authorised provided the source is acknowledged

Printed in Italy

Foreword

I am pleased to present this updated Risk Assessment Report on 5-TERT-BUTYL-2,4,6-TRINITRO-M-XYLENE which is the result of in-depth work carried out by experts in The Netherlands, 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) 793/93¹ 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.

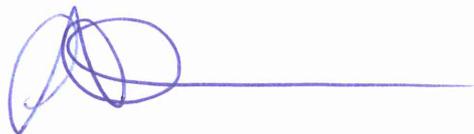
There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each 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) 1488/94², which is supported by a technical guidance document³. 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 and confirmed in the Johannesburg Declaration on Sustainable Development at the World Summit on Sustainable Development, held in Johannesburg, South Africa in 2002.

This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. I 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.



Elke Anklam
Director
Institute for Health and
Consumer Protection

¹ O.J. No L 084, 05/04/199 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]

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

CAS Number: 81-15-2
EINECS Number: 201-329-4

Addendum to the final report (2005)
of the risk assessment (PBT assessment)

FINAL APPROVED VERSION

January 2008
-The Netherlands

Rapporteur: The Netherlands

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

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

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

Executive Summary

The report provides the PBT assessment of the substance 5-tert-butyl-2,4,6-trinitro-m-xylene (musk xylene). It has been prepared by The Netherlands in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to humans and the environment, laid down in Commission Regulation (EC) No. 1488/94. In 2005 the environmental risk assessment for musk xylene concluded that there was a need for further information and/or testing because the substance is considered to be a PBT candidate chemical and a further PBT – testing strategy was proposed.

Conclusion regarding persistency: the extractable part of musk xylene in sediment is subject to anaerobic degradation with half-lives of equal to or below 60 days. Musk xylene is therefore considered to be not persistent in sediment. In this assessment the observed irreversible binding to sediment is considered as dissipation.

Given the fact that the metabolites in the ocean die-away test stayed in the water phase while the parent compound musk xylene volatilized and the fact that the ratio metabolites : parent compound was still close to one after 159 days, it is concluded that the half-life for biodegradation in seawater is more than 150 days, which significantly exceeds the criterion of 60 days. Musk xylene is therefore considered to be very persistent in water.

Conclusion regarding bioaccumulation: The experimental bioaccumulation studies for musk xylene in fish showed a wide range of BCFs, among which values above the vB criterion of 5,000 l/kg. Based on the evaluation of the critical study and its results it can be concluded that musk xylene is very bioaccumulative.

Conclusion regarding toxicity: there is no substantiated evidence that musk xylene can cause endocrine disrupting effects. However, musk xylene is classified as Carcinogenic Category 3), although it is realised that it is a borderline case. The overall conclusion for musk xylene is that regarding toxicity it is considered to be a borderline case. However, in view of the vPvB properties of musk xylene, no additional testing is required.

It is concluded in the Addendum that musk xylene is a very persistent and very bioaccumulative substance and is considered to be borderline T. This should be further considered under the REACH Regulation.

0

OVERALL RESULTS OF THE ADDENDUM TO THE RISK ASSESSMENT

CAS No: 81-15-2
EINECS No: 201-329-4
IUPAC name: 1-tert-butyl-3,5-dimethyl-2,4,6-trinitrobenzen

Environment

(x) **iii)** There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion iii) is reached, because MUSK XYLENE is concluded to be a vPvB chemical. In addition, it should be noted that the substance is considered to be borderline T.

CONTENTS

1	ADDITIONAL INFORMATION ON PERSISTENCY	9
1.1	INTRODUCTION	9
1.2	ABIOTIC DEGRADATION	9
1.2.1	Photolysis in water	9
1.2.2	Oxidation and photolysis in air.....	10
1.3	BIOTIC DEGRADATION.....	10
1.3.1	Screening data	10
1.3.2	Simulation studies	10
1.4	ENVIRONMENTAL DISTRIBUTION.....	12
1.5	CONCLUSION REGARDING PERSISTENCY.....	13
2	INFORMATION ON BIOACCUMULATION	14
2.1	DESCRIPTION OF KEY STUDIES.....	14
2.2	CONCLUSION REGARDING BIOACCUMULATION	19
3	INFORMATION ON TOXICITY	20
3.1	AQUATIC COMPARTMENT	20
3.2	CARCINOGENICITY	20
3.3	ENDOCRINE INTERACTIONS	20
3.4	CONCLUSION REGARDING TOXICITY	21
4	RISK CHARACTERISATION, CONCLUSION OF THE PBT ASSESSMENT	22
5	REFERENCES	23

Introduction

The EU risk assessment report (RAR) of the fragrance compound musk xylene was finalised in 2005 (EC, 2005). With respect to the environment a conclusion was drawn indicating a need for further information and/or testing. The conclusion was drawn based upon the fact that the substance was considered a PBT candidate chemical (TGD 2003). A further PBT-testing strategy was proposed, focussing on the refinement of the P-assessment.

In the risk assessment report (section 3.3.6) it was concluded that the Persistence screening criterion is fulfilled for musk xylene. The results of two biodegradation tests clearly showed no (ready) biodegradability. Other available information was limited to some inconclusive degradation studies with influent/effluent and modelling data (BIOWIN) showing lack of aerobic biodegradability.

The Bioaccumulation (B) criterion and Toxicity (T) criterion were concluded to be fulfilled. A testing strategy was laid down for persistency. In the risk assessment it was stated that a simulation test on biodegradability (half-life in the marine environment) should be considered for refining the P-criterion (see TGD (2003)).

The final testing strategy consisted of studies investigating the photolysis in water, degradation in water, degradation in a sediment/water system and adsorption to marine sediment. The testing programme was designed in the course of 2004 and subsequently carried out in 2005 with report completion in 2006.

Based on the results of the testing program for Persistency (P) it was tentatively concluded that musk xylene is (very) persistent in marine water, since the half-life for biodegradation in seawater is more than 150 days (see section 2.3.2). This conclusion was agreed on in the TCNES PBT-subgroup in May 2007. The industry was given the opportunity to provide additional information about transport through air, however no such information was submitted within the timeframe set. Therefore, the PBT working group factsheet was transformed into a draft addendum to the RAR (EC, 2005) and presented as such for adoption by the Technical Committee on New and Existing Substances (TC NES) in September 2007.

At the TCNES meeting in September 2007, the industry stated that the influence of photodegradation is underestimated, which was supported by several member states. However, this point of discussion will not be addressed for musk xylene specifically as agreed upon during the TCNES, but will be addressed in a more general approach with the development of new guidance on this subject. In addition, the industry stated that the current TDG (2003) indicates that substances classified as Carcinogenic Category 3 need to be reviewed on a case-by-case basis whether the evidence is sufficient for the substance to be considered as toxic. It was then decided that the addendum should be written as a stand-alone document with respect to the PBT-assessment, with a short motivation for B, a motivation for T and the discussion on P.

At the TCNES PBT-subgroup meeting in November 2007 it was indicated that the re-evaluation of the T-criterion was still under progress. Musk xylene is concluded to be borderline T based on human health toxicity (Carc. Cat. 3; R40), according to section 4.4.5.2 of the TGD (2003). Based on toxicity data for fresh water and marine organisms, musk xylene is considered inconclusive with respect to T. The overall outcome of this assessment is that the substance is considered as borderline T (see section 4.4). Therefore also the B has been re-evaluated, taking the (newly provided) critical BCF-studies into account, with BCF-values above 5,000 l/kg, to see if next to the B criterium also the vB criterium is met.

This addendum summarises the new information regarding the persistency, bioaccumulation and the toxicity of the compound musk xylene. Based on the new information and the overall PBT/vPvB assessment the environmental risk assessment is revised.

1 ADDITIONAL INFORMATION ON PERSISTENCY

1.1 INTRODUCTION

In order to investigate the fate properties of musk xylene a number of (simulation) studies have been performed as listed in the table below. These studies have been evaluated by the rapporteur. Based on the test results together with additional environmental fate information and the use of a multimedia fate model, it has been evaluated whether musk xylene fulfils the P-criterion.

Summary of the test program:

Photolysis in water (sterile sea water)	- Photolytic rate and route of musk xylene degradation in water. - Fate of photolytic degradation products in water and water sediment systems in the dark.
Degradation in water	OECD 309, water phase test adapted to marine conditions.
Degradation in sediment/water	OECD 308, adapted to marine conditions.
Adsorption to marine sediment	OECD 106, adapted to marine conditions.

1.2 ABIOTIC DEGRADATION

1.2.1 Photolysis in water

A photolysis study with musk xylene was performed in sterilized natural seawater under simulated sunlight conditions. Recoveries were generally between 90 and 110%. The half-life under laboratory conditions was 9.4 min. From this half-life under laboratory conditions a quantum yield was calculated. Based on this quantum yield an extrapolation was made to the environment. The estimated half-lives at 50°N are 2.8 d in spring, 1.8 d in summer, 5.4 d in autumn and 12 d in winter. The calculated half-lives are day-averaged values. The formula used to calculate the environmental degradation rate is, however, only valid for shallow water depths under clear sky conditions (Hamwijk and Oldersma, 2006).

Similar photolysis rates were found in other studies. In one study 95% of musk xylene in water was converted after 60 minutes of irradiation ($\lambda > 280$ nm) (Zhao and Schwack, 1999). This corresponds to a half-life in the order of 10 minutes. In another study the half-life of musk xylene irradiated by an UV lamp ($\lambda > 265$ nm) was 2 minutes. The light intensity used was about ten times that of natural daylight in northern Germany in midsummer at midday under cloudless conditions (Butte *et al.*, 1999).

In the study by Zhao and Schwack the reaction pathway and transformation rates were studied in different media. The solvents used were cyclohexane, methanol, cyclohexene, and water resulting in half-lives of musk xylene of 8.3 hours, 4.1 hours, 51 minutes, and approximately 10 minutes, respectively. In the inert solvent cyclohexane the slowest photolysis rate was observed. This solvent has no influence on the intramolecular reaction of the nitro group with the tert-butyl group. In methanol both inter- and intramolecular reactions occur. Cyclohexene has hydrogen donor properties, and therefore, photodegradation in this solvent is much faster. In water the same single reaction product was found as in cyclohexane. However, the reaction in water is much faster (Zhao

and Schwack, 1999). From this observation it can be concluded that the photodegradation process in water is mediated by the hydrogen donating and accepting properties of the solvent water.

Conclusion

Environmentally relevant exposure to musk xylene occurs in the whole water column. Photodegradation of musk xylene could be a relevant removal pathway in the environment, but its relevancy should be evaluated as a general issue, which has to be covered in new guidance to be developed in the near future. At this moment, aquatic photodegradation in general is considered to have no relevant impact on the overall persistency of musk xylene in the environment.

1.2.2 Oxidation and photolysis in air

The program AOPwin (U.S. Environmental Protection Agency, 2007) calculates a half life of 12.8 days for degradation of musk xylene in air based on intermolecular reactions with hydroxyl radicals or ozone. In a discussion paper attention is drawn to the fact that the program AOPwin is not suitable to predict the half-life of a substance that is subject to an intramolecular reaction (Van Bergen and Theewis, 2006). The half-life calculated by AOPwin will therefore probably be a worst-case estimate. However, given the fact that no solvent is present in air makes the reaction rate in air probably more comparable to the reaction rate in the apolar solvent cyclohexane instead of that in water, where the reaction is accelerated by the hydrogen donating and accepting properties of the solvent. Given the fact that the half-life in cyclohexane is 8.3 hour under continuous irradiation, it can be assumed that intramolecular transformation in air will not result in a half-life in air shorter than 1 day.

Conclusion

The half-life of musk xylene in air is expected to be in the range of 1 to 12.8 days.

1.3 BIOTIC DEGRADATION

1.3.1 Screening data

Musk xylene is not readily biodegradable. In a MITI I test (BOD) with 107 mg/l musk xylene (Calame and Ronchi, 1989) or in a CO₂ evolution test with 10 and 100 µg/l musk xylene (Marks and Marks, 1987) no degradation was observed.

Nevertheless, monitoring data of musk xylene in the influent and effluent of sewage treatment plants (STPs) showed that the removal was rather efficient (~95%) indicating that (partial) degradation might occur as one of the removal processes (Simonich et al., 2000). In several other studies amino derivatives were observed in sewage treatment samples, indicating that anaerobic reduction of the nitro groups occurs in STPs (Gatermann et al., 1998; Herren and Berset, 2000; Rimkus et al., 1999).

1.3.2 Simulation studies

In a GLP study with radiolabelled musk xylene the degradation rate in both a marine water sediment system (according to OECD guideline 308) and a marine water-only system (according to OECD guideline 309) were determined (Hanstveit, 2006b). Both tests were performed under dark conditions at 15±2 °C.

Marine sediment-water test (OECD 308)

The quality of the marine water-sediment test is good (valid without restrictions).

The water sediment ratios were 3:1 to 4:1. Two sediments were used for the test, both collected in the Scheldt delta area in the Netherlands, which receives water from the North Sea. One of the sediments (collected at Colijnsplaat) is silty fine sand sediment with an organic matter content of ca. 3.2% and a silt clay fraction of ca. 45%. The other sediment (collected at Zandkreekdam) is fine sand sediment with an organic matter content of ca. 1.2% and a silt clay fraction of ca. 14%. Both sediment-water systems had a salinity of 30‰.

Similar to the water-only system the substance was added from a stock solution in filtered sterile seawater. After addition of the stock solution, musk xylene partitioned within one week to the sediment. Only 10% of the total applied radioactivity remained in the aqueous phase in the system with 3.2% organic matter (Colijnsplaat) and 15% remained in the aqueous phase of the system with 1.2% organic matter (Zandkreekdam). These levels stayed constant during the remainder of the test.

The sediments were extracted each time with 100 ml of acidified water (0.01 M H₂SO₄), hexane, hexane/ethylacetate (1:1) and ethyl acetate, successively. The amount of radioactivity that is not extracted with this procedure is considered as non-extractable bound residue. The extraction method had proven to be a suitable method for the extraction of musk xylene from sediment. Additional extraction with EDTA in the samples from the Colijnsplaat system showed that some of the radioactivity (3-6%) could be recovered in this way. This suggests that some musk xylene or one of its metabolites is strongly but reversibly bound, probably to the clay particles within the sediment (Hanstveit, 2006c).

The total recovery of radioactivity was between 90 and 110% for the Zandkreekdam system. For the Colijnsplaat system the recovery was between 80 and 90% from day 28. This might be related to a less efficient determination of the amount of bound residue. In the accompanying letter (Hanstveit, 2006c) it is stated that this might be due to the fact that the combustion of clay is less efficient and that part of the bound residue is sorbed to clay.

The parent compound disappears from the system by formation of bound residue, biodegradation and volatilization. The latter process has a relatively minor contribution (<6% at all sampling times and most pronounced in the Zandkreekdam system). The individual contribution of these processes can not easily be distinguished. The dissipation of the summed concentration for the parent compound and the main metabolite (probably 4-amino musk xylene, see below) from the sediment is 4.6 days for the Colijnsplaat sediment and 16.1 days for the Zandkreekdam sediment.

For the Zandkreekdam system the amount of bound residue remains constant at 30.4±1.6% between days 61 and 176, which means that this route of dissipation is not interfering within this timeframe. The extractable parent compound in the sediment decreases exponentially from 7.6 to 2.4% in this timeframe. This corresponds with a half-life in the order of 60 days, which can be attributed to biodegradation. However, in the Colijnsplaat system the formation of metabolites appears to be much faster. After 7 days already, the ratio of the main metabolite (probably 4-amino musk xylene) and the parent compound is 1. From day 28 only minor amounts (≤ 1.1%) of parent compound could be extracted from the sediment.

Besides a number of minor degradation products, the major degradation product formed is probably 4-amino musk xylene, based on retention times, although the identification has yet to be confirmed by LC/MS. In Zandkreekdam sediment where the formation of the main metabolite was much higher than in Colijnsplaat sediment, the main metabolite seems rather stable.

In both systems a low percentage of carbon dioxide was formed (8% in Colijnsplaat system and 2% in Zandkreekdam system), indicating that some mineralization took place.

The fact that the transformation of musk xylene took place via a reduction step leading to the formation of 4-amino musk xylene, suggests that the degradation of musk xylene mainly took place in the anaerobic part of the sediment. This would explain the results from water degradation study that under aerobic conditions musk xylene is persistent.

In both tests systems a significant amount of bound residue was observed. In the organic rich Colijnsplaat sediment already after 7 days more than 58% (non extractable) bound residue was formed which remained more or less constant (about 60%) up to the end of the experiment. In the sandy Zandkreekdam sediment the bound residue increased from 13% (at day 7) to 32% by the end of the experiment (day 176). Whether the formation of bound residue should be considered as dissipation is still subject of discussion. In view of the high recovery after one day of incubation, the extraction method is considered to be appropriate to remove the maximum of musk xylene and metabolites adsorbed to sediment. In an attempt to remove material that is bound to clay particles/minerals in sediment, an additional extraction with EDTA was performed. This resulted in additional extraction of <6% of the total radioactivity. The amount of radioactivity in the extracts was too low for further analysis by HPLC, if possible at all with an EDTA solution, so it is not known which compounds were present. Overall, based on these considerations, the bound residue fraction could be considered as loss and not be accounted for as parent compound.

Ocean die-away test (OECD 309)

The quality of the performed water-only test is good (valid without restrictions).

The test was performed with water collected at Colijnsplaat, Scheldt delta area, the Netherlands. This location receives its water mainly from the North Sea. The water had a salinity of 30‰. The substance was added from a stock solution in sterile, filtered (0.22 µm) seawater. The total recovery of radioactivity was in two individual samples slightly below 90% and in all other samples between 90 and 110%. A reference test with benzoic acid resulted in more than 60% degradation within 14 days.

In the test, it appeared that in the testing period of 159 days most of the amount of musk xylene in water is volatilized with only 10-15% of the total radioactivity remaining in the water phase from day 99 and onwards. The radioactivity in the gas phase trapped in paraffin coated glass wool could be completely attributed to the parent compound. Only trace amounts of radiolabelled carbon dioxide trapped in a soda-lime column could be detected.

In the water phase musk xylene and two metabolites, most likely two amino-musk xylene metabolites, could be detected. The amount of the parent compound in the water phase after 159 days was still relatively high (i.e. 46% relative to the total amount of radio activity present in the water phase). The metabolites did not volatilize, because in the gas phase only parent musk xylene was detected. The metabolites were not mineralized, because carbon dioxide was only formed in trace amounts at the promille level.

Also a sterile control was included. For this purpose the seawater was autoclaved for 15 minutes at 119 °C and filtered over a 0.45 µm filter. The absence of any significant biodegradation in the water phase is underlined by the fact that the sterile control shows the same distribution pattern, both expressed as total radioactivity and as parent compound, as the non-sterile samples.

1.4 ENVIRONMENTAL DISTRIBUTION

For musk xylene the parameters relevant for the environmental distribution are summarized below:

- Vapour pressure: 0.00003 Pa at 25 °C
- Solubility: 0.15 mg/l at 25 °C

- Organic-carbon partition coefficient: 15500 L/kg (Hanstveit, 2006a)
- Log Kow: 4.9

Together with degradation rate constants in air, water, sediment, and soil, the distribution of musk xylene can be calculated with a multimedia fate model (SimpleBox v.3.01, which has been incorporated in EUSES). The overall environmental distribution of musk xylene is strongly dependent on the degradation rate constants in air and especially in water.

Assuming half-lives of 1 day in air, 1 year in water, 15 days in sediment and 10 years in soil and only local emissions to the fresh water compartment, the following conclusions can be drawn. Musk xylene appears to be transported mainly by advective transport through water (river discharge). Besides that, advective transport through air is also substantial (27% of the total inflow at continental level, 12% at moderate global scale). The exact ratio between the two processes is dependent on the chosen rate constants. However, within the expected range it appears that a significant amount of the total hold-up of musk xylene (tens of percentages) will reach the sea and ocean water compartments (continental and global scale). With the settings described above this is 29% of the total global mass of the substance. The amount in fresh surface water is accounting for another 19%. The mass fraction in soil is 40% and in sediments 10%. If the half-life in air would be 12.9 days, the percentage in the sea and ocean water compartment would be considerably higher.

1.5 CONCLUSION REGARDING PERSISTENCY

The extractable part of musk xylene in sediment is subject to anaerobic degradation with half-lives of equal to or below 60 days. Musk xylene is therefore considered to be not persistent in sediment. In this assessment the observed irreversible binding to sediment is considered as dissipation.

Given the fact that the metabolites in the ocean die-away test stayed in the water phase while the parent compound musk xylene volatilized and the fact that the ratio metabolites : parent compound was still close to one after 159 days, it is concluded that the half-life for biodegradation in seawater is more than 150 days, which significantly exceeds the criterion of 60 days. Musk xylene is therefore considered to be very persistent in water.

Because sea and ocean water are compartments with a significant hold-up of the total amount of musk xylene, musk xylene should be regarded as fulfilling both the P and vP criterion

2

INFORMATION ON BIOACCUMULATION

2.1

DESCRIPTION OF KEY STUDIES

Several bioaccumulation studies with musk xylene in fish have been performed as reported in the RAR (EC, 2005). The resulting BCF values ranged from 640 to 6,740 l/kg ww. For the purpose of the assessment of B/vB, this section focuses on the most critical study resulting in BCFs above 5,000 originating from the MITI database (1992), as cited by Geyer et al. (1994). The study has now been provided by the Japanese Ministry of Economy, Trade and Industry (METI) and evaluated in detail. It should be noted that in the study of Kuhlmann et al. as cited by Rimkus et al. (1997) a maximum BCF was calculated of 5,100 l/kg ww, which is the only study next to the study mentioned by Geyer et al. (1994) resulting in a BCF-value above 5,000 l/kg, although that study of Kuhlmann et al has never been published.

Geyer et al. (1994) cites a reference (MITI, 1992) as mentioned in the RAR. These BCF studies reported in summary on the website of the Japanese National Institute of Technology and Evaluation (NITE) have been evaluated based on the original study reports (in Japanese), which were obtained from METI. The studies with musk xylene comprise two individual studies both performed with carp (*Cyprinus carpio*).

The first test was carried out using a flow-through system in 100-l glass aquaria and a flow rate of 1152 l/day (800 ml/min). Musk xylene was tested at two concentrations, 1 µg/l and 10 µg/l. Both dispersing agents (HCO-10 and HCO-60) were present at 25 and 250 µg/l, at the test concentrations of 1 and 10 µg/l, respectively. The test fish had an average body weight of 37.0 g, and average body length of 11.0 cm and a lipid content of 3.4%. The temperature was 25±1 °C. No further detail on the test item and the used water were provided. Fish were monitored in duplicate at 1, 2, 4, 6, 8, and 10 weeks after the start of the exposure. The water concentration was monitored twice a week. Further, remaining fish were transferred to clean water after 10 weeks of exposure. Fish from this depuration phase were monitored after 3 (duplicate) and 7 days (individual fish). The used analytical method was GC-ECD (gas chromatography with electron capture detector), which enables the analysis of the parent compound.

Water concentrations were fairly constant (**Figure 1**). Average concentrations were 0.91±0.12(s.d.) µg/l at the lower concentration and 8.65±0.70(s.d.) µg/l at the higher concentration. The lowest water concentrations were observed during the first few days. With a one phase exponential model the plateau values were slightly higher than the average values that include the first two days when partial depletion was observed. The plateau values can be considered as the average concentrations during the remainder of the uptake phase. Consequently these values have been used to calculate the BCF values. The plateau concentrations were at 0.94±0.02(s.d.) µg/l at the lower concentration and 8.77±0.68(s.d.) µg/l at the higher concentration.

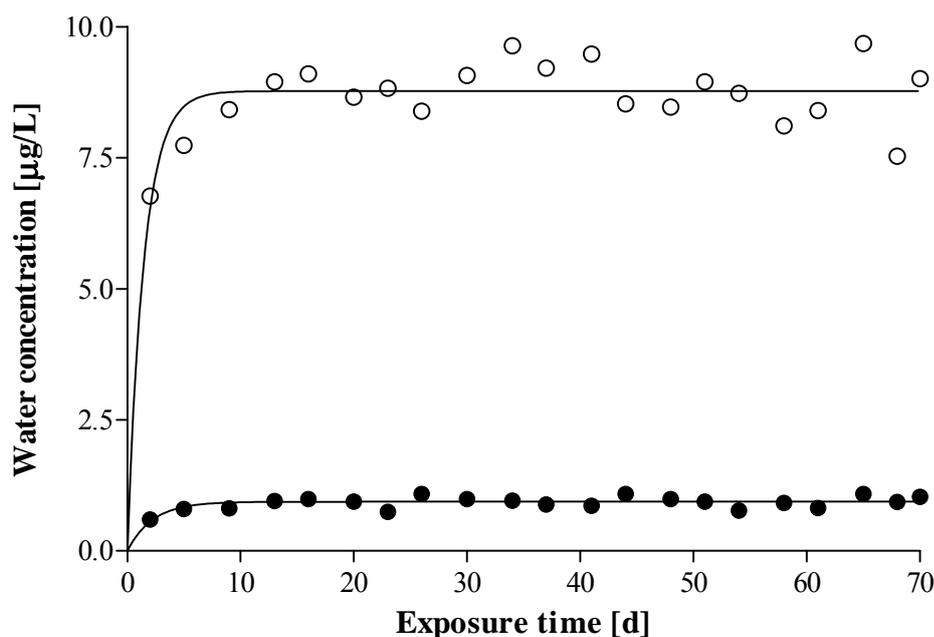


Figure 1: Water concentrations in a flow-through study with musk xylene at nominal concentrations of 1 (●) and 10 (○) µg/l.

The concentrations in fish increased over the exposure period (**Figure 2**). However, in the high concentration the concentrations in fish were highly variable. In the low concentrations, this variability was not observed. Accumulated concentrations did not reach equilibrium in the 10 weeks of exposure to the low concentration. The plateau levels, estimated by a one compartment model, were 9.89 and 32.7 mg/kg bw. The BCF values that result from the concentrations in water and in fish extrapolated to equilibrium are 3,730 and 10,500 l/kg for the high and low concentration respectively.

BCF values calculated from non-steady state concentrations in individual fish in the nominal water concentration of 10 µg/l were mostly higher than 2,000 L/kg from 4 weeks of exposure and beyond and exceeded the value of 5000 l/kg in one occasion. For the lower concentration of 1 µg/l, a BCF of 2,000 l/kg was already reached after two weeks and all data for a longer period of time exceeded the value of 2,000 l/kg. Both values at 10 weeks of exposure exceeded the value of 5,000 l/kg.

In the depuration phase (**Figure 2**), musk xylene was excreted with estimated half-lives of 4.2 and 2.8 days in the high and low concentrations, respectively. However, the number of data for the depuration phase are very limited and the variability in the data is rather high, especially in the high concentration.

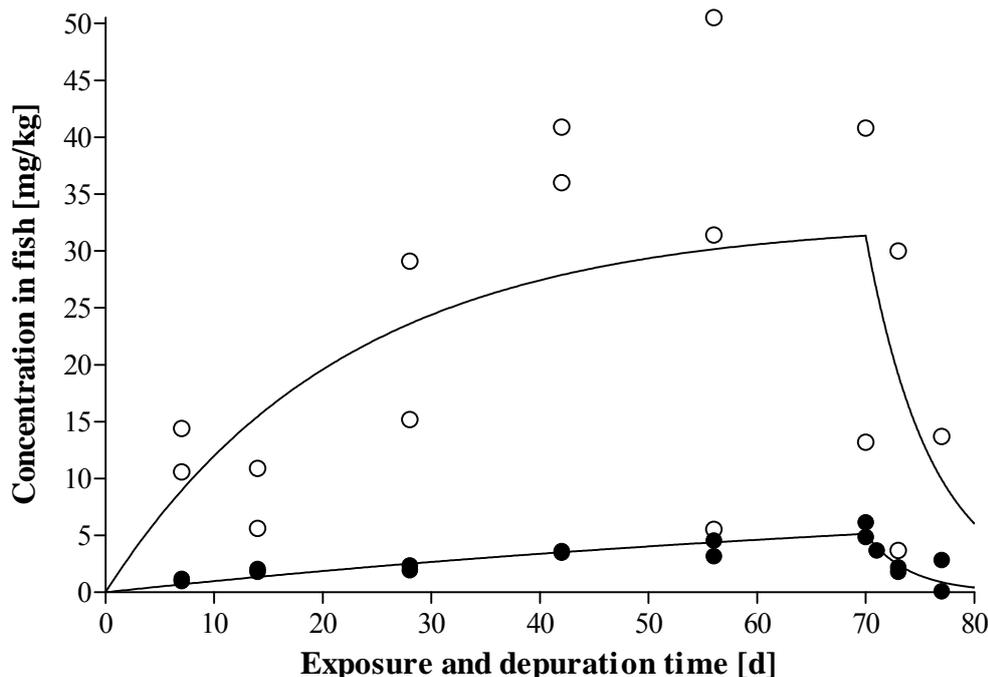


Figure 2: Whole body fish concentrations in a flow-through study with musk xylene at nominal concentrations of 1 (●) and 10 (○) µg/l.

In a second test, the BCF test at the low concentration of 1 µg/l was repeated. Experimental conditions were essentially the same. The flow rate was 1164 l/day (800 ml/min) in 100-l glass aquaria. Concentrations of the dispersing agents, temperature and analytical methods were the same as well. The test fish had an average body weight of 32.6 g, and average body length of 10.9 cm and a lipid content of 5.1%. No further detail on the test item and the used water were provided. Fish were monitored in triplicate at 4, 6, 8, 10, and 12 weeks after the start of the exposure. The water concentration was monitored twice a week. Remaining fish were transferred to clean water after 12 weeks of exposure. Fish from this depuration phase were monitored in triplicate after 2, 4, 8, and 14 days. The used analytical method was again GC-ECD.

Water concentrations were 0.89 ± 0.15 (s.d.) µg/l. Concentrations in the first three weeks were somewhat lower than in the first experiment but stabilized to the same plateau value of 0.94 ± 0.02 (s.d.) µg/l afterwards (**Figure 3**). This values has been used to calculate the BCF value.

The concentrations in fish only slightly increased over the exposure period, but were already relatively high at 4 weeks of exposure compared with the first study (Error! Reference source not found.). The plateau level, estimated by a one compartment model, was 4.75 mg/kg bw in contrast to 9.89 mg/kg bw from the first study. The BCF value that results from the concentrations in water and in fish extrapolated to equilibrium is 5,030 l/kg. BCF values calculated from non-steady state concentrations in individual fish were all higher than 2,000 l/kg at all exposure times and exceeded the value of 5,000 l/kg in 6 of 18 cases and in 5 out of 9 in the last three sampling times.

In the depuration phase (Error! Reference source not found.), musk xylene was excreted with estimated half-lives of 5.9 days. The variability in the data is again rather high.

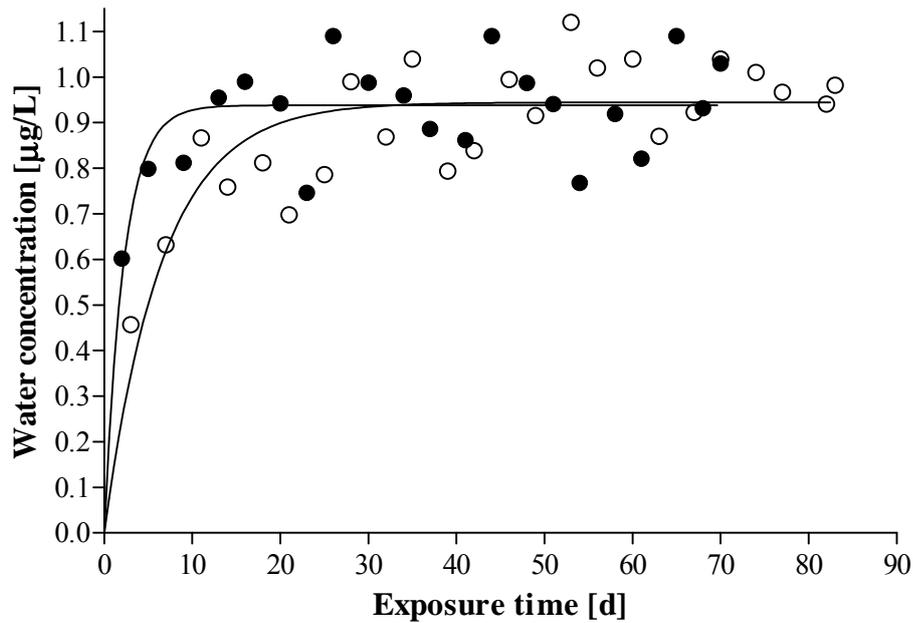


Figure 3: Water concentrations in two individual flow-through studies (● first study and ○ second study) with musk xylene at a nominal concentration of 1 µg/l.

BCF values for the individual samples at the different time intervals were calculated from the concentration in fish divided by the concentration in water estimated at that time interval as explained above. These values are slightly lower than the BCF values reported in the original studies, because in there the average water concentration over the relevant time interval were used to calculate the BCF. The BCF values for the individual samples are presented in

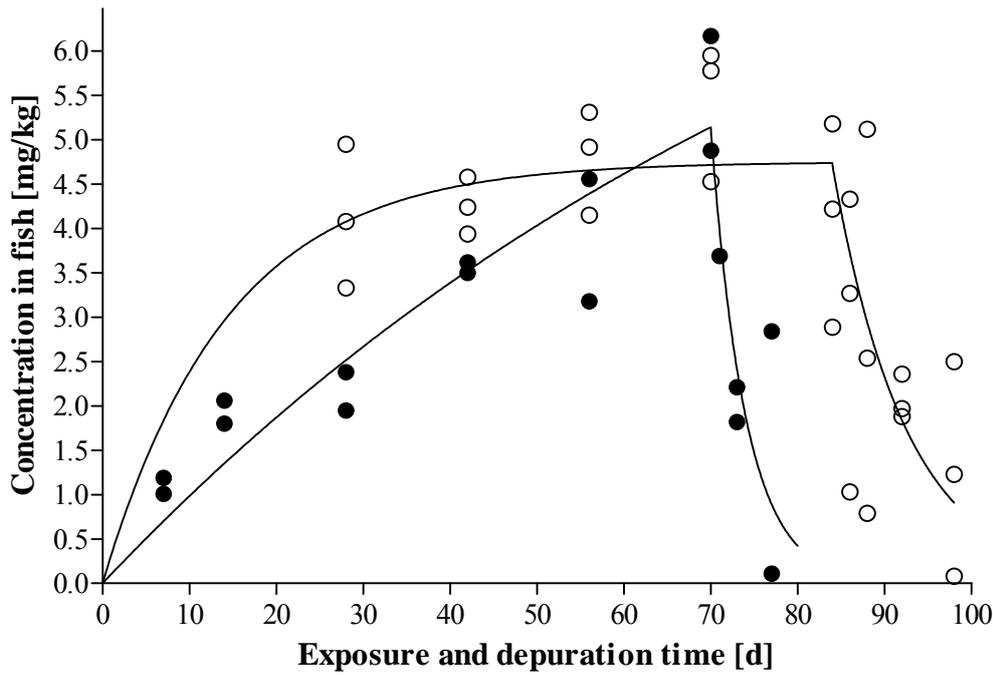


Figure 4: Whole body fish concentrations in two individual flow-through studies (● first study and ○ second study) with musk xylene at a nominal concentration of 1 µg/l.

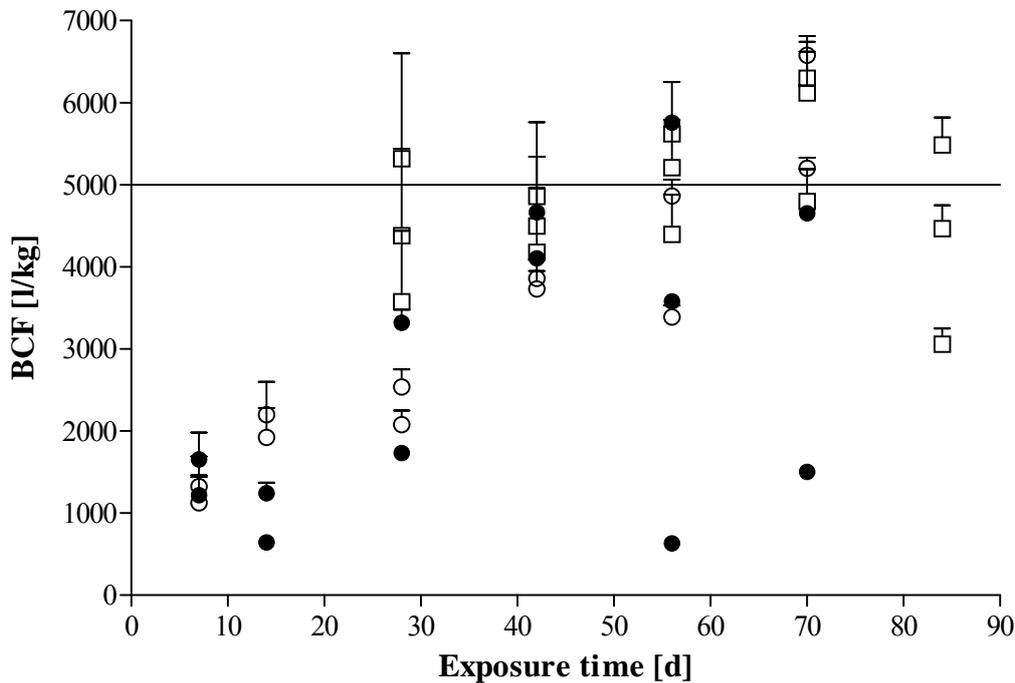


Figure 5: BCF values calculated for the individual fish samples (● 10 µg/L, first study; ○ 1 µg/L, first study; □ 1 µg/L, second study). Bars represent the difference with the values reported in the original studies.

It should be noted that these values are not normalised to lipid weight. In the study from Kuhlmann et al. mentioned by Rimkus et al. (1997) the fish used had a lipid content of 3-4%. In the first Japanese study the average lipid content was 3.4%. If data would be normalised to 5% lipid content the BCF values from both studies would amply exceed the value of 5,000.

Further, an additional study with bioaccumulation factors of musk fragrances in a sewage treatment pond was evaluated (Gatermann et al. 2002). Five aquatic species were analyzed. These were rudd (*Scardinius erythrophthalmus*), tench (*Tinca tinca*), crucian carp (*Carassius carassius*), eel (*Anguilla anguilla*), and zebra mussel (*Dreissena polymorpha*). The values for BAF based on wet weight obtained for musk xylene were 290, 2,400, 7,500, 40,000, and 1,800 l/kg for these five species respectively, corresponding to BAF values based on lipid weight of 32,000, 250,000, 328,000, 240,000, 130,000 l/kg, respectively. These values strongly contrast with the other studied musk fragrances musk ketone, HHCB, and AHTN for which much lower BAF values were calculated (see Table 5 in Gatermann et al. 2002). This finding is a strong indication of the very high bioaccumulation of musk xylene.

No experimental data are available on accumulation in earthworms.

2.2 CONCLUSION REGARDING BIOACCUMULATION

The experimental bioaccumulation studies for musk xylene in fish showed a wide range of BCFs, among which values above the vB criterion of 5,000 l/kg. Based on the evaluation of the critical study and its results it can be concluded that musk xylene is very bioaccumulative.

3 INFORMATION ON TOXICITY

3.1 AQUATIC COMPARTMENT

Results of toxicity tests with aquatic organisms are presented in Table 3.7 of the RAR on musk xylene (EC, 2005). For the purpose of the assessment of T, this section focuses on the most critical study, the study for which NOEC values at and below 10 µg/l are reported by Carlsson and Norrgren (2004). Because the study is considered to be a multi-stress experiment comprising starvation and the impact of the toxicant, the results are considered to be inconclusive with respect to the screening of Toxicity (T) for the purpose of the PBT assessment.

3.2 CARCINOGENICITY

A literature search was performed over the period 2002 until now to determine whether additional information is available on the carcinogenicity of musk xylene. One relevant publication (Apostolidis et al., 2002) was identified. Male NRMI mice were treated intraperitoneal with a single injection of lipopolysaccharide (125 µg/mouse) to activate the macrophages on day 0. Groups of mice (group size unknown) were treated on day 4 with a single intraperitoneal injection with musk xylene at a dose of 0, 100, 500 or 1000 mg/kg bw in combination with 100 ng 12-O-tetradecanoylphorbol-13-acetate (TPA) per mouse. Residential macrophages were collected by peritoneal lavage at 4 days after treatment and cultured in soft agar for 5-6 days and the frequency and size of the colonies were determined. Musk xylene induced a significant and dose-dependent increase in size and frequency of clones of transformed cells revealing a cell-transforming potential. Permanent cell lines developed from the transformed cells induced local tumours within three weeks after a subcutaneous injection in athymic nu/nu mice. As this is a non-OECD and not fully validated test system, the relevance of the test is doubtful and the results cannot be used for risk and hazard assessment. This study does not affect the previous conclusions on risk assessment and hazard assessment.

In conclusion, no additional information has been published since 2002 on the human toxicology of musk xylene which would change the previous conclusions on risk and hazard assessment.

3.3 ENDOCRINE INTERACTIONS

A literature search was performed over the period 2002 until now to determine whether additional information is available on the endocrine disrupting properties of musk xylene. No new information was found.

In the RAR (EC, 2005) it is stated that musk xylene and not 2-amino- and 4-amino-musk xylene, was demonstrated to be a very weak agonist in the E-screen assay (Bitsch et al., 2002). Binding to the estrogen receptor from rainbow trout and clawed frog showed binding of 2-amino- and 4-amino-musk xylene, and not for musk xylene itself (Chou and Dietrich, 1999). These results are in conflict with each other. Furthermore, this 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. It can be concluded that there is no substantiated evidence that musk xylene can cause endocrine disrupting effects.

3.4 CONCLUSION REGARDING TOXICITY

Although that some NOECs for a specific aquatic toxicity test were found to be at or below the threshold value of 10 µg/l, these results were considered inconclusive with respect to the screening of Toxicity (T) for the purpose of the PBT assessment. It can be concluded that there is no substantiated evidence that musk xylene can cause endocrine disrupting effects. However, musk xylene is classified as Carcinogenic Category 3), although it is realised that it is a borderline case. The overall conclusion for musk xylene is that regarding toxicity it is considered to be a borderline case. For a more thorough evaluation of T, a new FELS-test (according to OECD 210) could be considered as an option.

RISK CHARACTERISATION, CONCLUSION OF THE PBT ASSESSMENT

Musk xylene is considered to be not persistent in sediment. The half-life due to biodegradation in two estuarine sediments is estimated to be 60 days or less, based on the extractable part in the sediment phase. In this interpretation of the results of the simulation studies the observed irreversible binding to sediment is considered as dissipation. Musk xylene seems to degrade under anaerobic conditions.

Musk xylene is considered to be very persistent in water. In estuarine water, the half-life of musk xylene due to biodegradation appears to be longer than 150 days. The dissipation in the water simulation study can almost completely be attributed to volatilisation from the system (as the parent compound). When irradiated, musk xylene is subject to photolysis. This process appears to be rather efficient. However, its relevancy should be evaluated as a general issue, which has to be covered in new guidance to be developed in the near future. For the time being this route of degradation is considered to have no relevant influence on the overall persistence of musk xylene in the environment.

The estimated half-life in air due to reaction with radicals is estimated to be in the order of 13 days. This half-life might be an overestimation of the half-life in air because photolysis can be a substantial degradation pathway as well and this process is not taken into account in the estimation of the half-life. In water this process is very fast but in apolar solvents the half-life for photolysis is in the order of six hours under constant irradiation with maximum solar intensity. Consequently, the half-life in air due to direct photolysis is estimated to be more than 1 day. As calculated by the multimedia model, musk xylene will evaporate to air, which is confirmed by the observations in both the water and the water/sediment simulation tests. Therefore, a significant long-range transport can not be excluded. Hence, significant amounts of musk xylene will reach the sea and ocean water compartments. For this reason the degradation rate in marine water becomes essential in determining the persistency of musk xylene. In a deep water column, in the absence of sunlight and sediment, musk xylene is persistent.

Next to very persistent musk xylene is also considered to be very bioaccumulative based on the results of the critical BCF-study in fish. Musk xylene can therefore be considered to be a vPvB substance. In addition, it should be noted that the substance is considered to be borderline T. For a more thorough evaluation of T, a new FELS-test (according to OECD 210) could be considered as an option. However, in view of the vPvB properties of musk xylene, no additional testing is required.

Conclusion

Musk xylene fulfils both the vP as vB criterion and is considered to be borderline T.

Conclusion of the PBT assessment

(x) iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion iii) is reached, because MUSK XYLENE is concluded to be a vPvB chemical. In addition, it should be noted that the substance is considered to be borderline T.

- Apostolidis S., Chandra T., Demirhan I., Cinatl J., Doerr H., Chandra A. 2002 Evaluation of the carcinogenic potential of two nitro-musk derivatives, musk xylene and musk tibetene in a host-mediated in vivo/in vitro assay system. *Anticancer Research* 22: 2657-2662.
- Bitsch N., Dudas C., Korner W., Failing K., Biselli S., Rimkus G., Brunn H. 2002, Estrogenic activity of musk fragrances detected by the E-screen assay using human mcf-7cells. *Arch Environ Contam Toxicol*. 43: 257-64.
- Butte W., Schmidt S., Schmidt A. 1999. Photochemical degradation of nitrated musk compounds. *Chemosphere* 38: 1287-1291.
- Calame R., Ronchi W. 1989. Musk xylene: determination of the ready biodegradability. Private communication to RIFM. Givaudan-Roure, Switzerland. Test report 33-89.
- Carlsson G., Norgren L. 2004. Synthetic musk toxicity to early life stages of zebrafish (*Danio rerio*). *Arch Environ Contam Toxicol* 46: 102-105.
- Chou Y.-J. and Dietrich DR. (1999). Interactions of nitromusk parent compounds and their amino-metabolites with the estrogen receptors of rainbow trout (*Oncorhynchus mykiss*) and the South African clawed frog (*Xenopus laevis*). *Toxicology Letters* 111, 27-36.
- European Commission, 2005. European Union Risk Assessment Report of 5-TERT-BUTYL-2,4,6-TRINITRO-M-XYLENE (MUSK XYLENE) (EUR 21506 EN).
- Gatermann R., Hühnerfuss H., Rimkus GG., Attar A., Kettrup A. 1998. Occurrence of musk xylene and musk ketone metabolites in the aquatic environment. *Chemosphere* 36: 2535-2547.
- Gatermann R., Biselli S., Hühnerfuss H., Rimkus GG., Hecker M., Karbe L. 2002. Synthetic musks in the environment. Part 1: Species-dependent bioaccumulation of polycyclic and nitro musk fragrances in freshwater fish and mussels. *Arch Environ Contam Toxicol* 42: 437-446.
- Geyer H. J., Rimkus, G., Wolf, M., Attar, A., Steinburg, C., and Kettrup, A. 1994. Synthetic Nitro Musk Fragrances and Bromocyclen - New Environmental Chemicals in Fish and Mussels as Well as in Breast Milk and Human Lipids, *Z. Umweltchem Ökotox* 61(1), 8-17.
- Hamwijk C., Oldersma H. 2006. A study on the direct photolysis of musk xylene (CAS # 81-15-2) in sterilized seawater using [ring-U-14C] musk xylene (Setac, OECD draft). Zeist, The Netherlands: TNO Quality of Life. V5592/02.
- Hanstveit R. 2006a. A study on the adsorption of [14C]musk xylene in two marine sediment types (modified OECD 106, and 95/36/EC). Zeist, The Netherlands: TNO Quality of Life. V5592/05.
- Hanstveit R. 2006b. Determination of the degradation of [14C]-musk xylene (CAS # 81-15-2) in marine water/sediment systems and in seawater (OECD guideline 308 and 309). Zeist, The Netherlands: TNO Quality of Life. V5592/03 Interim report.
- Hanstveit R. 10 October 2006c Letter to Theewis J., Zeist, The Netherlands, Extraction procedure MX .
- Herren D and Berset JD. 2000. Nitro musks, nitro musk amino metabolites and polycyclic musks in sewage sludges. Quantitative determination by HRGC-ion-trap-MS/MS and mass spectral characterisation of the amino metabolites. *Chemosphere* 40: 565-574.
- Maekawa A., Matsushima Y., Onodera H., Shibutani M., Ogasawara H., Kodama Y., Kurokawa Y. and Hayashi Y. 1990. Long-term toxicity/carcinogenicity of the musk xylol in B6C3F1 mice. *Food Chem. Toxicol.*, 28, 581-586.
- Marks K, Marks P. 1987. Biodegradation of the test substances (X0438.01R, musk xylene) and controls in activated sludge. Private communication to RIFM. Weston, USA. Project No. 87-009.
- Ministry of International Trade and Industry (MITI). 1992. MITI-List, Tokyo, Japan.
- National Institute of Technology and Evaluation (NITE) 2005. Chemical Risk Information Platform (CHRIP). Available at <http://www.safe.nite.go.jp/english/db.html>.
- Rimkus GG., Butte W., Geyer HJ. 1997. Critical considerations on the analysis and bioaccumulation of musk xylene and other synthetic nitro musks in fish. *Chemosphere* 35: 1497-1507.
- Rimkus GG., Gatermann R., Hühnerfuss H. 1999. Musk xylene and musk ketone amino metabolites in the aquatic environment. *Toxicology Letters* 111: 5-15.

Simonich SL., Begley WM., Debaere G., Eckhoff WS. 2000. Trace analysis of fragrance materials in wastewater and treated wastewater. *Environmental Science and Technology* 34: 959-965.

TGD (2003). Technical Guidance Documents in support of Commission Directive 93/67/EEC on Risk assessment for new notified substances, Commission Regulation (EC) 1488/94 on Risk assessment for existing substances and Directive 98/8/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market.

U.S. Environmental Protection Agency. 2007. AOPwin [computer program]. Version v1.92.

Van Bergen TJ., Theewis JG. 2006. Photochemical behaviour of musk xylene.

Zhao X., Schwack W. 1999. Photodegradation of musk xylene. *International Journal of Environmental Analytical Chemistry* 74: 179-189.

An environmental risk assessment of musk xylene produced in accordance with Council Regulation (EEC) 793/93 was published in 2005. This report provides an Addendum to the risk assessment report dealing with the PBT assessment.

It is concluded in the Addendum that musk xylene is a very persistent and very bioaccumulative substance. This should be further considered under the REACH Regulation.

European Commission

EUR 24607 EN – Joint Research Centre – Institute for Health and Consumer Protection

Title: PBT Assessment of 5-Tert-Butyl-2,4,6-Trinitro-M-Xylene (Musk Xylene) Addendum to the Final Risk Assessment Report

Author(s): VERBRUGGEN Eric, SIJM Dick, VAN IERSEL Peter, SCHWARZ-SCHULZ Beatrice, OLSSON Heidi, PAYA PEREZ Ana

Luxembourg: Office for Official Publications of the European Union

2010 –24 pp. – 17.0 x 24.0 cm

EUR – Scientific and Technical Research series – ISSN 1831-9424

ISBN 978-92-79-18438-3

doi 10.2788/45153

Abstract

The report provides the PBT assessment of the substance 5-tert-butyl-2,4,6-trinitro-m-xylene (musk xylene). It has been prepared by The Netherlands in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to humans and the environment, laid down in Commission Regulation (EC) No. 1488/94. In 2005 the environmental risk assessment for musk xylene concluded that there was a need for further information and/or testing because the substance is considered to be a PBT candidate chemical and a further PBT – testing strategy was proposed.

Conclusion regarding persistency: the extractable part of musk xylene in sediment is subject to anaerobic degradation with half-lives of equal to or below 60 days. Musk xylene is therefore considered to be not persistent in sediment. In this assessment the observed irreversible binding to sediment is considered as dissipation.

Given the fact that the metabolites in the ocean die-away test stayed in the water phase while the parent compound musk xylene volatilized and the fact that the ratio metabolites : parent compound was still close to one after 159 days, it is concluded that the half-life for biodegradation in seawater is more than 150 days, which significantly exceeds the criterion of 60 days. Musk xylene is therefore considered to be very persistent in water.

Conclusion regarding bioaccumulation: The experimental bioaccumulation studies for musk xylene in fish showed a wide range of BCFs, among which values above the vB criterion of 5,000 l/kg. Based on the evaluation of the critical study and its results it can be concluded that musk xylene is very bioaccumulative.

Conclusion regarding toxicity: there is no substantiated evidence that musk xylene can cause endocrine disrupting effects. However, musk xylene is classified as Carcinogenic Category 3), although it is realised that it is a borderline case. The overall conclusion for musk xylene is that regarding toxicity it is considered to be a borderline case. However, in view of the vPvB properties of musk xylene, no additional testing is required.

It is concluded in the Addendum that musk xylene is a very persistent and very bioaccumulative substance and is considered to be borderline T. This should be further considered under the REACH Regulation.

How to obtain EU publications

Our priced publications are available from EU Bookshop (<http://bookshop.europa.eu>), where you can place an order with the sales agent of your choice.

The Publications Office has a worldwide network of sales agents. You can obtain their contact details by sending a fax to (352) 29 29-42758.

The mission of the JRC is to provide customer-driven scientific and technical support for the conception, development, implementation and monitoring of EU policies. As a service of the European Commission, the JRC functions as a reference centre of science and technology for the Union. Close to the policy-making process, it serves the common interest of the Member States, while being independent of special interests, whether private or national.

