Substance name: 5-tert-butyl-2,4,6-trinitro-m-xylene
(musk xylene)
EC number: 201-329-4
CAS number: 81-15-2

MEMBER STATE COMMITTEE

SUPPORT DOCUMENT FOR IDENTIFICATION OF 5-TERT-BUTYL-2,4,6-TRINITRO-M-XYLENE AS A SUBSTANCE OF VERY HIGH CONCERN

Adopted on 8 October 2008

CONTENTS

TABLES

FIGURES

Substance Name: 5-tert-butyl – 2,4,6 – trinitro-m-xylene (musk xylene)

EC Number: 201-329-4

CAS number: 81-15-2

The substance is identified as a vPvB substance according to article 57(e) of Regulation (EC) No 1907/2006 (REACH).

Summary of the evaluation:

Musk xylene is concluded to be a vPvB substance.

The Persistence (P) screening criterion is fulfilled for musk xylene. The results of two biodegradation tests clearly showed no (ready) biodegradability. In an ocean die-away test, the metabolites stayed in the water phase while the parent compound musk xylene volatilized. In addition, the ratio metabolites: parent compound was still close to one after 159 days, which shows no rapid degradation and therefore the half-life in water 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 that act as a sink for a significant fraction of the total amount of musk xylene, musk xylene should be regarded as fulfilling both the P and vP criterion.

The Bioaccumulation (B) screening criterion is fulfilled for musk xylene. Musk xylene has a log Kow of 4.9. 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 (vB).

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Chemical name: Musk xylene

EC Number: 201-329-4

CAS Number: 81-15-2

IUPAC Name: 1-tert-butyl-3,5-dimethyl-2,4,6-trinitrobenzene

1.2 Composition of the substance

Chemical name: Musk xylene

EC Number: 201-329-4

CAS Number: 81-15-2

IUPAC Name: 1-tert-butyl-3,5-dimethyl-2,4,6-trinitrobenzene

Molecular Formula: $C_{12}H_{15}N_3O_6$

Structural Formula:

Molecular Weight: 297.3

Typical proportion %: >99

Real proportion (range) in %: 99-100

1.3 Physico-chemical properties

Table1-1: Summary of physico- chemical properties

REACH ref Annex, §	Property	Value	
VII, 7.1	Physical state at 20º C and 101.3 kPa	Solid, powder	
VII, 7.2	Melting / freezing point	112-114˚C	Treff, 1926; Le Fèvre and Le Fèvre, 1935 ; Opfer-Schaum and Piristi, 1944
VII, 7.3	Boiling point	Not applicable	Tas and Van de Plassche, 1996; Givaudan, 1990.

VII, 7.5	Vapour pressure	0.00003 Pa at 20˚C	Grain, 1990; Tas and Van de Plassche, 1996
VII, 7.7	Water solubility	0.15 mg/l	Tas and Van de Plassche, 1996; Schramm et al., 1996
VII, 7.8	Partition coefficient n-octanol/water (log value)	4.9*	Rudio, 1996; Schramm et al., 1996; Tas and Van de Plassche, 1996; Johnson et al., 1984

* Recommended value based on test report

2 CLASSIFICATION AND LABELLING

2.1 Classification in Annex I of Directive 67/548/EEC

Classification

E, R2; Carc. Cat. 3, R40; N, R50/53

Labelling

E, Xn, N

R2, R40, R50/53

S2, S36/37, S46, S60, S61

2.2 Self classification(s)

Not applicable.

3 ENVIRONMENTAL FATE PROPERTIES

3.1 Degradation

3.1.1 Stability

From the EU RAR, section 3.1.1.1 (2005):

Abiotic degradation:

Studies on hydrolysis of musk xylene are not available. Based on the structure of the compound it is assumed that hydrolysis does not take place. According to Lyman et al. (1990) aromatic nitro compounds contain functional groups that are resistant to hydrolysis. Photolysis of musk xylene was studied by Butte et al. (1999). Under laboratory conditions using an UV immersion lamp, photolysis of musk xylene was observed in which an initial phase where the reaction followed first order kinetics (k: 0.344 minutes^{-1} and $t_{1/2}$: 2.0 minutes) was followed by a phase with a longer half life. Using GC/MS the metabolites 3,3,5,7-tetramethyl-4,6-dinitro-3H-indole and 3,3,5,7-tetramethyl-4,6-dinitro-2-indolinone were identified. Degradation was slower in an outdoor experiment in midsummer at midday under cloudless conditions (no results presented). Model estimation (SRC AOPWIN) of photodegradation for reaction with OH-radicals results in a half life of approximately 19 days when using the TGD OH concentration ($5 . 10^5$ molec.cm^{-3}/24 hours). It can be concluded on structural grounds that photolysis of musk xylene occurs. However, extrapolation of these results to a field situation is difficult, e.g. UV radiation intensity decreases with the depth of the water. In addition, in eutrophic surface waters algae and humic substances will adsorb most of the UV radiation (Kalf et al., 1995). The estimated DT50 for photodegradation for reaction with OH-radicals also indicates that this is not a major degradation route. Therefore, in the environmental risk assessment no photodegradation will be assumed.

From the PBT draft addendum (2008) to the final report of the risk assessment (2005):

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 50ºN 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 photodegration process in water is mediated by the hydrogen donating and accepting properties of the solvent water.

Conclusion: In the environment, musk xylene is distributed over the whole water column. At this moment, aquatic photodegradation is considered to have no relevant impact on the overall persistency of musk xylene in the environment.

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.

3.1.2 Biodegradation

3.1.2.1 Biodegradation estimation

From the EU RAR, section 3.1.1.1 (2005)

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

Simonich et al. (1998) measured fragrance material removal during activated sludge and trickling filter sewage treatment. From influent and effluent measurements they calculated a total removal of 98.7% for musk xylene. Simonich et al. (2000) and Sabaliunas et al. (2001) confirmed that the removal musk xylene within a STP is high i.e. app. 95%. The calculated removal is (again) based on influent and effluent measurements within both an activated sludge and trickling filter sewage treatment plant. This high removal rate indicates that besides adsorption also a biotransformation route (or routes) may be present. A plausible explanation for this could be that during an anaerobic phase of the sewage treatment a reduction of one or more of the nitro groups occur (expert judgement RIVM). Recently, Gatermann et al. (1998) and Rimkus et al. (sub.) presented measurements in influent and effluent of STPs, surface waters and biota for metabolites of musk xylene assuming that nitro musks will be transformed to the corresponding amino compounds. They

analysed and detected the 2-amino and 4-amino metabolites (chemical names: 1-tert-butyl-3,5-dimethyl-2-amino-4,6-dinitrobenzene and 1-tert-butyl-3,5-dimethyl-4-amino-2,6-dinitrobenzene), but were unable to detect diamino-musk xylene. Herren and Berset (2000) also detected amino metabolites of musk xylene in STP water. These data support in a qualitative way the findings of Simonich et al. (1998 and 2000) and Sabaliunas et al. (2001). Reduction of the nitro group is a well known transformation route for nitroaromatic compounds (Higson, 1992). It has for example been shown for the related chemical structure 2,4,6-trinitrotoluene (TNT), those white rot fungi or ectomycorrhizal basidiomycetes can degrade TNT (Gorontzy et al., 1994; Meharg et al., 1997). For musk xylene no such experimental data are available. However, the measurements described above show that reduction of nitro groups occurs for musk xylene in sewage treatment plants and fish.

3.1.2.2 Screening tests

From the EU RAR, section 3.1.1.1 (2005)

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

From the PBT draft addendum (2008) to the final report of the risk assessment (2005):

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 CO_2 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).

3.1.2.3 Simulation tests

From the PBT draft addendum (2008) to the final report of the risk assessment (2005):

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 ºC.

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 H_2SO_4), 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 volatization. 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 ºC 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.

3.1.3 Summary and discussion of persistence

From the PBT draft addendum (2008) to the final report of the risk assessment (2005):

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.

3.2 Environmental distribution

3.2.1 Adsorption/desorption

See section '3.1.1.2 Distribution' of the RAR for musk xylene

From the EU RAR, section 3.1.1.2 (2005)

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

$Koc = 1.26 . K_{ow}^{0.81}$ (eq. 1)

This results in the following partition coefficients:

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

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

3.2.2 Volatilisation

From the EU RAR, section 3.1.1.2 (2005)

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

3.2.3 Distribution modelling

From the EU RAR, section 3.1.1.2 (2005)

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

From the PBT draft addendum (2008) to the final report of the risk assessment (2005):

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

Vapour pressure: 0.00003 Pa at 25 ºC

Solubility: 0.15 mg/l at 25 ºC

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) SimpleBox (v. 3.01) is the multimedia model that has been included in EUSES. SimpleBox was used on its own because it also gives insight in the mass flows between compartments, whereas the EUSES output only provides information on the concentrations in each compartment and the relative contribution over the compartments.

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 12.8 days, the percentage in the sea and ocean water compartment would be considerably higher.

3.3 Bioaccumulation

3.3.1 Aquatic bioaccumulation

3.3.1.1 Bioaccumulation estimation

See section 3.3.1.2

3.3.1.2 Measured bioaccumulation data

From the EU RAR, section 3.1.1.2 (2005)

Bioaccumulation

The BCF can be calculated using the QSAR mentioned in the TGD: log BCF(wet weight) = 0.85 . log K_{ow} - 0.70. Using a log K_{ow} of 4.9 a BCF of 2900 L/kg is obtained. In addition to the calculated BCF a number of experimental data are available for musk xylene. These bioaccumulation studies, including their technical shortcomings, are discussed below.

Musk xylene was tested in bluegill sunfish (*Lepomis macrochirus*) (Paradice and Suprenant, 1984). Details of this test are given in Table 2. The radioactivity was determined in edible and non-edible portions of the fish, and a whole body concentration was calculated. The whole body concentration in fish was stable between 7 and 16 days of exposure, while the concentration in water showed some fluctuation. The uptake rate constant has not been calculated from the concentration in fish due to the rapid stabilisation of the concentration.

The log-transformed elimination shows a slightly bent curve. However, the correlation coefficient is sufficiently high to assume first order elimination. Depuration half-lives were approximately 2.5 days. The elimination rate constants correspond nicely for the two tested concentrations. The bioconcentration factor has been derived from C_{fish} /C_{water}, with C_{fish} determined between day 3 to 16, and C_{water} as the overall mean. There was no attempt to identify whether the radiolabel was parent compound or metabolite. In the test a solubiliser (DMF, Tween) was used to prepare the stock solution, but the test-concentrations were well below the water solubility. The test was carried out using a radiolabel, without identification of the parent compound in the fish. In water the parent compound is identified by HPLC for musk xylene. It should be realised that the BCF based on parent material will be lower than the current value of 1,600 l/kg.

Table 2 Bioconcentration of musk xylene (low and high refer to high and low dose of 0.98 and 13 µg/1, respectively) (Paradice and Suprenant, 1984).

^{14}C-radiolabel identified	No
species	bluegill sunfish
low dose [µg/1]	0.98 ± 0.26
high dose [µg/1]	13 ± 11
period of exposure [d]	16
period of elimination [d]	12
uptake rate constant [l/kg/day]	not determined
elimination rate constant [d^{-1}]	0.26 (low), r^2=0.84
	0.29 (high), r^2=0.98

$t^{1/2}$ elimination [d^{-1}]	2.7[a] (low); 2.4[a] (high)
bioconcentration factor (whole fish, wet weight) [l/kg]	1,600[b]

a Recalculated from the original data;

b Based on radio labelled residue in fish.

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

Geyer et al. (1994) cites a reference (MITI, 1992) describing that in carp with 3.4% lipid contents, the BCF was between 640-5,820 l/kg ww when exposed to 10 µg/l for 10 weeks and between 1,440-6,740 l/kg ww when exposed to 1 µg/l. The test was carried out in a flow through system and analytics were performed based on the parent compound. The test was carried out according to "305C. Bioaccumulation: Degree of Bioconcentration in Fish, stipulated in the OECD Guidelines for testing of Chemicals (May 12, 1981)". There is no information whether a steady state was reached in the test and, additionally, the relatively large variability in BCF values was not discussed. A recent study by Boleas et al. (1996) on the bioaccumulation of musk xylene in rainbow trout (44 g) was carried out under semi-static conditions with daily water renewal. Musk xylene was solved in ethanol and concentrations were 1, 10 and 100 µg/l. The plateau level was reached within a week and bioconcentration factors were between 10 and 60 l/kg for the edible portion. The analytical method used to measure musk xylene in the fish samples was the one as described by Fernandez et al. (1996).

In this method the fat containing extract of the fish samples is injected directly – without further clean-up - into the GC/MS. The study of Boleas et al. (1996) has been criticised by Rimkus et al. (1997):

- as no clean-up procedure is used after some injections the injector, column and the MS system including the detector will be contaminated;

- a static system instead of a flow-through system was used;

- water concentrations were not measured, but calculated from the dilution of the added stock solutions;

- relatively high concentrations in control fish up to 10 µg/kg fw were measured. This may be due to contamination in the laboratory during the fish experiment or analytical procedures. Helbling et al. (1994) has shown that organic solvents, paper tissues, rubber gloves and the hands of laboratory analysts can be potential sources of laboratory contamination with musk xylene.

These arguments are considered convincing reasons to reject the BCF values obtained by Boleas et al. (1996). Rimkus et al. (1997) report a study of Kuhlmann et al. (in press) in which rainbow trouts were exposed for several months to average water concentrations of 22.5 ng/l. The BCF was estimated at 4,400 l/kg ww. Further calculations on the same data resulted in BCF values of 4,200-5,100 l/kg ww and 115,000-122,000 l/kg on the basis of fat. No musk xylene could be detected in fish after 140 days in tests with spiked feed using concentrations of 1 and 10 µg/kg feed. No further details were presented in Rimkus et al. (1997). Information on the lipid content of the fish during

the study and the stability of the very low test concentration during the test phase is for example not available. According to one of the principal co-authors, Dr. Rimkus, the study meets all requirements, including the analytics, for a reliable estimate of the BCF of musk xylene in fish (pers. comm. Dr. Rimkus).

From the PBT draft addendum (2008) to the final report of the risk assessment (2005):

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. Based on a weight of evidence approach the upper values (> 4000) were considered to be most relevant in the RAR from 2005.

For the purpose of the assessment of vB, this section focuses on the most critical study resulting in BCFs above 5,000 l/kg 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*). Detailed analysis of these BCF studies is provided below in order to re-calculate reliable BCF values.

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 ºC. 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.

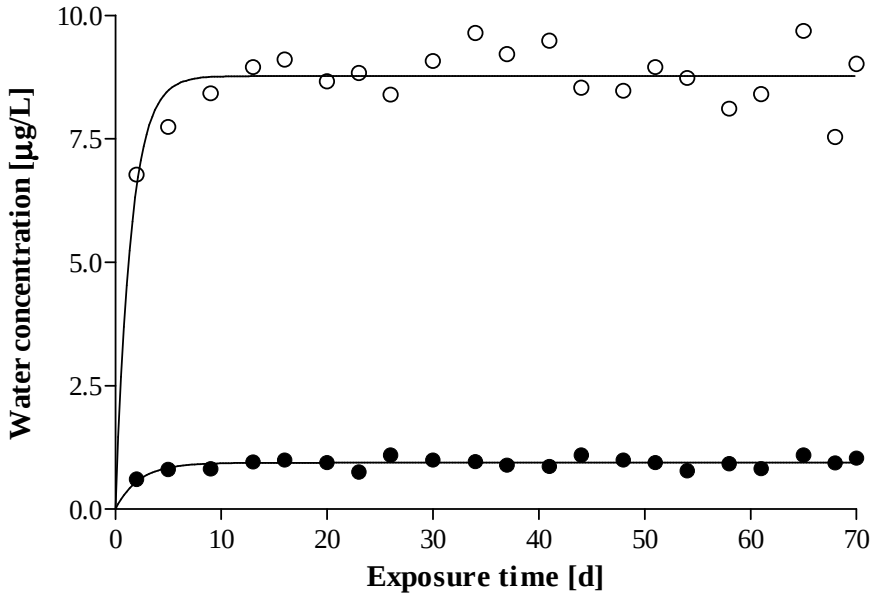

Figure 1: Water concentrations in a flow-through study with musk xylene at nominal concentrations of 1 (●) and 10 (○) µg/l.

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.

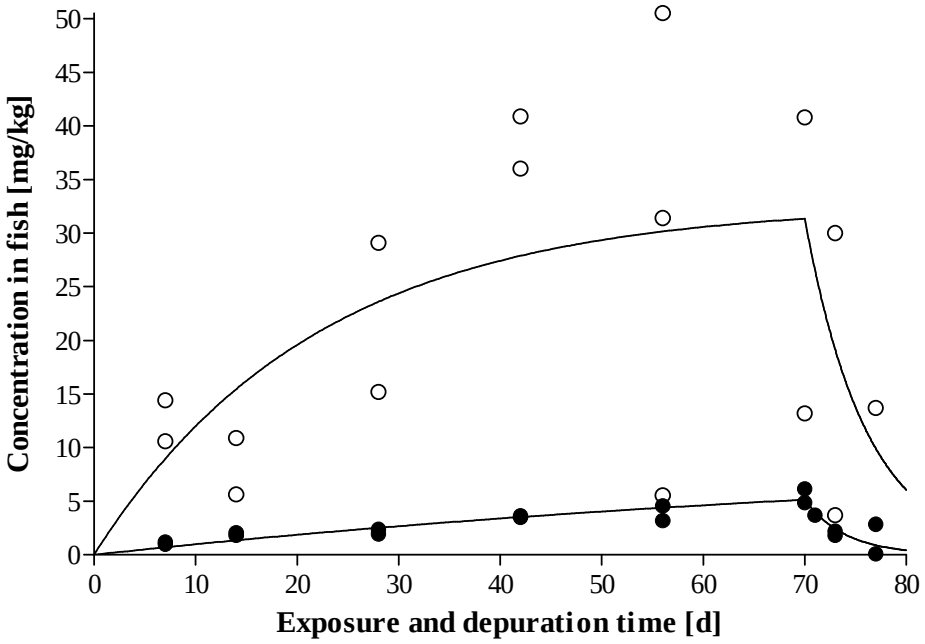

Figure 2: Whole body fish concentrations in a flow-through study with musk xylene at nominal concentrations of 1 (●) and 10 (○) µg/l.

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. 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 (Figure 4). 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, musk xylene was excreted with estimated half-lives of 5.9 days. The variability in the data is again rather high.

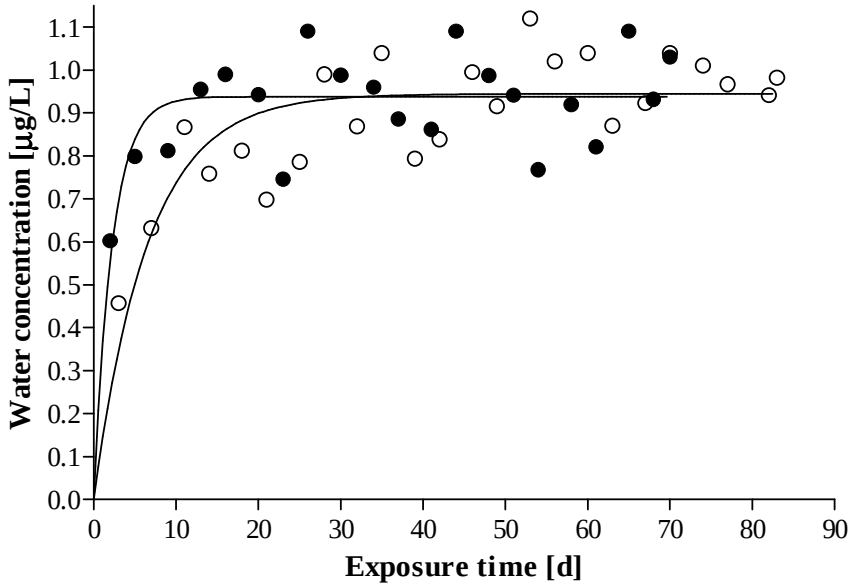

Figure 3: Water concentrations in two individual flow-through studies (● first study and ○ second study) with musk xylene at a nominal concentration of 1 µg/l.

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 5.

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 (*Scardinus 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.

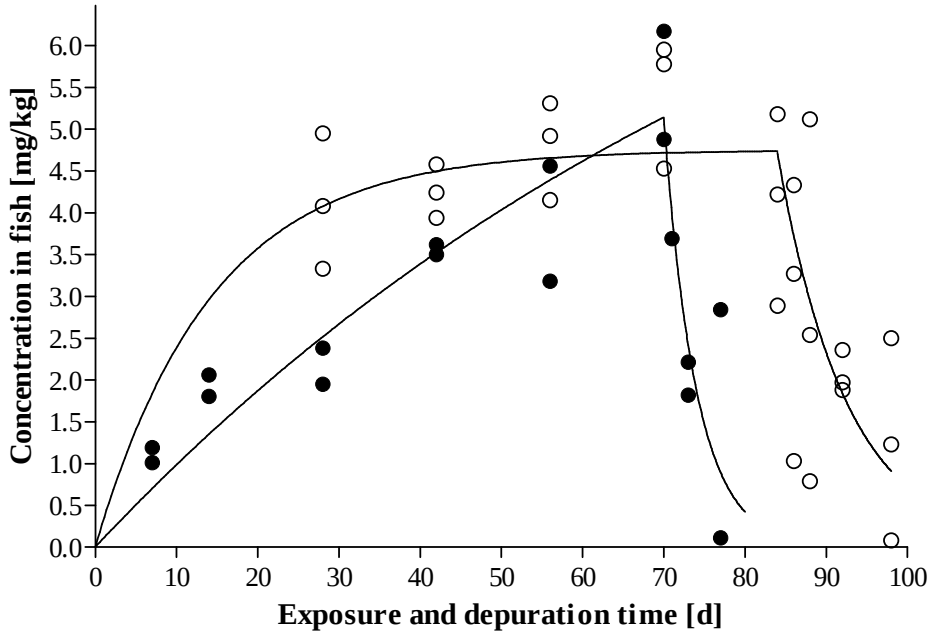

Figure 4: Whole body fish concentrations in two individual flow-through studies (● first study and ○ second study) with musk xylene at a nominal concentration of 1 µg/l.

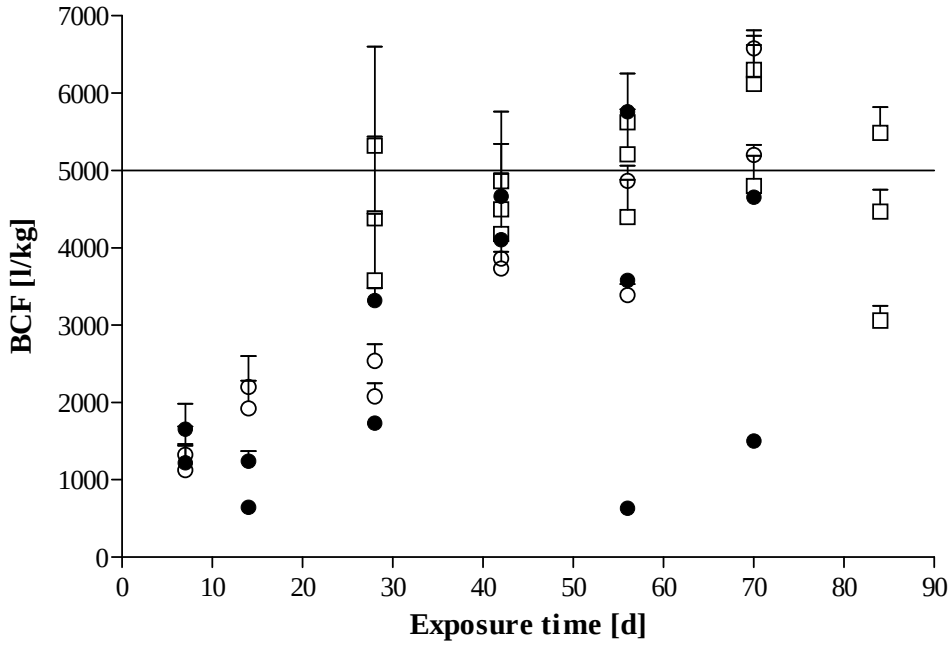

Figure 5: BCF values calculated for the individual fish samples (● 10 µg/L, first study; ○ 1 µg/L, first study; □ 1 µg/L, second study). Bars represent the difference with the values reported in the original studies.

3.3.2 Terrestrial bioaccumulation

From the EU RAR, section 3.1.1.2 (2005)

$BCF_{earthworm} = 0.84 + 0.012\ K_{ow} / RHO_{earthworm}$

where for $RHO_{earthworm}$ by default a value of 1 ($kg_{wwt}.L^{-1}$) can be assumed.

The formula for the $BCF_{earthworm}$ in $kg_{soil}/\ kg_{worm}$ then becomes:

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

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

From the PBT draft addendum (2008) to the final report of the risk assessment (2005):

No experimental data are available on accumulation in earthworms.

3.3.3 Summary and discussion of bioaccumulation

From the EU RAR, section 3.1.1.2 (2005)

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

From the PBT draft addendum (2008) to the final report of the risk assessment (2005):

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 detailed re-evaluation of the critical study of the MITI database and on the additional study by Gatermann et al. (2002), it can be concluded that musk xylene is very bioaccumulative.

4 HUMAN HEALTH HAZARD ASSESSMENT

4.1 Carcinogenicity

Conclusion from the EU RAR, section 4.1.2.7.3 (2005)

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

- only one species has been tested, i.e. the B6C3F1 mouse;

- this strain of mice is particularly prone to develop certain types of tumours, especially liver tumours;

- the mechanism behind the tumour development is not entirely understood, although it is clear that musk xylene has no genotoxic potential and that enzyme induction plays an important role in the development of the liver tumours observed.

Although musk xylene has not been tested for carcinogenicity in rats, there is a concern that it might be carcinogenic in rats as well, given the comparable enzyme induction properties of musk xylene in mice and rats. Further testing in e.g. rats or in mice to further elucidate the mechanism is, however, not considered to contribute much to the risk assessment of the carcinogenic risk of musk xylene to humans. This because the available data do allow the conclusions that musk xylene is a carcinogen in mice, that it acts by a non-genotoxic mode of action, and that the most serious type of tumour for which the incidence was statistically significantly increased (i.e. liver carcinomas in male mice) is mechanistically related to microsomal enzyme induction. Hence, for risk characterisation a threshold approach is considered justified, given that musk xylene is nongenotoxic and that enzyme induction is a threshold phenomenon. By taking the oral LOAEL of 70 mg/kg bw/day for tumour development (liver tumours in particular) as basis for the risk characterisation and by taking the mouse NOEL for enzyme induction into account in interpretation of the margin of safety (MOS), this will already result in a rather conservative approach when realising that the B6C3F1 mouse is especially prone to develop liver tumours. As to classification, IARC concluded in 1996 that there is limited evidence for the carcinogenicity of musk xylene in animals, but that the substance is not classifiable as to its carcinogenicity to humans (group 3) (IARC, 1996). However, the effects on the liver observed with musk xylene resemble those that can be seen after dosing rats and mice with phenobarbital. Phenobarbital is clearly a (liver) carcinogenic substance in rodents and often used to promote the development of tumours that were initiated by preceding treatment with genotoxic substances. Although the relevance of the carcinogenicity of phenobarbital for humans has been questioned (e.g. Williams and Whysner, 1996; IARC, 2001), IARC (2001) nevertheless just recently classified phenobarbital as a group 2B substance ("possibly carcinogenic to humans"). Hence, given the resemblance to phenobarbital, it is now concluded that the non-genotoxic compound musk xylene is to be classified as a carcinogen category 3 (R40), although it is realised that it is a borderline case. The CMR Working Group of May 2002 decided positive on classification as carc. cat. 3 (R40), and confirmed this at their September 2002 meeting.

Final conclusion on human hazard assessment

Based on these carcinogenicity data and other mammalian toxicology data (e.g. reproductive toxicity) presented in the RAR (2005), it is concluded that musk xylene does not fulfill the T criteria according to Annex XIII.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Aquatic compartment (including sediment)

From the EU RAR, section 3.2.1.1 (2005)

Table 3: Summary of toxicity data for aquatic organisms (information from the risk assessment report for musk xylene)

Species	Test	Result (mg/l)	Remark[a]	Reference
Vibrio fischeri	30-minutes	EC50 = >0.12	DIN 38412, part 34	Schramm et al., 1996
Selenastrum capricornutum	5-day static	NOEC > 0.56	Method Payne and Hall (1979); carrier: acetone; range: 0.1-5.6 mg/L (n=6) nominal concentrations	Hughes and Krishnaswami, 1985a
Mycrocystus aeruginosa	5-day static	NOEC > 1.0	Method Payne and Hall (1979); carrier: acetone; range: 0.1-10 mg/l (n=7) nominal concentrations	Hughes and Krishnaswami, 1985b
Scenedesmus subspicatus	72-hour static	EbC50 = >0.15	OECD TG 201	Schramm et al., 1996
Daphnia magna	48-hour static	EC50 = >0.15	OECD TG 202	Schramm et al., 1996
Daphnia magna	48-hour static	EC50 > 5.6 NOEC = 0.32 (swimming behaviour)	EECDir. 79/831, Annex V part C.2; static; carrier: DMSO; range: 0.18-5.6 mg/L (n=6); nominal concentrations	Adema and Langerwerf, 1985a
Daphnia magna	21-day semi-static	LC50 = 0.68 NOEC = 0.056 (reproduction)	EEC Ring test method 1985; carrier: DMSO; range: 0.01-1.0 mg/l (n=9); nominal concentrations	Adema and Langerwerf, 1985b

Bluegill sunfish *Lepomis macrochirus*	96-hour static	LC50 = 1.2 (0.55-1.7)	US-EPA-660/3-75-009; fish weight: 0.45 (0.17-0.77) g; length: 32 (23-39) mm; carrier: DMF; range: 0.78-6.0 mg/l (n=5); nominal concentrations; O2 declined in test below 60%	Sousa and Suprenant, 1984
Zebrafish *Brachydanio rerio*	14-day semi-static	LC50 = 0.4 (0.32-0.5) NOEC <0.1 (growth)	OECD TG 204; 3xweekly renewal; carrier: DMSO; range 0.1-5.6 mg/l (n=8); nominal concentrations; fish weight; 94±26 mg; length: 22.4+2.1 mm	Adema and Langerwerf, 1985c
Rainbow trout alevins *Oncorhynchus*	96-hours	LC50 = >1,000	APHA 1980; 5-10 g; range: up to 1,000 mg/l; screening test	Boleas et al., 1996
Rainbow trout *Oncorhynchus mykiss*	21-day semi-static	no effects on EROD activity in hepatic S9 fractions and retinol levels in hepatic plasma	fish weight: 44.2 + 2.8 g; range: 1-100 µg /1; solvent: ethanol	
Fish Zebra fish	48-hour 96-hour	LC50 = 3.75 LC50 « 0.2 mg/l	no information DMSO sonicated dispersions	MITI (1992) Unilever, 1995

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

The RAR (2005) presents detailed analysis of the aquatic hazard data summarized in the table above. None of these data was sufficient to support the T criterion (NOEC < 0.01 mg/l). In one study (Carlsson and Norrgren 2004), values below the threshold were reported but this study was considered to be a multi-stress experiment comprising starvation and the impact of the toxicant. Therefore this study is considered to be inconclusive with respect to the T criterion.

Final conclusion on environmental hazard assessment

Based on the environmental toxicity data presented in the RAR (2005) the substance does not meet the T criterion.

6 PBT, VPVB AND EQUIVALENT LEVEL OF CONCERN ASSESSMENT

6.1 Comparison with criteria from annex XIII

From the PBT draft addendum (2008) to the final report of the risk assessment (2005):

Musk xylene is not considered to be 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 based on the present data the substance does not meet the T criterion with regard to environmental toxicity criteria. The substance is also considered to be not T with regard to human toxicity criteria.

6.2 Conclusion of PBT and vPvB or equivalent level of concern assessment

Musk xylene fulfils the vP and the vB criteria.

REFERENCES

Addendum to the European Union Risk Assessment Report on 5-tert-butyl-2,4,6-trinitro-m-xylene (musk xylene) (2008)

Adema DMM and Langerwerf JSA (1985a). The acute toxicity of E-2642.01 (musk xylene) to *Daphnia magna.* Private communication to RIFM. TNO, Delft. Report R 85/116.

Adema DMM and Langerwerf JSA (1985b). The influence of E-2642.01 (musk xylene) on the reproduction *of Daphnia magna.* Private communication to RIFM. TNO, Delft. Report R85/128.

Adema DMM and Langerwerf JSA (1985c). The subchronic (14-d exposure) toxicity of E-2642.01 (musk xylene) to *Brachydanio rerio.* Private communication to RIFM. TNO, Delft. Report R 85/127.

Boleas, S, Fernandez C and Tarazona JV (1996). Toxicological and kinetic study of musk xylene in rainbow trout, *Oncorhynchus mykiss.* Buil. Environ. Contam. Toxicol. 57, 217-222.

Butte W, Schmidt S and Schmidt A (1999). Photochemical degradation of nitrated musk compounds. Chemosphere 38 (6), 1287-1291.

Calame R and Ronchi W (1989). Musk xylene: determination of the ready biodegradability. Private communication to RIFM. Givaudan-Roure, Switzerland. Test report 33-89.

Carlsson G and Norgrenn L (2002). Synthetic musk toxicity to early life stages of zebrafish (Danio rerio). Arch Environ Contam Toxicol 46: 102-105.

Downing JL (1994). Toxicity of musk xylene X0438.02 to the Earthworm *Eisenia foetida.* Private communication to RIFM. ABC Laboratories Inc, USA. Report No. 41582.

European Union Risk Assessment Report on 5-tert-butyl-2,4,6-trinitro-m-xylene (musk xylene) (2005).

Fernandez C, Carballo M and Tarazona JV (1996). A new method to determine musk xylene in water sewages fish and related products. Chemosphere 32, 1805-1811.

Gatermann R, Hühnerfuss H, Rimkus GG, Attar A and Kettrup A (1998). Occurrence of musk xylene and musk ketone metabolites in the aquatic environment. Chemosphere 36 (11), 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 HJ et al. (1994). Sunthetische Nitromoschus-Dufstoffe und Bromocyclen, UWSF-Z. Unweltchem. Ökotox. 6 (1), 9-17.

Givaudan (1990). Safety Test Results. November 12 1990.

Gorontzy T, Drzyzga MW Kahl D, Bruns-Nagel J, Breitung E, von Loew and Blotevogel KH (1994). Microbial degradation of explosives and related compounds. Crit. Rev. Microbiol. 20(4), 265-284.

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 .

Helbling KS, Schmid P and Schlatter C (1994). The tracé analysis of musk xylene in biological samples: problems associated with its ubiquitous occurrence. Chemosphere 29, 477-484.

Herren D and Berset JD (2000). Nitro musks, nitro musk amino metabolites and polycylci musks in sewage sludges. Quantitative determination by HRGC-ion-trap-MS/MS and mass spectral characterisation of the amino metabolites. Chemosphere 40, 565-574.

Higson FK (1992). Microbial degradation of nitroaromatic compounds. Adv. Appl. Microbiol. 37, 1-19.

Hughes JS and Krishnaswamin SK (1985a). The toxicity of B0817.01 to *Selenastrum capricornutum.* Private communication to RIFM. Malcolm Pirnie, New York. Project 165-06-1100-1.

Hughes JS and Krishnaswami SK (1985b). The toxicity of B0817.01 to *Microcystis aeruginosa.* Private communication to RIFM. Malcolm Pirnie, New York. Project 165-06-1100-2.

IARC (2001). IARC monographs on the evaluation of carcinogenic risks to humans. Volume 79: Some thyrothropic agents. WHO, International Agency for Research on Cancer, Lyon, France. Phenobarbital and its sodium salt 161-288.

Johnson LD (1984). Determination of octanol/water partition coëfficiënt of P1618. Private communication to RIFM. ABC Laboratories Inc, USA. Report No. 31640.

Kalf DF et al. (1995). Integrated environmental quality objectives for polycyclic aromatic hydrocarbons (PAHs). RIVM Report No. 679101018. Bilthoven, The Netherlands.

Le Fèvre CG and Le Fèvre RJW (1935). The dipole moments of 1:4-Dinitro-, 1:3:5-Trinitro-, and certain 2:4:6-Trisubstituted-l:3:5-trinitro-benzenes, 957-65.

Lyman WJ, Reehl WF and Rosenblatt DH (1990). Handbook of chemical property estimation methods. American Chemical Society, Washington DC.

Marks KH and Marks PJ (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.

Meharg AA, Dennis GR and Gairney JWG (1997). Biotransformation of 2,4,6-trinitrotoluene (TNT) by ectomycorrhizal basidomycetes. Chemosphere 35 (3), 513-521.

Ministry of International Trade and Industry (MITI). 1992. MITI-List, Tokyo, Japan.

Opfer-Schaum R and Piristi M (1944). Über die Schmelzpunkte des Xylolmoschus und des Athylvanillins. Fetteund Seifen, 133-4.

Paradice AP and Suprenant DC (1984). Accumulation and elimination 14C-residues by bluegill *(Lepomis macrochirus)* exposed to P1618.01R (musk xylene). Private communication to RIFM. Springborn bionomics, USA. Report No. BW-84-7-1602.

Payne AG and Hall RN (1979). A method for measuring algal toxicity and its application to the safety assessment of new chemicals. In *Aquatic Toxicology, ASTM STP 667.* Marking LL and Kimerle RA (eds), American Society for Testing and Materials, 171-180.

Rimkus GG, Butte W and 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, Wolf M, Attar A, Gatermann R, Hühnerfuss H (1998): Nitro musk metabolites in biota samples from the aquatic environment. Proceedings of the 20th International Symposium on Capillary Chromatography, Riva del Garda, Italy, 1998, M.09, 11 pp.

Rimkus GG, Gatermann R and Huhnerfuss H (1999) Musk xylene and musk ketone amino metabolites in the aquatic environment. December 20 1999. Toxicol Lett. 111 (1-2), 5-15.

Rudio J (1996). Partition coëfficiënt n-octanol/water of musk xylene according to OECD GuidelineNo. 117. Givaudan-Roure, Test Report No. 96-E02.

Sabaliunas D, Webb SF, Eckhoff WS and Simonich SL (2001). Recent analyses of nitromusks in sewage and river water in the UK (poster 2001).

Schramm KW, Kaune A, Beek B, Thumm W, Behechti A, Kettrup A, and Nicolova P (1996). Acute toxicities of five nitromusk compounds in Daphnia, Algae and photoluminescent bacteria. Wat. Res. 30, 2247-2250.

Simonich SL et al. (1998). Removal of fragrance materials during sewage treatment. SETAC presentation 1998.

Simonich SL, Begley WM, Debaere G and Eckhoff WS (2000). Tracé Analysis of Fragrance Materials in Wastewater and Treated Wastewater. Env. Science and Tech. 34 (6).

Sousa V and Suprenant DC (1984). Acute toxicity of P1618.02 (musk xylene) to bluegill *(Lepomis macrochirus).* Private communication to RIFM. Bionomics, USA. Report No. BW-84-2-1549.

Tas JW and Van de Plassche EJ (1996). Initial environmental risk assessment of musk ketone and musk xylene in The Netherlands in accordance with the EU-TGD, Report 601503 002. National Institute of Public Health and the Environment, Bilthoven, The Netherlands.

Treff W (1926). Kritische betrachtungen über Vorschriften zur Untersuchung atherischer Öle und Riechstoffe. Zeitschrift für angewandte Chemie, 1306-9.

Unilever (1995). Bioavailability. Research Contract. Sponsored by The Department of the Environment. Final Report June 1995.

Van Bergen TJ., Theewis JG. 2006. Photochemical behaviour of musk xylene. A summary & evaluation of the available literature studies on the photodegradation of musk xylene provided by the industry: International Flavours & Fragrances (IFF), The Netherlands.

Williams GM and Whysner J (1996). Epigenetic carcinogens: evaluation and risk assessment. Exp. Toxic. Pathol. 48, 189-195.

Winkler M, Kopf G, Hauptvogel C and Neu T (1998). Fate of artificial musk fragrances associated with suspended particulate matter (SPM) from the river Elbe (Germany) in comparison to other organic contaminants. Chemosphere 37, 1139-1156.

Yamagishi T, Miyazaki T, Horii S and Akiyama K (1983). Synthetic musk reisidues in biota and water from Tama River. Arch. Environ. Contam. Toxicol. 12, 83-89.