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Submitted by: The Netherlands
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PROPOSAL FOR IDENTIFICATION OF A SUBSTANCE AS A CMR CAT 1 OR 2, PBT, VPVB OR A SUBSTANCE OF AN EQUIVALENT LEVEL OF CONCERN

Substance name: Musk xylene
EC number: 201-329-97
CAS number: 81-15-2

- *It is proposed to identify the substance as a vPvB according to Article 57 (e).*

Summary of how the substance meets the CMR (Cat 1 or 2), PBT or vPvB criteria, or is considered to be a substance of an equivalent level of concern

Musk xylene is concluded to be a vPvB substance. In addition, it should be noted that the substance is considered to be borderline T.

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).

Musk xylene is considered to be borderline Toxic (T). Some NOECs for specific aquatic toxicity tests were found to be at or below the threshold value of 10 µg/l. However, 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.

Registration number(s) of the substance or of substances containing the substance:

EC Number: 201-329-97

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifier of the substance

Chemical name: Musk xylene
EC Number: 201-329-94
CAS Number: 81-15-2
IUPAC Name: 1-tert-butyl-3,5-dimethyl-2,4,6-trinitrobenzen

1.2 Composition of the substance

Chemical name: Musk xylene
EC Number: 201-329-94
CAS Number: 81-15-2
IUPAC Name: 1-tert-butyl-3,5-dimethyl-2,4,6-trinitrobenzen
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

Table 1 Summary of physico-chemical properties

REACH ref Annex, §	Property	Value	
V, 5.1	Physical state at 20º C and 101.3 kPa	Solid, powder	Musk xylene RAR Table 1.1
V, 5.2	Melting / freezing point	112-114˚C	Musk xylene RAR Table 1.1
V, 5.3	Boiling point	Not applicable	Musk xylene RAR Table 1.1
V, 5.5	Vapour pressure	0.00003 Pa at 20˚C	Musk xylene RAR Table 1.1
V, 5.7	Water solubility	0.15 mg/l	Musk xylene RAR Table 1.1
V, 5.8	Partition coefficient n-octanol/water (log value)	4.9	Musk xylene RAR Table 1.1

2 MANUFACTURE AND USES

Not relevant for this type of dossier.

3 CLASSIFICATION AND LABELLING

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

Symbols: E, Xn, N
R sentences: R2, R40, R50/53
S sentences: 2, 36/37, 46, 60, 61

3.2 Classification according to GHS

Not applicable

3.3 Self classification(s)

Not applicable.

4 ENVIRONMENTAL FATE PROPERTIES

4.1 Degradation

4.1.1 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: 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.

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.

4.1.2 Biodegradation

4.1.2.1 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.

4.1.2.2 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).

4.1.2.3 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.

4.1.3 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.

4.2 Environmental distribution

4.2.1 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.

4.2.2 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.

4.2.3 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, 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.

4.3 Bioaccumulation

4.3.1 Aquatic bioaccumulation

4.3.1.1 Bioaccumulation estimation

See section 4.3.1.2

4.3.1.2 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 [ju.g/1]	0.98 ± 0.26
high dose [ju.g/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" (low); 2.4" (high)
bioconcentration factor (whole fish, wet weight) [l/kg]	1,600"

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. 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 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.

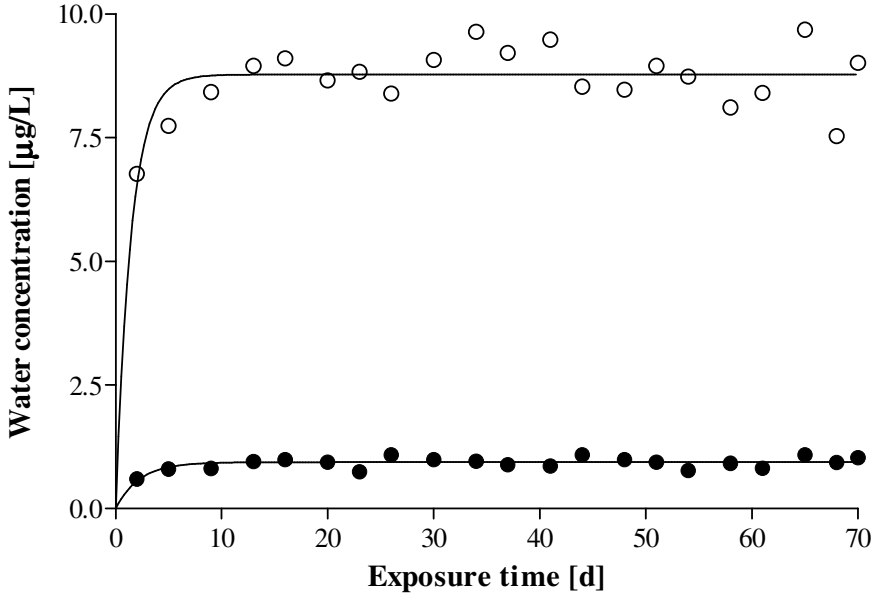

Figure 1: Water concentrations in a flow-through study with musk xylene at nominal concentrations of 1 (●) and 10 (○) µg/l.

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.

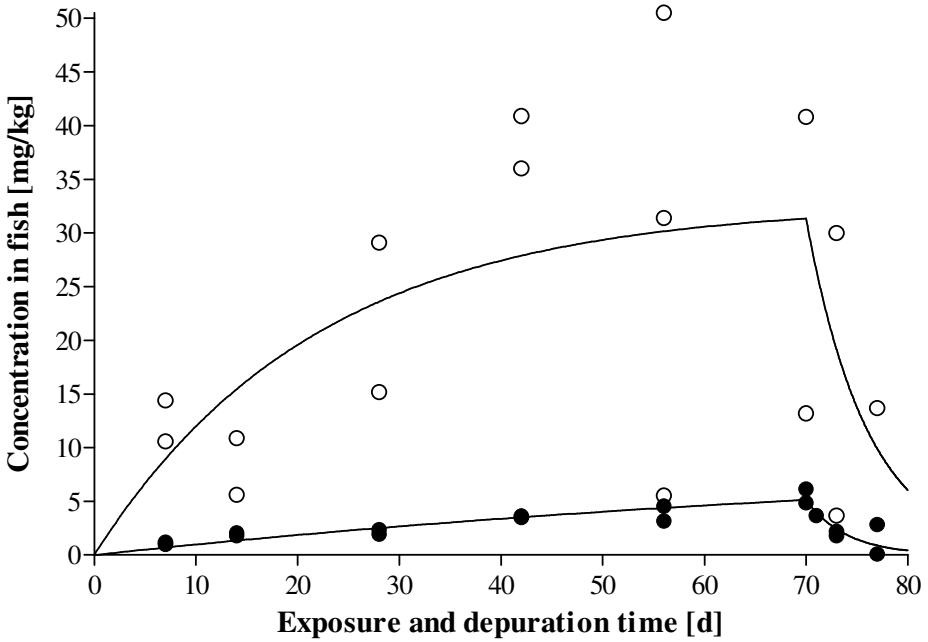

Figure 2: Whole body fish concentrations in a flow-through study with musk xylene at nominal concentrations of 1 (●) and 10 (○) µg/l.

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. 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.

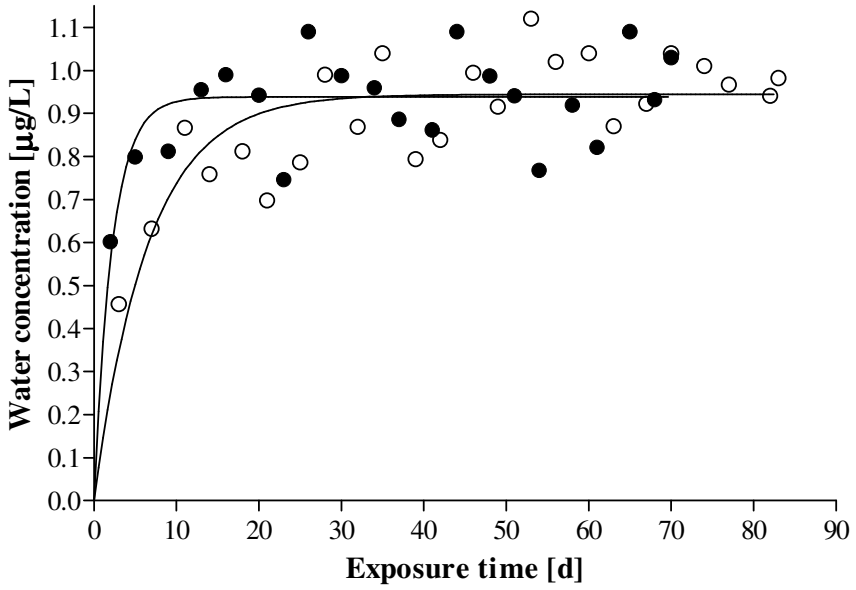

Figure 3: Water concentrations in two individual flow-through studies (● first study and ○ second study) with musk xylene at a nominal concentration of 1 µg/l.

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

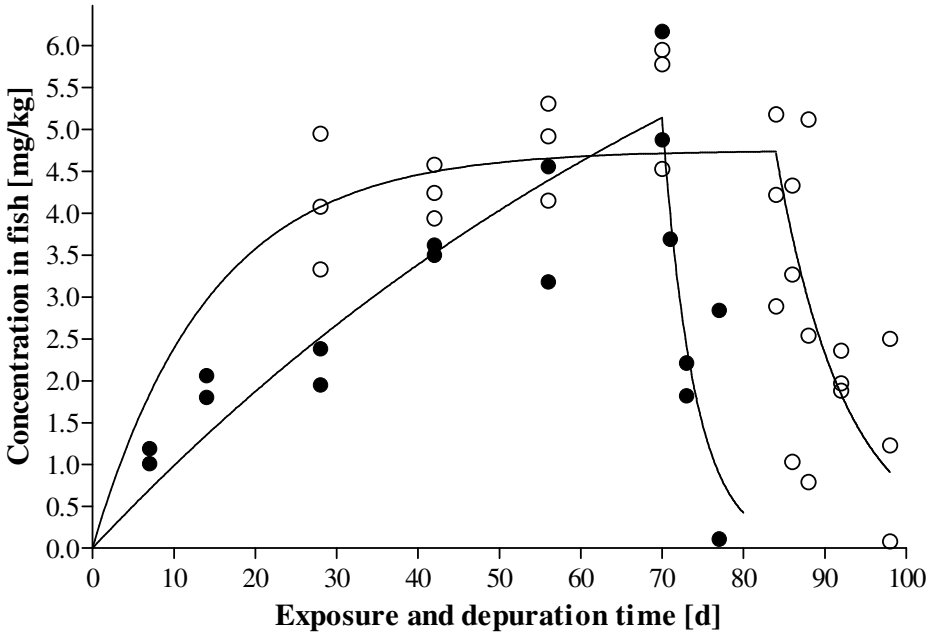

Figure 4: Whole body fish concentrations in two individual flow-through studies (● first study and ○ second study) with musk xylene at a nominal concentration of 1 µg/l.

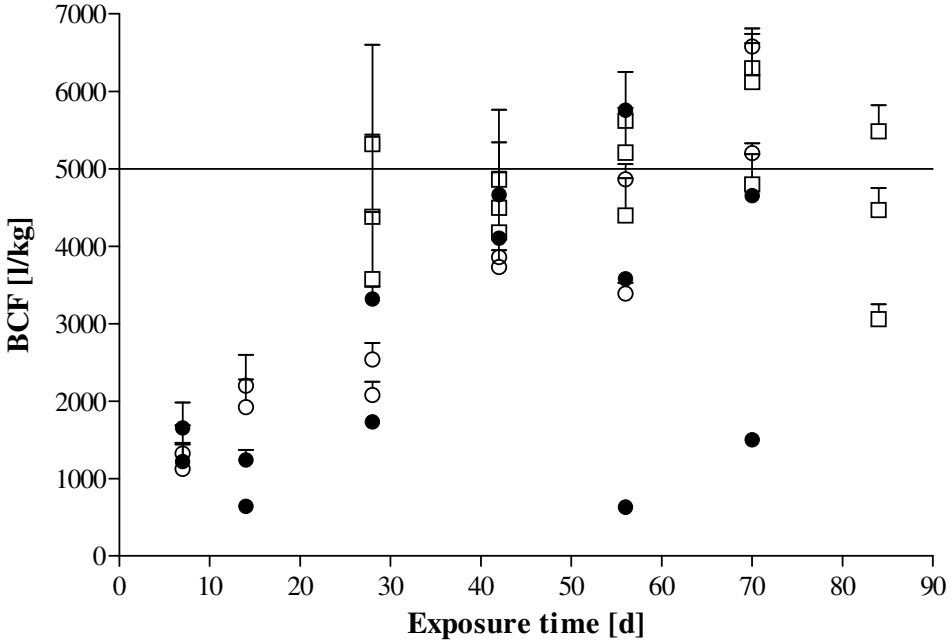

Figure 5: BCF values calculated for the individual fish samples (● 10 µg/L, first study; ○ 1 µg/L, first study; □ 1 µg/L, second study). Bars represent the difference with the values reported in the original studies.

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 (*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.

4.3.2 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:
$(0.84 + 0.012\ K_{ow} .\ RHO_{soil})/(K_{soil\text{-}water} .\ CONV_{water})$
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.

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

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 evaluation of the critical study and its results it can be concluded that musk xylene is very bioaccumulative.

4.4 Secondary poisoning

From the EU RAR, section 3.1.1.2 (2005)
No toxicological data are available for (top-)predators. No specific toxicological data area vailable on e.g. (fish-eating) birds. The PNEC for secondary poisoning will therefore be based on mammalian toxicity data for musk xylene. The oral NOAEL of 7.5 mg/kg bw/day for peri/postnatal toxicity in rats is used for this purpose. As toxicity is expressed on the P-generation (rats > 6 weeks) a food conversion factor of 20 has to be used. As this study equals a 28 days test, applying an AF of 300 on the ground of the exposure time should be considered here (TGD, 1996). However, a number of arguments can be adduced why the use of such factor of 300 may be over-protective in this case. One reason is that even at the next concentration in the test, i.e. 22.5 mg/kg bw/day, only marginal (6%) effects were seen on the body weight gain of the pups. This makes this LOAEL, and, implicitly, the selected NOAEL, rather conservative. Additionally, an 80 weeks mice oral carcinogenicity study is available (Maekawa et al., 1990) showing no effects on reproductive organs. However, no NOAEL could be derived from this study and other, ecologically relevant, effects were not addressed. A semi-chronic dermal rat study is further available (Ford et al., 1990) from which an oral NOAEL of 9.6 mg/kg bw/day can be calculated (route-to-route). This value is in line with the value of 7.5 mg/kg bw/day indicating that the extrapolation step from sub-acute to semi-chronic does not necessarily demand an additional uncertainty factor. A weak point here is that the TGD is clear in that only oral or dietary exposures should be used to derive a PNEC for secondary poisoning (and thus not an extrapolated dermal exposure).
From the above it is clear that the data set contains more useful information than 'just' the results of the 28-days test (AF <300), but that this extra information is not sufficient to fully equate this test with a semi-chronic NOAEL from a feeding study (AF > 90). When using the NOAEL of 7.5 mg/kg bw/day as a starting point for the $PNEC_{oral}$ derivation of musk xylene makes it is therefore suggested to use an AF of 150 as a reasonable 'compromise' between 90 and 300. The $PNEC_{oral}$ then becomes: 7.5 . 20/150 = 1 mg/kg food.

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

5 HUMAN HEALTH HAZARD ASSESSMENT

5.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

Not applicable for this dossier.

5.2 Acute toxicity

5.2.1 Acute toxicity: oral
Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.2.2 Acute toxicity: inhalation
Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.2.3 Acute toxicity: dermal
Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.2.4 Acute toxicity: other routes
Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.2.5 Summary and discussion of acute toxicity
Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.3 Irritation
Not relevant for this type of dossier.

5.4 Corrosivity
Not relevant for this type of dossier.

5.5 Sensitisation
Not relevant for this type of dossier.

5.6 Repeated dose toxicity
Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.6.1 Repeated dose toxicity: oral
Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.6.2 Repeated dose toxicity: inhalation
Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.6.3 Repeated dose toxicity: dermal
Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.6.4 Other relevant information
Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.6.5 Summary and discussion of repeated dose toxicity
Not applicable for this dossier. The data available do not provide any relevant information on the

toxic (T) properties of this substance.

5.7 Mutagenicity

Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.7.1 *In vitro* data

Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.7.2 *In vivo* data

Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.7.3 Human data

Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.7.4 Other relevant information

Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.7.5 Summary and discussion of mutagenicity

Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.8 Carcinogenicity

5.8.1 Carcinogenicity: oral

From the EU RAR, section 4.1.2.7.1 (2005)

Oral

Three groups of 50 male and 50 female mice (B6C3F1) received a diet containing 0, 0.075 or 0.15% musk xylene (i.e. 0, 750 or 1500 mg/kg) for 80 weeks. The dietary intakes were 70-125 and 141-228 mg musk xylene/kg bw/day for males and 80-143 and 166-259 mg musk xylene/kg bw/day for females in the low and high dose group, respectively. After 80 weeks the administration of musk xylene was stopped and the mice were then maintained on a basal diet without musk xylene until week 90 when all survivors were killed. All tissues, including reproductive organs were microscopically examined. Males fed 0.15% showed reduced body weights during weeks 4 to 80. At the end of the study there was no difference between the groups. There were no significant differences between controls and treated groups in mean survival time. Tumours developed in several organs and tissues in both sexes of all groups, including controls. Musk xylene at both dose levels tested statistically significantly increased the incidence of liver adenomas in both sexes and of liver carcinomas in males. In male mice, the incidence of Harderian gland adenomas was also statistically significantly increased in both treated groups. Positive trends (not statistically significant) were noted for the occurrence of lung tumours (adenomas) in both sexes and Harderian gland tumours (adenomas) and lymphomas in females. The incidences for the above mentioned tumours are given in Table 3. As can be seen from this table, these tumours also occurred in controls. For other tumours there was no difference in incidence between treated and control animals. The lowest dose tested (0.075%, equivalent to 70-125 mg/kg bw/day in male mice and 80-143 mg/kg bw/day in female mice) is an effect dose. In this study no effects were seen on the reproductive organs (Maekawa et al.,

1990).

Table 3 Tumour incidences in mice treated orally with musk xylene for 80 weeks.

Site and type of tumour	No. of mice with tumours					
	Males			Females		
Concentration in diet (mg/kg)	0	750	1,500	0	750	1,500
Effective no. of mice	49	50	47	46	50	49
Liver						
- adenoma	9	19*	20**	1	14***	13***
- carcinoma	2	8*	13**	0	1	2
- adenoma/carcinoma	11	27**	33***	1	15***	15***
- haemangioma	0	0	0	2	0	0
- haemangioendothelioma	2	0	0	0	0	0
Lung						
- adenoma	3	5	6	0	2	2
- carcinoma	0	0	0	0	1	0
Haematopoietic organs						
- lymphomas (lymphocytic)	4	4	2	3	5	6
Harderian gland						
- adenoma	2	9*	10*	3	5	5
- carcinoma	1	1	0	0	0	0

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

5.8.2 Carcinogenicity: inhalation

No data available

5.8.3 Carcinogenicity: dermal

No data available

5.8.4 Carcinogenicity: human data

No data available

5.8.5 Other relevant information

From the EU RAR, section 4.1.2.7.1 (2005)

Special investigations on enzyme induction

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

In order to characterise the inducing effects of musk xylene on phase I and phase II drugmetabolising enzymes, male Wistar rats (4 per group) were intraperitoneally dosed with 50, 100 or 200 mg musk xylene/kg bw for 5 consecutive days. Treatment with musk xylene resulted in increased relative liver weights and increased microsomal protein levels. Dose related increases in

benzo[a]pyrene hydroxylation and 7-ethoxycoumarin de-ethylation were observed from 50 mg/kg upwards. 7-pentoxyresorufin dependentylation was increased at all dose levels as compared to control but the increase diminished at the higher dose levels. Other activities (aniline hydroxylase, aminopyrine demethylase, benzphetamine demethylase and erythromycin demethylase) were not affected. Dose-related increases at 50 mg/kg and above were also observed for glutathione S-transferase (GST; two types), DT diaphorase (two types), and UDP-glucuronyl transferase but not for N-acetyl transferase. For GST type Ya and both DT diaphorase forms these increases were also reflected in cytosolic protein contents. Other enzymes mentioned were not quantified by immunochemical methods (Iwata et al*.,* 1993a).

Male wistar rats (5 weeks old; 4 per group) were injected intraperitoneally with 0.1 mmol (30 mg)/kg bw musk xylene or with equimolar amounts of 3-methyl cholanthrene (3MC) or 2,3-tertbutylhydroxyanisole (BHA) or with 0.1 or 0.93 mmol/kg bw isosafrole. After 5 days of treatment livers were removed and studied for induction of phase I and phase II biotransformation enzymes. Results in treatment groups were compared to those of a vehicle treated control group. At the dose levels mentioned only 3MC produced a profound increase in total cytochrome P450. Western blot analyses revealed that musk xylene, isosafrole and BHA induced more strongly cytochrome P450 1A2 (CYP1A2) in microsomes than CYP1A1, while 3MC induced CYP1A1 in preference to CYP1A2. Musk xylene induced ethoxyresorufin O-deethylase (EROD), equipotent to low dose isosafrole, erythromycin N-demethylation (ERD) and aniline hydroxylation, but did not affect benzphetamine demethylation. EROD was far more strongly induced by 3MC and high dose isosafrole. These two treatments also enhanced pentoxyresorufin-O-dependentylase (PROD) activity, but depressed benzphetamine demethylation. Treatment with musk xylene at this dose level did not affect phase II enzyme activities (DT-diaphorase, glutathione S-transferases or UDP-glucuronyl transferase). These enzymes were strongly induced by 3MC and to a lesser extend also by high dose isosafrole. According to the authors musk xylene is at these dose levels a more specific inducer for CYP1A2 than 3MC (Iwata et al., 1993b). Remark: although the authors measured PROD activity, effects on CYP2B protein were not directly studied.

Groups of 10 male B6C3F1 mice received daily for 7 days musk xylene dissolved in trioctanoin by i.p. injections at doses of 0, 50, 100 or 200 mg/kg. On day 8 the animals were sacrificed. Sections of the liver were taken for histological examination and protein and enzyme activity was determined. After i.p. administration total microsomal protein content, EROD, and methoxy resorufin O-demethylase (MROD) activities were dose relatedly increased in all treated groups when compared to the control group. No changes were observed in PROD or ERD activities. Electron microscopic examination of the livers showed proliferation of smooth and especially rough endoplasmic reticulum at 50 mg musk xylene/kg bw as well as signs of mitochondrial damage. These microscopical changes became more pronounced at the higher dose levels. Other groups of 20 male mice (same strain)/dose were given musk xylene in the diet at 0, 0.015, 0.045, or 0.15% (equivalent to 21.4, 64.3, 214.3 mg/kg bw) for 4 weeks. 10 Mice of each group were sacrificed and their kidneys and brains removed and weighed. The liver was examined histologically and microsomal protein and enzyme activities were determined. The remaining 10 mice in each group were withdrawn from musk xylene containing diets for 14 days. Then the mice were sacrificed and the livers were examined as above.

After 28 days feeding relative liver weight, total liver microsomal protein, EROD, and MROD but not PROD were dose relatedly and significantly increased at 0.045 and 0.150%, whether expressed as an activity per mg microsomal protein per minute or as substrate turnover (nmole of substrate per nmole P450 per minute). Immunoblotting showed that musk xylene induced cytochrome P450 1A2 enzyme. The cytochrome P450 1A1 was much less enhanced. After a 14-day recovery all increases were reversed and not significantly different when compared to controls (Caldwell and Thatcher, 1994).

Male B6C3F1 mice were dosed orally with musk xylene dissolved in corn oil at dosages of 10 and 200 mg/kg bw for 7 days. At the highest dose, increases were found in relative liver weight (38%), microsomal protein yield (125%), total P450 content (62%) and cytochrome P450-2B (CYP2B) protein level (about 20-fold). At 10 mg/kg bw no general hepatic effects consistent with cytochrome P450 induction were seen. Although musk xylene increased CYP2B protein levels, it did not

increase CYP2B enzyme activity (as determined by PROD activity). When given over a range of 1 to 200 mg/kg bw for 7 consecutive days, musk xylene dose-relatedly increased immunoreactive CYP2B protein levels (at the highest dose about 20-fold over control levels) but did not reveal any change in CYP2B enzyme activity from control levels at any dose (Stuard et al., 1996).

To characterise the effects of musk xylene on mouse hepatic microsomal enzyme activities groups of 5 male B6C3F1 mice were dosed by gavage for 7 days with 0 or 200 mg musk xylene/kg bw after which microsomes were prepared. A series of enzyme assays was used to determine total cytochrome P-450 content, NADPH cytochrome P-450 reductase, and the activities of cytochromes P-450 1A1, 1A2 and 2B. Immunoblotting procedures were also used to evaluate the changes in protein levels for CYP1A1, 1A2 and 2B. Liver weight was increased by 40%. The increased liver weight was reflected histologically as centrilobular hepatocellular hyperthrophy. Treatment increased the total microsomal cytochrome P450 content about 2-fold and NADPH cytochrome-c-reductase was increased about 4-fold over the control values. Induction of CYP1A1 and 1A2 protein levels was 2.50- and 2-fold, respectively. These results were consistent with increased 1A1 and 1A2 protein levels determined by immunoblotting (Lehman-McKeeman et al., 1995). As a follow-up to Lehman-McKeeman et al. (1995), a dose-response study was conducted in which musk xylene was dosed by gavage to groups of 5 male B6C3F1 mice for 7 days at dosages of 1, 5, 10, 20, 50, 100 and 200 mg/kg bw. Treatment with musk xylene resulted in a dose related increase in absolute and relative liver weights at 20 mg/kg and 50 mg/kg and higher, respectively. Total microsomal protein and total cytochrome P450 were dose-relatedly increased at 50 and 20 mg/kg, respectively. Induction of cytochrome P450 1A2 (about 3-fold) and 3A proteins was observed, together with a small but unquantified induction of cytochrome P4501A1 at 100 and 200 mg/kg bw. Enhanced protein levels were reflected in increased activities of EROD (4-fold), MROD (2-fold), and ERD (2.5-4 fold) at 200, 200, and 50-200 mg/kg, respectively. From 20 mg/kg bw and upwards, cytochrome P450 2B protein was also induced up to 25-fold at 200 mg/kg, but this was not reflected in increased PROD activity. Induction of cytochrome P450 2B was also confirmed by a 10-fold increase in P450 2B mRNA levels at 200 mg/kg within four h post dosing. The NOEL for effects on liver enzyme induction in this study is 10 mg/kg bw/day for 7 days (Lehman- McKeeman et al., 1997a).

In an additional experiment male B6C3F1 mice (5 per group) were exposed to the classical CYP2B inducer, phenobarbital (PB; 500 mg/l in drinking water) for 5 days and then given a single oral dosage of corn oil or 200 mg musk xylene (in corn oil)/kg bw at 2 or 18 hours prior to necropsy. By immunoblotting, there was no difference in the CYP2B protein levels in the PB treated animals dosed with either corn oil or musk xylene. CYP2B enzyme activity in the PB/corn oil treated mice was about 1,200 to 1,300 pmol/min/mg protein, representing about 20-fold induction over uninduced levels. When musk xylene was dosed 2 hours before necropsy, CYP2B enzyme activity was similar to the PB/corn oil treated mice (1,221 ± 76 pmol/min/mg protein). However, when dosed 18 hours before necropsy, CYP2B enzyme activity in the musk xylene-treated mice (99 ± 12 pmol/min/mg protein) was decreased 90% relative to the PB/corn oil treated mice. According to the authors these results demonstrated that musk xylene is a potent inhibitor of the CYP2B enzymes, but since the inhibitory effect was seen only at 18 hours after dosing, the results suggested that the inhibition of the CYP2B enzymes *in vivo* required metabolism of musk xylene (Lehman-McKeeman et al., 1997a).

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

dosed mice. These results indicate that the biotransformation of musk xylene by intestinal microflora is involved in the inhibition of CYP2B enzymes seen with musk xylene treatment (Lehman-McKeeman, 1997a).

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

In order to identify the substance responsible for induction of CYP2B in mouse liver, male B6C3F1 mice (5 per group) were treated with 0.67 mmol/kg of musk xylene, o-NH_2-musk xylene, or p-NH_2-musk xylene dissolved in corn oil (200 mg/kg bw for musk xylene or 180 mg/kg bw for both amino derivatives) by gavage. Control animals received only corn oil. At 4, 8, 16, 24, or 48 h post dosing livers were removed and studied for CYP2B and CYP1A2 mRNA, cytochrome P450 enzyme activities (PROD, EROD, MROD), CYP2B protein contents and several more general parameters for xenometabolism induction (liver weight, microsomal protein, total cytochrome P450, and cytochrome c reductase). In separate studies the effect of pretreatment with broad-spectrum antibiotics on cytochrome P450 induction was also studied. The induction pattern by musk xylene and metabolites was compared to the induction pattern by PB. PB and musk xylene and o-NH_2 musk xylene induced CYP2B mRNA by a factor of 5 over control levels with a maximum response at 6 to 18 h post dosing. With p-NH_2-musk xylene also a five fold induction of mRNA expression was observed, but this increase lasted for up to 48 hours post dosing. With PB, musk xylene and metabolites also an induction of CYP1A2 mRNA was observed, but this was only by a factor of 1.5 above control level.

When animals were pretreated with broad-spectrum antibiotics to eliminate metabolism by intestinal flora, no evidence of microsomal enzyme induction was obtained following dosing with musk xylene. Furthermore, while musk xylene when given alone induced CYP2B and CYP1A proteins it did not do so when given after a dose of antibiotics. It was therefore concluded that intestinal nitro-reduction products of musk xylene were responsible for induction of microsomal biotransformation enzymes (Lehman-McKeeman et al., 1997c). Male F344 rats (6 per group) were orally dosed with 0, 10, 50 or 200 mg musk xylene /kg bw for 7 days. The substance was dissolved in corn oil. Following sacrifice of the animals, livers were assayed for induction of microsomal enzymes by determination of enzyme activities, for CYP1A1/2, CYP2B1/2 and CYP3A, and by determination of protein levels and mRNA expression for CYP2B1/2.

Up to 200 mg/kg bw, clinical signs of toxicity were not observed. Induction of microsomal enzymes was reflected in a dose-related increase in absolute liver weight (statistically significantly at 200 mg/kg bw), and dose-related increases in total microsomal cytochrome P450, cytochrome b5 and NADPH-cytochrome P450 reductase which were statistically significant at 50 and 200 mg/kg bw. CYP2B activity measured as PROD was significantly increased at all dose levels up to 3-fold

maximally at 200 mg/kg bw. However, the concentration of the corresponding CYP2B enzyme was increased by about 50-fold at this dose level and the increase in steady-state CYP2B mRNA level amounted to 10-fold.
Exposure to musk xylene resulted also in increases in CYP1A1/2 and CYP3A1 enzyme activities which were reflected in dose related increases in EROD and MROD activities (statistically significant at all dose levels) and testosterone 6β-hydroxylation, which was statistically significant at 50 and 200 mg/kg bw. The induction profile of the biotransformation enzymes was similar to that observed with phenobarbital at 500 mg/l in the drinking water for 7 days. This study provided a LOEL of 10 mg/kg bw /day for microsomal liver enzyme induction in the ratafter 7 days of dosing (Lehman-McKeeman et al., 1999).
Female and male Long Evans rats (5-6 weeks old) were fed musk xylene through their diet at dose levels of 0 10, 100 or 1,000 mg musk xylene/kg feed (corresponding to 0, 0.7 to 0.8, 7-8 or 70 to 80 mg/kg bw/day). The four different treatment groups consisted of 6 to 8 animals per group, half of which were females and half of which were males. After 10 to 11 weeks, animals were weighed, killed, livers collected and weighed analysed for PROD, MROD and EROD activities, while microsomal proteins were submitted to gel-electroforesis followed by immunoblotting for CYP1A, 2B and 3A proteins, which were quantified by densitometry. No treatment related effects on absolute or relative liver weight or crude microsomal protein contents was observed. In the mid dose group MROD and EROD activities were enhanced by 2.5- to 4-fold in both sexes, while PROD activities were only enhanced by 1.7 fold. Immunoblot densitometry showed a 2- and 4-fold increase in CYP1A proteins at 10 and 100 mg/kg feed while at 1,000 mg/kg feed these proteins were about 7 times as high as in the controls. CYP3A was induced 1.1- to 1.6-fold in the exposed groups, while CYP2B proteins were enhanced *ca.* 10- and 6-fold in the 10 and 100 mg/kg feed groups, respectively. In the 1,000 mg/kg feed group CYP2B proteins were increased by a factor of about 180. Statistical significance for increased protein levels was only reached for CYP1A at 100 and 1,000 mg/kg feed and for CYP2B at 1,000 mg/kg feed (Suter-Eichenberger et al., 2000).
Female and male Long Evans rats (5-6 weeks old) were fed musk xylene through their diet at dose levels of 0 or 100 mg musk xylene/kg feed (corresponding to 7 to 8 mg/kg bw/day) for 18 weeks or 100 mg/kg feed for 16 weeks followed by 2 weeks of control diet or 100 mg/kg feed for 10 weeks followed by 8 weeks of control diet. The four different treatment groups consisted of 2 to 4 animals. ELISA-determined protein levels of liver microsomal cytochrome P450 1A1 and 1A2 were enhanced about 1.5 to 2 times in males and about 2 times in females. Enzyme activities as expressed by EROD and PROD were increased about 3 times in both males and females. After 16 or 10 weeks of exposure to musk xylene diet followed by either 2 or 8 weeks of control diet no appreciable differences with the 18 weeks control groups could be found. Other cytochrome P450 forms were not studied (Suter-Eichenberger et al., 2000).

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

5.8.6 Summary and discussion of carcinogenicity

From the EU RAR, section 4.1.2.7.3 (2005)

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

Special investigations into the mechanism behind the mouse liver tumours indicated that musk xylene treatment caused a very significant induction of liver enzymes, including cytochromes P450 1A1, 1A2 and 2B and cytochrome b5. Levels of CYP2B protein in liver are as high as those seen with the classical CYP2B inducer phenobarbital. However, the metabolite p-NH2- musk xylene selectively inactivates the enzyme CYP2B. The toxicological significance of this induction/inhibition phenomenon is unclear. In a 7 days study in the mouse the NOEL for effects on liver enzymes was 10 mg/kg bw/day. Similar induction phenomena have been observed in rat liver and for this species a LOEL of 10 mg/kg bw/day after 7 days of exposure could be derived. Even at dietary levels as low as 10 mg/kg feed, corresponding to 0.7 to 0.8 mg/kg bw/day, a slight inducing effect on CYP2B protein could be observed after *ca.* 75 days, while for CYP1A and 3A a ten times higher dose level appeared to be a LOEL. The induction phenomena were reversible and occured without simultaneous changes in liver weights. In absence of any other indication of liver toxicity the slight changes in levels of biotransformation enzyme activities are considered to be of an adaptive nature rather than adverse. Therefore this effect as such and the NOEL/LOEL for it will not be taken forward to the risk characterisation.

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

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

As to the Harderian gland tumours, only benign malformations developed. This gland and tissue type does not occur in humans and therefore these benign tumours are difficult to interpret with respect to their relevance to humans. Like the liver and Harderian gland tumours, the tumours in the

lung and haematopoietic system occurred spontaneously in the B6C3F1 mouse strain, with only slightly higher incidences in the treated animals.

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

Although musk xylene has not been tested for carcinogenicity in rats, there is a concern that it might be carcinogenic in rats as well, given the comparable enzyme induction properties of musk xylene in mice and rats. Further testing in e.g. rats or in mice to further elucidate the mechanism is, however, not considered to contribute much to the risk assessment of the carcinogenic risk of musk xylene to humans. This because the available data do allow the conclusions that musk xylene is a carcinogen in mice, that it acts by a non-genotoxic mode of action, and that the most serious type of tumour for which the incidence was statistically significantly increased (i.e. liver carcinomas in male mice) is mechanistically related to microsomal enzyme induction. Hence, for risk characterisation a threshold approach is considered justified, given that musk xylene is 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.

5.9 Toxicity for reproduction

5.9.1 Effects on fertility
Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.9.2 Developmental toxicity
Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.9.3 Human data
Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.9.4 Other relevant information
Not applicable for this dossier.

5.9.5 Summary and discussion of reproductive toxicity
Not applicable for this dossier. The data available do not provide any relevant information on the toxic (T) properties of this substance.

5.10 Other effects
Not applicable for this dossier.

5.11 Derivation of DNEL(s) or other quantitative or qualitative measure for dose response
Not applicable for this dossier.

5.11.1 Overview of typical dose descriptors for all endpoints
Not applicable for this dossier.

5.11.2 Correction of dose descriptors if needed (for example route-to-route extrapolation)
Not applicable for this dossier.

5.11.3 Application of assessment factors
Not applicable for this dossier.

5.11.4 Selection / identification of the critical DNEL(s) / the leading health effect
Not applicable for this dossier.

6 HUMAN HEALTH HAZARD ASSESSMENT OF PHYSICOCHEMICAL PROPERTIES
Not relevant for this type of dossier.

7 ENVIRONMENTAL HAZARD ASSESSMENT

7.1 Aquatic compartment (including sediment)

7.1.1 Toxicity test results

From the EU RAR, section 3.2.1.1 (2005)

Table 4: 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 mykiss*	96-hours	LC50 = >1,000	APHA 1980; 5-10 g; range: up to 1,000 mg/l; screening test	Boleas et al., 1996
Rainbow trout *Oncorhynchus mykiss*	21-day semi-static	no effects on EROD activity in hepatic S9 fractions and retinol levels in hepatic plasma samples	fish weight: 44.2 + 2.8 g; range: 1-100 µg /1; soivent: ethanol	
Fish Zebra	48-hour	LC50 = 3.75 LC50	no information DMSO	MITI (1992)

a the number of concentrations tested (n) is without controi and soivent controi

7.1.1.1 Fish

From the EU RAR, section 3.2.1.1 (2005):

Short- and long-term toxicity to fish

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

In the 14-day fish test with *Brachydanio rerio* test according to OECD Guideline 204 by Adema and Langerwerf (1985c) 30% mortality occurred at 0.32 mg/l, 100% at 1.0, 1.8 and 3.2 mg/l, and

one fish survived in the highest concentration of 5.6 mg/l. The estimated LC50 declined from 0.76 at day 2 to 0.40 mg/l at day 14. Since the 7-d LC50 is 0.42 mg/l, it is assumed that the value at day 14 represents the incipient LC50. The NOEC for swimming behaviour was 0.1 mg/l. Growth was affected in all concentrations: in the lowest concentration of 0.1 mg/l, the weight of the fish was 80% of the weight of the controls, while at the highest concentration of 0.56 mg/l this was 60%.

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

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

7.1.1.2 Aquatic invertebrates

From the EU RAR, section 3.2.1.1, (2005):

Short- and long-term toxicity to aquatic invertebrates

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

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

7.1.1.3 Algae and aquatic plants

From the EU RAR, section 3.2.1.1 (2005):

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

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

7.1.1.4 Sediment organisms

No data available.

7.1.1.5 Other aquatic organisms

From the EU RAR, section 3.2.1.1 (2005):

Schramm et al. (1996) tested the acute toxicity of musk xylene with photoluminescent bacteria (*Vibrio fischeri*), *D. magna* and *Scenedesmus subspicatus*. They found no effects up to the highest concentration tested, i.e. 80% of the water solubility of 0.15 mg/l for bacteria and the water solubility for algae and daphnids. From the tests on ready biodegradability it can be concluded that musk xylene was not toxic to the inoculum used at test concentrations up to 107 mg/l resulting in an NOEC of > 107 mg/l (Calame and Ronchi, 1989). This NOEC is much higher than the water solubility of 0.15 mg/l for musk xylene.

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

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

7.1.2 Calculation of Predicted No Effect Concentration (PNEC)

7.1.2.1 PNEC water

From the EU RAR, section 3.2.1.2 (2005):

For the determination of the PNEC both short and long-term toxicity test results studies are available for musk xylene. The 5 day-growth test with algae and the 21 day-reproduction test with *Daphnia magna* are considered long term tests. The 14-day fish test was considered too short for a long-term test, leaving two-long term studies for this substance. Subsequently, an assessment factor of 50 is applied to the long-term NOEC for *Daphnia magna* giving a $PNEC_{water}$ of 1.1 µg/l. The 14-day fish toxicity data may be used to support this PNEC. That is, if the LOEC of the 14-day fish

growth study is extrapolated using a factor of 10 to a chronic NOEC and an assessment factor of 10 is used, the resulting PNEC is almost identical to the PNEC obtained without using the fish data.

7.1.2.2 PNEC sediment

From the EU RAR, section 3.2.1.2 (2005):

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

$$PNEC_{sed} = [K_{susp\text{-}water} / RHO_{susp}] - PNEC_{water}$$

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

7.2 Terrestrial compartment

7.2.1 Toxicity test results

7.2.1.1 Toxicity to soil macro-organisms

From the EU RAR, section 3.2.3.1 (2005):

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

7.2.1.2 Toxicity to terrestrial plants

No data available.

7.2.1.3 Toxicity to soil microorganisms

No data available.

7.2.1.4 Toxicity to other terrestrial organisms

No data available.

7.2.2 Calculation of Predicted No Effect Concentration (PNEC soil)

From the EU RAR, section 3.2.3.2 (2005):

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

7.3 Atmospheric compartment

No data available.

7.4 Microbiological activity in sewage treatment systems

7.4.1 Toxicity to aquatic microorganisms

From the EU RAR, section 3.2.1.1 (2005):

For micro-organisms one test was available with bacteria where no effect was observed at the highest test concentration of 0.12 mg/l. However, according to the TGD these tests with

photoluminescent bacteria cannot be used for deriving a $PNEC_{STP}$. From the test on inherent biodegradability a NOEC of > 107 mg/l could be derived. Applying an assessment factor of 10 leads to a minimum $PNEC_{STP}$ of >10.7 mg/l. It is realised that this value is much higher than the water solubility of musk xylene of 0.15 mg/l.

7.4.2 PNEC for sewage treatment plant

From the EU RAR, section 3.2.1.2 (2005):

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

7.5 Calculation of Predicted No Effect Concentration for secondary poisoning (PNEC oral)

From the EU RAR, section 3.2.4 (2005):

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

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

7.6 Conclusion on the environmental classification and labeling

From the EU RAR, section 1.4 (2005):

Environmental classification and labelling according to the 29th ATP of Directive 67/548/EEC4:

Classification

N; R50-53 Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment

Specific concentration limits: None

Labelling
N
R: 50/53
S: 60-61 - This material and its container must be disposed of as hazardous waste
- Avoid release to the environment. Refer to special instructions/Safety data sheets.

8 PBT, VPVB AND EQUIVALENT LEVEL OF CONCERN ASSESSMENT

8.1 Comparison with criteria from Annex XIII

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

8.2 Assessment of substances of an equivalent level of concern

Not applicable for this type of dossier

8.3 Emission characterisation

Information currently not included

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

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

INFORMATION ON USE, EXPOSURE, ALTERNATIVES AND RISKS

1 INFORMATION ON EXPOSURE

Information currently not included

2 INFORMATION ON ALTERNATIVES

2.1 Alternative substances

No information currently available.

2.2 Alternative techniques

No information currently available.

3 RISK-RELATED INFORMATION

Information currently not included.

OTHER INFORMATION

Not relevant for this dossier.