

Annex XV Report

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: 1,2,4-Trichlorobenzene

EC Number: 204-428-0

CAS Number: 120-82-1

Submitted by: Germany

Version: August 2010

CONTENTS

PROPOSAL FOR IDENTIFICATION OF A SUBSTANCE AS A CMR CAT 1 OR 2, PBT, VPVB OR A SUBSTANCE OF AN EQUIVALENT LEVEL OF CONCERN	3
PART I.....	4
JUSTIFICATION	4
1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES	4
1.1 Name and other identifiers of the substance	4
1.2 Composition of the substance	4
1.3 Physico-chemical properties.....	4
2 HARMONISED CLASSIFICATION AND LABELLING	7
3 ENVIRONMENTAL FATE PROPERTIES.....	8
3.1 Degradation	8
3.1.1 Abiotic degradation	8
3.1.1.1 Hydrolysis	8
3.1.1.2 Phototransformation / photolysis.....	8
3.1.1.2.1 Phototransformation in air.....	8
3.1.1.2.2 Phototransformation in water.....	9
3.1.2 Biodegradation	10
3.1.2.1 Screening tests.....	10
3.1.2.2 Simulation tests	11
3.1.3 Summary and discussion on degradation.	13
3.2 Environmental distribution	14
3.2.1 Adsorption/desorption	14
3.2.2 Volatilisation	14
3.2.3 Distribution modelling	14
3.3 Bioaccumulation.....	19
3.3.1 Aquatic bioaccumulation.....	19
3.3.1.1 Bioaccumulation estimation	19
3.3.1.2 Measured bioaccumulation data	19
3.3.2 Terrestrial bioaccumulation.....	21
3.3.3 Summary and discussion of bioaccumulation	21
4 HUMAN HEALTH HAZARD ASSESSMENT	22
5 ENVIRONMENTAL HAZARD ASSESSMENT	23
5.1 Aquatic compartment (including sediment).....	23
5.1.1 Toxicity data.....	23
5.1.1.1 Fish.....	23
5.1.1.1.1 Long term toxicity to fish.....	23
5.1.1.2 Aquatic invertebrates.....	24
5.1.1.2.1 Long term toxicity to aquatic invertebrates.....	24
5.1.1.3 Algae and aquatic plants.....	24
5.1.1.4 Sediment organisms	25
5.1.1.5 Other aquatic organisms.....	25

6	CONCLUSIONS ON THE SVHC PROPERTIES	27
6.1	PBT, vPvB assessment	27
6.2	CMR assessment.....	27
6.3	Substances of equivalent level of concern assessment	27
6.3.1	Assessment of PBT/vPvB properties - comparison with criteria of Annex XIII.....	27
6.3.1.1	Persistence.....	27
6.3.1.2	Bioaccumulation.....	27
6.3.1.3	Toxicity	27
6.3.1.4	Further supporting evidence.....	28
6.3.2	Summary and overall conclusions on the PBT/vPvB properties.....	28
	PART II	29
	INFORMATION ON USE, EXPOSURE, ALTERNATIVES AND RISKS	29
	REFERENCES	30

TABLES

Table 1:	Summary of physico- chemical properties	4
Table 2:	Photodegradation rate constants and atmospheric half-lives	8
Table 3:	Removal of 1,2,4-TCB in mesocosms (Wakeham et al., 1983).....	11
Table 4:	Biodegradation rates for 1,2,4-trichlorobenzene as derived by Bartholmew and Pfaender and the corresponding half-lives (Bartholmew and Pfaender, 1983)	12
Table 5:	Half-lives of TCB in the sewage sludge-amended soil and standard spiked soil in microcosm experiments (Wang and Jones, 1994)	13
Table 6:	Distribution in WWTP (Simple Treat 3.0)	14
Table 7:	Input parameters for both models	15
Table 8:	Characteristic Travel Distances [km]	16
Table 9:	Spatial ranges.....	17
Table 10:	Available bioconcentration factors (BCF).....	19
Table 11:	Valid data on long-term ecotoxicity to fish. For details and references, see European Commission (2003).....	23
Table 12:	Valid data on long-term toxicity to aquatic invertebrates. For details and references, see European Commission (2003)	24
Table 13:	Toxicity to algae (see European Commission 2003)	24
Table 14:	Short-term toxicity to other aquatic organisms (see European Commission 2003).....	26

FIGURES

Figure 1:	Characteristic Travel Distance (CTD) and overall persistence (P_{ov}) of 1,2,4-Trichlorobenzene	15
Figure 2:	Characteristic Travel Distances of 1,2,4-TCB compared to PCB 28, PCB 153 and PCB 101.....	16
Figure 3:	Relative steady-state concentration distribution of 1,2,4-Trichlorobenzene.	18
Figure 4:	Relative steady-state concentration distribution of PCB 28.	18

**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: 1,2,4-Trichlorobenzene

EC Number: 204-428-0

CAS Number: 120-82-1

- *It is proposed to identify the substance as substance of equivalent concern according to Article 57 (f).*

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

Taking into account all available information on persistent, bioaccumulative and toxic properties of 1,2,4-trichlorobenzene, it is concluded that 1,2,4-trichlorobenzene is a PBT-substance. 1,2,4-trichlorobenzene was already identified as a PBT by the TC NES subgroup on identification of PBT and vPvB substances in 2003 and this conclusion was supported by the TCNES and CA-Meeting.

The substance is considered to fulfil the P criterion, and also the vP-criterion. In addition to that, also the B criterion is fulfilled. In case that lipid normalization is performed, 1,2,4-trichlorobenzene would not clearly fulfil the B criterion. However, if doing so the maximum BCF will still be close to the B criterion of Annex XIII. Although 1,2,4-trichlorobenzene is highly toxic for aquatic organisms tested on the long-term, it does not formally meet the T-criterion. The overall conclusion is however drawn in a weight of evidence approach, taking supporting evidence into account relating to the very high long-range environmental transport potential of this substance. It raises an equivalent concern according to Article 57 (f).

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

PART I

JUSTIFICATION

1 IDENTITY OF THE SUBSTANCE AND PHYSICAL AND CHEMICAL PROPERTIES

1.1 Name and other identifiers of the substance

Chemical Name: Benzene, 1,2,4-trichloro-

EC Name: 1,2,4-trichlorobenzene

EC Number 204-428-0

CAS Number: **120-82-1**

IUPAC Name: 1,2,4-trichlorobenzene

1.2 Composition of the substance

The detailed composition of the substance is confidential and provided in the technical dossier.

Chemical Name: Benzene, 1,2,4-trichloro-

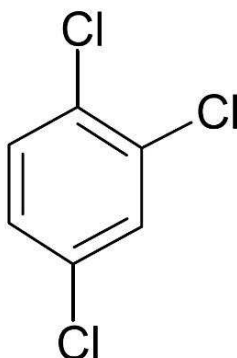
EC Number: 204-428-0

CAS Number: 120-82-1

IUPAC Name: 1,2,4-trichlorobenzene

Molecular Formula: $C_6H_3Cl_3$

Structural Formula:



Molecular Weight: 181.45 g/mol

Typical concentration (% w/w): Min. > 99 % (w/w)

Concentration range (% w/w): -

1.3 Physico-chemical properties

Table 1: Summary of physico- chemical properties

REACH ref Annex, §	Property	IUCLID section	Value	[enter comment/reference or delete column]
VII, 7.1	Physical state at 20°C and 101.3 kPa	4.1	liquid	European Communities, 2003
VII, 7.2	Melting/freezing point	4.2	16-17 °C ¹⁾	European Communities, 2003
VII, 7.3	Boiling point	4.3	213.5 °C at 1013 hPa	European Communities, 2003
VII, 7.5	Vapour pressure	4.6	36 Pa at 20 °C ²⁾	European Communities, 2003
VII, 7.7	Water solubility	4.8	36 mg/L at 20 °C	European Communities, 2003
VII, 7.8	Partition coefficient n-octanol/water (log value)	4.7 partition coefficient	log K _{ow} 4.05	European Communities, 2003
XI, 7.16	Dissociation constant	4.21	---	
VII, 7.4	Density	4.4	1.456 g/cm ³ at 20 °C	European Communities, 2003
VII, 7.9	Flash point	4.11	110 °C, (close cup)	PTB-AG 3.43, CHEMSAFE, 2009
VII, 7.10	Flammability	4.13	<p>Pyrophoric properties: The classification procedure need not be applied because the organic substance is known to be stable into contact with air at room temperature for prolonged periods of time (days).</p> <p>Flammability in contact with water: The classification procedure need not to be applied because the organic substance does not contain metals or metalloids.</p> <p>Flammability upon ignition (solids, gases): Testing is technically not possible, substance is a liquid.</p>	BAM, II.2 (2010)
VII, 7.11	Explosive properties	4.14	The classification procedure needs not to be applied because there are no chemical groups present in the molecule which are associated with explosive	BAM, II.2 (2010)

			properties.	
VII, 7.12	Auto-ignition temperature (Liquids and Gases)	4.12	571 °C	PTB-AG 3.43, CHEMSAFE, 2009
VII, 7.13	Oxidising properties	4.15	The classification procedure need not to be applied because contained chlorine atoms are chemically bonded only to carbon.	BAM, II.2 (2010)

- 1) The value 17°C is used in the risk assessment
- 2) measured; used for risk assessment

2 HARMONISED CLASSIFICATION AND LABELLING

1,2,4-trichlorobenzene (1,2,4-TCB) entry in Table 3.1 of Annex VI of Regulation (EC) 1272/2008 as amended by the 1st ATP:

Index Number: 602-087-00-6

Acute Tox. 4*	H302 (Harmful if swallowed)	GHS07
Skin Irrit. 2	H315 (Causes skin irritation)	GHS09
Aquatic Acute 1	H400 (Very toxic to aquatic life)	Wng H410
Aquatic Chronic 1	H410 (Very toxic to aquatic life with long lasting effects)	

1,2,4-TCB entry in Table 3.2 of Annex VI of Regulation (EC) 1272/2008 as amended by the 1st ATP:

Index Number: 602-087-00-6

Xn; R22 (Harmful if swallowed)

Xi; R38 (Irritating to skin)

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

3 ENVIRONMENTAL FATE PROPERTIES

3.1 Degradation

A large part of the data presented in this section are taken from the Risk Assessment Report (European Commission, 2003)

3.1.1 Abiotic degradation

3.1.1.1 Hydrolysis

Based on the experimental data (Korte and Freitag, 1986) on hydrolysis at 50°C, 1,2,4-trichlorobenzene (1,2,4-TCB) is not expected to hydrolyse under normal environmental conditions (Howard, 1989; Schmidt-Bleek et al., 1982).

3.1.1.2 Phototransformation / photolysis

3.1.1.2.1 Phototransformation in air

The atmospheric half-life of 1,2,4-TCB due to photodegradation by hydroxyl radicals is estimated to be in the order of a month. The following table summarises the photodegradation data.

Table 2: Photodegradation rate constants and atmospheric half-lives

Rate constant cm ³ /molecule/s	Half-life (days)	Method	Reference
5.32·10 ⁻¹³	18.5	Experimental	(European Commission, 2003)
5.32·10 ⁻¹³	30.2	Measured, 296°K	(Rinke and Zetzsch, 1984)
2.82·10 ⁻¹³	38	Calculated by AOP programme	(AOPWIN, 1995)

The reaction of 1,2,4-TCB with OH radicals was investigated in the presence of helium at pressures from 5 to 800 mbar using a pulsed vacuum UV photolysis-resonance fluorescence apparatus. At 23°C and 133 mbar helium, the rate constant k was observed to be 0.5·10⁻¹² cm³/s (Rinke and Zetzsch, 1984). Assuming an average tropospheric OH radical concentration of 5.10⁵ molecules/cm³, the half-life is about 30 days (BUA, 1987).

In the atmosphere, the estimated vapour phase half-life of 1,2,4-TCB was 18.5 days, estimated as a result of reaction with photochemically produced hydroxyl radicals at 8.10⁵ molecules/cm³, giving a reaction rate of 0.532·10⁻¹² cm³/molecules/s (European Commission, 2003).

The photochemical oxidation was estimated using the structure analysis by the model AOPWIN for comparison (AOPWIN, 1995). The estimated half-life of 38 days is based on an OH-radical concentration of 1.5·10⁶ molecules/cm³ and a 12-hour daylight period. The previous model used 5.10⁵ molecules/cm³ which was a 24-hour average value that included night-time. Using 5.10⁵ molecules/cm³ (24 hours) would result in a DT₅₀ of 57 days estimated by AOPWIN.

Using an experimental value for the OH rate constant of $0.55 \cdot 10^{-12} \text{ cm}^3/\text{molecules/s}$ and by setting the OH radical concentration to $5 \cdot 10^5 \text{ molecules/cm}^3$, a DT_{50} of 29 days would be estimated (Rippen, 1995) The value is based on the geometric mean of the measured absolute K_{OH} value (296 K) $0.5 \cdot 10^{-12} \text{ cm}^3/\text{s}$ and a relative measured K_{OH} value (300 K) $0.6 \cdot 10^{-12} \text{ cm}^3/\text{s}$.

Measured and calculated OH rate constants are in good agreement and the rate constant of $0.532 \cdot 10^{-12} \text{ cm}^3/\text{molecules/s}$ (European Commission, 2003) is used as input for calculations on the long-range transport potential.

The removal of 1,2,4-TCB from air may be due to degradation by chemical- or sunlight-catalysed reactions or by absorption onto particles that settle or are removed from the atmosphere by rain. A measure of the effectiveness of these factors is the atmospheric residence time. In a field study in California and Arizona, air samples during a two-week period included unspecified trichlorobenzene. The estimated residence time was 116 days, assuming an average daily (24 hours) abundance of OH radicals of $10^6 \text{ molecules/cm}^3$ (Singh et al., 1981).

3.1.1.2.2 Phototransformation in water.

Degradation by direct photolysis is not expected to be essential because the maximum absorption value is 286 nm (Bayer spectral data). The half-life for sunlight photolysis in pure surface water at 40° latitude in summer was 450 years (Dulin et al., 1986). The recovery of 1,2,4-TCB from isopropanol solution in Pyrex glass tubing (with a cut-off at 285 nm) irradiated with 300 and 310 nm fluorescent lamps for 30 minutes was 89.4% under anaerobic conditions where O_2 was replaced with N_2 and 8.1% under aerobic conditions. The products of photodegradation were 1,3- and 1,4-dichlorobenzene (Akermark et al., 1976).

In a laboratory study using artificial light (high pressure mercury vapour lamp at wavelengths $> 290 \text{ nm}$), the photodegradation was studied at a concentration of 2-5 ppm. 1,2,4-TCB was dissolved in distilled water and filtered river water. The photolytic half-life was estimated to be 16.7 hours in distilled water and 12.2 hours in river water. The photolysis products in river water were 1,4-dichlorophenol and 4-chlorophenol (Mansour et al., 1989).

The photolysis was studied by exposing 5 ml 1,2,4-TCB at $4 \mu\text{g/ml}$ at a distance of 30 cm from artificial light (two Fluorochemical lamps $20\text{W} \times 2$) at 25°C . After 144 hours, 0% was degraded (Kondo et al., 1988).

In a test where 1,2,4-TCB was adsorbed on silica gel and irradiated with light at wavelengths $> 290 \text{ nm}$ for 17 hours, 9.8% of the applied amount was degraded to CO_2 (Freitag et al., 1985).

In addition to the direct photolysis, the photodegradation may also follow an indirect photolysis by sensitisation or by secondary reactions with OH- and O_2 -radicals. The photolysis was studied in water solutions (1,2,4-TCB conc.: 4-20 mg/600ml) irradiated for 3 hours. The photoreactivity in solutions in the presence of nitrite was observed to increase the photodegradation rate. The rate constant in pure water was $1.5 \cdot 10^{-4} \text{ s}^{-1}$ resulting in DT_{50} to be 1.2 hours. In water added nitrite, the rate constant k was $3.1 \cdot 10^{-4} \text{ s}^{-1}$ and the resulting DT_{50} 0.4 hours (Kotzias et al., 1982). The study was conducted under artificial conditions but indicates that photolysis may be affected by the contents in water. In the study, salts were used but other organic substances and organic matter may also affect the photodegradation rate.

Although some photodegradation of 1,2,4-TCB may occur in water it is deemed a very slow process (European Commission 2003) and not expected to be a relevant degradation process, probably apart from very shallow clear waters in the first few centimetres of the water column. However, the substance is dispersed in the whole water column. Therefore aquatic photodegradation is not considered to have a relevant impact on the overall persistency of 1,2,4-TCB in the environment. In addition, because of the adsorption behaviour of the substance, it will predominantly adsorb to suspended organic matter and sediment (and to soil because of atmospheric washout).

Summary

On the basis of the available data, abiotic degradation of 1,2,4-TCB in the atmosphere is expected to be slow (DT_{50} between 18.5 and 38 days) and the substance is not expected to hydrolyse under environmental conditions.

3.1.2 Biodegradation

3.1.2.1 Screening tests

The ready biodegradability was studied with a method corresponding to the OECD TG 301C, Modified MITI (I) test (MITI-List, 2002). The test concentration was 100 mg/l and activated sludge concentration 30 mg/l. In the aerobic study, the degradation measured as Biochemical Oxygen Demand (BOD) was 0% after 14 days.

The BOD_{20} value for 1,2,4-TCB was studied using microorganisms from an industrial wastewater treatment plant normally exposed to phenolics and other industrial chemicals (i.e. adapted inoculum) (Simmons et al., 1977). The concentration of 1,2,4-TCB was 1.69 and 2.61 mg/l. Although no apparent biodegradation was indicated by the BOD oxygen uptake until day 10, GC-ECD analysis of the remaining 1,2,4-TCB indicated that the substance began to disappear within 1 to 5 days. 99% and 100% of the 1,2,4-TCB had disappeared in 10 days as determined by the GC-ECD analysis while the BOD test indicated 55% of the theoretical oxygen demand (ThOD). The remaining 45% ThOD was stated to be incompletely oxidised metabolites of 1,2,4-TCB. BOD_{10} and BOD_{20} were 55% and 55% using 1.7 mg/l and 19% and 55% using 2.6 mg/l, respectively. The test with the initial concentration of 2.6 mg/l showed a delayed degradation, which could be an advice for toxicological effects. Furthermore, it is not clear if volatilization was considered.

A static culture flask biodegradation screening study was used to determine the biodegradability at two concentrations of 1,2,4-TCB (5 and 10 mg/l), a 7-day static incubation at 25°C followed by three weekly subcultures of yeast and settled domestic wastewater (non-adapted) as microbial inoculum (Tabak et al., 1981). A gradual adaptation process followed by a de-adaptive process in subsequent subcultures (reduced degradation and accumulation of 1,2,4-TCB in the media) was observed. For the original culture 54% and 43% degradation were observed after 7 days at 5 and 10 mg 1,2,4-TCB/l, respectively. This test is less stringent than a standard ready biodegradability test because of the presence of an extra carbon source (yeast) allowing for co-metabolism. Further, it is not clear whether the reported biodegradation percentages are referring to the removal of parent compound or to mineralisation.

On the basis of these three available laboratory biodegradation screening tests, 1,2,4-TCB is not readily biodegradable but biodegradation occurs in conditions favouring biodegradation (adapted sludge as inoculum or presence of an additional carbon source).

3.1.2.2 Simulation tests

In a study by Simmons et al., using ^{14}C -labelled substance at 0.345 mg/l, the degradation was studied in activated sludge from a textile plant wastewater (i.e. adapted) by measuring $^{14}\text{CO}_2$ development (Simmons et al., 1977). After 5 days, 56% was recovered as $^{14}\text{CO}_2$, 23% as polar metabolites, 7% was evaporated and 13% remained as 1,2,4-TCB. The reduced volatilisation is stated to be caused by the high organic sludge environment since 80% was adsorbed to solids and 20% was actually in the water. The amount of ^{14}C -1,2,4-TCB converted to $^{14}\text{CO}_2$ was 33% in 1 day and 56% in 5 days. These results show that the substance is partly susceptible to biodegradation by adapted micro-organisms.

The removal from seawater was studied in mesocosm studies, which included consideration of volatilisation (Wakeham et al., 1983). The tanks were 5.5 m high and 1.8 m in diameter and contained 13 m³ seawater. In the study, a mixture of volatile organic compounds was added. The dissipation was studied at conditions spring (8-16°C), summer (20-22°C) and winter (3-7°C). The initial concentration of 0.5 µg/l was equivalent to the concentration measured in a moderately polluted bay. The concentrations were measured during 1-2 months.

Table 3: Removal of 1,2,4-TCB in mesocosms (Wakeham et al., 1983)

	Temperature (°C)	Initial concentration (mg/l)	Rate constant	DisDT ₅₀ (days)
Spring (Apr. 15-Jun. 18)	8-16	0.5	-0.032	22
Summer (Aug. 19-Sep. 8)	20-22	0.2	-0.066	11
Winter (Mar. 4-May 4)	3-7	2.2	-0.058	12
Water with HgCl ₂ (Sept. 9th to 15th)		0.2	-0.073	9.5
Water without HgCl ₂		0.3	-0.066	10.6

The dissipation was relatively independent of temperature with half-lives of 2-3 weeks regardless of the season. Retardation of the biological activity by adding HgCl₂ (2 mg/l) did not increase the summer dissipation time. Therefore, the dissipation was assumed to be primarily dissipation by volatilisation and not biodegradation. Thus, volatilisation dominates the dissipation of 1,2,4-TCB whereas biodegradation is of less importance according to the authors.

Trichlorobenzenes are chemically stable in both aerobic and anaerobic environments. In studies on the degradation in anaerobic sediments, trichlorobenzenes were reductively dechlorinated to monochlorobenzenes via dichlorobenzenes. 1,2,4-TCB was transformed via 1,4-dichlorobenzene (Bosma et al., 1988) and via 1,2- and 1,3- dichlorobenzenes (Peijnenburg et al., 1992). The study by Bosma et al. was performed as a study using 25 cm high and 5.5 cm internal diameter columns wet packed with sediment from the River Rhine near Wageningen. The columns were percolated continuously at a flow rate of 1 cm/h in an upflow mode. It was concluded that the observed removal was due to biological processes because of the long lag-phase preceding the onset of disappearance and because there was no elimination in a batch of anaerobic autoclaved sediment. The study by Peijnenburg et al. was performed in a methanogenic sediment-water system maintained at 22°C in a nitrogen atmosphere. The sediments were taken from a slow flowing river and a eutrophic pond. The anaerobic degradation rates were $\log k = -5.64 \text{ min}^{-1}$ and $\log k = -5.62 \text{ min}^{-1}$ (corresponding to half-lives of 212 days and 202 days), respectively. 1,2-, 1,3- and 1,4-

dichlorobenzenes were formed in ratios of approximately 1.5:1:1.5, as confirmed by GC. Almost immediately after incubation began, monochlorobenzene could be detected.

1,2,4-TCB can be degraded in soil, although very slowly (Marinucci and Bartha, 1979). The aerobic mineralisation was studied using ^{14}C -labelled 1,2,4-TCB and a mineralisation rate measured as CO_2 development/day. In a study using a sandy loam (pH 6.5) added 1,2,4-TCB at a concentration of 50 $\mu\text{g/g}$ soil, the degradation in soil was observed to be slow. The incubation was performed at 20°C for 3 to 12 weeks. 1,2,4-TCB was subject to mineralisation as soil poisoned with 1% HgCl_2 or NaN_3 reduced the CO_2 evolution consistently. Anaerobic conditions either continuously or alternated weekly with aerobic incubation periods markedly depressed the mineralisation. The mineralisation rate was 0.181 $\mu\text{g/day}/20$ g soil equivalent to 9 $\mu\text{g/d/kg}$. The turnover rate (% 1,2,4-TCB converted to $\text{CO}_2/\text{day} = 0.075\%$) was maximal at 10 $\mu\text{g/g}$ soil and sharply declined at higher concentrations. Haider et al. used 10 $\mu\text{g/g}$ (in 100 g soil) and observed a mineralisation rate about twice as high (Haider et al., 1974).

In addition to the experiments reviewed in the Risk Assessment Report (European Commission, 2003), two other biodegradation studies are available. Bartholomew and Pfaender conducted simulation type of tests measuring mineralisation ($^{14}\text{CO}_2$ respiration) and uptake of ^{14}C labelled metabolites (Bartholomew and Pfaender, 1983; Pfaender and Bartholomew, 1982). Kinetic values for the total biodegradation of 1,2,4-TCB covering both incorporation of ^{14}C into biomass and $^{14}\text{CO}_2$ -production, are illustrated in Table 4 The tests have also been reviewed by Battersby (Battersby, 1990).

Table 4: Biodegradation rates for 1,2,4-trichlorobenzene as derived by Bartholmew and Pfaender and the corresponding half-lives (Bartholomew and Pfaender, 1983)

Site	Date	Temperature ($^\circ\text{C}$)	$V_{\max}/K_m=k_1$ (d^{-1}) ¹⁾	$T_{1/2}$ (d)
River, upstream	7 Nov .80	12	0.028	24.8
River, upstream	26 May 81	24	b.d. ²⁾	-
Estuary	7 Nov.80	14	b.d. ²⁾	-
Estuary	26 May 81	28	0.027	25.7
Marine	7 Nov.80	17	b.d. ²⁾	-
Marine	26 May 81	24	0.012	57.8

1) Original data from Bartholomew and Pfaender (1983); calculations similar to those in Battersby (1990)

2) b.d., below detection limit (V_{\max} was less than 1 ng liter⁻¹ h⁻¹ and less than the metabolic rate that could be detected by the experimental method)

The data in Table 4 indicate that 1,2,4-TCB is degraded under some conditions with half-lives of approximately 25 days in fresh and estuarine waters and below 60 days in marine water. However, biodegradation rates were too low to be determined in half of the experiments. No explanation for the different results was provided by the authors. The $^{14}\text{CO}_2$ production was reported to be very low in general (no values provided by the authors), which implies that the ^{14}C incorporated by the biomass consisted of metabolites that were not further degraded during the experiment. It can be assumed, that the results of these two experiments do not change the overall picture provided by the other simulation type of studies available.

The behaviour and fate of chlorobenzenes in spiked and sewage sludge-amended soil was tested by Wang and Jones (Wang and Jones, 1994). For testing the fate, four different experimental conditions were investigated: (1) normal; (2) sterilized; (3) sterilized and shaded; (4) sterilized, shaded and sealed. Under condition (4) no decrease of concentration was observed. Whereas under condition (1)-(3) the concentration of TCB decreased. This indicated volatilization as main loss process. The loss process in soil occurs in two steps (two step first order kinetic model). In the first step the TCB volatilize to the atmosphere over short periods. The second step was much slower and was presumably controlled by the rate of compound desorption from soil. A proportion of the TCB will stay in the soil for much longer periods. The concentration in spiked soil decreased faster than in sludge amended soil.

Table 5: Half-lives of TCB in the sewage sludge-amended soil and standard spiked soil in microcosm experiments (Wang and Jones, 1994)

Compound	First step				Second step			
	Sewage sludge-amended soil		Standard spiked soil		Sewage sludge-amended soil		Standard spiked soil	
	Loss (%)	DisDT ₅₀ (d)	Loss (%)	DisDT ₅₀ (d)	Loss (%)	DisDT ₅₀ (d)	Loss (%)	DisDT ₅₀ (d)
1,2,4-TCB	83.2	22.5	64.5	12.5	2.26	49500	19.8	194
1,2,3-TCB	88.6	22.2	73.0	15.0	4.45	1370	12.8	222
1,3,5-TCB	85.3	23.7	71.2	10.5	6.22	239	14.8	238

A marine risk assessment on 1,2,4-TCB was carried out by van Wijk et al. (van Wijk et al., 2006). In contrast to the EU risk assessment report (European Commission, 2003) and the results of the EU PBT working group this paper characterised 1,2,4-TCB as non persistent.

The assessment of van Wijk et al. (van Wijk et al., 2006) contains no new tests, but is based on a review of existing data. However, in the majority of cases the experiments showing biodegradability were realized with isolated and enrichment cultures (Adrian et al., 2000; Brunsbach and Reineke, 1994; Nowak et al., 1996; Rapp and Timmis, 1999; Sander et al., 1991; Van der Meer et al., 1987) or the material was pre-exposed (Masunaga et al., 1996). Hence, the respective microorganisms were adapted. Furthermore, the studies showing persistence in soil and sediment (Wang and Jones, 1994; Peijnenburg et al., 1992) are not considered in this assessment.

3.1.3 Summary and discussion on degradation.

1,2,4-TCB has a low degradation rate in the atmosphere (DT₅₀ between 18.5 and 38 days) and is not expected to hydrolyse under environmental conditions.

Screening studies give evidence, that 1,2,4-TCB is not readily biodegradable. Further studies show relatively long dissipation time (DisDT_{50soil} = 194 days) and prove slow degradation in soil. For an evaluation of persistence, the degradation half life is required which is either greater than or equal to the dissipation time. Thus, the degradation half life in soil is considered to be greater than or equal to 194 days. In freshwater sediment, degradation half-lives of more than 200 days were detected.

In conclusion, 1,2,4-TCB is considered to fulfil the P and the vP criterion according Annex XIII of the REACH regulation.

3.2 Environmental distribution

3.2.1 Adsorption/desorption

Based on model estimates a K_{oc} of 2400 L/kg has been estimated (ECHA, 2008). Therefore a high adsorption potential and a low mobility in soil is expected for 1,2,4-Trichlorobenzene. Due to the slow degradation in soil, the substance may contaminate groundwater by leaching through sandy soils with low organic carbon content.

3.2.2 Volatilisation

Calculation of the Henry constant using the equation $HENRY=VP*MOLW/SOL$ from the Guidance document for Chemical Safety Assessment (ECHA, 2008) and the substance properties from table 1 results in a Henry constant of $181.45 \text{ Pa}\cdot\text{m}^3\cdot\text{Mol}^{-1}$. This indicates that the substance is highly volatile from water. Due to the high adsorption potential to organic matter, volatilisation from soil and sludge is expected to be lower.

The distribution coefficient $K_{air,water}$ (Henry coefficient) calculated from the Henry constant is $7.448\cdot 10^{-2}$.

3.2.3 Distribution modelling

Distribution in Waste Water Treatment Plant

The modelling of the distribution in a municipal Waste Water Treatment Plant was done with SimpleTreat 3.0 (debugged version, 7 Feb 1997) and the result of the Screening Test on ready biodegradation (substance is not biodegradable; $k=0/h$).

Table 6: Distribution in WWTP (Simple Treat 3.0)

Summary of distribution	(Percent)
To air	61.8
To water	20.1
Via primary sludge	16.1
Via surplus sludge	1.9
Degraded	0.0
Total	100

The result of the distribution modeling in a WWTP indicates that nearly 62 percent of the 1,2,4-TCB found in the influent waste water is emitted to air.

Long-range Transport

An important indicator for the environmental persistence can be the long-range transport to remote areas. For the calculation of the long-range transport potential of 1,2,4-Trichlorobenzene two different multimedia models have been used. On the one hand the OECD P_{ov} and LRTP Screening Tool (OECD, 2006) has been used and on the other hand the model ChemRange (Scheringer, 1996). Both models require only a few input parameters. These are the partition coefficients octanol/water, K_{ow} , the dimensionless Henry's Law Constant, K_{aw} and the environmental degradation half-lives in air, water and soil.

Unfortunately only very few measured half-lives are available for 1,2,4-Trichlorobenzene. These are the half-life in air and a measured DisT50 in soil. Although, it is well known that this DisT50 represents not only degradation this value was used in the calculation. 1,2,4-Trichlorobenzene is benchmarked as “non-biodegradable”. According to TGD (ECHA 2008) the half-lives in soil and water have to be considered as infinite. However the models require a discrete value for the simulation. Due to this fact the half-lives according to the TGD for inherent biodegradation in water of 150 d and 3000 d in soil have been used. Furthermore, the half-lives in water have been calculated on the one hand according to Arnot (Arnot et al., 2005) and on the other hand using the QSAR-model CATALOGIC (OASIS Catalogic v5.10.7, 2009). Finally, four calculations with different half-lives for water and soil has been carried out to show the influence of these half-lives on the long-range transport potential of 1,2,4-Trichlorobenzene.

Table 7: Input parameters for both models

Input parameter	1,2,4-TCB (TGD)	1,2,4-TCB (Arnot)	1,2,4-TCB (Catalogic)	1,2,4-TCB (measured soil)
logKow	4.05	4.05	4.05	4.05
logKaw	-1.128	-1.128	-1.128	-1.128
$t_{1/2}$ (air) [d]	18.5	18.5	18.5	18.5
$t_{1/2}$ (water) [d]	150	91.025	82	150
$t_{1/2}$ (soil) [d]	3000	273.075	246	194

Results using the OECD P_{ov} and LRTP Screening Tool:

Figure 1 shows that all four calculations yield nearly the same values for characteristic travel distance (CTD) and overall persistency (P_{ov}). Substances with CTD and P_{ov} values of this magnitude are considered to show a high long-range transport potential.

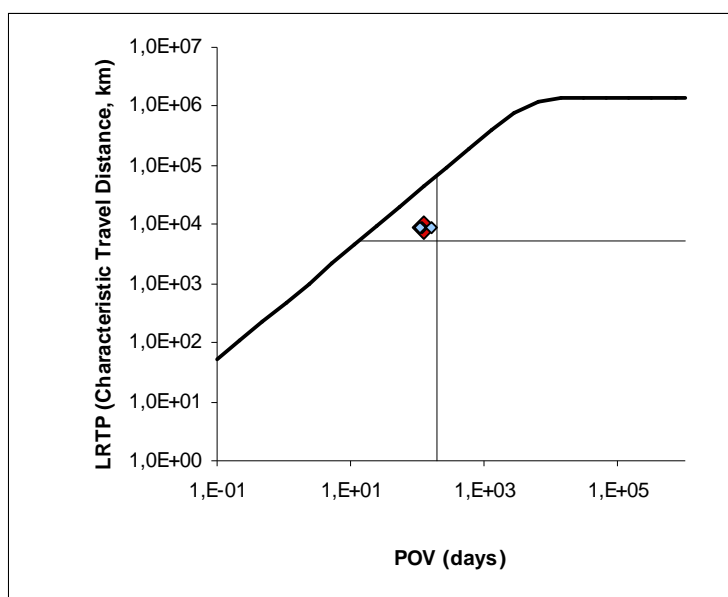


Figure 1: Characteristic Travel Distance (CTD) and overall persistence (P_{ov}) of 1,2,4-Trichlorobenzene

In order to benchmark the calculated long-range transport potential of 1,2,4-Trichlorobenzene the results have been compared with the long-range transport potential of the well known POPs PCB 28, PCB 101 and PCB 153, regulated under the Stockholm Convention.. In Table 8, the characteristic travel distances (CTD) calculated with the OECD P_{ov} and LRTP Screening Tool” are listed:

Table 8: Characteristic Travel Distances [km]

Substance	CTD [km]
1,2,4-Trichlorobenzene (Arnot)	9018
1,2,4-Trichlorobenzene (Catalogic)	9010
1,2,4-Trichlorobenzene (measured soil)	9050
1,2,4-Trichlorobenzene (TGD)	9053
PCB 153	12629
PCB 101	8595
PCB 28	3298

Figure 2 illustrates that the long-range transport potential of 1,2,4-Trichlorobenzene is in the same order of magnitude as the long-range transport potential of all three PCBs.

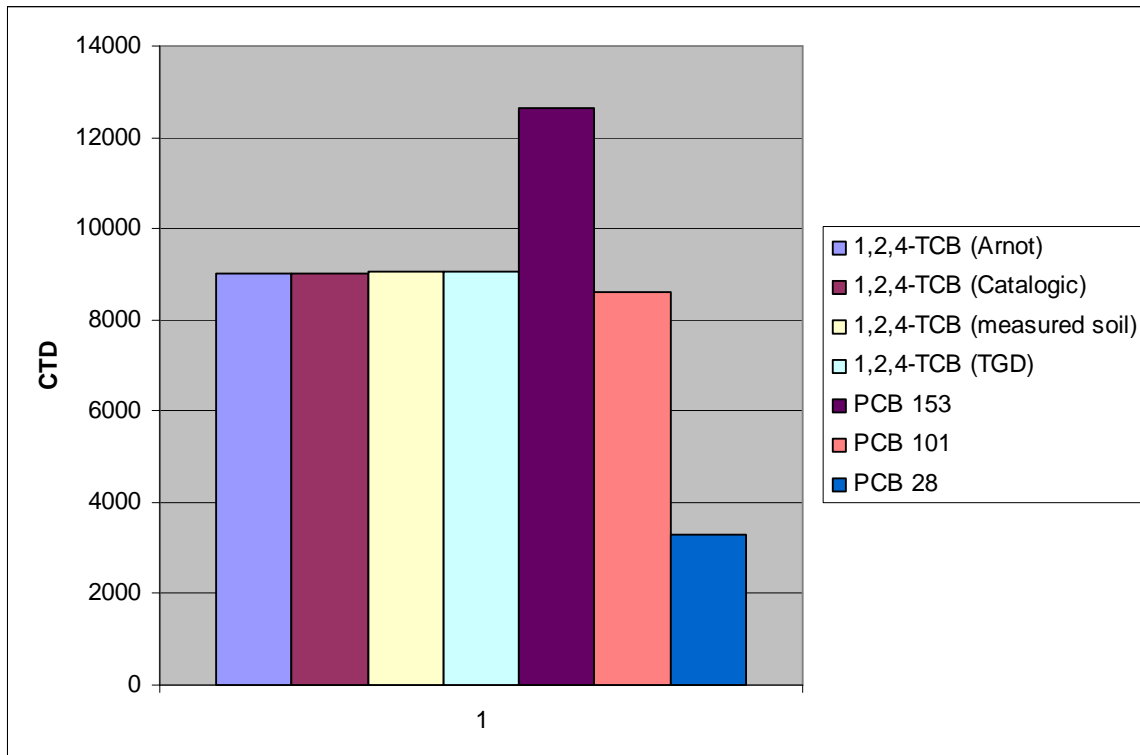


Figure 2: Characteristic Travel Distances of 1,2,4-TCB compared to PCB 28, PCB 153 and PCB 101.

Results using ChemRange:

In contrast to the OECD P_{ov} and LRTP Screening Tool, ChemRange calculates the spatial range of a chemical. The spatial range is calculated as the fraction of earth circumference which can be reached by a substance. The long-range transport is calculated for the surface compartments soil, water and air. In order to benchmark the calculated long-range transport potential of 1,2,4-Trichlorobenzene the results have been compared with the long-range transport potential of the well known POPs PCB 28, PCB 101 and PCB 153 regulated under the Stockholm Convention. Using a release scenario to air the calculated spatial ranges are listed in Table 9:

Table 9: Spatial ranges

Substance	Range soil (Fraction of earth circumference)	Range water (Fraction of earth circumference)	Range air (Fraction of earth circumference)
1,2,4-Trichlorobenzene (Arnot)	0.32	0.32	0.32
1,2,4-Trichlorobenzene (Catalogic)	0.32	0.32	0.32
1,2,4-Trichlorobenzene (measured soil)	0.32	0.32	0.32
1,2,4-Trichlorobenzene (TGD)	0.32	0.32	0.32
PCB 28	0.29	0.30	0.29
PCB 101	0.32	0.33	0.32
PCB 153	0.40	0.40	0.40

Comparison of results

The calculated spatial ranges for 1,2,4-TCB using a release scenario to soil are very similar. The high long-range transport potential of 1,2,4-Trichlorobenzene and PCB 28 is illustrated by Figure 3 and Figure 4: Both diagrams are similar and show a shape which is characteristic for substances with a high long-range transport potential. In case of a substance with a low long-range transport potential the shape of the plot would show just a sharp peak at the source point.

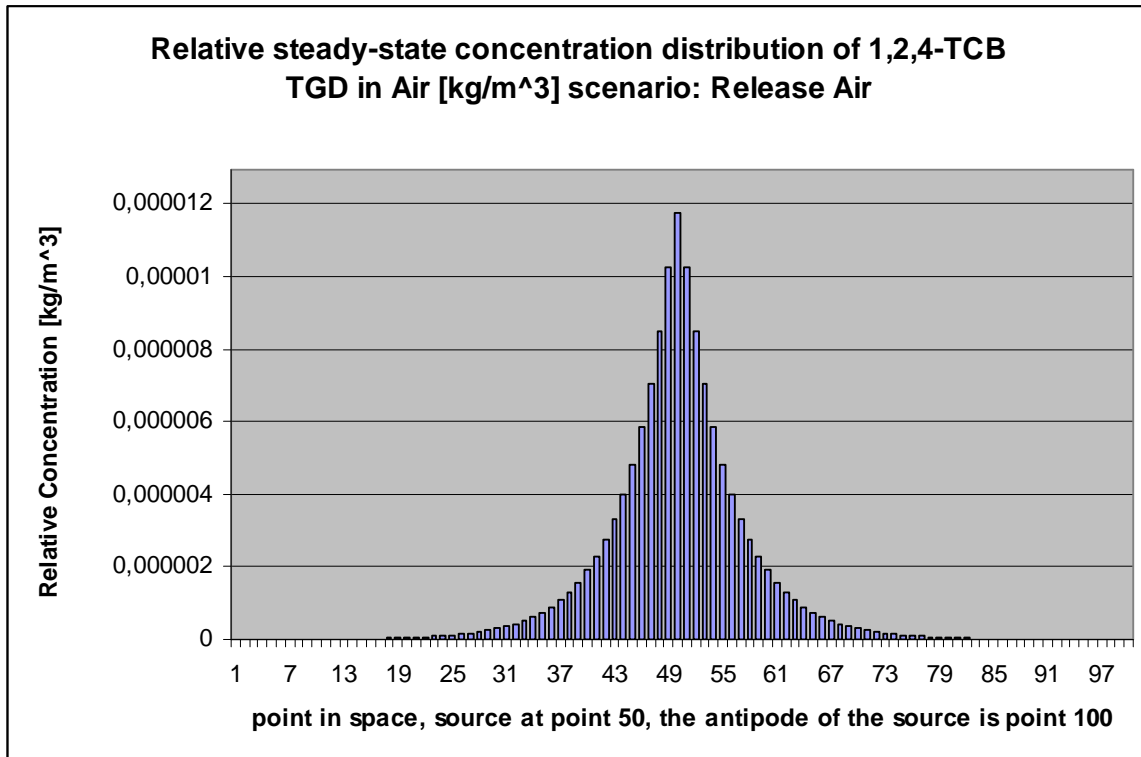


Figure 3: Relative steady-state concentration distribution of 1,2,4-Trichlorobenzene.

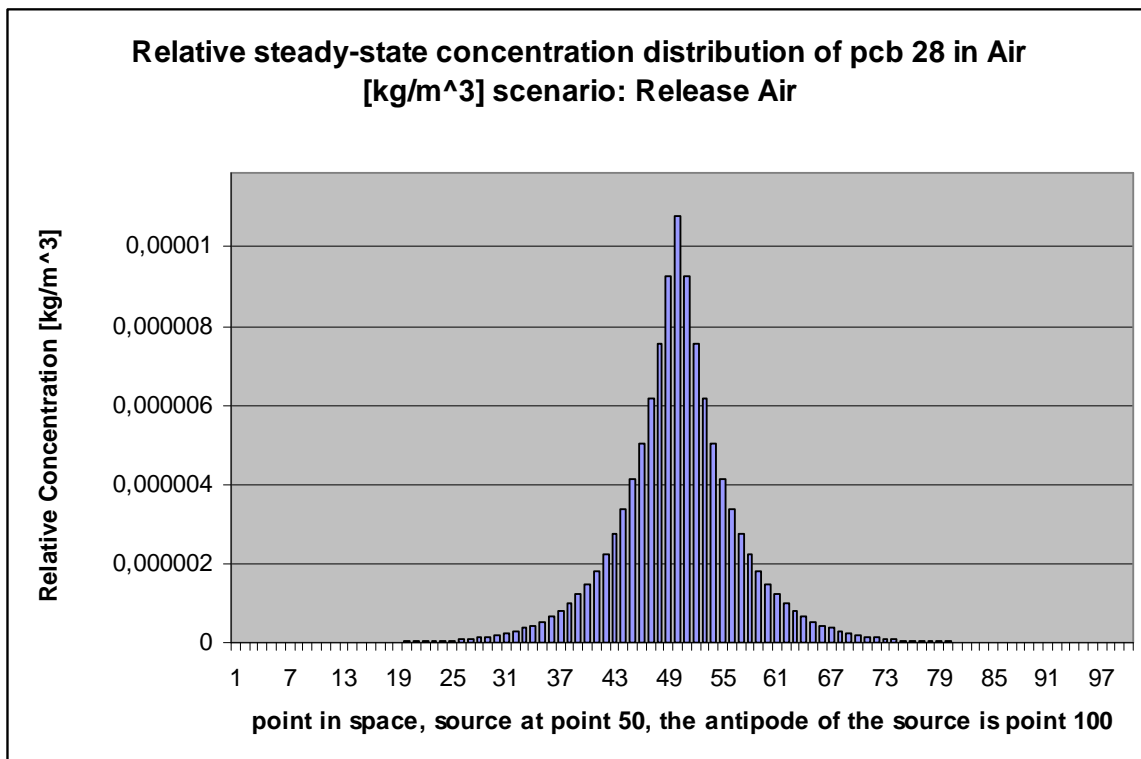


Figure 4: Relative steady-state concentration distribution of PCB 28.

Conclusions:

Due to its high volatility (Henry constant of $181.45 \text{ Pa}\cdot\text{m}^3\cdot\text{Mol}^{-1}$), 1,2,4-TCB is expected to be distributed from water to air. In the air, 1,2,4-TCB is persistent (estimated half lives between 18.5 and 38 days) and may be transported via air for several thousand kilometres. Distributed by this pathway also to remote areas, 1,2,4-TCB will be deposited there again to soil or water where it may be even more persistent e.g. due to lower temperatures.

Model calculations confirm the expected potential for long-range transport, as both the OECD P_{ov} and LRTP Screening Tool and ChemRange yield a long-range transport potential that is in the same order of magnitude for 1,2,4-Trichlorobenzene and for the POPs PCB 101, 153 and PCB 28.

Thus, it can be concluded that 1,2,4-Trichlorobenzene is persistent in air and has a high long-range transport potential.

3.3 Bioaccumulation**3.3.1 Aquatic bioaccumulation****3.3.1.1 Bioaccumulation estimation**

The log K_{ow} of 4.05 is indicating that bioconcentration in aquatic organisms may occur.

3.3.1.2 Measured bioaccumulation data

Table 10: Available bioconcentration factors (BCF)

Organism	Exposure mg/L	Exposure days	BCF whole body	Lipid content	Reference	Rel.
<u>Fish:</u>						
<i>Jordanella floridae</i>	0.0038	28	2026 (kin.)	11.4%	(Smith et al., 1990)	2
<i>Salmo gairdneri</i>	$3.2 \cdot 10^{-6}$	119	1300^l (ss)	8.3%	(Oliver and Niimi, 1983)	3
<i>Salmo gairdneri</i>	$52 \cdot 10^{-6}$	105	3200^l (ss)	8.8%		
<i>Cyprinus carpio</i>	0.005	42	$120-1320^2$	not given	(MITI-List, 2002)	2
<i>Cyprinus carpio</i>	0.05	42	$420-1140^2$	given		
<i>Brachydanio rerio</i>	0.0085	28	1412	not given	(Ballhorn et al., 1984)	4
<i>Brachydanio rerio</i>	0.2141	28	574	given		
<i>Cyprinus carpio</i>	0.004	56	830	4.8%	(Broecker et al., 1984)	3
<i>Cyprinus carpio</i>	0.04	56	805	4.8%		
<i>Poecilia reticulata</i>	0.136	17	1139 (ss) 1142 (kin.)	not given	(van Eck et al., 1997)	4
<i>Salmo gairdneri</i>	note ³	not given	124^3	1.8%	(Geyer et al., 1985)	4
<i>Pimephales promelas</i>	note ³	not given	2100^3	10,5%		
<u>Crustaceans:</u>						
<i>Daphnia</i>	0.003	not given	142	not given	(Callahan et al., 1979)	4

<u>Algae:</u> <i>Chlorella fusca</i>	0.05	1	250	not given	(Geyer et al.,1984)	4
-----------------------------------------	------	---	-----	-----------	---------------------	---

Reliability scores: 1) reliable without restriction, 2) reliable with restriction, 3) not reliable, 4) not assignable.

¹ This study was rated not reliable because fish were exposed simultaneously to 12 substances and the overall concentration may have elicited adverse effects on fish (conc. of 1,2,4-trichlorobenzene = 3.2 / 52 ng/L, overall conc. = 116 / 2544 ng/L); ² A mixture of three isomeric trichlorobenzenes was administered and the individual BCF were calculated. There is no further information given on lipid normalization of BCF; ³ Comparison of literature-reported BCF values for diverse fish species and varying lipid contents. Original literature not reviewed.

There is a moderate number of studies available, investigating the bioaccumulation potential of 1,2,4-trichlorobenzene. Most of these studies have already been evaluated in the TC NES subgroup on identification of PBT and vPvB substances and the EU Risk Assessment (European Commission, 2003).

In the study on juvenile American flagfish (*Jordanella floridae*) conducted in compliance with ASTM 1978 guideline juvenile fish, four to six month old and with a lipid content of 11.4% were kept in a flow through system at 25°C. After being exposed to a test concentration of 3.8 µg/L for 28 days, the elimination time half-life was calculated for 1.21 days and the BCF (whole body) reached 2026 (Smith et al., 1990).

In a study conducted by Oliver (Oliver and Niimi, 1983) trout with an initial weight of about 250 g were exposed to 2 different overall concentrations of a mixture containing 12 chlorinated benzenes and aliphatic compounds. The uptake period was extended to 119 days and 105 days, respectively, and the trout reached a final weight of about 400 g. Food was also analyzed for contamination with trichlorobenzenes and blank values were considered in BCF calculation. For the higher exposure concentration a BCF of 3200 was reported. However, this study was deemed not reliable because 12 substances were simultaneously administered and effects regarding mixture toxicity influencing bioaccumulation can not be excluded.

The relationship between lipid content of fish and their bioconcentration potential was investigated by Geyer et al. (1985). Within a selection of 23 literature data BCF for 1,2,4-trichlorobenzene ranged from 124 (trout with 1.8% lipid) to 2100 (fathead minnow with 10.5% lipid). Unfortunately further information on exposure conditions is not listed.

The MITI database also provides BCF values for 1,2,4-trichlorobenzene determined for carp and using a standard test system. Though, a mixture of all three isomeric trichlorobenzenes was administered in two different overall concentrations and individual concentrations of each isomer in exposure medium as well as in fish were analyzed. The reported BCF values range between 120 and 1320. Unfortunately no information on lipid content or lipid normalization is explicitly given.

The study on carp (*Cyprinus carpio*) was performed as a flow through test according to OECD 305 C (Broecker et al., 1984). The fish showed a lipid content of 4.8% and a BCF of about 800 (whole body) was calculated. No time dependent increase was observed and the steady state is expected to be attained within seven days. However, the report also noted that a great variation in exposure concentrations of 1,2,4-trichlorobenzene was observed due to the inappropriate test design and the substance's volatility. Equally, great variations of concentrations in fish were also observed due to individual variability. The authors thus suggested a modification in test design and the usage of smaller fish such as Zebrafish (*Brachydanio rerio*) to better maintain constant exposure concentration and to enhance the number of test fish per vessel.

The study on Zebrafish (*Brachydanio rerio*) was performed as a semi-static test (daily water renewal) almost following the OECD 305 B guideline (Ballhorn et al., 1984). The concentrations

were measured initially and at water changes. The recovery rate was determined for 50-90 % of the initial test concentration after two days. Additionally, a reverse dependency of BCF values on exposure concentration occurred showing higher BCF at lower exposure concentration. This indicates an underestimation of BCF in case of the higher test concentration due to overestimation of the bioavailable aqueous concentration.

Further studies on invertebrates and algae revealed a low bioaccumulation of 1,2,4-trichlorobenzene (Callahan 1979 and Geyer 1984).

In summary it can be stated that BCF values with the highest reliability (scoring 2) are likely to range from 120 to 2026. Although having lower reliability, in two publications (Geyer et al., 1985; Oliver and Niimi, 1983) higher BCF of 2100 and 3200 were also reported. Therefore a BCF of >2000 can be derived as a realistic worst case estimate. In case that lipid normalization to 5% is applied the BCF values still reach up to 1818 (Oliver and Niimi, 1983), indicating a high bioaccumulation potential.

3.3.2 Terrestrial bioaccumulation

No data.

3.3.3 Summary and discussion of bioaccumulation

1,2,4-trichlorobenzene has a log K_{ow} of 4.05 indicating a high bioaccumulation potential. The high bioaccumulation potential is confirmed by several bioconcentration tests using different fish species. Reasonable BCF values range from 124 to 2026. In one study a higher BCF of 2100 and 3200 were reported. Although showing higher uncertainty in data quality, these data may be used in a weight of evidence approach and support a realistic worst case BCF of >2000, which is also used in the PBT assessment.

In case that lipid normalization is performed, the BCF values still reach 1818, indicating a high bioaccumulation potential.

4 HUMAN HEALTH HAZARD ASSESSMENT

Not relevant for this dossier.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Aquatic compartment (including sediment)

5.1.1 Toxicity data.

In this section only test results of long-term studies are reported as those are relevant for comparison with the aquatic T-criterion of Annex XIII of the Reach Regulation. Acute toxicity data can be found in the Risk Assessment Report (European Commission, 2003).

5.1.1.1 Fish

5.1.1.1.1 Long term toxicity to fish

Valid studies on long-term toxicity to fish are available for five fish species (see Table 11). All results are based on measured concentrations.

Table 11: Valid data on long-term ecotoxicity to fish. For details and references, see European Commission (2003)

Species	Duration	EC ₅₀ (mg l ⁻¹)	NOEC (mg l ⁻¹)	Method, conditions	Reference
<i>Pimephales promelas</i>	32 days		0.29	Flow-through, ESF (egg and sac fry)-test	(McCarty and Reinhard, 1980; U.S.EPA, 1985)
<i>Pimephales promelas</i>	32 days		0.5	Flow-through, ELS-test, measured conc.	(Ahmad et al., 1984)
<i>Pimephales promelas</i>	32 days		0.50	Lake water, flow-through, ELS-test, EPA	(Carlson and Kosian, 1987)
<i>(Brachy)danio rerio</i>	21 days	2.4	0.04	Flow-through, mortality, behaviour, OECD 204	Broecker et al. (1984)
<i>Salmo gairdneri</i>	85 days		0.13	ELS (fry)	(Carlson and Kosian, 1987)
<i>Poecilia reticulata</i>	14 days	2.4	0.11	Semi-static (daily renewal), juvenile fish: growth	(Könemann, 1981)
<i>Cyprinodon variegatus</i>	-			ESF-test, MATC = 0.222 mg l ⁻¹	Suter and Rosen (1988)

Abbreviations: ESF-test (Egg and Sac Fry) embryo-larvae test. ELS: Early Life Stage test

The most sensitive species seems to be *Danio rerio* with a NOEC of 0.04 mg/L for the endpoint behaviour in a prolonged toxicity test (Broecker et al. 1984). From tests with *Poecilia reticulata* and *Salmo gairdneri* results a NOEC from 0.11 or 0.13 mg/L (Carlson and Kosian, 1987) and (Könemann, 1981).

5.1.1.2 Aquatic invertebrates

5.1.1.2.1 Long term toxicity to aquatic invertebrates

Valid studies on long-term toxicity to invertebrates are available for *Daphnia magna* and *Mysidopsis bahia* (see Table 12). Results of *Daphnia magna* are based on measured concentrations.

Table 12: Valid data on long-term toxicity to aquatic invertebrates. For details and references, see European Commission (2003)

Species	Duration	EC ₅₀ (mg l ⁻¹)	NOEC (mg l ⁻¹)	Method, conditions	Reference
<i>Daphnia magna</i>	14 days	0.45	(0.32)	Semi-static, (closed)	(Calamari et al., 1983) (NOEC = EC16)
<i>Daphnia magna</i>	16 days	0.16 (reproduction) 0.32 (mortality)*	0.06 (reproduction) 0.19 (mortality)*	Semi-static (3 times a week);	(Hermens et al. 1984)
<i>Daphnia magna</i>	21 days		0.4	Semi-static, EEC-Ann.V-c; reproduction	(Broecker et al. 1984)
<i>Daphnia magna</i>	28 days		0.36	Semi-static (closed), ASTM 1980	(Richter et al. (983)
<i>Mysidopsis bahia</i>	28 days		≤0.064	Flow-through, measured, EPA standard	(US EPA 1988) (LOEC = 0.033 mg l ⁻¹)

* from RAR2003: EC₅₀-values and NOECs are corrected values of the Rapporteur.

The RAR 2003 treats the data of a study by Hermens et al. (1984) on the sublethal toxicity to *Daphnia magna* in the following way: the 16-day LC₅₀ on mortality was 0.56 mg/l and the EC₅₀ on reproduction was 0.27 mg/l (reported as µmol/l in the reference). In an identical 16-day experiment, measurements of the test concentrations are stated to average 58% of nominal (Hermens et al., 1985) and the 16-day EC₅₀ was 0.52 mg/l. The results are not corrected for the measured concentrations. Assuming the average concentration was the same in the two experiments, the 16-day LC₅₀ was 0.32 mg/l, EC₅₀ 0.16 mg/l and the NOEC on mortality and reproduction 0.19 mg/l and 0.06 mg/l, respectively.

5.1.1.3 Algae and aquatic plants

Results of four standard ecotoxicity tests on algae are available.

Table 13: Toxicity to algae (see European Commission 2003)

Species	Duration	EC ₅₀ (mg l ⁻¹)	NOEC (mg l ⁻¹)	Method, conditions	Reference	Valid
---------	----------	-------------------------------------------	-------------------------------	--------------------	-----------	-------

<i>Pseudokirchneriella subcapitata</i> (reported as: <i>Selenastrum capricornutum</i>)	96 h	1.4	0.37	Static, closed system, US EPA, measured conc.	(Calamari et al., 1983) (Galassi and Vighi 1981)	yes
<i>Scenedesmus subspicatus</i>	96 h	18.9	2.2 (EC ₁₀)	Static (open system), 22°C, UBA guideline 1982, nominal conc.	(Broecker et al. 1984)	no
<i>Scenedesmus subspicatus</i>	96 h	8.4	3.0 (EC ₁₀)	Static (open system), 22°C, UBA guideline 1982, initial conc.	(Geyer et al. 1985)	no
<i>Chlorella vulgaris</i>	7 d	5.6		OECD TG 201 *	(Yoshioka and Ose 1993)	no
<i>Cyclotella meneghiniana</i> (diatom)	48 h	2.83		Static, 15°C (effects on DNA content reduction)	(Figuroa and Simmons 1991)	no

* Stated to be carried out according to OECD guideline. However, OECD recommends 72-h tests to consider the growth inhibition within the exponential growth stage

Some of the tests did not consider the volatility of the substance. Thus, the real values are probably lower than most of the mentioned. It may be assumed that results from static and open system tests underestimate the toxicity due to evaporation and photolysis, respectively. Therefore, only the results from the test using a closed system are accepted to be used in the risk assessment procedure: Algae EC₅₀ (96 h): 1.4 mg/l and NOEC (96 h): 0.37 mg/l.

5.1.1.4 Sediment organisms

Two studies have looked at the effects of 1,2,4-TCB on benthos. In an 8-week study employing macrobenthos community and spiked sediment, the lowest concentration influencing the number of animals was 100 mg kg⁻¹ sand (ww) (added 1,2,4-TCB, nominal) for molluscs, echinoderms and the most abundant arthropod *Corophium acherusicum* corresponding to 6 mg kg⁻¹ measured at the end of exposure. In the same study planctonic larvae of macrobenthos were exposed 6 days to 1,2,4-TCB via water under flow-through conditions. The lowest effect concentration (mortality) of 0.04 mg l⁻¹ was observed for mollusc larvae. In another study, grass shrimp (*Palaemonetes pugio*) and amphioxus (*Branchiostoma caribaeum*) were tested via water and sediment exposure, respectively. During a 10-day sediment exposure, no mortality was observed for grass shrimp at 10 mg kg⁻¹ sand (wet weight). For amphioxus LC₅₀ was observed to be 200 mg kg⁻¹ sand (ww) and NOEC 75 mg kg⁻¹ sand (ww).

5.1.1.5 Other aquatic organisms

Results from ecotoxicity tests with *Tetrahymena poryformis* (protozoa), *Tanytarsus dissimilis* (chironomid) and *Aplexa hypnorum* (snail) gave EC₅₀ values between 0.91-3.16 mg l⁻¹.

Table 14: Short-term toxicity to other aquatic organisms (see European Commission 2003)

Species	Duration	EC ₅₀ (mg l ⁻¹)	Method, conditions	Reference
<i>Tetrahymena pyriformis</i> *	24 h	0.91	30°C, 2% protease peptone medium, growth inhibition	(Yoshioka et al. 1985)
<i>Tanytarsus dissimilis</i>	48 h	0.93	Flow through, lake water, 17°C, 43 mg CaCO ₃ /l measured conc.	(Holcombe et al., 1987)
<i>Aplexa hypnorum</i>	96 h	3.16	Flow through, lake water, 17°C, 43 mg CaCO ₃ /l measured conc.	(Holcombe et al., 1987)

* These ciliate data are also included in the section below because they are regarded representative for ciliate species in sewage treatment plants

6 CONCLUSIONS ON THE SVHC PROPERTIES

6.1 PBT, vPvB assessment

Not relevant for this dossier. See section 6.3.

6.2 CMR assessment

Not relevant for this dossier.

6.3 Substances of equivalent level of concern assessment

6.3.1 Assessment of PBT/vPvB properties - comparison with criteria of Annex XIII

6.3.1.1 Persistence

1,2,4-trichlorobenzene (1,2,4-TCB) is not subject to hydrolysis. The screening biodegradation tests indicate that 1,2,4-TCB is not readily biodegradable. Based on data from soil and sediment degradation tests half-lives of more than 200 days have been determined.

Due to its high volatility (Henry constant of $181.45 \text{ Pa}\cdot\text{m}^3\cdot\text{Mol}^{-1}$), 1,2,4-TCB is expected to be distributed from water to air. In the air, 1,2,4-TCB is persistent (estimated half lives between 18.5 and 38 days) and may be transported by air for several thousand kilometres. Distributed by this pathway also to remote areas, 1,2,4-TCB will be deposited again to soil or water being even more persistent in regions with low temperatures.

Thus, considering all information available, 1,2,4-TCB is concluded to fulfil the P-criterion and the vP criterion.

6.3.1.2 Bioaccumulation

1,2,4-trichlorobenzene has a log K_{ow} of 4.05 indicating a high bioaccumulation potential. The high bioaccumulation potential is confirmed by several bioconcentration tests using different fish species. BCF values with the highest reliability range from 120 to 2026. Although having lower reliability, in two publications higher BCF of 2100 and 3200 were also reported. Therefore a BCF of >2000 can be derived as a realistic worst case estimate. Thus, based on weight of evidence it is concluded that 1,2,4-trichlorobenzene fulfils the B-criterion of Annex XIII.

In case that lipid normalization to 5% is applied the BCF values still reach up to 1818 (Oliver and Niimi, 1983), indicating a high bioaccumulation potential. This high BCF still supports an equivalent concern according to Article 57 (f) of REACH regulation.

6.3.1.3 Toxicity

Aquatic ecotoxicity tests with chronic exposure are available for fish, aquatic invertebrates and algae. The lowest NOEC is 0.04 mg l⁻¹ for zebra fish. The NOECs measured for invertebrates and algae are in the same order of magnitude. Although these NOECs indicate a high toxicity for aquatic organisms on the long-term, the T-criterion is formally not fulfilled.

6.3.1.4 Further supporting evidence

Long-range transport potential: Due to its volatility and persistence to atmospheric degradation, 1,2,4-TCB has a very high potential for long-range atmospheric transport. Based on model calculations, estimated travelling distances are several thousand kilometres.

6.3.2 Summary and overall conclusions on the PBT/vPvB properties

Taking into account all available information on persistent, bioaccumulative and toxic properties of 1,2,4-trichlorobenzene, it is concluded that 1,2,4-trichlorobenzene is a PBT-substance. The substance is considered to fulfil the P criterion, and also the vP-criterion. In addition to that, also the B criterion is fulfilled. In case that lipid normalization is performed, 1,2,4-trichlorobenzene would not clearly fulfil the B criterion. However, if doing so the maximum BCF will still be close to the B criterion of Annex XIII. Although 1,2,4-trichlorobenzene is highly toxic for aquatic organisms tested on the long-term, it does not formally meet the T-criterion. The overall conclusion is however drawn in a weight of evidence approach, taking supporting evidence into account relating to the very high long-range environmental transport potential of this substance. It raises an equivalent concern according to Article 57 (f).

The PBT-properties of 1,2,4-trichlorobenzene have already been agreed in the TC NES subgroup on identification of PBT and vPvB substances in 2003 (and supported by the TCNES and CA-Meeting), and as a result 1,2,4-trichlorobenzene was assessed as PBT-substance.

PART II

INFORMATION ON USE, EXPOSURE, ALTERNATIVES AND RISKS

INFORMATION ON MANUFACTURE, IMPORT/EXPORT AND USES – CONCLUSIONS ON EXPOSURE

1,2,4-TCB is mainly used as intermediate and as a process solvent in closed systems. In addition, the RAR identified the following minor uses that are by now restricted in the EU (Directive 2005/59/EC): solvent, dye carrier, corrosion inhibitor and lubricant. In the past, 1,2,4-TCB was used as an additive in dielectric fluids. 1,2,3-TCB and 1,3,5-TCB have a lower production volume, but are assigned to the same uses. As trichlorobenzenes are priority substances under the Water Framework Directive, their occurrence in rivers is controlled by monitoring programmes.

All substances are preregistered with an envisaged registration deadline of 2010. Though the preregistration data have to be interpreted cautiously, this indicates a potential production volume of more than 100 tons for 1,2,4-TCB (classified as R50-53 according to directive 67/548/EEC) and more than 1000 tons for 1,2,3-TCB and 1,3,5-TCB.

The main uses as an intermediate and as a process solvent in closed applications are not considered to result in a release of 1,2,4-TCB into the environment. All other uses are restricted.

Despite the extensive restriction on the marketing and use of 1,2,4-TCB in the EU, the substance may enter the EU market via imported articles. For example, textiles may contain 1,2,4-TCB from use as a dye carrier. However, there is no information on the amount of imported articles containing 1,2,4-TCB.

Furthermore, 1,2,3-TCB and 1,3,5-TCB are not restricted and may be used as substitutes for 1,2,4-TCB. Again, updated information would be helpful to clarify this concern.

With regard to the known uses of the three Trichlorobenzenes, current exposure is expected to be low, but trichlorobenzenes should be considered for further regulatory steps due to their SVHC properties: Both 1,2,4-TCB and 1,2,3-TCB have been identified as PBTs by the TCNES PBT subgroup. 1,3,5-TCB shows similar properties and should thus also be regarded as PBT. These substances pose a risk to human health and the environment that is not predictable in the long-term. Once released into the environment, adverse effects caused by PBTs cannot be remediated. Consequently, strong precautionary measures are necessary to prevent the release of these substances, though it is expected that only small amounts of trichlorobenzenes are emitted in the EU. As no safe concentration may be derived for PBTs, even low emissions give rise to concern and need to be limited further. Nonetheless, the production volume has to be considered when setting priorities for regulatory measures for concurrent SVHCs. In addition to that, the long-range transport potential of the trichlorobenzenes needs to be considered in a weight of evidence assessment of the PBT-properties including all available information.

CURRENT KNOWLEDGE ON ALTERNATIVES

RISK-RELATED INFORMATION

In 2003, the RAR concluded a need of limiting the risks posed by the use of 1,2,4-TCB to human health and the environment. For environment, a risk was identified regarding the use as intermediate, solvent or dye carrier. It was recommended to investigate if the substance should be considered in relation to national or international programmes addressing persistent organic pollutants.

For workers, risks were identified for the production of 1,2,4-TCB or of pigments containing 1,2,4-TCB, and for the use of certain products containing 1,2,4-TCB. For consumers, a risk was identified regarding the use of anti-corrosive paint and maintenance products containing 1,2,4-TCB. Furthermore, concern was expressed that guideline values in drinking water might be exceeded for local use scenarios. As a consequence, the use of 1,2,4-TCB was restricted by Directive 2005/59/EC (EU Risk Assessment and Existing Legal Requirements).

REFERENCES

- Adrian L, Szewzyk U, Wecke JG, Ârisch H. 2000. Bacterial dehalorespiration with chlorinated benzenes. *Nature* 408(6812): 580-583.
- Ahmad N, Benoit D, Brooke L, Call D, Carlson A, DeFoe D, Huot J, Moriarity A, Richter J, Shubat P, Veith G, Wallbridge C. 1984. Aquatic toxicity tests to characterize the hazard of volatile organic chemicals in water: a toxicity data summary -- Parts I and II. Environmental Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Duluth, Minnesota. 1-111.
- Akermark B, Baeckstöm P, Westlin UE, Göthe R, Wachtmeister CA. 1976. Photochemical dechlorination of 1,2,4-Trichlorobenzene. *Acta Chemica Scandinavia* 30(b): 49-52.
- AOPWIN. 1995. Atmospheric oxidation program for Microsoft Windows 3.2 (AOPWIN). prepared by Meylan W and Howard P, Syracuse Research Corporation, Merrill Lane, Syracuse, NY.
- Arnot J, Gouin T, Mackay D. 2005. Practical method for estimating environmental biodegradation rates.
- Ballhorn L, Freitag D, Geyer H, Quast I, Rott B, Scheunert I, Spieser h, Vishwanathan R (1984). Überprüfung der
- Durchführbarkeit von Prüfungsvorschriften und der Aussagekraft der Stufe I und II des E. Chem. G.
- Umweltforschungsplan des Bundesministeriums des Innern. Chemikalien, Forschungsbericht 106 04 011/02. 1984.
- Bartholomew GW, Pfaender FK. 1983. Influence of spatial and temporal variations on organic pollutant biodegradation rates in an estuarine environment. *Appl Environ Microbiol* 45(1): 103-109.
- Battersby NS. 1990. A review of biodegradation kinetics in the aquatic environment. *Chemosphere* 21(10-11): 1243-1284.

- Bengtsson BE, Tarkpea M. 1983. The acute aquatic toxicity of some substances carried by ships. *Mar Pollut Bull* 14: 213-214.
- Bobra AM, Shiu WY, Mackay D. 1983. A predictive correlation for the acute toxicity of hydrocarbons and chlorinated hydrocarbons to the water flea (*Daphnia magna*). *Chemosphere* 12 (9/10): 1121-1129.
- Bosma TNP, van der Meer JR, Schraa G, Tros ME, Zehnder AJB. 1988. Reductive dechlorination of all trichloro- and dichlorobenzene isomers. *FEMS Microbiol Lett* 53(3-4): 223-229.
- Broecker B, Fischer R, Gerber HG, Markert M, Wellens H. 1984. Überprüfung der Durchführbarkeit von Prüfungsvorschriften und der Aussagekraft der Stufe I und II des Chemikaliengesetzes. Umweltforschungsplan des Bundesministeriums des Innern. Umweltchemikalien, Forschungsbericht 106 04 011/07.
- Broderius S, Kahl M. 1985 Jul. Acute toxicity of organic chemical mixtures to the fathead minnow. *Aquatic Toxicology* 6(4): 307-322.
- Brunsbach FR, Reineke W. 1994. Degradation of chlorobenzenes in soil slurry by a specialized organism. *Applied Microbiology and Biotechnology* 42(2-3): 415-420.
- BUA. 1987. 1,2,4-Trichlorbenzene. GDCh-Advisory Committee on Existing Chemicals of Environmental Relevance (Beratergremium für Umweltrelevante Altstoffe).
- Calamari D, Galassi S, Setti F, Vighi M. 1983. Toxicity of selected chlorobenzenes to aquatic organisms. *Chemosphere* 12(2): 253-262.
- Callahan MA, Slimak MW, Gabl NW, May JP, Fowler CW, Freed JR, Jennings P, Durfee RL, Whitmore FC, Maestri B, Mabey WR, Holt BR, Gould C (1979). Water related environmental fate of 129 priority pollutants. US
- Environmental Protection Agency, Dec 1979, EPA 440/4-79-029.
- Carlson AR, Kosian PA. 1987. Toxicity of Chlorinated Benzenes to Fathead Minnows (*Pimephales promelas*). *Arch Environ Contam Toxicol* 16(2): 129-135.
- Clark JR, Patrick JM, Moore JC, Lores EM. 1987. Waterborne and sediment-source toxicities of six organic chemicals to Grass shrimp (*Palaemonetes pugio*) and amphioxus (*Branchiostoma caribaeum*). *Arch Environ Contam Toxicol* 16: 401-407.
- Douglas MT, Chanter DO, Pell IB, Burney GM. 1986 Sep. A proposal for the reduction of animal numbers required for the acute toxicity to fish test (LC50 determination). *Aquatic Toxicology* 8(4): 243-249.
- Dulin D, Drossman H, Mill T. 1986. Products and quantum yields for photolysis of chloroaromatics in water. *Environmental Science and Technology* 20(1): 72-77.
- ECHA. 2008. Guidance on information requirements and chemical safety assessment: Chapter R.16: Environmental Exposure Estimation.
- European Commission. 2003. European Union Risk Assessment Report 1,2,4-trichlorbenzene. Series: 2nd Priority List, Volume: 26; EUR 20540 EN.

- Figueroa IC, Simmons MS. 1991. Structure-activity relationships of chlorobenzenes using DNA measurement as a toxicity parameter in algae. *Environ Toxicol Chem* 10: 323-329.
- Freitag D, Ballhorn L, Geyer H, Korte F. 1985. Environmental hazard profile of organic chemicals : An experimental method for the assessment of the behaviour of organic chemicals in the ecosphere by means of simple laboratory tests with ¹⁴C labelled chemicals. *Chemosphere* 14(10): 1589-1616.
- Galassi S and Vighi M. 1981. Testing toxicity of volatile substances with algae. *Chemosphere* 10(19): 1123-1126.
- Geyer H, Scheunert I, Korte F. 1985. Relationship between the lipid content of fish and their bioconcentration potential of 1,2,4-trichlorobenzene. *Chemosphere* 14(5): 545-555.
- Geyer H, Scheunert I, Korte F. 1985. The effects of organic environmental chemicals on the growth of the alga *Scenedesmus subspicatus*: A contribution to environmental biology. *Chemosphere* 14(9): 1355-1369.
- Haider K, Jagnow G, Kohnen R, Lim SU. 1974. Degradation of chlorinated benzenes, phenols and cyclohexane derivatives by benzene and phenol utilizing soil bacteria under aerobic conditions. *Arch Microbiol* 96(3): 183-200.
- Hermens J, Canton H, Janssen P, de Jong R. 1984. Quantitative structure-activity relationships and toxicity studies of mixtures of chemicals with anaesthetic potency: acute lethal and sublethal toxicity to *Daphnia magna*. *Aquatic Toxicology* 5: 143-154.
- Holcombe GW, Phipps GL, Sulaiman AH, Hoffman AD. 1987. Simultaneous multiple species testing: Acute toxicity of 13 chemicals to 12 diverse freshwater amphibian, fish, and invertebrate families. *Arch Environ Contam Toxicol* 16(6): 697-710.
- Howard. 1989. Handbook of environmental fate and exposure data for organic chemicals. Vol. 1: Large production and priority pollutants. Lewis Publ. Inc., Michigan.
- Hulzebos EM, Adema DMM, Dirven-van Breemen EM, Henzen L, van Dis WA, Herbold HA, Hoekstra JA, Baerselman R, van Gestel CAM. 1993. Phytotoxicity studies with *Lactuca sativa* in soil and nutrient solution. *Environ Toxicol Chem* 12: 1079-1094.
- Knie J, Hälke A, Juhnke I, Schiller W. 1983. Ergebnisse der Untersuchungen von chemischen Stoffen mit vier Biotests. *Deutsche Gewässerkundliche Mitteilungen* 27(3): 77-79.
- Kondo M, Nishihara T, Shimamoto T, Ichikawa T, Fujii M. 1988. Screening test for degradation of chemicals in water. Degradation test by photoirradiation. *Eisei Kagaku* 34(1): 41-47.
- Könemann H. 1981. Quantitative structure-activity relationships in fish toxicity studies Part 1: Relationship for 50 industrial pollutants. *Toxicology* 19(3): 209-221.
- Korte F, Freitag D. 1986. Kriterien zur Auswahl umweltgefährlicher alter Stoffe: Mobilität einschließlich Abbaubarkeit und Akkumulation. *Umweltforschungsplan des Bundesministerium des Inneren, Chemikalien*.
- Kotzias D, Parlar H, Korte F. 1982. Photoreactivity of organic chemicals in water in the presence of nitrate and nitrite. *Naturwissenschaften* 69(9): 444-445.

- Mansour M, Feicht E, Méallier P. 1989. Improvement of the photostability of selected substances in aqueous medium. *Toxicological & Environmental Chemistry* 20(1): 139-147.
- Marinucci AC, Bartha R. 1979 Nov. Biodegradation of 1,2,3- and 1,2,4-trichlorobenzene in soil and in liquid enrichment culture 3. *Appl Environ Microbiol* 38(5): 811-817.
- Masunaga S, Susarla S, Yonezawa Y. 1996. Dechlorination of chlorobenzenes in anaerobic estuarine sediment. *Wat Sci Tech* 33(6): 173-180.
- McCarty PL, Reinhard M. 1980. Trace organics removal by advanced wastewater treatment. *Journal of the Water Pollution Control Federation* 52(7): 1907-1922.
- MITI-List. 2002. Biodegradation and Bioaccumulation of Existing Chemical Substances under the Chemical Substance Control Law. National Institute of Technology and Evaluation, Japan.
- Neuhauser EF, Durkin PR, Malecki MR, Anatra M. 1986. Comparative toxicity of ten organic chemicals to four earthworm species. *Comparative Biochemistry and Physiology* 83(part C): 197-200.
- Nowak J, Kirsch NH, Hegemann W, Stan HJ. 1996. Total reductive dechlorination of chlorobenzenes to benzene by a methanogenic mixed culture enriched from Saale river sediment. *Applied Microbiology and Biotechnology* 45(5): 700-709.
- OASIS Catalogic v5.10.7. 2009.
- OECD. 2006. The OECD Pov and LRTP Screening Tool 2.0. Software and Manual. OECD, Paris.
- Oliver BG, Niimi AJ. 1983. Bioconcentration of chlorobenzenes from water by rainbow trout: correlations with partition coefficients and environmental residues. *Environ Sci Technol* 17(5): 287-291.
- Peijnenburg WJGM, 'T Hart MJ, Den Hollander HA, van de Meent D, Verboom HH, Wolfe NL. 1992. Reductive transformations of halogenated aromatic hydrocarbons in anaerobic water-sediment systems: Kinetics, mechanisms and products. *etc* 11(3): 289-300.
- Pfaender FK, Bartholomew GW. 1982. Measurement of aquatic biodegradation rates by determining heterotrophic uptake of radiolabeled pollutants. *Appl Environ Microbiol* 44(1): 159-164.
- Rapp P, Timmis KN. 1999. Degradation of chlorobenzenes at nanomolar concentrations by *Burkholderia* sp. strain PS14 in liquid cultures and in soil. *Applied and Environmental Microbiology* 65(6): 2547-2552.
- Richter JE, Peterson SF, Kleiner CF. 1983. Acute and chronic toxicity of some chlorinated benzenes, chlorinated thanes, and tetrachloroethylene to *Daphnia magna*. *Arch Environ Contam Toxicol* 12: 679-684.
- Rinke M, Zetzsch C. 1984. RATE CONSTANTS FOR THE REACTIONS OF OH RADICALS WITH AROMATICS: BENZENE, PHENOL, ANILINE, AND 1,2,4-TRICHLOROBENZENE. *Berichte der Bunsengesellschaft/Physical Chemistry Chemical Physics* 88(1): 55-62.

- Rippen G. 1995. Handbuch Umweltchemikalien: Stoffdaten, Prüfverfahren, Vorschriften. Ecomed Verlagsgesellschaft, Landsberg/Lech Germany.
- Sander P, Wittich RM, Fortnagel P, Wilkes H, Francke W. 1991. Degradation of 1,2,4-trichloro- and 1,2,4,5-tetrachlorobenzene by *Pseudomonas* strains. *Applied and Environmental Microbiology* 57(5): 1430-1440.
- Scheringer M. 1996. Persistence and spatial range as endpoints of an exposure-based assessment of organic chemicals. *Environmental Science and Technology* 30(5): 1652-1659.
- Schmidt-Bleek F, Haberland W, Klein AW, Caroli S. 1982. Steps towards environmental hazard assessment of new chemicals (including a hazard ranking scheme, based upon directive 79/831-EEC). *Chemosphere* 11(4): 383-415.
- Simmons P, Branson D, Bailey R. 1977. 1,2,4 Trichlorobenzene: biodegradable or not? *Textile Chemist and Colorist* 9(9): 61-63.
- Singh HB, Salas LJ, Smith AJ, Shigeishi H. 1981. Measurement of some potentially hazardous organic chemicals in urban environments. *Atmospheric Environment* 15(4): 601-612.
- Smith AD, Bharath A, Mallard C, Orr D, Smith K, Sutton JA, Vukmanich J, McCarty LS, Ozburn GW. 1991. The Acute and Chronic Toxicity of Ten Chlorinated Organic Compounds to the American Flagfish (*Jordanella floridae*). *Arch Environ Contam Toxicol* 20(1): 94-102.
- Tabak HH, Quave SA, Mashni CI, Barth EF. 1981. Biodegradability studies with organic priority pollutant compounds. *Journal of the Water Pollution Control Federation* 53(10): 1503-1518.
- US EPA. 1984. Aquatic toxicity tests to characterize the hazard of volatile organic chemicals in water. A toxicity Data summary. Parts 1 and 2. US-Environmental Protection Agency, Research Lab. Duluth, MN, Jan. 1984. EPA 600/3-84-009, PB84-141-506.
- U.S.EPA. 1985. Health Assessment Document for chlorinated benzenes. U.S. Environmental Protection Agency, Office of Emergency and Remedial Response; Office of solid waste and Emergency Response, Washington. 1-614.
- Van der Meer JR, Roelofsen W, Schraa G, Zehnder AJB. 1987. Degradation of low concentrations of dichlorobenzenes and 1,2,4-trichlorobenzene by *Pseudomonas* sp. strain P51 in nonsterile soil columns. *FEMS Microbiology Ecology* 45(6): 333-341.
- van Eck JMC, Koelmans AA, Deneer JW. 1997. Uptake and elimination of 1,2,4-trichlorobenzene in the guppy (*Poecilia reticulata*) at sublethal and lethal aqueous concentrations. *Chemosphere* 34(11): 2259-2270.
- van Wijk D, Cohet E, Gard A, Caspers N, van Ginkel C, Thompson R, de Rooij C, Garny V, Lecloux A. 2006. 1,2,4-Trichlorobenzene marine risk assessment with special emphasis on the OSPAR region North Sea. *Chemosphere* 62: 1294-1310.
- Veith G, Call DJ, Brooke LT. 1983. Structure - toxicity relationships for the fathead minnow, *Pimephales promelas*: narcotic industrial chemicals. *Canadian Journal of Fisheries and Aquatic Sciences* 40(6): 743-748.

Wakeham SG, Davis AC, Karas JL. 1983. Mesocosm experiments to determine the fate and persistence of volatile organic compounds in coastal seawater. *Environmental Science and Technology* 17(10): 611-617.

Wang MJ, Jones KC. 1994. Behavior and fate of chlorobenzenes in spiked and sewage sludge-amended soil. *Environmental Science and Technology* 28(11): 1843-1852.

Yoshioka Y, Ose Y. 1993. A quantitative structure-activity relationship study and ecotoxicological risk quotient for the protection from chemical pollution. *Environ Toxicol Water Qual* 8(1): 87-101.

Yoshioka Y, Ose Y, Sato T. 1985. Testing for the toxicity of chemicals with *Tetrahymena pyriformis*. *Sci Total Envir* 43: 149-157.