

**UPDATING OF DATA CONCERNING
THE IMPACT ON THE AQUATIC
ENVIRONMENT OF CERTAIN
DANGEROUS SUBSTANCES,
SECOND PART**

Part II — Chlorinated benzenes

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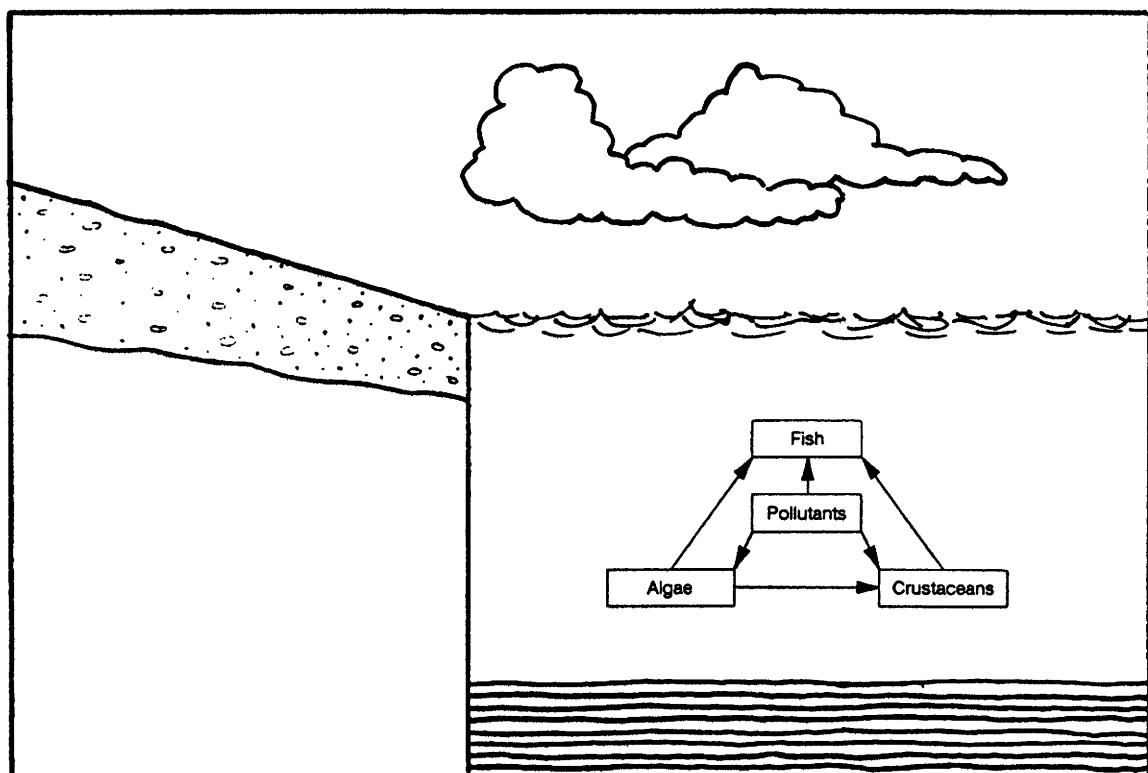
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Updating of data concerning the impact on the aquatic environment of certain dangerous substances, 2nd part

Part II Chlorinated benzenes



May, 1992

PREFACE

The Commission of the European Communities, Directorate-General for Environment, Nuclear Safety and Civil Protection has commissioned BKH Consulting Engineers to carry out Study Contract B6612-90-006688:

Updating of data concerning the impact on the aquatic environment of certain dangerous substances, 2nd part: benzenes.

The study deals with the following dangerous substances which belong or should belong to List I of Directive 76/464/EEC:

benzene (7)
ethylbenzene (79)
isopropylbenzene (87)
chlorobenzene (20, 53, 54, 55, 109, 117, 118)
chloronitrobenzene (27, 28, 29, 30, 63)
chloroanilines (17, 18, 19, 52)

The study updates information on the description of the substances, the level of contamination of the aquatic environment, the persistence of the substances in the aquatic environment and other media, the toxicity (mainly in the aquatic environment) and bioaccumulation. On the basis of the available data, water quality standards are proposed.

The study is presented in four parts:

Part I	Benzene, ethylbenzene and isopropylbenzene
Part II	Chlorinated benzenes
Part III	Chloronitrobenzenes and 4-chloro-2-nitroaniline
Part IV	Chloroanilines

The study has been carried out by F. Balk, P.C. Okkerman, M. Hof, A. van de Bovekamp and J.W. Dogger. Critical remarks of J. Blok were greatly appreciated.

SUMMARY

The impact of chlorobenzenes on the aquatic environment is evaluated in order to provide a toxicological basis to derive a proposal for water quality standards. Data concerning physical and chemical properties, degradability, toxicity, chemical detection and environmental concentrations have been reviewed and updated. Table i presents an overview of the availability of data on the different subjects.

Chlorobenzenes are used in the production of chloronitrobenzene and diphenyl ether; in rubber intermediates and as a solvent.

The major uses of 1,2-dichlorobenzene and 1,2,4-trichlorobenzene are as a process solvent and as intermediates in the synthesis of dyestuffs, herbicides and degreasers. 1,2-dichlorobenzene and 1,4-dichlorobenzene are used as deodorizer. 1,2,4,5-Tetrachlorobenzene is the only tetrachloro-isomer used in industrial quantities. Other isomers are not used in significant quantities.

Total production levels (t/year) in the EC are for monochlorobenzene 70-80000 in 1982; for dichlorobenzenes 80000 in 1987 and trichlorobenzenes (technical) 12-40000 in 1987.

Chemical analysis of chlorobenzenes is possible by isolation with purge and trap techniques, followed by GC separation and detection with ECD or FID. For complex mixtures an identification with MS is recommended.

Depending on the type of chlorobenzene and the detection method, the detection limits are between 6- 20 µg/l water up to about 500 µg/l.

Concentration levels in air, river water, sediment and biota have been reported scarcely. In urban air between 0.05 and 7 µg/m³ is measured, but in the neighbourhood of some industries 1 - 3 g /m³ may occur.

In sediments the range is 0.1 - 250 µg/kg, depending on the neighbourhood of pollution sources. In municipal sewage water up to several hundred µg/l have been measured. In surface water recent measurements in the river Rhine show maximum levels below 0.1 - 1 µg/l.

Levels in fish are between 0.01 and 20 µg/kg body weight and in fat tissue or milk of human values up to 130 µg/kg fat have been found. Values are rather variable and highly dependent on the neighbourhood of emitting industry.

The chlorobenzenes are not likely to be photodegraded in the aquatic environment; however, after volatilization photodegradation in the atmosphere will be the main pathway for disappearance.

The biodegradation of chlorobenzenes depends on suitable circumstances. Except for 1,4-dichlorobenzene all other isomers failed to pass the standard screening tests for ready biodegradability. Inhibition of the bacterial growth and a lack of adapted micro-organisms seems to be a reason for the negative test results. In different types of simulation tests and under experimental conditions most isomers have shown to be inherently degradable. Under anaerobic methanogenic conditions dehalogenation of the higher substituted benzenes is likely to occur but more information on the rates and end products is needed. Information on the actual biodegradation rates in the river sediment and other relevant places in the environment is incomplete.

The chlorobenzenes are water soluble to a level between 1 and 500 mg/l depending on the degree of chlorine substitution. They will disappear from the water phase by volatilization within a few months and in atmospheric conditions they will be

photodegraded. The higher substituted isomers are less soluble and show a higher sorption to sediment and a higher degree of bioaccumulation. For tetrachlorobenzene the bioaccumulation factor may be around 1000.

The toxicity of the chlorobenzenes is documented fairly well; however, the results have to be interpreted with care because of the variability due to volatilization under the test conditions. Acute toxicity for a variety of aquatic species varies between 4 and 22 mg/l for the monochlorobenzenes and between 0.1 and 31 mg/l for the di-, tri- and tetrachlorobenzenes. All available chronic toxicity data for aquatic organisms are above 0.1 mg/l.

Based on the low variability of sensitivity between different species and between acute and chronic effects a safe level can be proposed for all aquatic ecosystems at 10 µg/l. This implies that maximum levels in the river Rhine (1 µg/l) are one order of magnitude below this safe level.

The proposed water quality standards of 10 µg/l for the sum of all chlorobenzenes is within an order of magnitude of the standards proposed by the Rhine Action Program of 0.1 µg/l for each trichlorobenzene isomer.

The risk for human health through exposure of chlorobenzenes in water is negligible. For tetrachlorobenzene, however, data are incomplete.

Table 1 Information available for chlorobenzenes on the different subjects.

Subject:	CB	1,2-DCB	1,3-DCB	1,4-DCB	1,2,3-TCB	1,2,4-TCB	1,3,5-TCB	1,2,4,5-TcB
1. PHYSICO-CHEMICAL CHARACTERISTICS	+	+	+	+	+	+	+	+
Production levels	+	+	+	+	+	+	+	+
2. ANALYTICAL DETECTION TECHNIQUES								
Detection methods	+	+	+	+	+	+	+	+
Detection levels								
4. ENVIRONMENTAL LEVELS								
Residues in the atmosphere	+	+	+	+	+	+	+	+
Residues in soil and groundwater	+	+	+	+	+	+	+	+
Residues in surface water and sediment	+	+	+	+	+	+	+	+
Residues in aquatic organisms	+	+	+	+	+	+	+	+
Residues in terrestrial organisms	-	-	-	-	-	-	-	-
Residues in humans	+	+	+	+	+	+	+	+
5. PERSISTENCE AND DEGRADATION PATHWAYS								
Abiotic degradation								
Biological degradation and metabolism								
6. DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS								
Volatilization								
Sorption	+	+	+	+	+	+	+	+
Bioaccumulation:								
Aquatic organisms								
Terrestrial organisms	-	-	-	-	-	-	-	-
Biomagnification								
7. TOXICITY								
Aquatic toxicity:								
Acute toxicity (freshwater; marine)	++	++	++	++	++	++	++	++
Chronic toxicity (freshwater; marine)	++	++	++	++	++	++	++	++
Quantitative structure activity relations (QSARs)	+	+	+	+	+	+	+	+
Toxicity to terrestrial organisms	-	-	-	-	-	-	-	-
(semi)field studies	-	-	-	-	-	-	-	-
Toxicity to mammals (acute; chronic)	++	++	++	++	++	++	++	++
Carcinogenic, mutagenic and teratogenic effects	++	++	++	++	++	++	++	++

+= data available

+= insufficient data available

-= no data available

ZUSAMMENFASSUNG

Werte bezüglich physischen und chemischen Eigenschaften, Abbaubarkeit, Toxizität, chemischer Determinierung und Umweltkonzentrationen wurden revidiert aufgebessert für Chlorobenzolen. In Tafel i sind die Ergebnissen dargestellt über die verfügbare Daten der verschiedene Gegenstände.

Chlorobenzole werden angewendet in der Produktion von Chloronitrobenzol und Diphenylaether; in Gummi-zwischenverbindungen und als Lösungsmittel.

Die wichtigsten Anwendungen von 1,2-Dichlorbenzol und 1,2,4-Trichlorbenzol finden statt als Lösungsmittel in der Verfahrenstechnik und als Zwischenverbindung in der Synthese von Farbstoffe, Pflanzenschutzmittel und Entfetter. 1,2-Dichlorbenzol und 1,4-Dichlorbenzol werden eingesetzt als Deodorant. 1,2,4,5-Tetrachlorbenzol ist der einzige Tetrachlor-Isomer welche in industrieller Mengen angewendet wird. Die übrige Isomeren werden nicht in betrachtliche Mengen angewendet.

Die totale Produktion (Tonne/Jahr) in der EG wird eingeschätzt auf 70-80000 in 1982 für Monochlorobenzol, 80000 in 1987 für Dichlorbenzolen und 17-40000 in 1987 für Trichlorbenzol (Technisch).

Chemische Analyse von Chlorobenzol ist möglich durch die Isolierung mittels Reinigungs- und Einfangtechniken, fortgesetzt durch GC Trennung und Determinierung mit ECD oder FID. Für komplexe Mischungen wird eine Identifizierung mit MS empfohlen.

Abhängig von der Art Chlorobenzol und die Determinierungsmethode, liegen die Determinierungsgrenzwerten zwischen 6-20 µg/l Wasser bis ungefähr 500 µg/l.

Über Konzentrationsniveaus in Luft, Flusswässer, Sedimente und Biotopen wurde spärlich berichtet. In Städteluft wurde 0,05 bis 7 µg/m³ gemessen, aber in der Umgebung von einigen Industrien können 1-3 g/m³ auftreten.

In Sedimenten ist die Streuung zwischen 0,1-250 µg/kg, abhängig von der Umgebung der Verunreinigungsquellen. In kommunalen Abwässer sind bis zu mehrere Hunderte µg/l gemessen worden. In offenen Gewässern vereigneten rezente Messungen im Rhein maximalen Niveaus unterhalb 0,1-1 µg/l.

In Fischen liegen die Niveaus zwischen 0,01 und 20 µg/kg Körpermengen und in Fettgeweben oder Muttermilch wurden Konzentrationen bis zu 130 µg/kg Fett aufgezeignt.

Die Werte variieren ziemlich und sind in hohem Maße abhängig von der Umgebung der emittierende Industrie. Es ist nicht anzunehmen dass die Chlorobenzole in der aquatische Umwelt Photogen abgebaut werden; aber nach Verflüchtigung wird Photogenischer Abbau in der Atmosphäre der bedeutenster Entfernungsweg bilden. Biologischer Abbau von Chlorobenzole ist abhängig von dazu geeignete Umstände. Mit der Ausnahme von 1,4-Dichlorbenzol zeigten sich alle weiteren Isomere nicht im Stande die normierten Beurteilungstests zur direkter biologischer Abbau zu durchlaufen.

Die Verhinderung von Bakterienwachstum und das fehlen von angepassten Mikro-Organismen scheint der Ursache für die negativen Versuchsresultate zu sein.

In verschiedenen Arten von Simulierungsversuche und unter experimentelle Bedingungen zeigten die meisten Isomere inherent abbaubar zu sein. Unter anaerobe methanogenische Bedingungen könnte Dehalogenation der höher substituierten Benzole erwartungsgemäss stattfinden, aber weitere Hinweise nach Schnelligkeit und Endprodukte sind notwendig. Fakten über auftretender

biologischer Abbau an Flusssedimenten und übrigen im Frage kommenden Stellen in der Umwelt sind nicht vollständig verfügbar.

Die Chlorobenzole sind löslich in Wasser bis eine Höhe zwischen 1 und 500 mg/l, abhängig von der Grad an Chlорidensubstitution.

Die Chlorobenzole werden durch Verflüchtigung in wenigen Monaten aus dem Wasser entweichen und unter atmosphärischen Verhältnissen findet photogenischer Abbau statt.

Die höher substituierte Isomeren sind weniger löslich und zeigen ein höherer Haftungsgrad an Sedimenten und ein höherer Grad an Bioakkumulation.

Für Tetrachlorbenzol könnte der Bioakkumulationsfaktor ungefähr 1000 betragen.

Die Toxizität von Chlorobenzole ist recht gut dokumentiert; aber die Ergebnissen sind mit der nötigen Sorgfalt zu beurteilen durch die Variationen welche auftreten durch Verflüchtigung während den Versuchsumständen.

Akute Toxizität bezüglich einer Vielfalt von aquatischen Arten variiert zwischen 4 und 22 mg/l bezogen auf Monochlorbenzole und zwischen 0,1 und 31 mg/l bezogen auf die Di-, Tri- und Tetrachlorbenzole. Alle verfügbaren chronischen Toxizitätswerten für aquatische Organismen liegen über 0,1 mg/l.

Ausgehend von der niedrige Empfindlichkeitsvariation zwischen den verschiedenen Arten und zwischen akuten und chronischen Effekten könnte ein sicheres Niveau für alle aquatischen Umweltsystemen vorgeschlagen werden von 10 µg/l.

Das bedeutet, dass die maximalen Niveaus in Rhein (1 µg/l) um ein Faktor zehn unter dieses sichere Niveau liegt.

Das vorgeschlagene Wasserqualitäts Kriterium (10 µg/l für die Summierung von alle Chlorobenzene) ist innerhalb ein Faktor 10 vergleichbar mit dem Kriterium der Rhein Aktionsprogramm (0,1 µg/l für jedes Trichlorbenzolisomer).

Das Gesundheitsrisiko für Menschen durch Aussetzung an Chlorobenzolen in Wasser ist bedeutungslos.

Für Tetrachlorbenzol aber sind die Werten unvollständig.

RESUMÉ

Les données concernant les propriétés physiques et chimiques, la dégradabilité, la toxicité, la détection chimique et les concentrations dans l'environnement ont été recueillies et revisées pour les chlorobenzènes. Le tableau i montre un aperçu de la disponibilité des données concernant les sujets divers.

Les chlorobenzènes sont utilisés dans la production de chloronitrobenzène et de l'éther diphenyl, dans les intermédiaires de caoutchouc et comme dissolvant. On applique le 1,2-dichlorobenzène et le 1,2,4-trichlorobenzène principalement comme dissolvant et comme intermédiaire dans la synthèse de colorants, d'herbicides et comme moyen de dégraissage. Le 1,2-dichlorobenzène et le 1,4-dichlorobenzène sont utilisés comme désodorisant. Le 1,2,4,5-tetrachlorobenzène est le seul isomère tétrachlorique qui est utilisé en quantités industrielles. Les autres isomères ne sont pas utilisés en quantités significantes.

Les chiffres de la production dans la CEE sont pour le monochlorobenzène 70-80.000 tonnes/an en 1982, pour le dichlorobenzène 80.000 tonnes/an en 1987 et pour le trichlorobenzène (technique) 17-40.000 tonnes/an en 1987.

Une analyse chimique de chlorobenzènes est possible par moyen d'isolation par une technique de purger et d'attraper, suivie de la séparation par CG et de détection à capture d'électron ou de détection à ionisation de flamme. Pour les mélanges plus complexes une identification par spectrométrie de masse sera recommandée.

Les limites de détection dans l'eau varient entre 6-20 µg/l et 500 µg/l, selon le type de chlorobenzène et la méthode de détection.

Les niveaux de concentration dans l'air, dans les eaux de surface, dans les sédiments ou en matière biologique, n'ont été rapportés que rarement. On a mesuré un niveau entre 0,05 et 7 µg/m³ dans l'air urbain, mais un niveau de 1-3 g/m³ dans les zones industriels peut se produire.

Dans les sédiments ce niveau varie de 0,1 - 250 µg/kg, dépendant de la proximité de sources pollueuses. On a mesuré un niveau de quelque cents µg/l dans les eaux d'égout. Des sondages dans l'eau du Rhin montrent des niveaux sous 0,1 - 1 µg/l au maximum.

Dans les poissons les niveaux sont entre 0,01 et 20 µg/kg et dans la matière grasse ou dans le lait maternel on a trouvé des valeurs jusqu'à 130 µg/kg matière grasse. Les valeurs sont assez variables et dépendent largement de la proximité d'industries pollueuses.

Il n'est pas probable que les chlorobenzènes soient photodégradables dans l'environnement aquatique; pourtant la photodégradation dans l'atmosphère sera la voie la plus probable après que les chlorobenzènes sont évaporés.

La dégradation biologique des chlorobenzènes dépendra de circonstances favorables. Aucun des isomères n'a pu passer les "screening" tests d'examination de base. Seul le 1,4-dichlorobenzène pouvait être désigné comme facilement biodégradable ("readily").

A ce qu'il paraît la croissance bactérielle freinée et le manque de micro-organismes adaptés forment la raison de ce résultat négatif. La plupart des isomères prouve être bien dégradables sous autres formes de test simulé ou en de conditions expérimentales. Les benzènes substitués sur un plan plus élevé pourraient se

déhalogéniser en de conditions anaérobies méthanogènes, mais il nous faut l'information sur la vitesse et les résultats finals. L'information sur la vitesse de la biodégradation dans les sédiments fluviaux et dans d'autres situations pertinentes est incomplète.

Les chlorobenzènes sont solubles dans l'eau jusqu'à un niveau de 1 à 500 mg/l, selon le degré de substitution chlorique. Ils disparaîtront du niveau aquatique par vaporisation dans quelques mois et ils seront photodégradés en de conditions atmosphériques. Les isomères substitués sur un plan plus élevé sont moins solubles et montrent une capacité d'absorption en sédiments et un degré d'accumulation biologique plus large. Le facteur de bio-accumulation de tétrachlorobenzène peut être environ 1000.

La toxicité des chlorobenzènes a été documentée suffisamment, pourtant les résultats doivent être interprétés soigneusement à cause de la variabilité issue de la volatilisation conditionnée par les tests. La toxicité aigue de beaucoup d'espèces aquatiques varie entre 4 et 22 mg/l pour le monochlorobenzène et entre 0,1 et 31 mg/l pour les di-, tri- et tétrachlorobenzènes. Toutes les données qu'on a de la toxicité chronique pour les organismes aquatiques sont au-dessus de 0,1 mg/l.

Un niveau maximal de 10 µg/l peut être proposé pour tous les systèmes écologiques aquatiques, basé sur la variabilité mineure de sensibilité entre les espèces différentes et entre les effets directs et chroniques. Ceci signifie que les niveaux maximaux dans l'eau du Rhin (1 µg/l) sont un niveau de magnitude en dessous de ce niveau.

La proposition d'un "niveau maximal" de 10 µ/l pour l'ensemble des chlorobenzènes convient aux limites proposées par la Programma Rhin Action de 0,1 µ/l pour les trichlorobenzènes distincts.

Le risque pour la santé de l'homme par l'exposition aux chlorobenzènes dans l'eau peut être négligé. Pourtant pour le tétrachlorobenzène les données sont incomplètes.

SAMENVATTING

Voor chloorbenzenen zijn gegevens over de fysisch-chemische eigenschappen, afbreekbaarheid, toxiciteit, chemische detectie en concentraties in het milieu verzameld.

Gegevens aangaande de beschikbaarheid van data over de verschillende onderwerpen staan vermeld in Tabel i.

Chloorbenzenen worden gebruikt bij de produktie van chloornitrobenzenen en difenylether, van rubberussenprodukten en als oplosmiddel. 1,2-Dichloorbenzeen en 1,2,4-trichloorbenzeen zijn vooral belangrijk als industrieel oplosmiddel en als tussenprodukt in bij de synthese van kleurstoffen, herbiciden en ontvettingsmiddelen. 1,2-Dichloorbenzeen en 1,4-dichloorbenzeen worden toegepast als stankverdrijvers. Van de tetrachloor-isomeren wordt alleen 1,2,4,5-tetrachloorbenzeen in industriële hoeveelheden gebruikt; van de andere isomeren worden geen belangrijke hoeveelheden gebruikt.

De totale produktie in de EG wordt geschat op 70-80000 ton/jaar in 1982 voor monochloorbenzen, 80000 ton/jaar in 1987 voor dichloorbenzenen, en 17-40000 ton/jaar in 1987 voor technisch trichloorbenzeen.

Chloorbenzenen kunnen chemisch geanalyseerd worden door opwerking met "purge and trap" technieken, scheiding door GC en detectie met ECD of FID. Voor complexe mengsels wordt identificatie met behulp van MS aanbevolen. Detectiegrenzen in water variëren, afhankelijk van de structuur en de detectiemethode, tussen 6-20 µg/l tot 500 µg/l.

Er zijn maar weinig resultaten van concentratiemetingen in lucht, oppervlaktewater, sediment of biologisch materiaal gepubliceerd. In steden zijn concentraties in de lucht gemeten tussen 0,05 en 7 µg/m³, maar in de buurt van bepaalde industrieën kan 1 tot 2 g/m³ voorkomen. In het sediment kunnen de concentraties variëren van 0,1 tot 250 µg/kg, afhankelijk van de aanwezigheid van verontreinigingsbronnen. In rioolwater zijn concentraties tot enkele honderden microgrammen per liter gemeten. Recente metingen in Rijnwater leverden maximum waarden van minder dan 0,1 tot 1 µg/l op. In vis liggen de gehalten tussen 0,01 en 20 µg/kg lichaamsgewicht en in menselijk vetweefsel of moedermelk zijn concentraties tot 130 µg/kg vet gevonden. De meetresultaten variëren sterk en zijn afhankelijk van de aanwezigheid van industriële bronnen.

Fotodegradatie van chloorbenzenen in water is niet waarschijnlijk. Als chloorbenzenen echter door verdamping in de atmosfeer terechtkomen, is fotodegradatie het belangrijkste afbraakproces. Het optreden van biologische afbraak is afhankelijk van gunstige milieuomstandigheden. In de standaard screening testen kon alleen 1,4-dichloorbenzeen als gemakkelijk afbreekbaar (ready biodegradable) worden geklassificeerd. Mogelijk kunnen de negatieve testresultaten worden verklaard door remming van de bacteriegroei en het ontbreken van geadapteerde microorganismen. In diverse simulatiestesten en onder laboratorium- omstandigheden blijken de meeste chloorbenzenen uiteindelijk wel afbreekbaar te zijn (inherently biodegradable). Onder anaerobe methanogene omstandigheden worden hoger gesubstitueerde benzenen waarschijnlijk gedehalogeneerd, maar de snelheden en eindprodukten zijn niet bekend. De kennis over de werkelijke afbraaksnelheden in de rivierbodem en in andere belangrijke milieucompartimenten is onvolledig.

De oplosbaarheid van chloorbenzenen in water varieert tussen 1 en 500 mg/l afhankelijk van de mate van chlorering. De stoffen verdwijnen binnen enkele maanden uit het water door vervluchting en onder atmosferische omstandigheden treedt fotodegradatie op. De hoger gesubstitueerde isomeren lossen minder goed op, adsorberen sterker aan sediment en bioaccumuleren meer. Voor tetrachloorbenzeen zou de bioaccumulatiefactor circa 1000 bedragen.

Er is betrekkelijk veel gepubliceerd over de toxiciteit van de chloorbenzenen. In verband met de vluchtigheid van deze stoffen onder testomstandigheden moeten de onderzoeksresultaten echter zorgvuldig worden bekeken. Voor monochloorbenzeen varieert de acute toxiciteit voor groot aantal aquatische soorten tussen 4 en 22 mg/l en voor de di-, tri- en tetrachloorbenzenen tussen 0,1 en 31 mg/l. In alle chronische testen lag de toxiciteit voor aquatische organismen boven 0,1 mg/l.

Gezien het geringe verschil in gevoeligheid tussen de soorten en tussen acute en chronische effectconcentraties wordt voorgesteld het veilige niveau op 10 µg/l te stellen. Dit betekent dat de hoogste concentraties die in de Rijn gemeten zijn (1 µg/l) een factor onder het veilige niveau liggen.

De voorgestelde waterkwaliteitsnorm van 0,05 µg/l is binnen een factor 10 vergelijkbaar met normen opgesteld door het Rijn Actie Programma van 0,01-0,1 µg/l.

Het risico voor de volksgezondheid door blootstelling aan chloorbenzenen in water is te verwaarlozen. Voor tetrachloorbenzeen zijn de gegevens om dit te beoordelen echter niet volledig.

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1

DESCRIPTION

Chlorobenzenes are used in the production of chloronitrobenzene and diphenyl ether; in rubber intermediates; as a solvent in adhesives, dyestuffs paints, waxes, and polishers; and as an inert solvent it is also used in very small amounts in the production of some pesticides.

The major uses of 1,2-dichlorobenzene are as a process solvent in several certain chemical manufacturing processes and as intermediates in the synthesis of dyestuffs, herbicides and degreasers. 1,2-Dichlorobenzene is also used as a deodorizer.

1,3-Dichlorobenzene has no documented use.

1,4-Dichlorobenzene is used primarily as an air deodorant and insecticide.

1,2,4-Trichlorobenzene is used as a dye-carrier, a herbicide intermediate, a heat transfer medium, a dielectric fluid in transformers, a degreaser and a lubricant (EPA 1980^b). Other isomers are not used in significant quantities.

1,2,4,5-Tetrachlorobenzene is the only tetrachloro-isomer used in industrial quantities (EPA 1980^b) e.g. in the synthesis of 2,4,5-trichlorophenol; and as a fungicide (sources: CCREM, 1987 and Ware, 1988).

1.1

Identification

chlorobenzene

List I Dir. 76/464/EEC 20

EINECS-no. 2036285

CAS no. 108-90-7

Synonyms: phenylchloride

benzene chloride

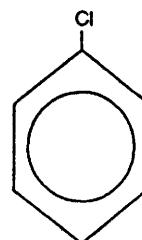
monochlorobenzene

monochloorbenzeen

MCB

Molecular formula: C₆H₅Cl

Structural formula:



WLN formula: GR

1,2-dichlorobenzene

List I Dir. 76/464/EEC 53

EINECS-no. 2024259

CAS no. 95-50-1

Synonyms: o(rtho)-dichlorbenzene

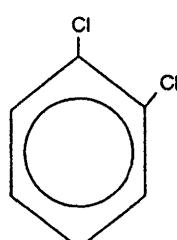
o-dichlor benzol

o-dichloorbenzeen

1,2-DCB

Molecular formula: C₆H₄Cl₂

Structural formula:



WLN formula: GR BG

1,3-dichlorobenzene

List I Dir. 76/464/EEC 54

EINECS-no.

CAS no.

541-73-1

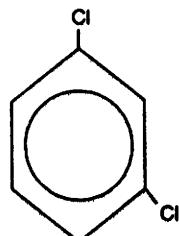
Synonyms:

m-di chlorobenzene
m-di chlorobenzol
m-dichlorbenzene
1,3-DCB

Molecular formula:

$C_6H_4Cl_2$

Structural formula:



WLN formula:

GR CG

1,4-dichlorobenzene

List I Dir. 76/464/EEC 55

EINECS-no.

2034005

CAS no.

106-46-7

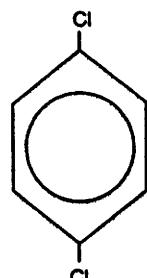
Synonyms:

p-dichlorobenzene
p-di chlorobenzol
p-dichlorbenzene
1,4-DCB

Molecular formula:

$C_6H_4Cl_2$

Structural formula:



WLN formula:

GR DG

1,2,3-trichlorobenzene

List I Dir. 76/464/EEC

EINECS-no.

87-61-6

CAS no.

1,2,3-trichlorbenzene

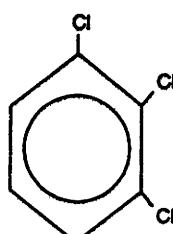
Synonyms:

1,2,3-TCB

Molecular formula:

$C_6H_3Cl_3$

Structural formula:

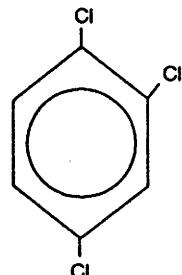


WLN formula:

GR BG CG

1,2,4-trichlorobenzene
List I Dir. 76/464/EEC 118
EINECS-no.
CAS no. 120-82-1
Synonyms: 1,2,4-trichloorbenzen
1,2,4-TCB

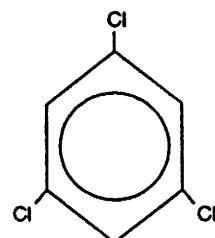
Molecular formula: $C_6H_4Cl_3$
Structural formula:



WLN formula: GR BG DG

1,3,5-trichlorobenzene
List I Dir. 76/464/EEC
EINECS-no.
CAS no. 108-70-3
Synonyms: 1,3,5-trichloorbenzen
1,3,5-TCB

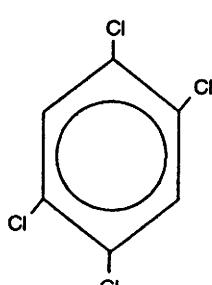
Molecular formula: $C_6H_4Cl_3$
Structural formula:



WLN formula: GR CG EG

1,2,4,5-tetrachlorobenzene
List I Dir. 76/464/EEC 109
EINECS-no.
CAS no. 95-94-3
Synonyms: 1,2,4,5-tetrachloorbenzen
1,2,4,5-TeCB

Molecular formula: $C_6H_4Cl_3$
Structural formula:



WLN formula: GR BG DG EG

1.2 Physicochemical characteristics

	CB	1,2-DCB	1,3-DCB	1,4-DCB
mol. weight	112.56	147.01	147.01	147.01
COD (mg O/mg substance)				
mg substance/mg organic C1				
melting point (°C)	-45	-17	-24.7	53.1
boiling point (°C)	131.7	180.5	173	174
density at 20°/4°C	1.106	1.305	1.288	1.458
vapour pressure (kPa) at 20-25°C	1.581	0.196	0.307	0.09
water solubility (mg/l) at 25°C	491	134	122	83,2
log K _{ow}	2.89	3.4	3.5	3.4
Henry's constant (kPa.m3/mol) at 20°C	0.363	0.195	0.366	0.160
Standard half-life (h) (volatilization)	260	550	290	670
log Koc	2.25	2.63	2.65	2.74

	1,2,3-TCB	1,2,4-TCB	1,3,5-TCB	1,2,4,5-TeCB
mol. weight	181.45	181.45	181.45	215.9
COD (mg O/mg substance)				
mg substance/mg organic C1				
melting point (°C)	53	16.95	63	140
boiling point (°C)	218	213.5	208	243
density at 20°/4°C				
vapour pressure (kPa) at 20-25°C	0.053	0.061	0.077	5.4*10 ⁻⁴
water solubility (mg/l) at 25°C	24	29.8	15.8	0.6
log K _{ow}	4.13	4.05	4.18	4.6
Henry's constant (kPa.m3/mol) at 20°C	0.234	0.379	0.161	0.261
Standard half-life (h) (volatilization)	510	320	730	500
pKa				
log Koc	3.10	3.04	3.20	4.03

References:

log K_{ow} and standard halflife: obtained from De Bruin, 1985

density: Verschueren, 1983

other references: Canadian Water Quality Guidelines, 1987

1.3

Production levels

Data on the production of chlorobenzenes in EC are presented in Table 1.1, derived form EURECO (1990).

Table 1.1 Production levels of chlorobenzenes in EC countries

Country	Capacity in t/y * actual production						1.2.4.-5-TcB	TCB (Techn.)	1.2.4.-5-TcB	Producer
	MCB	1,2-DCB	1,3-DCB	1,4-DCB	1,2,4-TCB					
Belgium										
Denmark	20000 (y87)	[1,2]	3000 y84	[2,3]		7000 y84 [2,3]			10000 (y87) [6]	+
FRG (Germany)	85000 (y87)	[1,2,3]	15000 y84	[3]	3800 (y88)	[5]	25000 y87 [2]		5000 (y86) [6,7]	+
	15000 y90	[4]	5000* y90	[4]	3800* y90	[4]	8000 y90 [4]		7000 (y86) [6]	[2,3]
Greece										Bayer AG (Leverkusen) Hoechtl
Ireland										
Italy	15000 y90	[4]	2200 y90	[4]	500 y90	[4]	1550 y90 [4]			
Luxembourg										
Netherlands										
Portugal									+	[4]
Spain										
United Kingdom										
Total production EC	70-80000* (y82)	[1]	25000 (y84)	[3]	8000 (y88)	[5]	49000 (y84)	[3]	15-20000 (y87) [2]	17-40000 (y87) [2]
Total use EC							27400 (y87)	[2]		

¹ Hoofman and De Kreuk (1981; 1983)² Water Research Centre (1987)³ IDET-EGOS (1983)⁴ Information obtained from industry (Eureco, 1990)⁵ SEMA METRA (1988)⁶ Gesellschaft Deutscher Chemiker (1987)⁷ Economist Intelligence Unit (1978)

- 5 -

Atochem SA (Jarrle)
Rhône-PoulencEnichem Sintesi
(Pierre-Vergote)Atochem (San-
Adrián de Besos)

2 ANALYTICAL DETECTION TECHNIQUES

2.1 Detection methods

In the previous report of the EC, Hooftman and De Kreuk (1981; 1983) reported on the detection method for all chlorobenzenes. They described a purge and trap method which is analogous to the EPA methods that will be described later. Nevertheless, they proposed GC/MS-detection for a better identification of chlorobenzenes in mixtures with many other substances.

EPA method 612 for chlorinated benzenes (EPA, 1984a) describes a detection method for chloroethane, hexachlorobutadiene, 2-chloronaphthalene, 1,2-dichlorobenzene, 1,2-dichlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene and hexachlorobenzene in municipal and industrial discharges.

The contaminated water is extracted with methylene chloride. The extract is dried by passing through a sodium sulphate column and concentrated to a volume of 10 ml or less. The substances are detected with a gas chromatograph suited for column injection with an electron capture detector (ECD). Table 2.1.1 lists the detection limits for EPA method 602.

Table 2.1.1 Detection limits for chlorobenzenes with EPA method 612 (EPA, 1984a)

Substance	Detection limit ($\mu\text{g/l}$)
1,3-dichlorobenzene	0.009
1,4-dichlorobenzene	0.018
1,2-dichlorobenzene	0.012
1,2,4-trichlorobenzene	0.006

The analytical conditions for the EPA method 602 are:

- column temperature: 75°C
- carrier gas: 5% methane/95% argon (30 ml/min)
- column: Supelcoport 80/100 mesh coated with 1.5% OV-1/2.4% OV-225 packed in a 1.8 m long x 2 mm ID glass column

In EPA method 602 for purgeable aromatics (EPA 1984b) a purge and trap chromatographic technique has been described for benzene, chlorobenzene, 1,2-dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, ethylbenzene and toluene in 6 types of water: distilled water, drinking-water, surface water and three types of wastewater. An inert gas is purged through a water sample in a chamber with a specific temperature. The aromatics are transferred to the vapour phase and are trapped in a sorbent trap. After purging is completed the trap is heated and backflushed with the inert gas to desorb the aromatics onto a gas chromatographic column. The gas chromatograph is temperature programmed to separate the aromatics which are detected with a photo-ionization detector (PID).

The analytical conditions are:

- temperature programme: 50°C-2 min., 6°/min to 90°C
- carrier gas: N₂ (20-40 ml/min)
- column packing: 5% SP-1200/1.75% Bentone 34

The chromatographic conditions and detection limits for this method are presented in Table 2.1.2.

Table 2.1.2 Method Detection Limits for EPA method 602 (EPA 1984^b)

Substance	Detection limit ($\mu\text{g/l}$)	Method standard deviation (%)
chlorobenzene	0.2	9.4
1,4-dichlorobenzene	0.3	20.4
1,3-dichlorobenzene	0.4	20.0
1,2-dichlorobenzene	0.4	27.7

Lopez-Avilla et al. (1989) evaluated EPA method 8120 (EPA, 1986) for the determination of chlorinated hydrocarbons in environmental samples, including the compounds discussed in this study: di-, tri-, and tetra-chlorobenzenes. After some adaptations on the chromatographic column satisfactory recovery rates were obtained. This resulted in a better separation of complex samples.

The GC was equipped with a ^{63}Ni -ECD and a fused-silica open tubular capillary column. Samples were extracted at neutral pH with methylene chloride. The extracts were dried and concentrated. Hexane was added and the volume again reduced. The samples were cleaned up with florisil, with petroleum ether and diethyl ether as eluents.

The precision of the method for water samples containing chlorinated hydrocarbons discussed in this study ranged from 3-9%, and recoveries were almost 100%. The detection limits of the method for chlorobenzenes are presented in Table 2.1.3.

Table 2.1.3 Detection limits for EPA Method 8120 (Lopez-Avilla et al., 1989)

Substance	Detection limit (ng/l)
1,2-dichlorobenzene	270
1,3-dichlorobenzene	250
1,4-dichlorobenzene	890
1,2,3-trichlorobenzene	39
1,2,4-trichlorobenzene	130
1,3,5-trichlorobenzene	12
1,2,4,5-tetrachlorobenzene	9.5

In Table 2.1.4 a review is given of the detection methods for chlorobenzenes in ground, water and air. For each technique the sample treatment, cleaning, detection method, congeners, detection limits and references are presented, as far as possible (Slooff et al., 1991).

Table 2.1.4 Detection methods for chlorobenzenes (Slooff et al., 1991)

Sample treatment	Cleaning/desorption	Detection method	Sample volume	Subst.	Detection limits (µg/l)	Ref.
<u>Ground:</u>						
purge and trap adsorbens: tenax purge gas: N ₂		GC-MS	2 g	mono di tri		1
<u>Water:</u>						
purge and trap adsorbens: graphitfluoride purge gas: helium	desorption with helium at 300°C	GC-FID	5 ml	mono di	100 500	2
purge and trap adsorbens: tenax purge gas: helium	desorption with helium at 180°C	GC-FID with cryotrap	50 ml	di	2	3
extraction with pentane hexane extract	chromatography over acid/base column (AgNO ₃ /-Al ₂ NO ₃)	GC-FID GC-MS	1 l	all di tri tetra	0.3-0.5	4
						5
<u>Air:</u>						
purge and trap adsorbens: graphitfluoride	desorption with helium at 300°C	GC-FID	3 l	mono		2
purge and trap adsorbens: tenax	desorption with H ₂ at 200°C	GC-FID/ECD		mono di tri		6
trapping on activated coal	desorption with carbondisulfide	GC-FID	1000 l	mono di tri	0.8	1

References:

- 1 Kliest and Van der Wiel (1987)
- 2 Yao and Zlatkis (1987)
- 3 Vandegrift (1988)
- 4 Strottmeister et al. (1988)
- 5 Cuiu et al. (1986)
- 6 Termonia and Alaerts (1985)

Alberti (1987) described a detection method for chlorinated hydrocarbons in water and in sediment. The water sample is extracted with n-pentane, n-hexane or acetone. The extract can be cleaned up by elution through silica gel, florisil or aluminumoxid. Detection can take place with GC-ECD. For non-volatile substances the sediment is dried at 110°C or with sodium- or magnesiumsulphate and subsequently extracted and detected like water samples.

With this method the following detection limits can be reached: 1,2-, 1,3- and 1,4-dichlorobenzene 0.5 µg/l; 1,2,4-trichlorobenzene 0.1 µg/l.

Barkly et al. (1980) studied the concentration of halogenated hydrocarbon in humans and in environmental media in the neighbourhood of a toxic chemicals dump in the US. They analyzed urine, blood, water, breath and air (indoors and outdoors). For detection they used a capillary gas chromatograph. The urine, blood and water samples were heated (50-90°C). The evaporated substances were trapped on a Tenax column and after desorption analyzed by GC-MS. The breath and air samples were collected by a respirometer. From hereof analysis was the same as for the blood, urine and water samples.

2.2

Conclusions

The most frequently applied technique for chlorobenzenes is proceeded by a purge-and trap technique. As environmental samples often contain a mixture of substances that can disturb the identification of single substances, this pretreatment is important. Generally the substances are detected by GC-ECD, GC-MS or GC-FID. In general, detection methods for chlorobenzenes are well described by the EPA. Other authors have often developed related methods for measurements under special environmental conditions.

In some cases the detection limit can be approximately 10 ng/l, depending on the applied technique and on the complexity of the mixture.

3

ENVIRONMENTAL LEVELS

In this chapter a review will be given of the concentration levels for the chlorobenzenes in various environmental compartments. These levels depend on environmental conditions like climate, the organic content of sediments, water flow rate of the rivers, etc. Therefore these levels will give only restricted information of the concentration levels that may occur at other locations.

3.1

Residues in the atmosphere

In their report on chlorobenzenes for the EC Hooftman and De Kreuk (1981; 1983) reported levels of chlorobenzenes in the air. A summary of these levels is presented in Table 3.1.1.

Table 3.1.1 Levels of chlorobenzenes in air (Hooftman and De Kreuk, 1981; 1983)

Substance	Location	Concentration	
monochlorobenzene	Los Angeles and Phoenix (USA)	0.2	µg/m ³
	New Jersey (USA)	0.25 - 0.8	µg/m ³
dichlorobenzenes (all)	Los Angeles (USA)	8 - 53	ng/m ³
1,2-dichlorobenzene	wool mills	60 - 1620	mg/m ³
1,4-dichlorobenzene	Tokyo (city)	2.7 - 4.2	µg/m ³
	Tokyo (suburban)	1.5 - 2.4	µg/m ³
	manufacture works	60 - 3300	mg/m ³
1,2,4-trichlorobenzene	urban air	3 - 7	µg/m ³

Table 3.1.2 Concentrations of indoor and outdoor air in The Netherlands (Slooff et al., 1991)

Substance	Indoor (µg/m ³) ¹	outdoor (µg/m ³) ¹
monochlorobenzene		(0.4) ²
1,2-dichlorobenzene	0.004	(0.013)
1,3-dichlorobenzene	0.002	(0.009)
1,4-dichlorobenzene	7.2 (299)	0.024 (0.110)
1,2,3-trichlorobenzene		
1,2,4-trichlorobenzene		(0.033)
1,3,5-trichlorobenzene		(0.3) ²
1,2,4,5-tetrachlorobenzene		(<0.081)

1 In parentheses the maximum measured concentrations are presented

2 Calculated maximal year average on a 500 meter's distance from a sewage treatment plant

Hooftman and De Kreuk (1981) presented references, but no data, for occurrence of 1,3-dichlorobenzene in the environment. 1,2,3-, 1,2,4- and 1,3,5-trichlorobenzene were also detected after combustion of PVC at 1 µg/g PVC.

Slooff et al., (1991) reported concentrations in air samples in The Netherlands. A summary of these air concentrations are presented in Table 3.1.2.

3.2 Residues in soil

Hooftman and De Kreuk (1981; 1983) reported concentrations of some chlorobenzenes in sediments. In the Great Lakes (Canada) trichlorobenzenes were detected: 1,2,3-trichlorobenzene in a range of 0.1 to 16 µg/kg; 1,2,4-trichlorobenzene in a range of 0.1 to 220 µg/kg; 1,3,5-trichlorobenzene in a range of 0.1 to 250 µg/kg.

In 1982 Alberti (1987) reported concentrations of 0.8-1.0 µg/kg in dry sediment samples in the river Rhine in Germany for 1,2,4-trichlorobenzene.

Masunaga et al. (1991a) studied the concentration of chlorobenzenes in the sediment of the Ise Bay in Japan on different locations. The concentrations were found to be higher in the vicinity of the two ports of the bay. The average concentrations for chlorobenzenes are presented in Table 3.2.1.

Table 3.2.1 Concentration of chlorobenzenes in the Ise bay sediment, Japan (Masunaga et al., 1991a)

Substance	Average concentration (ng/g)
1,2-dichlorobenzene	2.3
1,3-dichlorobenzene	1.9
1,2,4-trichlorobenzene	4.8
1,3,5-trichlorobenzene	0.04 - 0.15
1,2,3,5- and 1,2,4,5-tetrachlorobenzene	0.04 - 0.15

In general, chlorobenzenes can migrate in the soil and they may be found in the soil in the vicinity of waste landfills.

3.3 Residues in water

Hooftman and De Kreuk (1981; 1983) reported, in their literature search for the EC, concentration levels for chlorobenzenes in different types of water. In Table 3.3.1 a summary of these levels is presented.

Table 3.3.1 Concentrations of chlorobenzenes in different types of water according to Hooftman and De Kreuk (1981; 1983)

Substance	Water type and place	Concentration ($\mu\text{g/l}$)
monochlorobenzene	groundwater (USA)	1
	wastewater (USA)	0.1 - 27
	Rhine (Europe)	<0.01 - 1.96
	well water near chemical plant (Switzerland)	8
1,2,3-trichlorobenzene	surface water (Canada)	0.1 - 38
	drinking water	2 - 3
	wastewater (Canada)	2 - 3
	municipal discharge (USA)	21 - 46
1,2,4-trichlorobenzene	surface water (Canada)	0.6 - 107
	drinking water	1 - 4
	municipal discharge (USA)	<0.01 - 275
	municipal discharge (USA)	<0.01 - 26
1,3,5-trichlorobenzene	surface water (Canada)	0.2 - 8
	wastewater (Canada)	0.2 - 0.5
	Rhine	0.6 ± 1.5

Recently, concentrations in groundwater and surface water have also been reported for The Netherlands (Slooff et al., 1991). A summary of the available concentrations is presented in Table 3.3.2.

Table 3.3.2 Concentrations of chlorobenzenes in groundwater and surface water in The Netherlands (Slooff et al., 1991).

Substance	Groundwater ($\mu\text{g/l}$) ¹	Surface water ($\mu\text{g/l}$) ¹
monochlorobenzene	<0.5 (<0.5)	
1,2-dichlorobenzene	<0.1 (0.3)	0.03 (0.6)
1,3-dichlorobenzene	<0.1 (0.3)	0.02 (0.3)
1,4-dichlorobenzene	<0.1 (0.3)	0.04 (0.6)
1,2,3-trichlorobenzene	<0.1 (<0.1)	0.02 (0.09) ²
1,2,4-trichlorobenzene	<0.1 (<0.1)	0.02 (0.09) ²
1,3,5-trichlorobenzene	<0.1 (<0.1)	0.02 (0.09) ²
1,2,4,5-tetrachlorobenzene		(<0.001)

¹ In parentheses the maximum measured concentrations are presented

² Sum of all trichlorobenzenes

Petersen (1986) studied the concentration of chlorobenzenes at many locations in three rivers in Germany: the Rhine, Main and Elbe. For 1,4-dichlorobenzene the following concentration ranges were reported: Rhine 0.1 - 2.9 $\mu\text{g/l}$; Main 0.1 - 1.5 $\mu\text{g/l}$; Elbe 0.01 - 0.27 $\mu\text{g/l}$. For each of the trichlorobenzenes concentration ranges were found: Rhine 0.01 - 0.06 $\mu\text{g/l}$; Main 0.03 - 0.1 $\mu\text{g/l}$; Elbe 0.1 - 0.15 $\mu\text{g/l}$.

Masunaga et al. (1991b) reported concentrations of chlorobenzenes in the water of Ise Bay in Japan. The concentrations were measured at various locations and depths in the bay. A summary of the reported data is presented in Table 3.3.3. In general, the concentrations at the surface and on the bottom were relatively high as compared to the concentration in between.

Table 3.3.3 Concentrations of chlorobenzenes in the water of Ise Bay, Japan (Masunaga et al., 1991b)

Substance	Concentration range (ng/g)
1,2-dichlorobenzene	not detected - 145.8
1,3-dichlorobenzene	not detected - 9.7
1,2,3-trichlorobenzene	not detected - 542.1
1,2,4-trichlorobenzene	not detected - 29.1
1,3,5-trichlorobenzene	not detected - 11.9

3.4 Residues in aquatic organisms

In their evaluations for the EC, Hooftman and De Kreuk (1981; 1983) reported concentration levels for chlorobenzenes in fish. In Table 3.4.1 a review of these data is presented.

Table 3.4.1 Concentrations of chlorobenzenes in fish (Hooftman and De Kreuk, 1981; 1983)

Substance	Fish and place	Concentration
1,4-dichlorobenzene	horse mackerel	0.05 µg/g wet weight
trichlorobenzenes (all)	Herring (<u>Clupea harengus</u>)	<0.01 - 0.5 µg/g wet weight
1,2,4-trichlorobenzene	trout (Canadian Lakes)	0.5 - 5 µg/kg wet weight
	herring (Can. Lakes)	10 - 20 µg/kg fresh weight
1,3,5-trichlorobenzene	trout (Can. Lakes)	0.1 - 5 mg/kg

No recent data are available on the levels of chlorobenzenes in water organisms.

3.5 Residues in terrestrial organisms

No data are available on the concentration levels of chlorobenzenes in terrestrial organisms.

3.6 Residues in humans

Hooftman and De Kreuk (1981; 1983) reported some concentration levels of chlorobenzenes in humans. A review of the data available is presented in Table 3.6.1.

Table 3.6.1 Concentration levels of chlorobenzenes in humans (Hooftman and De Kreuk, 1981; 1983)

Substance	Occurrence	Concentration
1,4-dichlorobenzene	adipose tissues (Tokyo)	0.2 - 11.7 µg/g
	human blood (Tokyo)	4 - 16 ng/ml
1,2,3-trichlorobenzene	human milk (Yugoslavia)	128 µg/kg fat
	adipose tissues (Yugoslavia)	103 µg/kg fat
1,2,4-trichlorobenzene	human milk (Yugoslavia)	9 µg/kg fat
	adipose tissues (Yugoslavia)	25 µg/kg fat
1,3,5-trichlorobenzene	human milk (Yugoslavia)	25 µg/kg fat
	adipose tissues (Yugoslavia)	16 µg/kg fat
1,2,4,5-tetrachlorobenzene	adipose tissues (Tokyo)	8 - 39 µg/kg fat

Barkly et al. (1980) studied the concentration of chlorinated hydrocarbons in the vicinity of a toxic waste dump in the US. For this reason they measured these substances in the urine, blood and breath of 9 persons living near the dump. The concentration levels found are summarized in Table 3.6.2.

Table 3.6.2 Concentration of chlorobenzenes in humans and their environment near a toxic waste dump (US) (Barkly et al., 1980)

Substances	Concentration range (in 9 persons); average concentration in parentheses		
	breath (ng/m ³)	blood (ng/ml)	urine (ng/l)
chlorobenzene	0 - trace (0)	0 - 17 (1.9)	20 - 120 (41)
dichlorobenzenes	0 - 5000 (751)	0.15 - 68 (9.2)	0 - 39000 (4700)
trichlorobenzenes	0 - 90 (10)	-	-
tetrachlorobenzenes	0 - 180 (23)	0 - 14 (1.6)	-
Substances	drinking water (ng/ml)	air (inside) (ng/m ³)	air (outside) (ng/m ³)
chlorobenzene	10 - 60 (26)	0 - 600 (107)	0 - 120 (13)
dichlorobenzenes	10 - 800 (168)	trace - 31000 (4-033)	0 - 440 (170)
trichlorobenzenes	-	-	0 - trace (0)
tetrachlorobenzenes	-	-	0 - 300 (33)

3.7

Conclusions

A variety of data have been reported for the concentrations of chlorobenzenes in the atmosphere, soil and water. In general, relatively high concentrations are found in the neighbourhood of industry.

The data indicate that chlorobenzenes can accumulate in the human body. No data have been found on residues in terrestrial organisms and only few data have been reported on the levels of chlorobenzenes in aquatic organisms.

4 PERSISTENCE AND DEGRADATION PATHWAYS

4.1 Abiotic degradation in the aquatic environment

4.1.1 Abiotic degradation

In the previous evaluation for the EC, Hooftman and De Kreuk (1981; 1983) reported no chemical degradation for chlorobenzenes in the aquatic environment. It is expected that chlorobenzenes, which are relatively volatile, will be released to the atmosphere where they will be degraded by photolysis by means of hydroxyl radicals (Hooftman and De Kreuk, 1981; Ware, 1988).

Boule et al. (1987) found that chlorobenzenes can be photohydrolysed into (chloro)phenols. It was also found that the reaction rate was increased by quenchers as well.

Dichlorobenzenes are degraded slowly under environmental conditions: direct photolysis cannot take place, because dichlorobenzenes do not absorb UV-light above 290 nm (EPA 1981b).

The reactivity of chlorobenzenes is dependant on their substitution pattern. A higher degree of substitution will result in a lower reactivity (Boule et al., 1987; Slooff et al., 1991). For example 1,2,4-trichlorobenzene is a very unreactive molecule because the halogenation of this benzene derivative deactivates the reactivity of the ring to electrophilic and nucleophilic substitution (EPA 1981a).

Guittonneau et al. (1988) studied photo-oxidation of chlorobenzene, 1,2,4-trichlorobenzene and 1-chloro-4-nitrobenzene in water by UV-radiation (253 nm) and UV-radiation in the presence of hydrogen peroxide. They showed that these substances degraded much faster in the presence of the hydroxyl radical. The reaction products of the oxidation are hydroxylated derivatives. For chlorobenzene the degradation half-life with UV-light with and without hydroxyl radical is 10 and 2 minutes respectively. For 1,2,4-trichlorobenzene they found 49 and 2.5 minutes respectively. The speed of the photo-oxidation in their laboratory experiment, however, gives no indication of the oxidation rate in the natural environment.

From experimental data on some chlorobenzenes the photolysis half-lives in water can be estimated for other chlorobenzenes (Slooff et al., 1991). These are 2 - 5 days for monochlorobenzene, 12 - 28 days for 1,2- and 1,4-dichlorobenzene, 3 - 7 days for 1,3-dichlorobenzene, 50 - 110 days for the trichlorobenzenes and more than 150 days for tetrachlorobenzenes. The method for estimating these data is not clear.

No data are available on dichlorobenzenes, 1,2,3-tri- and 1,3,5-trichlorobenzenes and 1,2,4,5-tetrachlorobenzene.

4.1.2 Conclusions

Limited information is available on the chemical degradation of chlorobenzenes in the aquatic environment. Most of these substances will volatilize rapidly in air because of their high volatilization rate. In the air the substances will degrade rapidly by indirect photolysis with radicals.

Because of the variable environmental conditions, most of the presented data give only an indication of the photodegradation. Chlorobenzenes are very stable under environmental conditions. Therefore, chlorobenzenes will break down rapidly only

under special circumstances, like a high concentration of radicals and a high dose of sunlight.

4.2 Biological degradation and metabolism

The following literature study is based on a review report on biodegradability of priority chemicals by Keuning and Janssen (1987), which covers all chlorobenzenes except for 1,2,4,5-tetrachlorobenzene. In an additional search relevant international literature from 1986 until the beginning of 1991 was screened for publications on biodegradation of chloronitrobenzenes.

Standardized tests are available to determine biodegradation under aerobic conditions (Annex V in Dir. 79/831/EEC). However, most of the studies reported in literature do not comply with these standardized methods. Therefore, an attempt has been made to classify the substances in line with definitions used by OECD and EC on the basis of the available data on test conditions and results. The following definitions are used:

Primary degradation is the alteration of the chemical structure resulting in the loss of a specific property; or the loss of the parent compound. Ultimate biodegradation is achieved when the test compound is totally utilized by micro-organisms, producing carbondioxide, water, mineral salts and new biomass; or mineralization.

A substance is classified as readily biodegradable if it shows a positive result in some defined stringent test methods which provide limited opportunities for biodegradation and acclimatization. Readily biodegradable substances are assumed to undergo rapid and ultimate biodegradation in the environment. Substances that biodegrade under more favourable test conditions (prolonged exposure, high biomass concentration, low substance concentration) may be classified as inherently biodegradable. This does not necessarily mean that mineralization is complete, nor that rapid and reliable biodegradation in the environment will occur.

A negative result in a ready biodegradability test does not necessarily mean that the chemical will not be biodegraded under relevant environmental conditions. If in the test the concentration of the test substance is at a level which inhibits microbial activity, the method is not appropriate.

4.2.1 Monochlorobenzene

Biodegradation in standard screening tests

Monochlorobenzene was classified as not readily biodegradable (OECD TG 301C) by MITI (1985). Information on the toxicity of chlorobenzene for bacteria is limited to Microtox data (*Photobacterium phosphoreum*), which range between 11 and 20 mg/l, see Chapter 7. This implies that a negative result in a test for ready biodegradability could possibly be connected to inhibition of the microbial activity.

Biodegradation in simulation tests

It seems possible that monochlorobenzene is eliminated under favourable, aerobic conditions in the natural environment by biodegradation, although abiotic degradation (e.g. sorption and evaporation) is also important.

Bartholomew and Pfaender (1983) studied the uptake and degradation of ¹⁴C-labelled monochlorobenzene by micro-organisms in freshwater, estuarine water and seawater. Uptake of monochlorobenzene by micro-organisms took place in all three types of water and 30-40% of the ¹⁴C-labelled monochlorobenzene was transformed into ¹⁴CO₂. The rates of degradation of monochlorobenzene in freshwater, estuarine water and seawater were 13, 4.9-10 and less than 1-1.7 ng/l.h, respectively. The

degradation rates were not only strongly influenced by the salinity of the water, but also by the temperature. Below water temperatures of 10°C the degradation rates were less than 1 ng/l.h.

Bouwer (1989) performed aerobic batch studies at 20°C on samples from a strip-pit pond contaminated with alkylbenzenes and chlorobenzene from tank-truck washing operations. Over a 43-days incubation period 51 ± 5% chlorobenzene (initial concentration of about 100 µg/l) was biotransformed in pondwater, after a 25-days lag period. However, chlorobenzene was transformed without an apparent lag time in pond water amended with nutrients (10 mg/l acetate; 5 mg/l N; 5 mg/l P) as concentrations were below the detection limit after 12 days of incubation. The addition of nutrients and sediment (collected from the bottom of the strip-pit pond; concentration: 0.61 g/l) to the pond water produced similar rapid biotransformation. In the pond water amended with nutrients (with and without sediment) the major product of ¹⁴C-chlorobenzene biotransformation was ¹⁴CO₂ (62-88% after 15 days of incubation). An unidentified fraction increased in the samples and may have been due to incorporation of ¹⁴C into cell mass.

In a repeated batch experiment with pondwater (with and without sediment) amended with nutrients chlorobenzene (initial concentration: 100 µg/l) was completely biotransformed within 24 hours.

Battersby and Wilson (1989) tested the anaerobic biodegradation potential under methanogenic conditions by measurement of the total net gas production (CH₄ plus CO₂). Diluted sludge (2 to 3 g dry solids per litre medium) with the test chemical (50 mg TOC/l) at 35°C for at least 60 days. In this test the chemical to biomass ratio is relatively high. The test indicated that chlorobenzene is a persistent compound under methanogenic conditions and that its presence did not inhibit gas production. Chemicals which fail to be degraded in this type of test may not necessarily persist at a lower and therefore environmentally more realistic chemical to biomass ratio.

Biodegradation under experimental conditions

Micro-organisms which can use chlorobenzene as the sole source of carbon and energy are known in literature.

Klecka and Gibson (1981) isolated *Pseudomonas putida* from soil. *P.putida* was able to grow on toluene as the sole source of carbon and energy. Cometabolic degradation of chlorobenzene to 3-chlorocatechol was possible in the presence of toluene. Ring fission of 3-chlorocatechol did not occur because this substrate inhibited catechol 2,3-dioxygenase in *P.putida*.

Reineke and Knackmuss (1984) isolated a chlorobenzene degrading bacterium by continuous enrichment from a mixture of soil and sewage samples. This micro-organism was able to use chlorobenzene as the sole carbon and energy source. The maximum growth rates of this organism (strain WR1306) in batch culture were accomplished at substrate concentrations of ≤ 0.5 mM (56 mg/l) in the culture medium. The strain was highly sensitive towards higher concentrations of chlorobenzene. With increasing amounts of chlorobenzene a prolonged lag phase was observed. Correspondingly, a sudden increase in substrate concentrations (> 0.5 mM) severely disturbed exponential growth with chlorobenzene. Biodegradation of chlorobenzene in strain WR1306 took place via an ortho-cleavage pathway. The pure culture of strain WR1306, obtained after several passages of growth with chlorobenzene, was no longer able to use benzene as the growth substrate. Obviously benzene when present as a growth substrate counteracts total

suppression of its meta-cleavage pathway. This pathway, however, which is unproductive for haloaromatics must be prevented to allow productive breakdown of chlorocatechols via the ortho-pathway.

Oldenhuis et al. (1989) described the effect of addition of *Pseudomonas* strain GJ31 to soil slurries (at 30°C) containing approximately 500 µM chlorobenzene. Without inoculation, the indigenous microflora needed approximately 30 h for adaptation to degrade chlorobenzene and 100 h for complete removal. Inoculation with cultures of strain GJ31 (5 mg dry weight per l of slurry) realized rapid degradation and completely disappearance within 50 h. The results showed that degradation was due to growth of the inoculated cells using chlorobenzene as source of carbon and energy. Addition of activated sludge did not stimulate degradation. The degradation rate of aromatic solvents by the added bacteria in soil slurries was similar or higher than that observed in liquid cultures of the same organisms.

Pettigrew et al. (1991) studied the simultaneous biodegradation of chlorobenzene and toluene by *Pseudomonas* sp. strain JS6, which was originally selected for its ability to grow on 1,4-dichlorobenzene, although the catabolic pathways of these compounds are incompatible: methyl-substituted aromatic substrates are generally degraded via meta-ring fission catalyzed by catechol 2,3-dioxygenase, whereas chloro-substituted aromatic substrates are degraded via modified ortho-ring fission catalyzed by catechol 1,2-dioxygenase; catechol 2,3-dioxygenase of the meta cleavage pathway is inactivated by chlorocatechols. *Pseudomonas* sp. strain JS6 can grow on toluene and chlorobenzene simultaneously by means of an alternative modified ortho-ring fission pathway.

Goulding et al. (1988) studied the ability of a microbial mixture of five Pseudomonads, one Klebsiella, four Rhodococci and two fungal strains to degrade chlorobenzene. After 144 h of incubation the removal percentage of chlorobenzene (initial concentration 200 mg/l, 30°C, pH 7) was 100 (corrected for volatilization). In the degradation test no lag period was observed.

Bouwer and McCarty (1982, 1985) studied aerobic columns with immobilised micro-organisms growing on acetate (1 mg/l), which degraded chlorobenzene in low concentrations (10 µg/l); the acclimatization period was 20 days. During a period of 9 months, chlorobenzene was added to the influent and 10% was detected in the effluent. An increase of the acetate concentration (to 5 mg/l) lengthened the acclimatization period to 60 days, but the ultimate removal percentage still was 90%.

In general the first step in microbial degradation of aromatics is the production of catechols. These catechols then can be degraded via the ortho- or meta-ring fission pathway. For halogenated aromatic compounds the meta-pathway leads to halogenated dead-end or suicide metabolites (acylhalides which inhibit the ring fission enzyme dioxygenase). In case of microbial degradation of chlorobenzene via the meta-pathway accumulation of chlorocatechol will take place. Complete biodegradation of chlorobenzene occurs via a modified ortho-pathway. The enzymes of this ortho-pathway are characterized by high affinities for chlorinated substrates, while the enzymes of the "normal" ortho-cleavage pathway for degradation of simple aromatics are characterized by a narrow substrate specificity. Generally Cl⁻ release takes place after ring fission of catechols; usually Cl⁻-release occurs during or after lactonization.

Almost no data are available on the anaerobic degradation of chlorobenzene. Bouwer and McCarty (1985) described that immobilised micro-organisms in anaerobic methanogenic columns growing on 100 mg acetate/l, could not degrade simultaneously added chlorobenzene (approximately 20 µg/l). The columns were percolated during the period of 1 year.

A study of Horowitz et al. (1982) on anaerobic biodegradation of aromatics in sediments and sludge also indicated that chlorobenzene was a persistent compound under anaerobic conditions.

A summary of the data supplied by the present study is given in Table 4.2.1.

4.2.2 Conclusions

In the previous reports to EC (Hooftman and De Kreuk, 1981, 1983) it was concluded that monochlorobenzene could be biodegraded although only by preadapted micro-organisms. Therefore, biodegradation was not expected to play a significant role for the disappearance of monochlorobenzene at least from non-stagnant waters.

The additional data supplied by the present evaluation confirm that although chlorobenzene is not readily biodegradable, complete mineralization by adapted bacteria may take place.

Up till now, significant biodegradation of chlorobenzene under anaerobic conditions has not been detected.

4.2.3 Dichlorobenzenes

Biodegradation in standard tests

The isomer 1,4-dichlorobenzene was classified as readily biodegradable in the MITI test (OECD TG 301C, MITI, 1985) and in the OECD Closed Bottle Test (OECD TG 301B, Topping, 1987).

According to Canton et al. (1985) the Repetitive Die Away test (Blok, 1979) indicated that 1,2- and 1,3-dichlorobenzene cannot be biodegraded in this ready biodegradability test in which a non-adapted inoculum was used.

Information on the toxicity of dichlorobenzenes for bacteria is limited to Microtox data (*Photobacterium phosphoreum*), which range between 3 and 5 mg/l, see Chapter 7. This implies that a negative result in a test for ready biodegradability could possibly be connected to inhibition of the microbial activity.

Biodegradation in simulation tests

As for monochlorobenzene, biodegradation of dichlorobenzenes seems possible, depending on the prevailing environmental conditions. Kuhn et al. (1985) studied the aerobic microbial transformation of dichlorobenzenes (initial concentrations 0.2-0.8 µM) in laboratory aquifer columns simulating saturated-flow conditions typical for a river water/groundwater infiltration system at 20°C in the dark. The behaviour of dichlorobenzenes in the laboratory was qualitatively the same as that observed at a field site; dichlorobenzenes were biotransformed under aerobic conditions. Almost 50% of the original 1,4-dichlorobenzene was recovered in the effluent after 10 days of operation. The percentage of 1,4-dichlorobenzene removal and the rate of its transformation gradually increased with time, so after almost 3 weeks, the overall 1,4-dichlorobenzene level in the effluent was reduced to less than

10% of the input value. Most of the 1,4-dichlorobenzene removal (approx. 80%) occurred within the 4.7 cm of the column (total length 20 cm). Transformation of 1,2- and 1,3-dichlorobenzene under aerobic conditions never exceeded 25% of the input concentration and occurred only in that region of the column where 1,4-dichlorobenzene was transformed.

Topping (1987) investigated the fate of 1,4-dichlorobenzene (initial concentration approximately 0.4 mg/l) in a modified Porous Pot test (model activated sludge plant), operated at an aeration rate of 1.0 l.min⁻¹, temperatures of 8, 15 and 20°C and sludge retention times (SRT) of 3 and 6 days; a domestic settled sewage was used. The overall removal was found to be consistently >95%. Mass balance results suggested that under normal operating conditions the major proportion of 1,4-dichlorobenzene (>76%) had been removed by biodegradation. The amount of 1,4-dichlorobenzene removed by volatilization showed a slight dependence on aeration rate, but only became significant ($63 \pm 4\%$) when adverse operating conditions prevailed (i.e. 8°C, at a 3 day SRT). Radioisotopic studies confirmed the presence of 1,4-dichlorobenzene degrading bacteria in activated sludge taken from a porous pot continuously dosed with 1,4-dichlorobenzene. The results of a similar test using sludge from an undosed porous pot suggested that acclimatization to 1,4-dichlorobenzene was rapid, occurring within 2 days. No adverse effects on sewage treatment processes, due to the presence of 1,4-dichlorobenzene were observed.

Biodegradation under experimental conditions

Aerobic microbial degradation of dichlorobenzenes is described repeatedly. Bouwer and McCarty (1982, 1985) studied acetate grown biofilms in aerobic columns with respect to the microbial degradation of all three dichlorobenzene isomers. When the columns were operated at an acetate concentration of 1 mg/l and separate concentrations of 1,2-, 1,3- and 1,4-dichlorobenzene of approximately 10 µg/l the lag-phases were respectively 20, 500 and 10 days; the corresponding removal of the isomers during the first 9 months of percolation of the columns were 96, 22 and 98%, respectively. Even after a percolation period of 36 months only 71% of 1,3-dichlorobenzene was removed, whereas for 1,2- and 1,4-dichlorobenzene the removal percentage was 97 and 99%, respectively. An increase of the influent concentration of acetate to 5 mg/l resulted in a negative effect on the degradation of all dichlorobenzene isomers.

Goulding et al. (1988) studied the ability of a microbial mixture of five *Pseudomonas*, one *Klebsiella*, four *Rhodococci* and two fungal strains to degrade dichlorobenzenes. After 96 h of incubation the removal percentage of all dichlorobenzene isomers (initial concentration 200 mg/l, 30 °C, pH 7) was 100% (corrected for volatilization). In the degradation test no lag phase was observed.

Van der Meer et al. (1987) isolated *Pseudomonas* species, strain P51, growing aerobically on all dichlorobenzene isomers and on 1,2,4-trichlorobenzene as sole carbon and energy sources. Strain P51 was tested for its ability to mineralize these components also in a non-sterile soil environment. Untreated sand from the river Rhine in which none of the dichloro- and trichlorobenzenes were degraded was placed in a percolation column and inoculated with *Pseudomonas* sp. strain P51. The column was fed continuously with synthetic river water containing the chlorinated compounds at concentrations between 10 µg/l and 1 mg/l. The inoculated microorganisms were able to degrade the chlorinated benzenes and survived for at least 60 days in the column. For each compound a specific threshold concentration was observed below which no further degradation took place, and

which was independent of the initial concentration. These thresholds were 6 ± 4 $\mu\text{g/l}$ for 1,2-dichlorobenzene, 20 ± 5 $\mu\text{g/l}$ for 1,2,4-trichlorobenzene and more than $20 \mu\text{g/l}$ for the 1,3- and 1,4-isomers. Repeated inoculation of the column with strain P51 did not affect this minimal concentration. In non-inoculated soil columns the native microbial population adapted to degrade 1,2-dichlorobenzene after a long lag phase, and reduced it from $25 \mu\text{g/l}$ to a threshold concentration of $0.1 \mu\text{g/l}$.

De Bont et al. (1986) isolated from a mixture of soil and water samples an *Alcaligenes* sp. (strain OBB65) by using 1,3-dichlorobenzene as the sole carbon and energy source. During growth on 1,3-dichlorobenzene almost stoichiometric amounts of chloride were released. Simultaneous adaptation studies, as well as enzyme studies, indicated that 1,3-dichlorobenzene was metabolized via a dihydrodiol to 3,5-dichlorocatechol. Subsequently, the latter product was converted to 2,4-dichloromuconate.

Schraa et al. (1986) isolated *Alcaligenes* sp. strain A175 from an enrichment culture in which monochlorobenzene, 1,3-dichlorobenzene and 1,4-dichlorobenzene served as the sole carbon and energy source. Strain 175A was not capable of growth on 1,2-dichlorobenzene and 1,2,4-trichlorobenzene as a sole source of carbon and energy. During growth with 1,4-dichlorobenzene in pure culture, stoichiometric amounts of chloride were released; 3,6-dichlorocatechol and (2,5-dichloro)muconic acid could be detected as intermediates during the degradation of 1,4-dichlorobenzene. Incubation of strain A175 with 1,4-dichlorobenzene, as well as with other aromatic compounds sometimes resulted in the production of a red pigment in the medium which turned black over time. This colour change preceded an irreversible loss in activity of the cells is most probably the result of the accumulation of chlorocatechols which undergo autoxidation. It is proposed that dioxygenases are involved in the initial steps of 1,4-dichlorobenzene degradation, while ring opening proceeds via ortho cleavage.

Papers by Spain and Nishino (1987) and Oltmanns et al. (1988) describe a similar pathway for the degradation of 1,4-dichlorobenzene in *Pseudomonas* sp. and *Pseudomonas* and *Alcaligenes* sp., respectively.

Haigler et al. (1988) isolated *Pseudomonas* species strain JS100 by selective enrichment from activated sludge. *Pseudomonas* sp. strain JS100 was capable of growth on 1,2-dichlorobenzene or chlorobenzene as a sole source of carbon and energy. The results suggest that 1,2-dichlorobenzene is initially converted by a dioxygenase to a dihydrodiol, which is converted to 3,4-dichlorocatechol by an NAD^+ -dependent dehydrogenase. Ring cleavage of 3,4-dichlorocatechol is by a catechol 1,2-oxygenase to form 2,3-dichloro-cis,cis-muconate. Preliminary results indicate that chloride is eliminated during subsequent lactonization of the 2,3-dichloro-cis,cis-muconate, followed by hydrolysis to form 5-chloromaleylacetic acid. *Pseudomonas* species strain JS100 oxidized chlorobenzene and 1,2-dichlorobenzene at similar rates, and the initial enzymes seemed to be at least partially constitutive.

In general it is proposed that the aerobic microbial degradation of dichlorobenzenes is analogous to the degradation of chlorobenzene. That is the production of catechols by means of dioxygenase activity and the subsequent complete biodegradation via an ortho-cleavage pathway. The first elimination of chloride occurs most probably during lactonization; the second elimination of chloride is less clear.

Anaerobic reductive dechlorination by micro-organisms can transform chlorinated benzenes to intermediates (lower chlorinated benzenes) which can easily be

mineralized aerobically. Bosma et al. (1988) studied anaerobic transformation in columns packed with sediment of the River Rhine. Chlorinated hydrocarbons (30-50 nmol/l) were present as the only source of carbon and energy. After an initial lag-phase of 2-6 months (depending on the compound) 1,2,3-, 1,2,4- and 1,3,5-trichlorobenzene were removed from the column. The dechlorination was specific: 1,2,3- and 1,3,5-trichlorobenzene were solely transformed to 1,3-dichlorobenzene, while 1,4-dichlorobenzene was the only product of 1,2,4-trichlorobenzene transformation. The column results indicated that dechlorination of dichlorobenzene was inhibited in the presence of trichlorobenzene. Subsequently dichlorobenzenes were dechlorinated, yielding monochlorobenzenes.

Bouwer (1985) and Bouwer and McCarty (1985) described that immobilised micro-organisms in anaerobic, methanogenic columns, growing on acetate (100 mg/l) could not degrade simultaneously added 1,2-, 1,3- and 1,4-dichlorobenzene (initial concentration approximately 10 µg/l). The columns were percolated during a 1 year period.

A summary of the data supplied by the present study is given in Table 4.2.1.

4.2.4

Conclusions

In the previous reports to EC (Hooftman and De Kreuk, 1981; 1983) it was concluded that 1,2- and 1,3-dichlorobenzene are inherently biodegradable under aerobic conditions. Biodegradation can be slow and the relevance to the environment is not clear. Therefore more data are needed on biodegradation under conditions relevant to the environment.

The supplementary data in this report show that 1,4-dichlorobenzene may be classified as readily biodegradable. Furthermore, the data confirm the conclusion that all dichlorobenzene isomers can be metabolized as primary or secondary substrate under aerobic conditions by several micro-organisms.

Under anaerobic conditions dichlorobenzenes may be dechlorinated to form monochlorobenzene.

4.2.5

Trichlorobenzene

Biodegradation in standard tests

Trichlorobenzenes (unspecified) were classified as not readily biodegradable (OECD TG 301C) by MITI (1985). Information on the toxicity of trichlorobenzenes for bacteria is limited to Microtox data (*Photobacterium phosphoreum*), which range between 3 and 4 mg/l, see Chapter 7. This implies that a negative result in a test for ready biodegradability could possibly be connected to inhibition of the microbial activity.

Biodegradation in simulation tests

Almost no data are available on the biodegradation under natural conditions. It is proposed that trichlorobenzenes are rather persistent in the natural environment. It is assumed that 1,3,5-trichlorobenzene cannot be biodegraded under aerobic conditions. Biodegradation of chlorobenzenes is expected to take place via ring fission catalyzed by catechol dioxygenases and this pathway demands two adjacent positions at the aromatic ring, which are not substituted by chlorine (Slooff et al., 1991).

Bartholomew and Pfaender (1983) studied the uptake and degradation of ¹⁴C-labelled 1,2,4-trichlorobenzene in freshwater, estuary water and seawater. The uptake of trichlorobenzene varied from less than 1 to 7.9 ng/l.h; no transformation to ¹⁴CO₂ was observed. These results indicate that trichlorobenzene was either degraded cometabolically or accumulated unchanged by micro-organisms.

Swindoll et al. (1988) measured the biodegradation in aquifer solid slurries as both the conversion of radiolabeled substrate to ¹⁴CO₂ and the incorporation of label into cell biomass. Under aerobic conditions, the microbial community metabolized chlorobenzene and 1,2,4-trichlorobenzene. First-order rate constants ranged from 10⁻² to 10⁻⁴ h⁻¹ for the natural compounds (e.g. acetic acid, amino acids, glucose); for chlorobenzene and 1,2,4-trichlorobenzene this constant was respectively 10⁻⁶ and 10⁻³ h⁻¹. Turnover times ranged from 47 to more than 1,900 h for natural compounds, for chlorobenzene and 1,2,4-trichlorobenzene turnover times were respectively 60,000 and 15,000. Mineralization of chlorobenzene and trichlorobenzene represented respectively 20% and 4% of total metabolism.

Biodegradation under experimental conditions

Aerobic microbial degradation of 1,2,4-trichlorobenzene is described by Bouwer (1985) and Bouwer and McCarty (1982, 1985); immobilised micro-organisms in aerobic columns, growing on 1 mg/l acetate removed 90% of simultaneously added 1,2,4-trichlorobenzene (initial concentration approximately 10 µg/l) during the first 9 months of percolation of the columns and after a lag phase of 40 days. Increase of the influent concentration of acetate to 5 mg/l resulted in a negative effect on the degradation of 1,2,4-trichlorobenzene.

Goulding et al. (1988) studied the ability of a microbial mixture of five Pseudomonads, one Klebsiella, four Rhodococci and two fungal strains to degrade 1,2,3- and 1,2,4-trichlorobenzene. After 48 h of incubation the removal percentage of these isomers (initial concentration 200 mg/l, 30°C, pH 7) was 100% (corrected for volatilization). In the degradation test no lag phase was observed.

Van der Meer et al. (1987) isolated *Pseudomonas* species, strain P51, growing aerobically on all dichlorobenzene isomers and on 1,2,4-trichlorobenzene as sole carbon and energy sources. Strain P51 was tested for its ability to mineralize these components also in a nonsterile soil environment. Untreated sand from the River Rhine in which none of the dichloro- and trichlorobenzenes were degraded was placed in a percolation column and inoculated with *Pseudomonas* sp. strain P51. The column was fed continuously with synthetic river water containing the chlorinated compounds at concentrations between 10 µg/l and 1 mg/l. The inoculated micro-organisms were able to degrade the chlorinated benzenes and survived for at least 60 days in the column. For each compound a specific threshold concentration was observed below which no further degradation took place, and which was independent of the initial concentration. These thresholds were 6 µg/l for 1,2-dichlorobenzene, 20 ± 5 µg/l for 1,2,4-trichlorobenzene and more than 20 µg/l for the 1,3- and 1,4-isomers. Repeated inoculation of the column with strain P51 did not affect this minimal concentration.

Anaerobic reductive dechlorination by micro-organisms can transform chlorinated benzenes to intermediates (lower chlorinated benzenes) which can easily be mineralized aerobically. Fathepure et al. (1988) observed complete biotransformation of hexachlorobenzene in anaerobic sewage sludge within a period of 3 weeks; hexachlorobenzene (initial concentration approximately 50 mg/l) was

dechlorinated to tri- and dichlorobenzenes. The major route was the transformation of hexachlorobenzene via pentachlorobenzene, 1,2,3,5-tetrachlorobenzene and 1,3,5-trichlorobenzene. More than 90% of the added hexachlorobenzene was recovered as 1,3,5-trichlorobenzene, and there was no evidence for further dechlorination of 1,3,5-trichlorobenzene. The minor route was the transformation of hexachlorobenzene via pentachlorobenzene, 1,2,4,5-tetrachlorobenzene, 1,2,4-trichlorobenzene and dichlorobenzenes.

Bouwer (1985) described that immobilised micro-organisms in anaerobic methanogenic columns, growing on 100 mg/l acetate as the primary substrate could not degrade simultaneously added 1,2,4-trichlorobenzene (initial concentration approximately 10 µg/l). The columns were percolated during a 1 year period.

A summary of the data supplied by the present study is given in Table 4.2.1.

4.2.6 Conclusions

In the EC evaluation reports on trichlorobenzenes (Hooftman and De Kreuk, 1981; 1983) it was concluded that biodegradation was obtained with micro-organisms from soil and from activated sludge. Therefore trichlorobenzenes may be considered as inherently biodegradable. However, it is questionable whether biodegradation of these isomers in the environment will actually occur.

Aerobic microbial degradation of 1,2,3- and 1,2,4-trichlorobenzene is confirmed by the additional data supplied in this report. The isomer 1,2,4-trichlorobenzene can be used as sole carbon and energy source by pure cultures. For 1,3,5-trichlorobenzene no additional data were available.

Although the results with regard to anaerobic biodegradation are contradictory, it is proposed that transformations are possible by means of reductive dechlorination.

4.2.7 1,2,4,5-Tetrachlorobenzene

Biodegradation in standard tests

Standard tests with tetrachlorobenzenes have not been reported. Information on the toxicity of tetrachlorobenzenes for bacteria ranges between 3 and 20 mg/l, see Chapter 7. This implies that toxicity might inhibit the microbial activity in standard tests.

Biodegradation in simulation tests

No recent data are available on the biodegradation of 1,2,4,5-tetrachloro-benzene under natural conditions. With respect to the metabolic pathways it is assumed to be unlikely that 1,2,4,5-tetrachlorobenzene is biodegraded under aerobic conditions; chlorobenzenes are generally assumed to be biodegraded via ring fission catalyzed by catechol dioxygenases and this pathway demands two adjacent positions at the aromatic ring, which are not substituted by chlorine (Slooff et al., 1991).

Biodegradation under experimental conditions

Information on the degradation of 1,2,4,5-tetrachlorobenzene under experimental conditions is also limited. Sander et al. (1989) isolated a *Pseudomonas* strain PS14 from soil samples from an industrial waste deposit by enrichment techniques using chlorinated benzenes as the substrates. Strain PS14 was able to use 1,2,4,5-tetrachlorobenzene as the only source of carbon and energy under aerobic

conditions. The stoichiometric amounts of chloride which were measured in the medium of strain PS14 after conversion of chlorobenzene, all three isomeric dichlorobenzenes, 1,2,3- and 1,2,4-trichlorobenzenes and 1,2,4,5-tetrachlorobenzene, strongly suggest a complete mineralization of the substrate.

Fathepure et al. (1988) observed complete biotransformation of hexachlorobenzene in anaerobic sewage sludge within a period of 3 weeks; hexachlorobenzene (initial concentration approximately 50 mg/l) was dechlorinated to tri- and dichlorobenzenes. The major route was the transformation of hexachlorobenzene via pentachlorobenzene, 1,2,3,5-tetrachlorobenzene and 1,3,5-trichlorobenzene. More than 90% of the added hexachlorobenzene was recovered as 1,3,5-trichlorobenzene, and there was no evidence for further dechlorination of 1,3,5-trichlorobenzene. The minor route was the transformation of hexachlorobenzene via pentachlorobenzene, 1,2,4,5-tetrachlorobenzene, 1,2,4-trichlorobenzene and dichlorobenzenes.

A summary of the data supplied by the present study is given in Table 4.2.1.

4.2.8 Conclusions

In a previous report to EC (Hooftman and De Kreuk, 1981) it was concluded that, although it appears that 1,2,4,5-tetrachlorobenzene is biodegradable, more data on biodegradation will be needed, based on the relative significance of this compound for the environment.

The additional data supplied in this report are rather limited, but confirm that 1,2,4,5-tetrachlorobenzene is inherently biodegradable.

Evidence for anaerobic degradation is also available.

Table 4.2.1 Biodegradation of chlorobenzenes

Compound	Conditions	Results	Ref.
monochlorobenzene	M111 (OECD TG301C) fresh-, estuarine and seawater	not readily biodegradable 30-40% mineralisation ($^{14}\text{CO}_2$)	1 2
	adapted pond water samples, 100 $\mu\text{g/l}$	50% biodegradation, after 25 days lag, in 18 days	3
	adapted pond water samples + nutrients, 100 $\mu\text{g/l}$	62-88% mineralisation ($^{14}\text{CO}_2$) after 12-15 days, no lag phase, inherently biodegradable	3
	repeated addition, 100 $\mu\text{g/l}$	complete biodegradation in 24h, inherently biodegradable	3
	soil slurry sample, 30°C, 56 mg/l	30h adaptation; complete removal after 100h	4
	anaerobic, methanogenic, 2-3 g d.s/l and 50 mg TOC/l, 35°C	no degradation after 80 days	5
1,2-dichlorobenzene	R.D.A.-test ^a , no adaptation	not readily biodegradable	6
	aquifer material, simulated groundwater infiltration, 0.2-0.8 μMol , 20°C	25% degradation by bacteria adapted to 1,4-dichlorobenzene	8
	biofilms, acetate (1 mg/l) grown, 10 $\mu\text{g/l}$, unadapted	96% removal during 9 months (lag phase 20d), inherently biodegradable	9
	defined microbial mixture, 200 mg/l, 30°C, pH 7	100% removal (corrected for volatilisation), inherently biodegradable	10
1,3-dichlorobenzene	R.D.A.-test ^a , no adaptation	not readily biodegradable	6
	aquifer material, simulated groundwater infiltration, 0.2-0.8 μMol , 20°C	25% degradation by bacteria adapted to 1,4-dichlorobenzene	8
	biofilms, acetate (1 mg/l) grown, 10 $\mu\text{g/l}$, unadapted	22% removal after 9 months; 71% removal after 36 months (lag phase 500 days)	9
	defined microbial mixture, 200 mg/l, 30°C, pH 7	100% removal (corrected for volatilisation), inherently biodegradable	10
1,4-dichlorobenzene	R.D.A. test ^a , no adaptation	readily biodegradable, t%: 2-3 weeks	6
	M111 (OECD TG301C)	readily biodegradable	1
	Closed Bottle Test (OECD TG301C)	readily biodegradable	7

Table 4.2.1 Biodegradation of chlorobenzenes (continued)

Compound	Conditions	Results	Ref.
1,4-dichlorobenzene (continued)	aquifer material, simulated groundwater infiltration, 0.2-0.8 µMol, 20°C	after 3 weeks 90% degradation	8
	Modified Porous Pot (sim. act. sludge plant), 8°, 15° and 20°C, SRT 3d and 6d	>95% removal, 76% biodegradable; adaptation within 2 days	7
	biofilms, acetate (1 mg/l) grown, 10 µg/l, unadapted	98% removal during 9 months (lag phase 10 days)	9
	defined microbial mixture, 200 mg/l, 30°C, pH 7	100% removal (corrected for volatilization)	10
trichlorobenzenes	MITI (OECD T6301C)	not readily biodegradable	1
	defined microbial mixture, 200 mg/l, 30°C, pH 7	100% removal (corrected for volatilization), no lag phase, inherently biodegradable	10
1,2,3-trichlorobenzene	freshwater, estuary water, seawater, ¹⁴ C-labeled biofilms, acetate (1 mg/l) grown, 10 µg/l, unadapted	no transformation to ¹⁴ CO ₂ 90% removal during 9 months (lag phase 40 days), inherently biodegradable	2
	defined microbial mixture, 200 mg/l, 30°C, pH 7	100% removal (corrected for volatilization), no lag phase, inherently biodegradable	10
1,3,5-trichlorobenzene	anaerobic sewage sludge (major intermediate from hexachlorobenzene)	no further dechlorination (inherent biodegradability reported in Hooftman and de Kreuk, 1981, 1983)	11
1,2,4,5-trichlorobenzene	Pseudomonas strain PS14 isolated on chlor.benzenes, only C - and energy source	complete mineralization (stochiometric recovery of chloride)	12
	anaerobic sewage sludge (minor intermediate from hexachlorobenzene)	further dechlorination	11

*) conditions are according to OECD comparable to screening tests for ready biodegradability

- 1. MITI, 1985
- 2. Bartolomew and Pfaender, 1983
- 3. Bouwer, 1989
- 4. Oldenhuis et al., 1989
- 5. Battersby and Wilson, 1989
- 6. Canton et al., 1985
- 7. Topping, 1987
- 8. Kuhn et al., 1985
- 9. Bouwer and McCarthy, 1982, 1985
- 10. Goulding et al., 1988
- 11. Fathepure et al., 1988
- 12. Sander et al., 1989

5 DISTRIBUTION BETWEEN ENVIRONMENTAL COMPARTMENTS

5.1 Volatilization

In the previous evaluation for the EC, Hooftman and De Kreuk (1981) reported a volatilization half-life of 0.3 days for monochlorobenzene (experiment in the river Rhine). For 1,3-dichlorobenzene the volatilization half-life in other Dutch rivers varied from 2 to 50 days; for 1,2,3-trichlorobenzenes 1.9 to 30 days; for 1,2,4-trichlorobenzene 2.1 to 28 days; for 1,3,5-trichlorobenzene the half-life in the river Rhine amounted to 18 days.

Ince and Inel (1989) carried out laboratory experiments to measure the volatilization rate of different substances. For chlorobenzene a volatilization (first-order) rate constant of 0.67 h^{-1} (25°C) was found. Aeration of the solution resulted in a doubling of the volatilization rate.

Hellman (1987) studied the vaporization behaviour of chlorobenzenes. He developed a model for the quantification of the volatilization rate in the environment. The following volatilization rates were found: for chlorobenzene 0.00165 min^{-1} ; for 1,2-dichlorobenzene 0.0011 min^{-1} ; for 1,4-dichlorobenzene 0.0013 min^{-1} and for 1,3,5-trichlorobenzene 0.0093 min^{-1} .

Rippen et al. (1984) calculated a volatilization half-life of 21 hours for 1,4-dichlorobenzene from an aqueous solution for 1 meter water depth at 25°C .

No volatilization data are available for 1,2,4,5-tetrachlorobenzene.

In recent years several models have been developed to estimate the volatilization behaviour of substances in the natural environment. In this evaluation study the estimation method developed by De Bruin (1985) is used to estimate the volatilization half-life ("standard half-life"). The calculation is based on physical parameters like water solubility, vapour pressure, temperature (20°C), water flow rate (1 m/s), air velocity (3 m/s), and water depth (1 m). The calculation method is presented in Appendix 1. The calculated half-lives for the chlorobenzenes are presented in Table 5.1.1.

Table 5.1.1 The calculated volatilization rate (standard half-life) of the chlorobenzenes according to De Bruin (1985).

Substance	Half-life (h)
chlorobenzene	260
1,2-dichlorobenzene	550
1,3-dichlorobenzene	290
1,4-dichlorobenzene	670
1,2,3-trichlorobenzene	510
1,2,4-trichlorobenzene	320
1,3,5-trichlorobenzene	730
1,2,4,5-tetrachlorobenzene	500

A summary of published data on volatilization rates and half-lives data is given in Table 5.1.2.

Table 5.1.2 Experimentally determined volatilization rates and half-lives of chlorobenzenes.

Substance	Volatilization rate (h ⁻¹)	Half-life (h)	ref.
chlorobenzene	0.069	7.2	1
	0.99	0.7	2
	0.67	1.3	3
1,2-dichlorobenzene	0.67	1.0	2
	0.014 - 0.35 d ¹	2 - 50 d	1
1,3-dichlorobenzene	0.033	21	4
	0.76	0.91	2
1,2,3-trichlorobenzene	0.023 - 0.37 d ¹	1.9 - 30 d	1
	0.025 - 0.33 d ¹	2.1 - 28 d	1
1,3,5-trichlorobenzene	0.0016	18 d	1
	0.56	1.2	2

References:

- 1 Hooftman and De Kreuk (1981)
- 2 Hellmann (1987)
- 3 Ince and Inel (1989)
- 4 Ruppen et al. (1984)

Table 5.1.2 shows a high variety in volatilization half-lives of each substance, e.g. chlorobenzene, 1,4-dichlorobenzene and 1,3,5-trichlorobenzene. This is mainly caused by the differences in experimental conditions. Differences in the half-lives of the various compounds as reported by the same reference will be caused by the specific physical and chemical properties of the substances.

For a proper comparison of the volatility of the substances, Table 5.1.1 should be used, since those calculations are based on identical physical and chemical properties and assumptions.

In general, it can be concluded that the substances will volatilize within 1 to 2 months from water into the air.

5.2 Sorption

For the fate of a substance in the environment, sorption can play an important role. Substances may adsorb on suspended solids and on sediment where they can remain for a long time. After sorption a substance may return to the aquatic environment by desorption. It is possible to measure a distribution coefficient of a substance between sediment and water. This parameter is the sediment/water partition coefficient. The value of this coefficient is dependant on physical and chemical properties of the substances e.g. the solubility, and on the organic carbon content of the sediment. Usually, the partition coefficient is calculated for the carbon content of the sediment. This parameter is called the "organic carbon partition coefficient" (K_{oc}).

For many substances the K_{oc} has been measured. For other substances the K_{oc} can be estimated from the octanol/water partition coefficient (K_{ow}) or from the water solubility of that substance, these data are presented in chapter 1. In Table 5.2.1 the calculated K_{oc} are presented for the chlorobenzenes. The following formula has been used for the calculation (De Bruin, 1985): $\log K_{oc} = -0.557 \log S + 0.935$, where S = water solubility (mol/l).

Table 5.2.1 Estimated K_{oc} -values for chlorobzenes

	$\log K_{oc}$
monochlorobenzene	2.25
1,2-dichlorobenzene	2.63
1,3-dichlorobenzene	2.65
1,4-dichlorobenzene	2.74
1,2,3-trichlorobenzene	3.10
1,2,4-trichlorobenzene	3.04
1,3,5-trichlorobenzene	3.20
1,2,4,5-tetrachlorobenzene	4.03

From Table 5.2.1 it can be seen that the K_{oc} is higher with an increasing chlorination. The dichlorobzenes have rather low partition coefficients, hence adsorption to sediment will not be an important pathway for dichlorobzenes to leave the aqueous phase. For the trichlorobzenes and 1,2,4,5-tetrachlorobenzene the K_{oc} is higher indicating that sorption onto the sediment will be stronger than for the other chlorobzenes.

5.3

Bioaccumulation

Information on the accumulation of substances in organisms can be obtained from bioconcentration factors (BCF). The bioconcentration factor is the ratio between the concentration in a organism and the mean concentration in the testwater. The concentration in the organism (or in body tissue) should be determined at (near-) steady state. For the former method the test period must be sufficiently long to reach "steady state", otherwise the BCF will be underestimated. The BCF can also be calculated as the ratio between the rate constants of uptake (k_1) and depuration (k_2), assuming 1st order kinetics.

The time needed to reach 80% or 90% of steady state can be calculated by:

$$T(80\%) = 1.6/k_2 \quad k_2 = \text{elimination rate (d}^{-1}\text{)}$$
$$T(90\%) = 3.0/k_2$$

If k_2 -values for chlorobzenes (for fish) are known the minimum time required to reach a certain percentage of the steady state can be calculated (Kristensen and Tyle, 1990). These calculations are only indicative because the k_2 -values depend for instance on the size of the fish (large fish need a longer exposure period to reach steady state than small fish).

Calculation of the 80% steady state for chlorobzenes for fish based on measured k_2 -values resulted in values ranging from 1.6 days for 1,4-dichlorobenzene to 4 days for 1,2,4,5-tetrachlorobenzene.

Bioconcentration factors can be expressed based on wet weight or on lipid weight. As organisms show a variation in lipid weight, more variation is expected in BCF-values based on wet-weight than in BCF-values based on lipid weight.

Bioconcentration factors available for chlorobzenes are presented in Table 5.3.1. Most of the accumulation experiments were conducted with freshwater fish with the exception of two experiments with algae.

5.3.1 Monochlorobenzene

For monochlorobenzene the bioconcentration factors for algae were 50 for *Chlorella fusca vari vacuolata* and 2172 for *Selenastrum capricornutum*. The BCF for the fish *Leuciscus idus melanotus* was 70.

Monochlorobenzene is classified as being non-accumulative or low accumulative according to the MITI list (based on results obtained using OECD Test Guideline No 305 CL MITI, 1985).

5.3.2 Dichlorobenzenes

For the various dichlorobenzenes the bioconcentration factors based on wet weight ranged from 40 for *Salmo gairdneri* (alevin) to 1400 for *Salmo gairdneri* (at hatching). Both values are derived from the same experiment carried out by Calamari et al. (1982) who investigated the bioconcentration of 1,4-dichlorobenzene for different developmental stages of *Salmo gairdneri*. BCFs based on wet weight, derived from other experiments with fish, ranged from 50 for *Leuciscus idus melanotus* to 740 for *Salmo gairdneri*. BCFs based on lipid weight ranged from 3590 for *Jordanella floridae* to 8880 for *Salmo gairdneri*.

In experiments with algae a BCF (wet weight) of 19,700 was found for *Selenastrum capricornutum* and 90 for *Chlorella fusca var vacuolata*.

Dichlorobenzenes are confirmed to be non-accumulative or low accumulative according to the MITI list (MITI, 1985).

5.3.3 Trichlorobenzenes

BCFs for trichlorobenzenes varied from 39 for *Salmo gairdneri* alevis to 4100 for the adult. The "whole-body" BCF is very dependent on the lipid content of the organism. Galassi and Calamari (1983) found for 1,2,3-trichlorobenzene and 1,2,4-trichlorobenzene a similar relationship as Calamari (1982) for 1,4-dichlorobenzene between developmental stage and "whole-body" BCF in the eyed-egg stage the BCF for the hatching stage was higher (349) and at the end of the experiment the BCF decreased to 39 for the alevin. The BCF (lipid weight) varied only within a factor 2. For algae the only BCF-value available was 250, for *Chlorella fusca var vacuolata*. Trichlorobenzenes are confirmed to be accumulative on a medium level according to the MITI list (MITI, 1985).

5.3.4 1,2,4,5-Tetrachlorobenzene

BCF were only available for fish. BCF were 4054 for *Jordanella floridae* and 13000 for *Salmo gairdneri*. Based on lipid weight, BCF were 50300 and 15600.

1,2,4,5-Tetrachlorobenzene is classified as being medium accumulative according to the MITI list (MITI, 1985).

Table 5.3.1 Bioconcentration factors for chlorobenzenes

Substance Species	Lifestage age/size	Concentration ($\mu\text{g/l}$)	Test system	Solvent	Uptake period	Depuration period	k_1 (days^{-1})	k_2 (days^{-1})	BCF (wet weight)	BCF (lipid weight)	Reference/ guideliness
chlorobenzene											
Algae											
<i>Chlorella fusca</i> var <i>vacuolata</i>		50			24h				50		Freitag, 1985
<i>Selenastrum capricornutum</i>		10000			24h				2172		Cassery, 1983
Fish											
<i>Leuciscus idus melanotus</i>		50			3d				70		Freitag, 1985
1,2-dichlorobenzene											
Algae											
<i>Selenastrum capricornutum</i>		10000			24h				19700		Cassery, 1983
Fish											
<i>Salmo gairdneri</i>	250 g ¹⁴	0.94 ± 0.16	f	methanol	7-119d				560 ± 130 ¹⁵	6720 ¹⁶	Oliver, 1983
1,3-dichlorobenzene											
Fish											
<i>Pimephales promelas</i>	1000 - 2300	f							97		Carlson, 1987 b
<i>Poecilia reticulata</i>	206-283 mg	s			48h				6025		Opperhuizen (P., 1988
<i>Salmo gairdneri</i>	250 g ¹⁴	0.69 ± 0.15	f	methanol	7-119d				740 ± 170 ¹⁷	8080 ¹⁸	Oliver, 1983
1,4-dichlorobenzene											
Algae											
<i>Chlorella fusca</i> var <i>vacuolata</i>	50				24h				90		Freitag, 1985
Fish											
<i>Jordanella floridae</i>	4-6 months	2.68 ± 0.23	f	acetone	28d	14d	291 ± 26	0.98 ± 0.04296 ± 29	3590 ± 498	Smith, 1990;	
<i>Leuciscus idus melanotus</i>	50				3d				50		OECD, 1981; Freitag, 1985

Table 5.3.1 Bioconcentration factors for chlorobenzenes - continued

Substance Species	Lifestage age/size	Concentration ($\mu\text{g/l}$)	Test system	Solvent	Uptake period	Depuration k_t (days $^{-1}$)	k_2 (days $^{-1}$)	BCF (wet weight)	BCF (lipid weight)	Reference/ guidelines
1,4-dichlorobenzene										
Pimephales promelas		570 - 1000	F					110		Carlson, 1987b
Salmo gairdneri	alevin	15			7d	1d		40		Calamari, 1982
Salmo gairdneri	alevin	73			7d	1d		85		Calamari, 1982
Salmo gairdneri	egg-alevin		F		60d			100-1400 ^a		Calamari, 1982
Salmo gairdneri	alevin	3			7d	1d		112		Calamari, 1982
Salmo gairdneri	250g ^a	0.67 ± 0.18	F	methanol	7-119d			720 ± 130 ^b	8640 ^b	Oliver, 1983
1,2,3-trichlorobenzene										
Fish										
Salmo gairdneri	alevin	3.8 ± 0.45 ^{b2}	F		48h	96h	-	52	7761	Galassi, 1983
Salmo gairdneri	eyed-egg	5.5 ± 0.61 ^{b2}	F		48h	96h	0.3	108	5118	Galassi, 1983
Salmo gairdneri	hatching	6.05 ± 0.21 ^{b2}	F		48h	96h	0.34	710	22050	Galassi, 1983
Salmo gairdneri	250g ^a	0.072 ± 0.025	F	methanol	7-119d			2600 ± 460 ^b	31200 ^b	Oliver, 1983
1,2,4-trichlorobenzene										
Algae										
Chlorella fusca var vacuolata		50			24h			250		Freitag, 1985
Fish										
Jordanella floridae	4-6 months	3.8 ± 0.0013	F	acetone	28d	14d	1158 ± 84	0.57 ± 0.01	2026 ± 154	17750 ± 1481
Leuciscus idus melanotus		50				3d			490	Smith, 1990; OECD, 1981
Pimephales promelas	500 - 1000	F							410	Freitag, 1985
										Carlson, 1987 b

Table 5.3.1 Bioconcentration factors for chlorobenzenes - continued

Substance Species	Lifestage age/size	Concentration ($\mu\text{g/l}$)	Test system	Solvent	Uptake period	Depura- tion period	k_1 (days^{-1})	k_2 (days^{-1})	BCF (wet weight)	BCF (lipid weight)	Reference/ guideliness
1,2,4-trichlorobenzene											
<i>Salmo gairdneri</i>	alevin	6.9 ± 0.9 ²	F	48h	96h	-	-	-	5281	5281	Galassi, 1983
<i>Salmo gairdneri</i>	eyed-egg	4.43 ± 0.5 ²	F	48h	96h	0.75	85	4028	4028	Galassi, 1983	
<i>Salmo gairdneri</i>	hatching	9.6 ± 0.7 ²	F	48h	96h	0.85	349	10838	10838	Galassi, 1983	
<i>Salmo gairdneri</i>	250g ⁴	0.052 ± 0.02	F	methanol	7-119d		3200 ± 540 ⁵	3840 ⁶	3840 ⁶	3840 ⁶	Oliver, 1983
1,3,5-trichlorobenzene											
Fish											
<i>Poecilia reticulata</i>	206-233mg		S		48h				22387	22387	Opperhuisen ⁷ , 1988
<i>Salmo gairdneri</i>	250g ⁴	0.045 ± 0.02	F	methanol	7-119d		4100 ± 690 ⁵	49200 ⁶	49200 ⁶	49200 ⁶	Oliver, 1983
1,2,4,5-tetrachlorobenzene											
Fish											
<i>Jordanella floridae</i>	4-6 months	3.02 ± 0.29	F	acetone	28d	14d	1630 ± 246	0.4 ± 0.03	4054 ± 783	50300 ± 11427	Smith, 1990; OECD, 1981
<i>Salmo gairdneri</i>	250g ⁴	0.021 ± 0.013	F	methanol	7-119d		13000 ± 1700 ⁵	15600 ⁶	15600 ⁶	15600 ⁶	Oliver, 1983

¹) mass balance: loss during experiments <4%

²) concentrations determined daily; data refer to mean concentrations

³) BCF-value depends on the developmental stage; at hatching a BCF-value of 1400 was found, the BCF-value of 1400 was converted to 100, at the end of the experiment

⁴) initial weight

⁵) average lipid contents: 8.8%

⁶) converted BCF: "wet-weight" BCFx12

5.4

Environmental distribution

Some generalizations on the environmental distribution of chlorobenzenes can be made. Chlorobenzenes which are released into the aquatic environment will dissolve in water: monochlorobenzene less than 500 mg/l; dichlorobenzenes around 100 mg/l, trichlorobenzenes around 20 mg/l and tetrachlorobenzene less than 1 mg/l. On one hand, the substances will volatilize from the water compartment. Volatilization half-lives for chlorobenzenes vary between 1 hour to 30 days and the substances will volatilize from water into air within 1 or 2 months. In air they will degrade rapidly by indirect photolysis with radicals. On the other hand they will adsorb to organic matter in the sediment and maintain partition-equilibrium. Sorption will be important for tri- and tetrachlorobenzenes ($\log K_{ow} > 3$). Chlorobenzenes will also accumulate in fish with low bioconcentration factors for monochlorobenzene (< 100) en somewhat higher factors for the higher substituted benzenes (< 1000).

Limited data are available on the chemical degradation in the aquatic environment. However, it is not expected that chemical degradation is important because of the stability of the aromatic ring of the substances. Abiotic degradation can only be expected under special conditions like a high concentration of radicals and a high dose of sunlight. However, all chlorinated benzenes up to 1,2,4,5-tetrachlorobenzene are inherently biodegradable, whereas 1,4-dichlorobenzene is readily biodegradable. Therefore they may be degraded in the sediment but only under favourable conditions.

TOXICITY

A general remark should be made with regard to the interpretation of the data presented in the literature. Several chlorobenzenes are highly volatile substances, indicated by measured half-lives less than 8 hours (Table 5.1.2). As a consequence these substances will also disappear from the test vessels in toxicity studies.

According to Kühn et al. (1989) in a static renewal test where the test medium was refreshed every other day, the initial concentration of 0.63 mg/l was actually reduced by 95% to 0.03 mg/l. Therefore the tables give information on initial and actual concentrations and the way the values are reported.

6.1

Aquatic toxicity

All toxicity data for chlorobenzenes are summarized in Tables 6.1.1. to 6.1.14. The previous reports to EC of Hooftman and De Kreuk (1981; 1983) contain several tables with toxicity data. A selection of these data are included in the Tables.

6.1.1.

Monochlorobenzenes

Most of the acute E(L)C₅₀-values for bacteria, algae, crustaceans and fish range from 4.1 to 22.3 mg/l. One low EC₅₀-value of 0.58 mg/l found for *Daphnia magna* is considered as a outlier, as all other EC₅₀-values for *Daphnia magna* range between 4.3 and 12.9 mg/l.

Chronic NOEC-values are available for *Daphnia magna* the fish *Brachydanio rerio* and the marine alga *Skeletonema costatum* and echinoderm *Paracentrotus lividus*. For *Skeletonema costatum* a NOEC-value of 100 mg/l was found, which is considerably higher than NOEC-values ranging from 0.32 to 5.6 mg/l for *Daphnia magna* and *Brachydanio rerio*, respectively. Monochlorobenzene was most toxic to the marine Echinoderm *Paracentrotus lividus*, causing chromosomal abnormalities in embryos at a concentration of 0.14 mg/l.

6.1.2.

Dichlorobenzenes

From the available acute toxicity values it is concluded that 1,2-, 1,3- and 1,4-dichlorobenzenes are comparably toxic. Furthermore dichlorobenzenes appear to be slightly more toxic than monochlorobenzene, indicated by 2 to 3 times lower E(L)C₅₀-values found for *Photobacterium phosphoreum* and *Pimephales promelas* (On molecular bases this is even more pronounced). However, the lowest acute data for monochlorobenzene and dichlorobenzenes were comparable 0.58 and 0.78 mg/l respectively.

Chronic toxicity data confirm that dichlorobenzenes are equally toxic as most of the NOEC-values are ranging from 0.3 to 1.0 mg/l for the algae *Selenastrum capricornutum*, *Daphnia magna*, the fish species *Brachydanio rerio* and *Pimephales promelas*.

No difference could be distinguished between chronic toxicity data for monochlorobenzene and dichlorobenzenes. In experiments performed with the marine Echinoderm *Paracentrotus lividus*, both 1,2-dichlorobenzene and 1,3-dichlorobenzene also caused chromosomal abnormalities in embryos at a concentration of 0.14 mg/l.

6.1.3. Trichlorobzenes

In accordance with dichlorobzenes, 1,2,3-trichlorobenzene and 1,2,4-trichlorobenzene also have comparable toxicity. For *Photobacterium phosphorum*, *Daphnia magna* and *Brachydanio rerio* acute E(L)C₅₀-values were found, most of them ranging from 0.35 to 6.3 mg/l. For 1,2,4-Trichlorobenzene, eleven LC₅₀-values were available for fish, ranging from 1.32 mg/l for *Salmo gairdneri* to 21 mg/l for the saltwater fish *Cyprinodon variegates*. The latter LC₅₀-value was based on nominal concentrations and was very high as compared to the other values.

Chronic NOEC-values of 1,2,3-trichlorobenzene ranged from 0.22 mg/l for the alga *Selenastrum capricornutum* to 0.56 mg/l for *Brachydanio rerio*. Kühn et al., 1989 reported in a renewal experiment with *Daphnia magna* a nominal NOEC-value of 0.63 mg/l, whereas after analysis an initial concentration of 0.03 mg/l was measured. Therefore this experiment was not used for effect assessment (chapter 7.1).

The lowest NOEC of 0.1 mg/l was reported for 1,2,4-trichlorobenzene for *Daphnia magna*.

For 1,3,5-trichlorobenzene no additional new data were found. However, the data reported in Hooftman and De Kreuk (1981; 1983) indicate no difference in toxicity compared to other trichlorobzenes.

6.1.4. 1,2,4,5-tetrachlorobenzene

Three acute (4 d) LC₅₀-values were reported for fish, ranging from 0.33 mg/l to 1.2 mg/l. No other acute toxicity data were available.

The only reported chronic toxicity data for fish were a MATC of 0.12 mg/l for *Cyprinodon variegates* and a LC₅₀-value of *Pimephales promelas* of 0.3 mg/l.

In an ELS experiment conducted with *Salmo gairdneri* (Van Leeuwen, 1983), where concentrations were used which were around the water solubility, no mortality was found up to and including the sac fry stage.

No other toxicity data were reported for aquatic organisms.

Table 6.1.1 Acute single species toxicity data for monochlorobenzene

Species	Lifestage age/size	A/N	Test system	Purity/ solvent	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality
Bacteria											
Photobacterium phosphoreum (M)			Microtox			30min.	EC ₅₀		11.25	Ribo, 1983	
Photobacterium phosphoreum (M)			Microtox			10min.	EC ₅₀		20	Bazin, 1987	(AFNOR, 1974 T90-301)
Algae											
Ankistrodesmus falcatus	N	S	pure	CHU 10 medium acetone 0.1 %	8	4	EC ₅₀ growth	49		Wong, 1984	#2-3
Selenastrum capricornutum	A ⁵	S	pure	AAPBT medium		96	EC ₅₀ growth	12.5		Calamari, 1983	#2
Skeletonema costatum (M)	N	S	pure	ASP12 medium (revised)	7.7-9	5d	EC ₅₀	203 61-344		Congill et al., 1989	#2 (EPA, 1971)
Crustaceans											
Ceriodaphnia dubia	N ³	S	pure/ acetone	Lakewater	8.2-8.99	90.3	LC ₅₀		8.9 (7.5-10.5)	Congill et al., 1989	#2 (ASTM, 1980)
Daphnia magna	1.5 mm	N	pure		6-7		EC ₅₀ immob.	48	0.58	Bobra, 1985	#2
Daphnia magna	A	S	pure				EC ₅₀ immob.	24	4.3 (3.2-5.7)	Calamari, 1983	#2 (AFNOR, 1974)
Daphnia magna	Neonates	N	pure > 97%				EC ₅₀ immob.	48	5.8	Abernethy, 1986	#2
Daphnia magna	N ³	S	pure/ acetone	Lakewater	8±0.12	159.6 ± 7.1	EC ₅₀ immob.	48	12.9 (11.2-15.2)	Congill et al., 1985	(ASTM, 1980)
Daphnia magna	N	S	pure	Lakewater	8.0±0.2	157	EC ₅₀ immob.	48	12.9 ² (9.4-18.4)	Gersbach, 1986	#2 (ASTM, 1980)
Daphnia magna	A	S	pure	Lakewater	7±0.1		EC ₅₀ immob.	24	16	Bazin, 1987	(AFNOR, 1974 T90-301)
Fish											
Brachydenio rerio	A ⁴	S	pure		7.4	320	LC ₅₀	48		Calamari, 1983	(IRSA, 1973)
Cyprinodon veriegatus (M)	Juv.	N	pure > 80% (filtered)	Seawater			LC ₅₀	96	10 (8.8-12) (7.1-15.5)	Heitmuller, 1981	#2 (EPA, 1975)

Table 6.1.1 Acute single species toxicity data for monochlorobenzene - continued

Species	Lifestage age/size	A/N	Test system solvent	Purity/ mg CaCO ₃ /l	Testwater	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality
<i>Lepomis macrochirus</i>	0.9 g ± 0.22 N	S	pure	Dechlorinated tapwater	6-8	31.2	24	LC ₅₀	4.5 (4.1-4.9)	Baily, 1985	#2 (EPA, 1975)	
<i>Lepomis macrochirus</i>	0.9 g ± 0.22 N	S	pure	Dechlorinated tapwater	6-8	31.2	48	LC ₅₀	4.5 (4.1-4.9)	Baily, 1985	#2 (EPA, 1975)	
<i>Lepomis macrochirus</i>	0.9 g ± 0.22 N	S	pure	Dechlorinated tapwater	6-8	31.2	72	LC ₅₀	4.5 (4.1-4.9)	Baily, 1985	#2 (EPA, 1975)	
<i>Lepomis macrochirus</i>	0.9 g ± 0.22 N	S	pure	Dechlorinated tapwater	6-8	31.2	96	LC ₅₀	4.5 (4.1-4.9)	Baily, 1985	#2 (EPA, 1975)	
<i>Lepomis macrochirus</i>	0.9 g ± 0.22 A ⁵	F	pure	Dechlorinated tapwater	6-8	31.2	72	LC ₅₀	7.4 (6.9-7.9)	Baily, 1985	#2 (EPA, 1975)	
<i>Lepomis macrochirus</i>	0.9 g ± 0.22 A ⁵	F	pure	Dechlorinated tapwater	6-8	31.2	96	LC ₅₀	7.4 (6.9-7.9)	Baily, 1985	#2 (EPA, 1975)	
<i>Lepomis macrochirus</i>	0.9 g ± 0.22 A ⁵	F	pure	Dechlorinated tapwater	6-8	31.2	48	LC ₅₀	7.7 (7-11)	Baily, 1985	#2 (EPA, 1975)	
<i>Lepomis macrochirus</i>	juv.	A ⁵	pure	Dechlorinated tapwater	6-8	31.2	24	LC ₅₀	8.0 (7-11)	Baily, 1985	#2 (EPA, 1975)	
<i>Pimephales promelas</i>	subadult (60-100d)	S		Lakewater ¹	7.2-8.5	96-125	96	LC ₅₀	22.2 (19.2-26.6)	Mayes, 1983	#2 (ASTM, 1980)	
<i>Pimephales promelas</i>	fry (10-15d)	S		Lakewater ¹	7.2-8.5	96-125	96	LC ₅₀	22.3 (19.2-26.9)	Mayes, 1983	#2 (ASTM, 1980)	
<i>Pimephales promelas</i>	juv. (30-35d)	S		Lakewater ¹	7.2-8.5	96-125	96	LC ₅₀	35.4 (31.2-43.3)	Mayes, 1983	#2 (ASTM, 1980)	
<i>Salmo gairdneri</i>	A ⁴	S	pure		7.4	320	48	LC ₅₀	4.1 (3.87-4.23)	Calembi, 1983	#2 (IRSA, 1975)	

1) carbon filtered and UV irradiated

2) geometric mean of three replicate tests

3) estimated loss less than 10%

4) data refer to nominal concentrations, differences with actual concentrations were less than 10%

5) average of initial and final concentrations

Table 6.1.2 Chronic single species toxicity data for monochlorobenzene

Species	Lifestage age/size	A/N	Test system	Purity/ solvent	Testwater	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality
Algae <i>Skeletonema costatum</i> (N)	N	S	pure	ASP12 medium (revised)	7.7-9			5d	NOEC growth	100	Cougill et al., 1989	#2
Crustaceans												
Daphnia magna	A	R						sub-acute	NOEC growth	0.32	De Wolf, 1988	#2
Daphnia magna	A	F		Standard water				16d	NOEC reprod.	0.32	Hermens, 1984	#2
Daphnia magna	A'	R						sub-acute	NOEC reprod.	1	De Wolf, 1988	#2
Daphnia magna	A	F		Standard water				16d	EC ₅₀ reprod.	1.1	Hermens, 1984	#2
Daphnia magna	A'	R						sub-acute	EC ₅₀ reprod.	1.9	De Wolf, 1988	#2
Daphnia magna	12h	A'	pure	IRCHA Standard Water				14d	EC ₅₀ fertility	2.5	Catamari, 1983	#2

Table 6.1.2 Chronic single species toxicity data for monochlorobenzene - continued

Species	Lifestage age/size	A/N	Test system solvent	Purity/ Testwater	pH	Hardness mg CaCO ₃ /l	Expo- time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality
Echinodermes											
Paracentrotus lividus (M)	embryo	N	S	purity > 99% DMSO 0.01%	Seawater	8.2-8.4	48	LOEC development	11	Pagano, 1988	#2
Paracentrotus lividus (M)	embryo	N	S	purity > 99% DMSO 0.01%	Seawater	8.2-8.4	48	LOEC genotoxic	1.1	Pagano, 1988	#2
Paracentrotus lividus (M)	sperm	N	S	purity > 99% DMSO 0.01%	Seawater	8.2-8.4	48	LOEC inactivation	1.1	Pagano, 1988	#2
Paracentrotus lividus (M)	sperm	N	S	purity > 99% DMSO 0.01%	Seawater	8.2-8.4	48	LOEC develop- ment offspring	1.1	Pagano, 1988	#2
Fish											
Brechydanio rerio	fert. eggs	A ²	R ³	purity > 99% 0.01 % DMSO	Recon- stituted	7.4-8.4	210	ELS NOEC survival hatching growth	4.8	Van Leeuwen, 1990	#1
Brechydanio rerio	egg -> larvae	N	R (2d)	> 99%	DSML	7.4-8.4	210	NOEC hatch	10	Adema, 1987	#1
Brechydanio rerio	fert. eggs	A ²	R ³	purity > 99% DMSO 100 μ l/l	Recon- stituted	7.4-8.4	210	mortality growth morph.	5.6 5.6 5.6	Van Leeuwen, 1990	#1

1) actual concentrations were determined after and before renewal of the test concentration

2) mean concentrations; based on measured values before and at renewal (actual concentrations were 85% of nominal)

3) renewed three times a week

4) data refer to nominal concentrations, differences with actual concentrations were more than 10%

5) data refer to nominal concentrations, differences with actual concentrations were less than 10%

Table 6.1.3 Acute single species toxicity data for 1,2-dichlorobenzene

Species	Lifestage age/size	A/N	Test system	Purity/ solvent	Testwater	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality
- 42 -												
Bacteria <i>Photobacterium phosphoreum</i> (M)			Microtox						30min. EC ₅₀	4.0	Ribe, 1983	#2
Algae <i>Ankistrodesmus falcatus</i>	N	S	pure/ 0.1% acetone	CHU 10 medium	8		4	EC ₅₀ growth	19.9	Wong, 1984	#2-3	
<i>Scenedesmus pannonicus</i>	N		purity > 99.9%				96	EC ₅₀ growth	17	Canton, 1985	#2 (OECD, 1979)	
<i>Selenastrum capricornutum</i>	A ³	S	pure	AAPBT medium			96	EC ₅₀ growth	2.2	Calamari, 1983	#2 (EPA, 1971)	
Crustaceans <i>Artemia (M)</i>							24	LC ₅₀	10	Abernethy, 1986	#2	
<i>Daphnia magna</i>	A ¹		purity > 99.9%				24	EC ₅₀ immob.	0.74	Canton, 1985	#2 (OECD, 1979)	
<i>Daphnia magna</i>	A ²	S	pure				24	EC ₅₀ immob.	0.78	Calamari, 1983	#2 (AFNOR, 1974)	
<i>Daphnia magna</i>	24h	N	S	enriched deionized water + tapwater	8.0	250	24	EC ₅₀	1.7	Kühn et al., 1989	#2	
<i>Daphnia magna</i>	neonates	S					48	EC ₅₀ immob.	2.3	Abernethy, 1986	#2	
<i>Daphnia magna</i>	1.5 mm	N	S	pure	6-7		48	EC ₅₀ immob.	2.3	Bobre, 1985	#2	

Table 6.1.3 Acute single species toxicity data for 1,2-dichlorobenzene - continued

Species	Lifestage age/size	A/N	Test system	Purity/ solvent	Testwater	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality
Fish												
<i>Brachydanio rerio</i>		A ²	S	pure		7.4	320	48	LC ₅₀	6.8 (5.2-8.9)	Calamari, 1983	#2 (IRSA, 1973)
<i>Cyprinodon variegatus</i> (M)	juv.	N	S	purity > 80%	Seawater (filtered)		96	LC ₅₀	9.7 (9.0-10)	Heitmuller, 1981	#2 (EPA, 1975)	
<i>Salmo gairdneri</i>		A ²	S	pure		7.4	320	48	LC ₅₀	2.3 (2.2-2.4)	Calamari, 1983	#2 (IRSA, 1973)

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- 1) corrected for the actual concentrations during the tests
 2) data refer to nominal concentrations, differences with actual concentrations were less than 10%

Table 6.1.4 Chronic single species toxicity data for 1,2-dichlorobenzene

Species	Lifestage age/size	A/N	Test system	Purity/ solvent	Testwater	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality
Algae												
<i>Selenastrum capricornutum</i>	A ³	S	pure	AAPBT medium				96	NOEC growth	< 0.88	Calamari, 1983	#2 (EPA, 1971)
Crustaceans												
<i>Daphnia magna</i>	12h	A ²	R	pure	IRCHA Standard Water			14d	EC ₅₀ fertility	0.59	Calamari, 1983	#3
<i>Daphnia magna</i>	24h	N	R (2d)		enriched deionized water + tapwater	8.0	250	21d	NOEC repr. + mort.	0.63	Kühn et al., 1989	#2
Echinoderes												
<i>Paracentrotus lividus</i> (M)	embryo	N	S	purity > 99% DMSO 0.01%	Seawater	8.2-8.4		48 ¹	LOEC genotoxic	0.14	Pagano, 1988	#2
<i>Paracentrotus lividus</i> (M)	embryo	N	S	purity > 99% DMSO 0.01%	Seawater	8.2-8.4		48 ¹	LOEC development	1.47	Pagano, 1988	#2
<i>Paracentrotus lividus</i> (M)	sperm	N	S	purity > 99% DMSO 0.01%	Seawater	8.2-8.4		48 ¹	LOEC inactivation	1.47	Pagano, 1988	#2

1) after 48 h exposure, test is continued in clean water

2) data refer to nominal concentrations, differences with actual concentrations were more than 10%
 3) data refer to nominal concentrations, differences with actual concentrations were less than 10%

Table 6.1.5 Acute single species toxicity data for 1,3-dichlorobenzene

Species	Lifestage age/size	A/N	Test system solvent	Purity/ solvent	Testwater	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality
Bacteria												
Photobacterium phosphoreum (M)			Microtox					10min.	EC ₅₀	4.2	Bazin, 1987	#2 (AFNOR, 1974)
Photobacterium phosphoreum (M)			Microtox					30min.	EC ₅₀	5.0	Ribo, 1983	#2
Algae												
Ankistrodesmus falcatus	N	S	pure	CHU 10 medium	8			4	EC ₅₀ growth	22.9	Wong, 1984	#2-3
Scenedesmus pannonicus	N	99.4%	purity					96	EC ₅₀ growth	31	Canton, 1985	#2 (OECD, 1979)
Crustaceans												
Daphnia magna	A'		purity 99.4%					-	EC ₅₀ immob.	1.2	Canton, 1985	#2 (OECD, 1979)
Daphnia magna	adult	A	S	purity 95.99%	7.1-7.7	44.7		48	EC ₅₀ immob.	4.2 (3.3-5.9)	Richter, 1983	#2 (ASTM, 1980)
Daphnia magna		A	S	pure	Lakenster	7 ± 0.1		24	EC ₅₀ (immob.)	6	Bazin, 1987	(AFNOR, 1974)
Daphnia magna	24h	N	S	enriched deionized water + tapwater	8.0	250		24	EC ₅₀	7.0	Kühn et al., 1989	#2
Daphnia magna	adult	A'	S	purity 95.99%	7.1-7.7	44.7		48	LC ₅₀	7.4 (6.3-8.8)	Richter, 1983	#2 (ASTM, 1980)

Table 6.1.5 Acute single species toxicity data for 1,3-dichlorobenzene - continued

Species	Lifestage age/size	A/N	Test system	Purity/ solvent	Testwater	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality
Fish												
Cyprinodon variegatus (M)	juv.	N	S	purity > 80%	Seawater (filtered)			96	LC ₅₀	7.8 (6.8-8.7)	Weitmuller, 1981	#2 (EPA, 1975)
Pimephales promelas	30d	F	pure		Lakewater	7.3-7.6	44-46	96	LC ₅₀	7.8	Carlson, 1987 ^b	#2
Pimephales promelas	juv. 30d	A ³	F	pure	Lakewater	7.5	42.5-45.5	96	LC ₅₀	7.8	Veith, 1983	#2
Pimephales promelas	juv.	A ²	F	pure		7.6	44	96	LC ₅₀	9.12 (7.57-11.0)	Broderius, 1985	#2 (ASTM, 1980)

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1) average of initial and final test solution concentrations

2) measured daily, concentrations were averaged

3) analysed daily
4) corrected for the actual concentrations during the tests

Table 6.1.6 Chronic single species toxicity data for 1,3-dichlorobenzene

Species	Life stage age/size	A/N	Test system solvent	Purity/ Testwater	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality
Crustaceans											
<i>Daphnia magna</i>			pure/ ethanol	Dutch Standard Water			16d	NOEC growth	0.3	Deneer, 1988	#2 (HEN 6502)
<i>Daphnia magna</i>	adult	A ⁵	R ⁴	purity	6.6-7.9	44.7	28d	NOEC repro, length	0.69	Richter, 1983	#2 (ASTM, 1978)
<i>Daphnia magna</i>	24h	N	R (2d)	enriched deionized water + tapwater	8.0	250	21d	NOEC repr. + mort	0.8 ²	Kühn et al., 1989	#2
<i>Daphnia magna</i>	adult	A ⁵	R ⁴	purity 95-99%	6.6-7.9	44.7	28d	LOEC repro, length	1.5	Richter, 1983	#2 (ASTM, 1978)
Echinodermes											
<i>Paracentrotus lividus</i> (M)	embryo	N	S	purity > 99% DMSO 0.01%	Seawater	8.2-8.4	48	LOEC development	0.14	Pagano, 1988	#2
<i>Paracentrotus lividus</i> (M)	embryo	N	S	purity > 99% DMSO 0.01%	Seawater	8.2-8.4	48	LOEC genotoxic	1.47	Pagano, 1988	#2
Fish											
<i>Pimephales promelas</i> emb- juv.	A ³	F	pure	Lakewater ¹	7.3-7.6	44-46	32d	ELS NOEC	1.0	Carlson, 1987	#2
<i>Pimephales promelas</i> emb- juv.	A ³	F	pure	Lakewater ¹	7.3-7.6	44-46	32d	ELS LOEC	2.3	Carlson, 1987 ^b	#2

1) filtered and heated

2) actual concentration: 0.5 (mg/l)

3) toxicant concentrations were measured twice a week

4) renewed three times a week

5) mean effective concentrations based on measured values before and at renewal

Table 6.1.7 Acute single species toxicity data for 1,4-dichlorobenzene

Species	Lifestage	A/N	Test system	Purity/ solvent	Testwater	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality
Bacteria <i>Photobacterium phosphoreum</i> (M)			Microtox						30min. EC ₅₀	5.3	Ribo, 1983	#2
algae									EC ₅₀ growth	31	Canton, 1985	#2 (OECD, 1979)
<i>Scenedesmus pannonicus</i>	N		pure	99.7%							Calamari, 1983	48
<i>Selenastrum capricornutum</i>	A ⁵	S	pure		AABT medium		96	EC ₅₀ growth	1.6			
Crustaceans									LC ₅₀	13	Abernethy, 1986	#2
<i>Artemia salina</i>			S	purity			24					
<i>Daphnia magna</i>	A ⁴			purity 99.7%			24	EC ₅₀ immob.	0.7	Canton, 1985	#2 (OECD, 1979)	
<i>Daphnia magna</i>	A ³	S	pure				24	EC ₅₀ immob.	1.6 (1.5-1.7)	Calamari, 1983	#2 (AFNOR, 1974)	
<i>Daphnia magna</i>	24h	N	S		enriched deionized water + tapwater	8.0	250	EC ₅₀	3.2	Kühn et al., 1989	#2	
<i>Daphnia magna</i>			S	pure/ acetone	Lake Water	8.0	157	LC ₅₀	11.6 (9.5-15.5)	Gersich, 1986	#2 (ASTM, 1980)	
Fish											Calamari, 1983	
<i>Brachydanio rerio</i>	A ³	S	pure			7.4	320	LC ₅₀	4.25 (3.4-5.3)			
<i>Cyprinodon variegatus</i> (M)	juv.	N	S	purity > 80%	Seawater (filtered)		96	LC ₅₀	7.4 (6.8-7.9)	Heitmüller, 1981	(IRSA, 1973)	
<i>Pimephales promelas</i>	fry (10-15d)		S		Lakewater ¹	7.2-8.5	96-125	LC ₅₀	3.6 (3.3-3.7)	Mayes, 1983	(EPA, 1975)	
<i>Pimephales promelas</i>	juv. 30d	A ²	F	pure	Lake Water	7.5	42.2-45.5	LC ₅₀	4.0	Veith, 1983	#2 (ASTM, 1980)	
<i>Pimephales promelas</i>	juv. 30d	F	pure	Lakewater	7.3-7.6	44-46	96	LC ₅₀	4.2	Carlson, 1987		

Table 6.1.7 Acute single species toxicity data for 1,4-dichlorobenzene

Species	Lifestage age/size	A/N	Test system solvent	Purity/ Testwater	pH	Hardness mg CaCO ₃ /l	Parameter Expo time (h)	Results (mg/l) (95% C.V.)	Reference	Quality
Pimephales promelas	subadult (60-100d)	S	Lakewater ¹	7.2-8.5	96-125	96	LC ₅₀	11.7 (9.7-14.5)	Mayes, 1983	#2 (ASTM, 1980)
Pimephales promelas	juv. (30-35d)	S	Lakewater ¹	7.2-8.5	96-125	96	LC ₅₀	14.2 (12.1-17.3)	Mayes, 1983	#2 (ASTM, 1980)
Salmo gairdneri	A ³	S	pure > 97%	7.4	320	48	LC ₅₀	1.18 (1.08-1.28)	Calamari, 1983	#2 (IRSA, 1973)

1) carbon filtered and UV irradiated

2) analysed daily

3) data refer to nominal concentrations, differences with actual concentrations were less than 10%

4) corrected for the actual concentrations during the tests

5) data refer to nominal concentrations, differences with actual concentrations were less than 10%

Table 6.1.8 Chronic single species toxicity data for 1,4-dichlorobenzene

Species	Lifestage age/size	A/N	Purity/ System solvent	Testwater	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality
Algae <i>Ankistrodesmus</i> <i>falcatus</i>	N	S	pure/ 0.1 % acetone	CHU 10 medium	8		4	EC ₅₀ growth	19.9	Wong, 1984	#2-3
Selenastrum <i>capricornutum</i>	A ⁵	S	pure	AAPBT medium			96	NOEC growth	0.57	Calembri, 1983	#2 (EPA, 1971)
Crustaceans <i>Daphnia magna</i>	24h	N	R (2d)	enriched deionized water + tapwater	8.0	250	21d	NOEC repr. + mort.	0.5 ²	Kühn et al., 1989	#2
Daphnia magna	12h	A ⁴	R	pure	IRCHA Standard Water		14d	EC ₅₀ fertility	0.93	Calembri, 1983	#3
Echinoderes <i>Paracentrotus</i> <i>lividus</i> (M)	embryo	S	N	purity > 97% DMSO 0.01%	Seawater	8.2-8.4	48	EC genotoxic	0.14	Pegano, 1988	#2
Fish <i>Branchiostoma rerio</i>	eggs (fert.)	A	R	DMSO 100 μ l/l substituted	Reconstituted	7.4-8.4	210	ELS NOEC survival hatching growth	0.65	Van Leeuwen, 1990	#1

Table 6.1.8 Chronic single species toxicity data for 1,4-dichlorobenzene - continued

Species	Lifestage age/size	A/N	Test system solvent	Purity/ Testwater	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality	
<i>Branchydanio rerio</i>	eggs (fert.)	A	R	purity 99% DMSO 100 μ l/l	Recon- stituted	7.4-8.4	210	28d	ELS LC ₅₀	2.7 (1.2-3.6)	Van Leeuwen, 1990	#1
<i>Brachydanio rerio</i>	egg → larvae	N	R (2d)	99% DMSO (100 μ l/l)	DSHL	7.6-8.3	210	28d	NOEC hatch mortality growth morph.	≥ 5.6 3.2 1.0 3.2	Adema, 1987	#2
<i>Pimephales promelas</i>	emb- juv.	A ³	F	pure	Lakewater ¹	7.3-7.6	44-46	32d	ELS NOEC	0.57	Carlson, 1987	#2
<i>Pimephales promelas</i>	emb- juv.	A ³	F	pure	Lakewater ¹	7.3-7.6	44-46	32d	ELS LOEC	1.0	Carlson, 1987	#2
<i>Pimephales promelas</i>		A	F		Lakewater			8d	LC ₅₀	3.5	Hall, 1984	#2

1) filtered and heated

2) actual concentration 0.3 mg/l

3) toxicant concentrations were measured twice a week

4) data refer to nominal concentrations, differences with actual concentrations were more than 10%

5) data refer to nominal concentrations, differences with actual concentrations were less than 10%

Table 6.1.9 Acute single species toxicity data for 1,2,3-trichlorobenzene

Species	Life stage age/size	A/N	Test system solvent	Purity/ Testwater	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality
Bacteria											
Photobacterium phosphoreum (M)				Microtox			30min.	EC ₅₀	3.15	Ribo, 1983	#2
algae							4	EC ₅₀ growth	5.9	Wong, 1984	#2
Ankistrodesmus falcatus	N	S	pure/ acetone 0.1 %	CHU 10 medium	8						
Selenastrum capricornutum	A ²	S	pure	AABT medium			96	EC ₅₀ growth	0.9	Calamari, 1983	#2 (EPA, 1971)
Crustaceans											
Artemia	nauplii	S	purity > 97%				24	LC ₅₀	2.3	Abernethy, 1986	#2
Daphnia magna	A ¹	S	pure				24	EC ₅₀ immob.	0.35	Calamari, 1983	#2 (AFNOR, 1974)
Daphnia magna	24h	N	S	enriched deionized water + tapwater	8.0	250	24	NOEC repr.	0.63	Kuhn et al., 1989	#2
Daphnia Magna	neonates	S	purity > 97%				48	LC ₅₀	1.45	Abernethy, 1986	#2
Daphnia magna	1.5 mm	N	S	pure	6-7		48	EC ₅₀ mort	2.7	Bobra, 1985	#2
Fish											
Brachydanio rerio	A ¹	S	pure		7.4	320	48	LC ₅₀	3.1 (2.3-4.1)	Calamari, 1983	#2 (IRSA, 1973)
Salmo gairdneri	A ¹	S	pure		7.4	320	48	LC ₅₀	0.71 (0.65-0.77)	Calamari, 1983	#2 (IRSA, 1973)

1) data refers to nominal concentrations, differences with actual concentrations were less than 10%

2) data refer to nominal concentrations, actual concentrations differences less than 10%

Table 6.1.10 Chronic single species toxicity data for 1,2,3-trichlorobenzene

Species	Lifestage age/size	A/N	Test system	Purity/ solvent	Testwater	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	quality
Algae <i>Selenastrum capricornutum</i>		A ³	S	pure	AAPBT medium			96	NOEC growth	0.22	Calamari, 1983	#2 (EPA, 1971)
Crustaceans <i>Daphnia magna</i>	12h	A ²	R	pure	IRCHA Standard Water			14d	EC ₅₀ fertility	0.2	Calamari, 1983	#3
<i>Daphnia magna</i>	24h	N	R (2d)		enriched deionized water + tapwater		250	21d	NOEC repr. + mort. growth	0.63 ¹	Kühn et al., 1989	#2
Fish <i>Brachydanio rerio</i>	eggs	A ⁴	R	0.01 % DMSO	Recon- stituted	7.4-8.4	210	28d	ELS NOEC survival hatching	0.25	Van Leeuwen, 1990	#1
<i>Brachydanio rerio</i>	eggs	A ⁴	R	purity 99% 0.01 % DMSO	Recon- stituted	7.4-8.4	210	28d	ELS LC ₅₀	0.99 (0.81-1.44)	Van Leeuwen, 1990	#1
<i>Brachydanio rerio</i>	egg -> larf	N	R (2d)	99% 0.001 % DMSO	DSWL	7.7-8.2	210	28d	NOEC hatch mortality growth morph.	1.8 1.8 0.56 1.0	Adema, 1987	#2

- 1) actual concentration 0.03 (mg/l) |
- 2) date refer to nominal concentrations, actual concentrations differ more than 10 %
- 3) date refer to nominal concentrations, actual concentrations differences less than 10%
- 4) mean measured concentrations during the test were 65% of nominal concentrations

Table 6.1.11 Acute single species toxicity data for 1,2,4-trichlorobenzene

Species	Lifestage age/size	A/N	Test system	Purity/ solvent	Testwater	pH	mg CaCO ₃ /l	Hardness Parameter	Expo time (h)	Results (mg/l) (95% C.V.)	Reference	Quality
Bacteria												
Photobacterium phosphoreum (M)					Microtox			10min. EC ₅₀	3	Bazin, 1987	#2	(AFNOR, 1974)
Photobacterium phosphoreum (M)					Microtox			30min. EC ₅₀	3.9	Ribo, 1983	#2	
Algae												
Ankistrodesmus falcatus	N	S	pure/ acetone 0.1 % pure	CHU 10 medium	8			4	EC ₅₀ growth	5.9	Wong, 1984	#2
Selenastrum capricornutum	A ⁵	S		AAPBT medium				96	EC ₅₀ growth	1.4	Calemaris, 1983	#2
Crustaceans												
Orconectes immunis adults	A ⁴	F	pure	Lakewater	7.4±0.2	44.7	96	LC ₅₀		3.02 (2.55-3.57)	Holcombe, 1987	#2
Palamonetes pugio adult (M)	N	F	pure/ acetone	Seawater (filtered with 20 μ m)	7.8-8.2		96	LC ₅₀	0.54		Clark et al., 1987	#2
Daphnia magna	A ⁶	S	pure				24	EC ₅₀ immob.	1.2		Calemaris, 1983	#2
Daphnia magna	A	S	pure/ 0.1 % acetone		7±0.1		24	EC ₅₀ immob.	2		Bazin, 1987	(AFNOR, 1974)
Daphnia magna adult	A ²	S	purity 95-99%		7.1-7.7	44.7	48	LC ₅₀		2.1 (1.8-2.6)	Richter, 1983	(ASTM, 1980)
Daphnia magna 0-24h	A	F	pure	Lakewater	7.4±0.2	44.7	48	LC ₅₀		3.39 (3.10-3.69)	Holcombe, 1987	#2
Insects												
Tanystarsus dissimilis instar	3rd and 4th	A	F	pure	Lakewater	7.4±0.2	44.7	48	LC ₅₀	0.93 (0.76-1.16)	Holcombe, 1987	#2
Molluscs												
Aplexa hypnorum	adults	A ⁴	F	pure	Lakewater	7.4±0.2	44.7	96	LC ₅₀	3.16 (2.90-3.44)	Holcombe, 1987	#2

Table 6.1.11 Acute single species toxicity data for 1,2,4-trichlorobenzene - continued

Species	Lifestage age/size	A/N	Test system	Purity/ solvent	Testwater	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality
Fish												
Brachydanio rerio	A ⁶	S	pure		7.4	320	48	LC ₅₀	6.3 (4.4-9.0)	Calamari, 1983	(IRSA, 1973)	
Cyprinodon variegatus (M)	juv.	N	S	purify > 80%	Seawater (filtered)			96 LC ₅₀	21 (17-26)	Heitmuller, 1981	(EPA, 1975)	
Leponis macrochirus	0.6g	A ⁴	F	pure	Lakewater	7.4±0.2	44.7	96 LC ₅₀	3.02 (2.68-3.4)	Holcombe, 1987	#2	
Pimephales promelas	juv.	A ³	F	pure	Lakewater ¹	7.6	44	96 LC ₅₀	2.76 (1.67-4.34)	Broderius, 1985	#2 (ASTM, 1980)	
Pimephales promelas	30d	F	pure			7.3-7.6	44-46	96 LC ₅₀	2.8	Carlson, 1987	#2	
Pimephales promelas	A ⁵	F	pure	Lakewater	7.5	42.5-45.5	96 LC ₅₀	2.9	Veith, 1983	#2		
Pimephales promelas	A ⁴	F	pure	Lakewater	7.4±0.2	44.7	96 LC ₅₀	3.01 (2.65-3.40)	Holcombe, 1987	#2		
Salmo gairdneri	5.2g	A ⁴	F	pure	Lakewater	7.4±0.2	44.7	96 LC ₅₀	1.32 (1.24-1.40)	Holcombe, 1987	#2	
Salmo gairdneri	A ⁶	S	pure			7.4	320	48 LC ₅₀	1.95 (1.79-2.15)	Calamari, 1983	#2 (IRSA, 1973)	
Salmo gairdneri	juv.	S	pure/ Tween 80 in dimethyl formamide ⁷					96 LC ₅₀	4.04 (3.4-4.7)	Douglas et al., 1986	#2 (OECD, No. 203 1981)	
Salmo gairdneri	juv.	S	pure/ Tween 80 in dimethyl formamide ⁷					96 LC ₅₀	4.33 (3.68-4.94)	Douglas et al., 1986	#2 (OECD, No. 203 1981)	

1) filtered and heated

2) average of initial and final test solution concentration

3) measured daily, concentrations were averaged

4) analysed daily

5) data refer to nominal concentrations, differences with actual concentrations were less than 10 %

6) data refer to maximum final concentration of 40 µ l/l

7) 5 % Tween-80 in dimethylformamide at a maximum final concentration of 40 µ l/l

Table 6.1.12 Chronic single species toxicity data for 1,2,4-trichlorobenzene

Species	Lifestage age/size	A/N	Test system/ solvent	Purity/ Testwater	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality
Algae <i>Selenastrum capricornutum</i>		A ⁵	S	pure	AAPBT medium		96	NOEC growth	0.37	Calamari, 1983	#2 (EPA, 1971)
Crustaceans <i>Daphnia magna</i>		A ³	R				sub- acute	NOEC growth	0.1	De Wolf, 1988	#2
<i>Daphnia magna</i>		A	R	Standard water			16d	NOEC reprod.	0.1	Hermens, 1984	#2
<i>Daphnia magna</i>		A	R	Standard water			16d	EC ₅₀ reprod.	0.27	Hermens, 1984	#
<i>Daphnia magna</i>		A ³	R				sub- acute	EC ₅₀ reprod.	0.33	De Wolf, 1988	#2
<i>Daphnia magna</i>	adult	A ²	R ¹	puritY 95-99%	6.6-7.9	44.7	28d	NOEC repro., length	0.36	Richter, 1983	#2 (ASTM, 1980)
<i>Daphnia magna</i>	12h	A ⁴	R	pure	IRCHA Standard Water		16d	EC ₅₀ fertility	0.45	Calamari, 1983	#3
<i>Daphnia magna</i>		A	R	Standard water			16d	LC ₅₀	0.56	Hermens, 1984	#2
<i>Daphnia magna</i>	adult	A ²	R ¹	puritY 95-99%	6.6-7.9	44.7	28d	LOEC repro., length	0.69	Richter, 1983	#2 (ASTM, 1980)

Table 6.1.12 chronic single species toxicity data for 1,2,4-trichlorobenzene - continued

Species	Life stage age/size	A/N	Test system solvent	Purity/ Testwater	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality
Fish											
Pimephales promelas	emb- juv.	A F	pure	filtered	7.3-7.6	44-46	28d	ELS NOEC growth, survival	0.5	Carlson, 1987a	#2
Pimephales promelas	emb- juv.	A F	pure	filtered	7.3-7.6	44-46	28d	ELS LOEC growth, survival	0.92	Carlson, 1987a	#2

- 1) renewed three times a week
- 2) mean effective exposure based on measured values before and at renewal
- 3) actual concentrations were determined after and before renewal of the test concentration
- 4) data refer to nominal concentrations, differences with actual concentrations were more than 10 %
- 5) data refer to nominal concentrations, differences with actual concentrations were less than 10 %

Table 6.1.13 Acute single species toxicity data for 1,2,4,5-tetrachlorobenzene

Species	Lifestage age/size	A/N	Test System	Purity/ solvent	Testwater	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality
Fish <i>Cyprinodon variegatus</i> (M)	juv.	A ¹	F	pure	Seawater (filtered)		96	LC ₅₀		0.33 (0.12-0.94)	Ward, 1981	#3
<i>Cyprinodon variegatus</i> (M)	juv.	N	S	purity > 80%	Seawater (filtered)		96	LC ₅₀		0.8 (0.7-1.1)	Heitmuller, 1981	#2 (EPA, 1975)
<i>Salmo gairdneri</i>	N	R	97%	Standard water	7.2	50	96	LC ₅₀		1.2	Van Leeuwen, 1985	#2

1) mean measured concentrations, concentrations were 9-22 % of nominal concentrations, test temperature was 30° C

Table 6.1.14 Chronic single species toxicity data for 1,2,4,5-tetrachlorobenzene

Species	Lifestage age/size	A/N	Test System	Purity/ solvent	Testwater	pH	Hardness mg CaCO ₃ /l	Expo time (h)	Parameter	Results (mg/l) (95% C.V.)	Reference	Quality
Fish <i>Cyprinodon variegatus</i> (M)	hatch	A ¹	F	pure	Seawater (filtered)	7.3-8.3	28d	MTTC mort		0.12	Ward, 1981	#2
<i>Pimephales promelas</i>		A	F	Lakewater		8d	LC ₅₀			0.3	Hall, 1984	#2

1) mean measured concentrations were 26 to 53% of nominal concentrations

1 Well performed experiment with reliable test results; sufficiently documented

2 Experiment with reliable test results; insufficiently documented

3 Experiment with unreliable test results

6.1.5 Quantitative structure activity relations (QSARs)

Based on experimental data quantitative structure activity relations can be established for substances with similar structures and working mechanisms. For narcotizing substances like chlorobenzenes QSARs are often used for relations where toxicity is a function of their octanol-water partition coefficient. Therefore for several species QSARs are available as presented in Table 6.1.15.

Table 6.1.15 QSARs for the chlorobenzenes

Organism	QSAR equations ($\mu\text{mol/l}$)	r	s	Ref.
<i>Photobacterium phosphoreum</i> ^a	$\log 1/\text{EC}_{50} = 1.03 \log K_{ow} - 5.22$	0.899	0.53	1
<i>Selenastrum capricornutum</i> ^b	$\log 1/\text{LC}_{50} = 0.87 \log K_{ow} - 4.87$	0.988	0.24	2
<i>Daphnia magna</i> ^c	$\log 1/\text{NOEC} = 0.99 \log K_{ow} - 4.16$	0.974	0.50	3
<i>Brachydanio rerio</i> ^d	$\log 1/\text{LC}_{50} = 0.94 \log K_{ow} - 4.62$	0.997	0.07	4
<i>Brachydanio rerio</i> ^d	$\log 1/\text{NOLC} = 0.96 \log K_{ow} - 4.56$	0.991	0.13	4
<i>Brachydanio rerio</i> ^d	$\log 1/\text{NOEC} = 1.06 \log K_{ow} - 4.57$	0.987	0.17	4
<i>Poecilia reticulata</i> ^e	$\log 1/\text{LC}_{50} = 0.845 \log K_{ow} - 4.63$	0.980	-	2

r=correlation coefficient and s=standard error of estimate

References:	a 30 minutes
1 Hermens et al., (1985)	b 4 days
2 Könemann, (1981)	c 4 days
3 De Wolf et al., (1988)	d 28 days
4 Van Leeuwen et al. (1990)	e 2 days

6.2

Toxicity to terrestrial organisms

Data on the toxicity of chlorobenzenes to terrestrial organisms like plants, bacteria and invertebrates are summarized in Table 6.2.1. As lipophilic substances may strongly adsorb to organic matter in the soil, the bioavailability of the test substance for the test organism is strongly influenced by content of organic material in the soil. Therefore test concentrations are often recalculated and standardized for a so-called standard soil, which contains 10% of organic matter. NOEC-values for chlorobenzenes, dichlorobenzenes, trichlorobenzenes and 1,2,4,5-tetrachlorobenzene, converted to standard soil conditions ranged from <5 to 220 mg/kg dry weight.

6.3

(Semi)field studies

The effects of 1,2,4-trichlorobenzene were studied in a multi-species system (Lay et al., 1985). The concentration where the ecosystem was not affected was estimated at 57 $\mu\text{g/l}$ (Okkerman et al., 1990).

Table 6.2.1 LC₅₀- and NOEC-values for chlorobenzenes derived from laboratory experiments with various soil organisms, converted into standard soil (organic matter (OM) 10%) (Denneman et al., 1989; Hesse et al., 1991)

Compound\ test organism	Soil type	pH	Soil properties %OM	%clay	Expo. time	Criterion	Result (mg/kg) soil	Standard Ref. (mg/kg)
monochlorobenzene								
Plants								
Lactuca sativa OECD		7.8	2	12	14d	EC ₅₀ growth NOEC growth	>1000 10	>5000 50
1,4-dichlorobenzene								
Plants								
Lactuca sativa OECD		7.8	2	12	14d	EC ₅₀ growth NOEC growth	248 10	1250 50
1,2,3-trichlorobenzene								
Plants								
Lactuca sativa OECD		7.8	2	12	14d	EC ₅₀ growth NOEC growth	3.8 1	20 5
Oligochaetes								
Eisenia andrei humic		5.3	6.1	2.4	14d	LC ₅₀	240	393
						NOEC growth	100	164
humic sand		4.8	3.7	1.4	14d	LC ₅₀	134	362
						NOEC growth	56	150
peaty soil		6.5	8.1	8.1	14d	LC ₅₀	133	83
						NOEC growth	56	35
art. OECD soil		3.8	15.6	9	14d	LC ₅₀	547	675
						NOEC growth	180	220
Eisenia andrei humic		5.3	6.1	2.4	14d	LC ₅₀	207	340
						LC ₅₀	115	310
humic sand		4.8	3.7	1.4	14d	LC ₅₀	563	695
						LC ₅₀	195	122
peaty soil		6.5	8.1	8.1	14d	LC ₅₀		
art. OECD soil		3.8	15.6	9	14d	LC ₅₀		
1,2,4-trichlorobenzene								
Oligochaetes								
Eisenia fetida art. soil		6.0	10	20	14 d	LC ₅₀	197	197
Allobophora tuberculata					14 d	LC ₅₀	251	251
Eudrilus eugeniae					14 d	LC ₅₀	127	127
Perionyx excavatus					14 d	LC ₅₀	180	180
Plants								
Lactuca sativa OECD		7.8	2	12	14d	EC ₅₀ growth	48	240
					14d	NOEC growth	10	50
1,3,5-trichlorobenzene								
Plants								
Lactuca sativa OECD		7.8	2	12	14d	EC ₅₀ growth	123	615
					14d	NOEC growth	10	50
1,2,3,5-tetrachlorobenzene								
Plants								
Lactuca sativa OECD		7.8	2	12	14d	EC ₅₀ growth	1.3	6.5
					14d	NOEC growth	<1	<5

1 Adema and Henzen, 1990

2 Van Gestel et al., 1989

3 Van Gestel and Ma, 1990

4 Neuhauser et al., 1986

6.4

Toxicity to mammals

For chlorobenzenes the relevant routes of exposure of mammals are oral intake and inhalation exposure. Toxic effects in test organisms are aberrations of the liver, spleen and lymphatic system. For higher chlorinated benzenes also disturbance of the porphyrin metabolism is observed. The toxicity data are presented in Table 6.4.1.

Monochlorobenzene

On basis of a 2 year toxicity experiment with mouses an oral dose without effect is estimated at 30 mg/kg body weight. Using a safety factor of 100 the TDI (tolerable daily intake, for humans) is calculated at 0.3 mg/kg body weight (Hesse et al., 1991). Several subchronic inhalation studies with monochlorobenzene are available, but difficult to interpret. A no observed effect level in rat of 0.1 mg/m³ was estimated in an East-European study. In other experiments, however, 2000 mg/m³ caused no effects in the rat. In one experiment at 345 mg/m³ marginal effects were found. In other experiments neurotoxic effects in rat were found at 100 mg/m³ (EPA, 1984).

Dichlorobenzenes

For 1,2-dichlorobenzene a no-effect dose of 60 mg/kg body weight is found in oral chronic experiments with mouses and rats. Using a safety factor of 100 the TDI is calculated at 0.6 mg/kg body weight (Hesse et al., 1991). The lowest concentration causing effects in semichronic inhalation experiments (6-7 mths) was 560 mg/m³ in male guinea pigs with a reduced spleen weight. No effects were observed at 260 mg/m³ (61 mg/m³ continuously).

Liver porphyry was found in rat after 15 days exposure to 455 mg/m³ (EPA, 1984). Using a safety factor of 100 a toxicologically derived acceptable level in air for continuous exposure is calculated at 0.6 mg/m³ (Hesse et al., 1991). An odour threshold is established at 305 mg/m³ (Hesse et al., 1991).

For 1,3-dichlorobenzene there were too few data available to calculate a TDI. No inhalation studies are available.

For 1,4-dichlorobenzene on basis of semi-chronic experiments (6 mths) with rats a no effect dose of 19 mg/kg body weight is found using a safety factor of 100. The TDI is calculated at 0.19 mg/kg body weight (Hesse et al., 1991).

In semichronic (6-7 mths) inhalation experiments with rats, guinea pigs, mouses, rabbits and monkeys, no effects were observed at 577 mg/m³. Using a safety factor of 100, a toxicologically derived acceptable level in air for continuous exposure is calculated at 1.2 mg/m³. An odour threshold is established at 1 to 10 mg/m³ (Hesse et al., 1991).

Trichlorobenzenes

Most the experiments were performed with 1,2,4-trichlorobenzene. On basis of an oral experiment (120 days) with monkeys a no effect dose of 25 mg/kg body weight is found for 1,2,4-trichlorobenzene. In toxicity experiments with rats no effects on reproduction or teratogenic effects were found at 20 mg/kg body weight. Too few data were available to calculate a TDI. In an inhalation experiment (3 mths) with rats, 1,2,4-trichlorobenzene did not effect the porphyrin metabolism (EPA, 1984). In an inhalation experiment (3 mths, rats) with 1,3,5-trichlorobenzene in rats no effects were observed at 742 mg/m³ (EPA, 1984).

1,2,4,5-Tetrachlorobenzene

In a semi-chronic experiment a no effect dose was estimated at 0.4 mg/kg body weight. Due to a lack of data a TDI could not be calculated. No inhalation experiments with 1,2,4,5-tetrachlorobenzene were available.

Table 6.4.1 Acute toxicity of chlorobenzenes (Sax, 1989; Hesse et al. 1991)

Compound\test organism	Route of administration	Expo. time	Criteria	Result (mg/kg bw)
monochlorobenzene				
rat	oral		LD50	400
rat	oral		LD50	2,144
rabbit	oral		LD50	2,830
mouse	oral	2 y	NEL hep	30 **
rat	inhalation		LC50	2,965 *
rat	inhalation		sub-chron.	NEL hep. ren. 0.1 *
rat	inhalation		sub-chron.	TDLo hep. ren. 345 *
rat	inhalation		sub-chron.	NEL hep. ren. 2000 *
rat	inhalation		sub-chron.	EC neuro. 100 *
mouse	inhalation		LC50	1,886 *
dog	inhalation		sub-chron.	NEL hep. ren. 750 *
rabbit	dermal		LD50	>10,000
1,2-dichlorobenzene				
rat	oral		LD50	500
rat	oral		LD50	2,138
rabbit	oral		LD50	1,875
mouse	oral		LD50	2,000
guinea pig	oral		LD50	3,375
cat	oral	103 wks	NEL	60 **
mouse	oral	103 wks	NEL	60 **
rat	inhalation	15 d	TDLo lever	455 *
guinea pig	inhalation	6-7 mths	TDLo spleen weight	560 *
guinea pig	inhalation	6-7 mths	NEL	260 *
1,3-dichlorobenzene				
1,4-dichlorobenzene				
rat	oral		LD50	500
rat	oral	6 mths	NEL	19 **
rabbit	oral		LD50	2,812
rat	inhalation	6-7 mths	NEL	577 *
guinea pig	inhalation	6-7 mths	NEL	577 *
mouse	inhalation	6-7 mths	NEL	577 *
rabbit	inhalation	6-7 mths	NEL	577 *
monkey	inhalation	6-7 mths	NEL	577 *
1,2,3-trichlorobenzene				
1,2,4-trichlorobenzene				
rat	oral		LD50	756
rat	oral	3 mths	NEL ter.	20 **
mouse	oral		LD50	766
monkey	oral	4 mths	NEL org.	186 **
1,3,5-trichlorobenzene				
rats	inhalation	3 mths	NEL Org.	100 *
1,2,4,5-tetrachlorobenzene				
rat	oral	3 mths	NEL hep. ren.	0.4 *
mouse	oral		LD50	1,035
rat	oral		LD50	1,500

* mg/m³

** mg/kg body weight/day

hep. hepatocellar necrosis

ren. renal effects

neuro. neurotoxic effects

ter. teratogenic effects

org. organs

6.5

Carcinogenic, mutagenic effects and teratogenic effects

The Dutch National Institute of Public Health and Environmental Protection (RIVM) has evaluated chlorobenzenes for deriving toxicological guidelines (Vermeire et al., 1991). The most important results are presented here.

Monochlorobenzene

Monochlorobenzene is considered to have no carcinogenic properties. From the restricted in-vitro genotoxicity experiments, however, it was concluded that monochlorobenzene possibly has mutagenic properties.

Dichlorobenzenes

For 1,2-dichlorobenzene experiments with test organisms have shown no carcinogenic effects. Too few data were available to evaluate mutagenicity. No information was available for 1,3-dichlorobenzene for the evaluation of carcinogenicity or mutagenicity.

For 1,4-dichlorobenzene carcinogenic properties were found in experiments with test organisms.

Trichlorobenzenes

Experiments with test organisms have shown no carcinogenic effects. Due to a lack of data the mutagenicity could not be evaluated.

1,2,4,5-Tetrachlorobenzene

Experiments with test organisms have shown no carcinogenic effects. For the evaluation of mutagenicity too few data were available.

Information on teratogenicity was only available for 1,4-dichlorobenzene. In-vitro and in-vivo experiments indicated no genotoxic activity for this compound.

7 ENVIRONMENTAL IMPACT ASSESSMENT

7.1 Comparison of exposure and effects

After release into the aquatic environment, chlorobenzenes will dissolve in water: mono and dichlorobenzenes to concentrations of 100 mg/l or more, trichlorobenzenes up to 20 mg/l and tetrachlorobenzene less than 1 mg/l. While the water solubility is decreasing as the degree of chlorination increases, the lipophilicity and therefore sorptive and bioaccumulative properties increase. Bioaccumulation in fish can be predicted on the basis of $\log K_{ow}$ (2.9 - 4.6).

Chlorobenzenes are expected to be chemically stable in water. However, they are inherently biodegradable and therefore they may be degraded in the sediment if conditions are favourable. Another important elimination route from the aquatic system is by volatilization. In the atmosphere the substances will degrade rapidly by indirect photolysis with radicals.

Observed environmental concentrations for chlorobenzenes are limited, but vary from detection limits to 3 µg/l in the river Rhine (early, 1980's), whereas higher concentrations of several hundreds of µg/l are reported for surface water of contaminated locations.

The toxicity for most of these substances is well below their water solubility. Although for several isomers only a limited amount of data is available, it seems that in the groups of di- and trichlorobenzenes the isomers are about equally toxic to aquatic organisms, whereas the toxicity increases with increasing chlorination (this is more distinct if toxicity data are expressed as mol/l).

The lowest concentration of monochlorobenzene and dichlorobenzenes causing chronic effects was 0.14 mg/l with as most sensitive species the echinoderm *Paracentrotus lividus*. The lowest concentration without effect for trichlorobenzenes was 0.1 mg/l, for *Daphnia magna*. For 1,2,4,5-tetrachlorobenzene only one LOEC of 0.12 mg/l is reported. The margin of safety between these toxicity data and the above mentioned environmental levels for the river Rhine is at least a factor 30.

7.2 Water quality standard

For the estimation of a 'safe' level for the environment, an extrapolation factor can be applied to the available toxicity data which accounts for the different sensitivity of other, non-tested species in the ecosystem. Various approaches have been proposed to extrapolate from single species toxicity data to a concentration where the aquatic ecosystem is not affected (OECD, 1991). This NOEC_{ecosystem} may also be called maximal tolerable concentration or MTC.

The 'modified EPA method' can be applied even if only one LC₅₀-value is available. The method applies an assessment factor of 10 to account for each additional level of uncertainty: (1) from one or several chronic values to the NOEC_{ecosystem}, (2) from acute LC₅₀-values for at least algae, Daphnia and fish to a chronic NOEC-value, and (3) from only one acute LC₅₀-value to a chronic value. Thus, the assessment factors are 10, 100 and 1000, respectively.

Another approach is to use the variability in the sensitivity among the various test species as a means to calculate a concentration that is expected to be safe for most (e.g. 95%) of the species in the aquatic ecosystem. In other words, a concentration is calculated that is hazardous for only a small number (5%) of species. Two calculation methods are available, which differ in their basic assumptions regarding the shape of the distribution curve for the species sensitivity. One method assumes a log-logistic distribution (Aldenberg and Slob, 1991), whereas the other assumes a log-normal distribution (Wagner and Løkke, 1991).

The results of the two main approaches are presented in Table 7.2.1. As the different isomers within the group of di- and trichlorobenzenes are considered as about equally toxic, the data for all isomers are grouped and the extrapolation methods are applied to derive a MTC for the sum of all chlorobenzenes. The results are presented in Table 7.2.1:

Table 7.2.1 Maximum tolerable concentrations for chlorobenzenes

Extrapolation method	MTC ($\mu\text{g/l}$)	Remarks
monochlorobenzene		
Modified EPA	7	lowest NOEC ^a / 10
Aldenberg and Slob, 1991	7	
Wagner and Løkke, 1991	6	[95% protection level, (50% confidence,) 20 test species]
dichlorobenzenes		
Modified EPA	7	lowest NOEC ^a / 10
Aldenberg and Slob, 1991	80	[95% protection level, (50% confidence,) 20 test species]
Wagner and Løkke, 1991	77	
trichlorobenzenes		
Modified EPA	19	lowest NOEC ^a / 10
Aldenberg and Slob, 1991	73	[95% protection level, (50% confidence,) 20 test species]
Wagner and Løkke, 1991	69	
1,2,4,5-tetrachlorobenzene		
Modified EPA	6	lowest NOEC ^a / 10

a: NOEC is calculated by LOEC/2

The calculated MTC-values for the different groups range from 6 to 80 $\mu\text{g/l}$. Because of the uncertainty of the data, it is proposed that the sum of all chlorobenzenes should not exceed 10 $\mu\text{g/l}$.

Another safety factor should account for the combined effects of many other xenobiotic chemicals present in the environment. Chlorobenzenes and many other 'industrial' chemicals are neutral substances that show narcosis-type toxicity. The effects of these substances are supposed to be additive (Hermens et al., 1984). The order of magnitude for this safety factor is unknown at the present state of the art. One could suggest a safety factor of 10 when not many other environmental contaminants are expected, but when the environmental load is high, a safety factor of 1000 could be more appropriate.

The magnitude of this safety factor is not only a matter of science but political considerations are involved as well. Therefore no safety factor is applied to the MTC to derive a water quality standard for the sum of chlorobenzenes 10 $\mu\text{g/l}$. It should be realized that the proposed water quality standard does not take into account that other industrial pollutants may be present at the same time.

This proposed level is detectable by the analytical techniques as described in chapter 2.

In the previous report to EC (Hooftman and De Kreuk, 1981; 1983) No effect levels of 0.5 and 0.3 µg/l for monochlorobenzene and the sum of trichlorobenzenes, respectively were suggested to protect both freshwater and the marine environment against mono- and trichlorobenzene, respectively. In view of the recent approaches in hazard assessment, however, these limits seem to be too stringent.

When the proposed water quality standard of 10 µg/l is compared to the environmental levels found in the river Rhine (2 µg/l for total chlorobenzenes, see Chapter 3.1.3) the levels are lower but come close to the proposed water quality standard.

For trichlorobenzenes also standards were proposed by the Rhine Action Program (1991) of 0.1 µg/l for each isomer. These standards are based on the extrapolation of laboratory toxicity data of an algae, daphnia and/or fish.

Extrapolation factors of 10,000 or 1000 were used on the lowest toxicity test results. Considering the fact that the proposed water quality standard is based on the sum of all chlorobenzenes, the Rhine Action Program standards proposed for trichlorobenzene are of the same order of magnitude.

7.3

Human exposure

Exposure of man to substances in the aquatic environment may occur through oral intake from (drinking) water and from fish, shellfish or crayfish. Other possible exposure routes are dermal contact and inhalation. The route of dermal contact is relevant if a substance is lipophilic, whereas inhalation is important for highly volatile substances. For the sake of simplicity, intake by inhalation and dermal contact are considered to be negligible in relation to the oral uptake for chlorobenzenes. The human exposure analysis is therefore exclusively based on oral intake.

The calculations are performed for the concentration in water at the level of the proposed water quality standard: 10 µg/l.

Moreover it is assumed that only monochlorobenzene is present. Assuming a daily water consumption of 2 litre, the oral intake of chlorobenzene from the water will be 20 µg/day.

For monochlorobenzene the concentration in fish is estimated from the bioconcentration factor ($C_{\text{fish}}/C_{\text{water}} = 70$) to be 700 µg/kg fish. If furthermore a (relatively high) figure for the daily consumption of fish is assumed to be 100 g, the oral intake will be 70 µg/dy. The total oral intake of total chlorobenzenes is estimated at 90 µg/day or for a 60 kg person 1.5 µg/kg body weight/day. Comparison with the "tentative acceptable daily intake" for monochlorobenzenes of 0.3 mg/kg body weight/day (see chapter 6.4) shows that the margin of safety is rather high.

The 1.5 µg/kg body weight/day for monochlorobenzene can not be taken to be representative of the intake of higher substituted chlorobenzenes, as the bioconcentration factors are higher: 740 for dichlorobenzenes and 4100 for trichlorobenzene.

If it is assumed that only dichlorobenzenes or only trichlorobenzenes are present, the estimated maximum total daily intake is 13 and 69 µg/kg body weight/day, respectively. The intake of dichlorobenzenes (13 µg/kg body weight/day) is an order of magnitude below the lowest tentative acceptable daily intake of 190 µg/kg body weight/day for 1,2-dichlorobenzene. For trichlorobenzenes, however, no tentative "acceptable daily intake" is available. The estimated intake is, however, a factor 500 below no effect doses in chronic experiments with rats and monkeys (chapter 6.4).

For tetrachlorobenzene with a bioconcentration factor of 13000, the maximum daily intake is estimated at 220 mg/kg body weight/day, which is only a factor 2 above the only reported two effect dose.

The risk for human health through exposure to monochlorobenzene, dichlorobenzenes and trichlorobenzenes present in aquatic systems in a total concentration of 10 µg/l is expected to be negligible. This cannot be said for tetrachlorobenzene, which, however has not been detected in environmental samples.

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APPENDIX 1.

The calculation method for the volatilization standard half-life according to De Bruin (1985).

In the calculation method some assumptions have been made:

- water flow rate = 1 m/s
- air velocity = 3 m/s
- water depth = 1 m
- temperature = 10 °C

The calculation consists of 6 different steps that will be described below:

1 Henry Constant (H):

$$H = p/s$$

p = vapour pressure (atm.)

s = water solubility (mol/m³)

H = Henry Constant (atm·m³/mol)

2 dimensionless Henry constant (H'):

$$H' = H/RT$$

$$RT = 0.024$$

3 Liquid-Phase Exchange Constant (k(l)):

if M < 65:

$$k(l) = 20\sqrt{44/M}$$

if M > 65:

$$k(l) = 41.93\sqrt{32/M}$$

M = molecular weight

4 Gas-Phase Exchange Constant (k(g)):

$$k(g) = 4550\sqrt{18/M}$$

5 Overall liquid-phase mass transfer coefficient (K(L)):

$$K(L) = \{H' \cdot k(g) \cdot k(l)\} / \{H' \cdot k(g) + k(l)\}$$

6 Half-life (t(½)):

$$t(½) = 69.3/K(L)$$

t(½) = half-life (hours)

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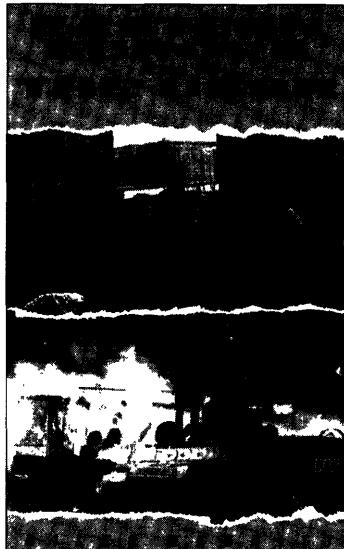
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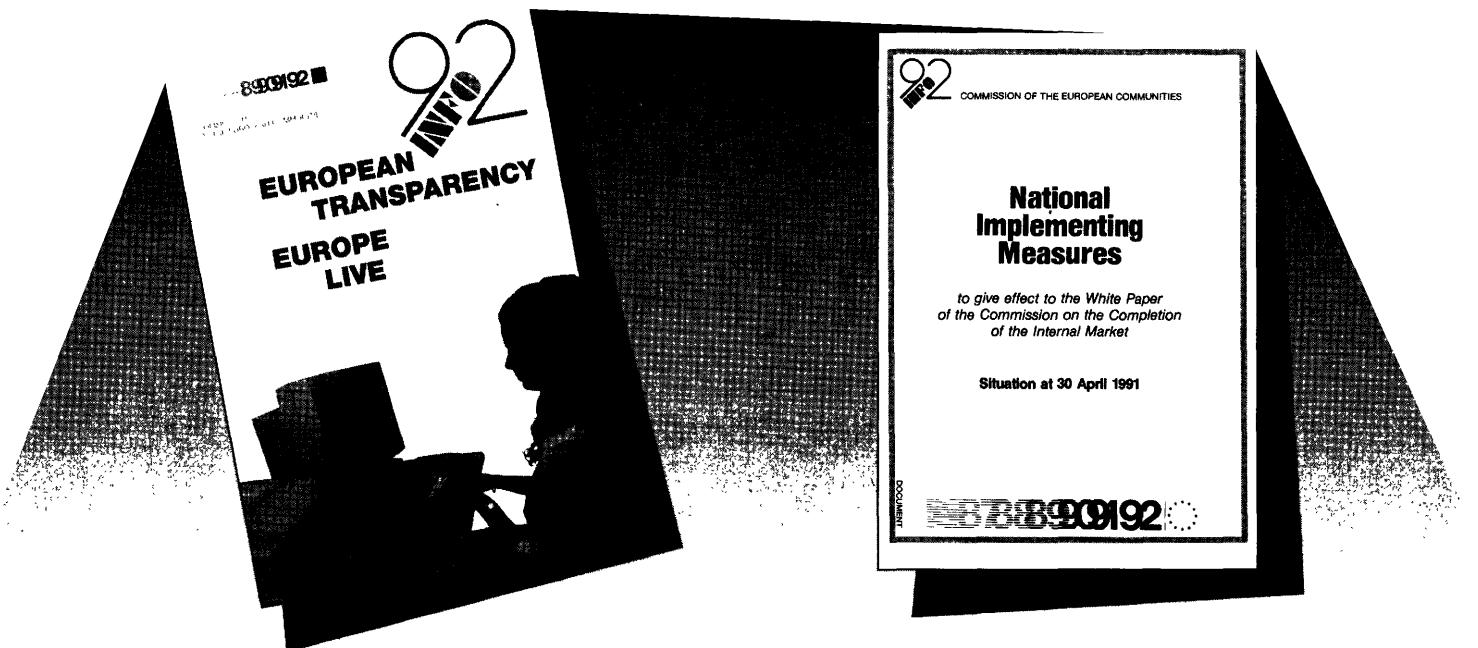
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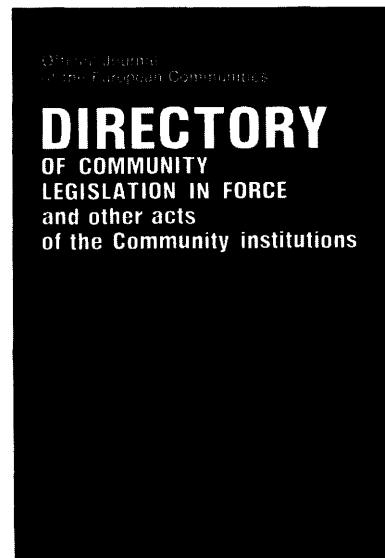
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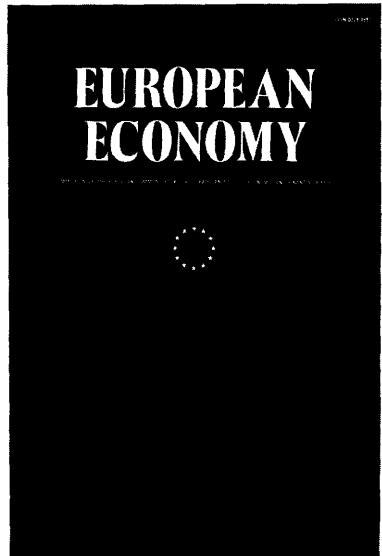
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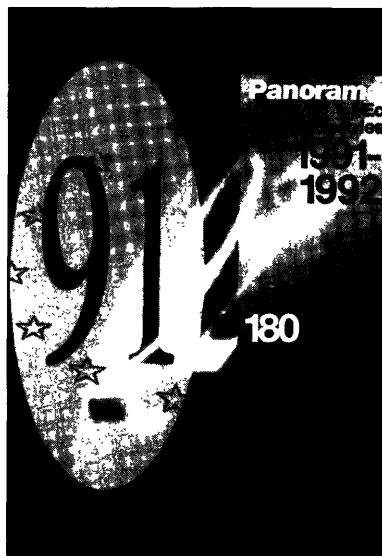
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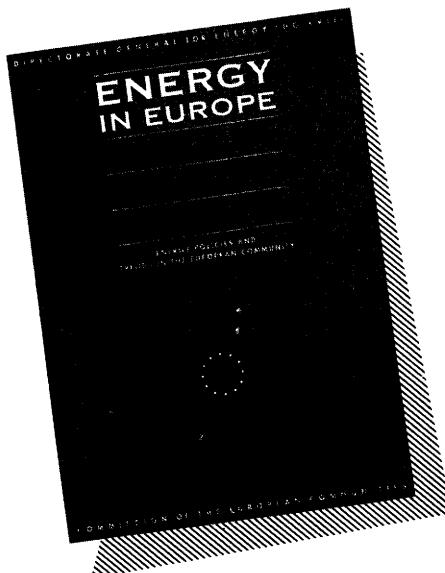
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