Euro Chlor Risk Assessment for the Marine Environment
OSPARCOM Region - North Sea

Hexachlorobenzene

January 2002

Euro Chlor
representing the chlor-alkali industry

EURO CHLOR RISK ASSESSMENT FOR THE MARINE ENVIRONMENT

HEXACHLOROBENZENE (HCB) – January 2002

OSPARCOM Region - North Sea

EXECUTIVE SUMMARY

Euro Chlor has voluntarily agreed to carry out risk assessments of 25 chemicals related to the chlorine industry. The risk assessments
were targeted on the marine environment, specifically for the North Sea. The assessments are carried out according to the methodology laid down in the EU Risk Assessment Regulation (1488/94) and the Guidance Documents of the EU Existing Substances Regulation (793/93). The exercise consists of the collection and evaluation of data on effects on aquatic organisms and environmental fate. Basically, the adverse effect data are derived from laboratory toxicity tests and the exposure data from monitoring programs. Finally, the risk is indicated by comparing the "predicted environmental concentrations" (PEC) as indices of exposure with the "predicted no effect concentrations" (PNEC) as indices of effect. This PEC/PNEC ratio is considered as the risk quotient (RQ) for the marine environment. If RQ < 1 it is presumed that the likelihood of an adverse effect is very low. An RQ > 1 is a cause for concern, necessitating a further refinement of the risk assessment and eventually for reducing the risks.

In addition to determining the risk of HCB to marine organisms in the aquatic phase, the physico-chemical properties of HCB (e.g. low water solubility, high lipophility) necessitate to consider the risks to other compartments as well.
HCB will readily partition into sediments and has the potential to accumulate in organisms. Therefore the risk to sediment organisms and the risk of secondary poisoning through the marine food chain are also considered.

The potential risks posed by HCB to the marine environment were considered by following several approaches.

1. Assessment of risk for aquatic organisms

The risk posed by HCB to organisms living in the marine environment was assessed by comparing the predicted no effect concentration (PNEC) from toxicological studies on representative aquatic organisms with the predicted environmental concentrations (PEC). Based on current ecotoxicological information, there is no reason to believe there is a difference in sensitivity for freshwater or marine species. A PNEC value of 0.37 µg/l was derived from the results of toxicological studies in organisms representing three different trophic levels, i.e. aquatic plants, invertebrates and fish.

The PEC value was determined from monitoring data for rivers discharging into the North Sea and from measurements in North Sea coastal and estuarine waters. Overall the data indicate that the typical PEC for HCB in both estuarine and marine waters is less than 0.001 µg/l (below 1 ng/l). HCB concentrations measured in river water indicate a 90-percentile of the distribution of measurements of 8 ng/l. These concentrations will be diluted when reaching the marine environment. This is supported by the typical HCB measured concentrations in coastal and marine waters of 1 ng/l. The observed distribution of concentrations also demonstrate that 8 ng/l should be considered as a worst case PEC.

The calculated worst-case PEC/PNEC ratio (RQ) for surface waters is 0.02 while the RQ based on the typical PEC is 0.003. These ratios indicate that the present levels of HCB in surface waters are unlikely to represent a risk to the marine environment in the North Sea region.

2. Assessment of risk to fish species as evaluated by bioconcentration and monitoring data

As an alternative approach to the method described above, the bioconcentration factor (BCF) and the long-term no effect concentration (NOEC) were used to calculate a critical body burden (CBB) which predicts the highest level of HCB that may be present within tissues of the organism without causing a toxic effect. This relationship is written as

$$CBB = NOEC \times BCF$$

Bioconcentration factors measured for HCB range from 300 to 35000 l/kg for which a representative BCF of 18621 l/kg was proposed (see 7.5 and Meylan *et al*., 1999). However, for this calculation a BCF of 2040 l/kg and the lowest long-term NOEC of 3.7 µg/l were used as a worst case scenario.
Using these figures the CBB for HCB is:

CBB = 3.7 µg/l x 2040 l/kg = 7548 µg/kg
or 7.5 µg/g (wet weight)

The risk posed by HCB to fish due to bioconcentration was assessed by comparing this calculated CBB value of 7.5 µg/g with the concentrations of HCB measured in marine fish of about 1 to 3 ng/g ww for edible flesh and liver, respectively. This comparison showed that the actual concentration of HCB in marine fish is about 2500 to 7500 fold lower based on liver and edible tissue concentrations, respectively than the critical body burden associated with a threshold for toxic effects. These data indicate that toxicity due to the observed bioconcentration of HCB in fish is unlikely, supporting the above conclusions on very low HCB risks for marine surface waters in the North Sea region.

3. Assessment of risk posed to organisms living in sediment

A $PEC_{sediment}$ determined by the Fraunhofer Institute (EU COMMPS Database, 1998) was used, reporting a 90-percentile value for HCB concentrations in sediment of 50 µg/kg dry

weight. A $PNEC_{sediment}$ was derived from the available toxicological data giving a $PNEC_{sediment}$ of 840 µg/kg dry weight.

The calculated worst-case RQ for the sediment compartment was 0.06, indicating that the present levels of HCB in marine sediments in the North Sea region are unlikely to represent a risk to sediment organisms.

4. Assessment of risk to fish-eating predators (biomagnification)

To assess the risk posed to predators eating fish contaminated with HCB the Predicted No Effect Concentration of HCB in these species via food uptake, i.e. the $PNEC_{oral/food}$, was compared with a calculation of the Estimated Daily Intake (EDI) of HCB for these species.

Three effect values have been used to determine the risks of HCB for fish-eating mammals via biomagnification:

- A $PNEC_{oral/food}$ for chronic toxicity in laboratory rodents of 8 µg/kg bw/d
- A $PNEC_{oral/food}$ for sub-chronic toxicity in Japanese quail of 10 µg/kg bw/d
- A $PNEC_{oral/food}$ for reproductive toxicity in the mink of 0.4 µg/kg bw/d

To assess the exposure, the EDI was calculated by multiplying the Predicted Environmental Concentration of HCB in fish, i.e. PEC_{fish} with the feeding rate (FR) of the predators.
Based on biomonitoring data the PEC_{fish} was estimated to be 1-3 µg HCB/kg bw. With feeding rates of 0.15 and 0.11 for the mink and eagle, respectively and using the worst-case PEC_{fish} of 3 µg HCB/kg bw, this gives EDIs of 0.45 µg HCB/kg bw/day for the mink and 0.33 µg HCB/kg bw/day for the eagle. The data indicate there is little risk of general toxicity occurring in fish-eating mammals or birds. However, it cannot be excluded that adverse reproductive effects may be occurring in highly sensitive species such as mink and ferret, when eating fish contaminated with HCB.

To estimate the risk posed to developing herring gull embryos the environmental concentrations of HCB measured in eggs, (PEC) of 30 to 120 ng/g ww was compared with the dose of HCB reported to reduce embryo weights, i.e. 1,500 ng/g ww (WHO IPCS, 1997). While the available information on embryo toxicity is limited, the results do indicate that the PEC of HCB in gull eggs is about 50 to 12-fold lower than the concentration of HCB in eggs reported to produce embryo toxicity. However, as a NOEC and resulting PNEC for chick embryo toxicity has not been determined it is not possible to fully exclude the possibility that concentrations of HCB in the eggs of fish-eating birds may cause toxic effects to the embryo's.

The risk of secondary poisoning posed by HCB to sea mammals or sea birds was also considered by determining bioaccumulation in such species. The available data indicate however that the concentration factors in warm-blooded animals relative to their dietary intake is not large. Quoted figures of 3 fold in liver or up to 150 fold may in fact represent a decrease in overall body burden concentrations relative to that in food. At low exposure rates the bioconcentration factor for warm-blooded animals is probably no more than 1-10 fold and may well be less than 1 in many cases, indicating that the risk of bioaccumulation and secondary poisoning in such species is low.

Overall conclusions

The calculated PEC/PNEC ratios for HCB for the various scenarios are summarised in the table below. It can be concluded that the present levels of HCB in surface water are unlikely to represent a risk to aquatic organisms in the North Sea region. This conclusion is supported by considering the bioconcentration in fish, which demonstrates that the exposures as determined by monitoring data are far below the critical body burden. Furthermore, the current levels of HCB in sediment are unlikely to pose unacceptable risks to organisms living in sediments.

The data also indicate that there is little risk of general toxicity occurring in fish eating mammals or birds. However, it cannot be excluded that adverse reproductive effects may occur in highly sensitive species such as mink, ferret, or other fish-eating mammals, since their dietary effect levels are only a few times higher than concentrations of HCB measured in various species of fish. To this end it must be noted that environmental concentrations of HCB continue to show a decreasing trend with time (Bailey, 2001), so there will be a corresponding further reduction in the risk of adverse effects in marine wild life.

Summary table for PEC/PNEC ratios for hexachlorobenzene in various environmental compartments based on worst-case scenarios

Compartment	**PEC**	**PNEC**	**PEC/PNEC**
Aquatic			
typical	0.001 µg/l	0.37 µg/l	0.003
worst case	0.008 µg/l	0.37 µg/l	0.02
Fish (CBB approach)	1-3 ng/g ww	7.5 µg/g ww	0.0001-0.0004
Sediment			
typical	8 µg/kg dw	840 µg/kg	0.01
worst case	50 µg/kg dw	840 µg/kg	0.06
	EDI	**PNEC**	**EDI/PNEC**
Predators			
- Rodent (chronic toxicity)	0.33-0.45 µg/kg bw	8 µg/kg bw/day	0.041-0.056
- Quail (sub chronic toxicity)	(for eagle-mink)	10 µg/kg bw/day	0.033-0.045
- Mink (reproductive toxicity)		0.4 µg/kg bw/day	0.825-1.13

1. INTRODUCTION: PRINCIPLES AND PURPOSES OF EURO CHLOR RISK ASSESSMENT

Within the EU a programme is being carried out to assess the environmental and human health risks for "existing chemicals", which also include chlorinated chemicals. In due course the most important chlorinated chemicals that are presently in the market will be dealt with in this formal programme. In this activity Euro Chlor members are co-operating with member state rapporteurs. These risk assessment activities include human health risks as well as a broad range of environmental scenarios.

Additionally Euro Chlor has voluntarily agreed to carry out targeted risk assessments for 25 prioritised chemicals related to the chlorine industry. These compounds are on lists of concern of European Nations participating in the North Sea Conference. The purpose of this activity is to explore if chlorinated chemicals presently pose a risk to the marine environment especially for the North Sea situation. This will indicate the necessity for further refinement of the risk assessments and eventually for additional risk reduction programmes.

These risk assessments are carried out specifically for the marine environment according to principles given in *Appendix 1*. The EU methodology is followed as laid down in the EU Risk Assessment Regulation (1488/94) and the Guidance Documents of the EU Existing Substances Regulation (793/93), (TGD, 1996). In addition the potential for HCB to produce toxicity as a result of bioconcentration has been assessed using the methodology described by Nendza (1997) based on the critical body burden. Moreover, as HCB has the potential to bioaccumulate the assessment includes an evaluation of the risk of secondary poisoning as a result of predators eating fish contaminated with HCB.

The exercise consists of the collection and evaluation of data on effects and environmental concentrations. Basically, the effect data are derived from laboratory toxicity tests and exposure data from analytical monitoring programmes. Where necessary, the exposure data are backed up with calculated concentrations based on emission models. Finally, the risk is indicated by comparing the "predicted environmental concentrations" (PEC) with the "predicted no effect concentrations" (PNEC) expressed as risk quotients (RQ) for the relevant compartments of the marine environment. This PEC/PNEC ratio is considered as the risk quotient (RQ) for the marine environment. If RQ < 1 it is presumed that the likelihood of an adverse effect is very low. An RQ > 1 is a cause for concern, necessitating a further refinement of the risk assessment and eventually for reducing the risks.

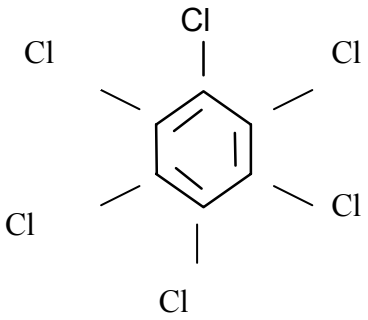
2. DATA SOURCES

The data used in this risk assessment are primarily derived from the published literature, from country-specific chemical monitoring programs (for exposure data), IPCS document on Hexachlorobenzene (WHO IPCS, 1997), and IUCLID data (2000).

3. COMPOUND IDENTIFICATION

3.1. Description

CAS number:	118-74-1
EINECS No.:	204-273-9
IUPAC Name:	hexachlorobenzene
Appearance:	white crystals or crystalline solid
Molecular Formula:	C_6Cl_6
Structural formula:	



3.2. EU labelling

According to Annex 1 of Directive 98/98/EEC hexachlorobenzene is classified as category 2 R45 (may cause cancer) and R48/25 (danger of serious damage to health by prolonged exposure if swallowed). It is also classified as dangerous for the environment (N) with risk phrases R50/53 (very toxic to aquatic organisms, may cause long term adverse effects in the aquatic environment).

4. PHYSICO-CHEMICAL PROPERTIES

Table 1 gives the major chemical and physical properties of the compound which were adopted for the purpose of this risk assessment.

Table 1: Physical and chemical properties of hexachlorobenzene
(Adopted from IUCLID data sheet)

Property	**Value**
Molecular weight	284.8
Aspect	White crystalline solid
Vapour pressure	0.0023 Pa at 25°C
Log-octanol-water partition coefficient (log K_{ow})	5.5 (5-6.92)
Koc	36,308 (3,000-180,000)
Water solubility	5 µg/l at 25°C
Henry's Law constant (calculated from solubility and vapour pressure)	131 Pa/mol per m^3

5. COMPARTMENT OF CONCERN BY MACKAY LEVEL I MODEL

The risk assessment presented here focuses on the marine environment, with special attention for the North Sea conditions where appropriate. Although this risk assessment focuses on the aquatic environment, it should be borne in mind that all environmental compartments are inter-related.

An indication of the partitioning tendency of a compound can be defined using a Mackay level I calculation obtained through the ENVCLASS software distributed by the "Nordic Council of Ministers". This model describes the ultimate distribution of the compound in the environment (Mackay & Patterson, 1990, Pedersen *et al.*, 1994).

The results describe the potency of a compound to partition between water, air, soil and sediment. Practically, it is an indicator of the potential compartments of concern. The data used for the calculation are shown in *Appendix 2* and the results of the calculation for HCB are given in Table 2.

Table 2 : Partition of hexachlorobenzene (HCB) into different environmental compartments according to Mackay level I calculation
(Mackay & Patterson, 1990)

Compartment	**%**
Air	48.3
Water	1.1
Soil	26.2
Sediment	24.4

6. PRODUCTION, USES AND EMISSIONS

6.1. Production and uses

Historically HCB had a variety of applications in agriculture being used as a seed dressing and fungicide for a variety of crops. It has also been used in a number of manufacturing processes such as aluminium and graphite rods. Due to concerns about persistence, potential to bioaccumulate and toxicological properties the use of HCB in such applications has now virtually ceased in Europe and the US. However, HCB may still be in use in some parts of the world.

6.2. Emissions

While the commercial production and use of HCB has been virtually eliminated in North America and Europe, its continued presence in the environment suggests some current releases. Today the major sources of HCB emissions are reported (WHO IPCS, 1997) to be:

- trace contaminant in certain pesticides.
- emissions from chemical processes such as production of perchloroethylene, chlorobenzenes and other chlorinated organics. (Note: nowadays in Europe HCB formed in such manufacturing processes is separated and incinerated with a high degree of efficiency).
- emissions from metal industries.
- emissions from combustion processes.
- volatilisation and leaching from landfills.

A review of HCB atmospheric emissions in the 15 OSPARCOM countries in 1990 has been published by Berdowski *et al.* (1997) and is summarised in Table 3.

Table 3: Emissions of HCB by different sectors in
15 OSPARCOM countries in 1990 (Berdowski *et al*., 1997)

Source	Amount (kg per year, 1990)
Agriculture (pesticide impurities)	650
Organic chemistry	657
Other processes	480
Industrial combustion	448
Solvent use	314
Iron and steel industry	239
Waste and disposal	35
Public power and heating	28
Total	**2851**

Data for the whole of Europe (38 countries) mentioned an estimated emission level of 8040 kg in 1990 (Berdowski *et al*., 1997) with emissions from agriculture representing 72% of the total.

Since 1985, emissions from Euro Chlor member company sites (more than 80 European sites) have decreased to 100 kg in water and 4 kg in air in 1997 (Euro Chlor, 2001). This represents a reduction of 87 and 97% respectively.

The world-wide emissions of HCB in the mid 1995 are estimated by Bailey (2001) to be in the range of 12,000 to 92,000 kg/year. The identified HCB emissions are calculated to be insufficient to explain the observed atmospheric concentration. Part of the HCB in the atmosphere is thought to come from unidentified sources along with volatilisation of "old" HCB on the soil from past contamination. Current HCB emission rates from the identified sources suggest that its concentration in the atmosphere, and thus in the rest of the environment, will slowly decline as historic HCB is degraded in the atmosphere and anaerobic sediments.

6.3. Applicable regulations

In the European Union, hexachlorobenzene emissions to water are governed by EC Directive 76/464 on pollution caused by certain dangerous substances and by Directive 88/347 setting limits to environmental releases of certain hazardous chemicals including HCB.
The European Council of Vinyl Manufacturers (ECVM) set a voluntary emission limit value of 10 µg/l in waste water discharge from EDC/VCM/PVC production plants to be committed before end 2003 (ECVM, 1998).
A number of regulatory standards for acceptable levels of HCB in water have been established by different countries and authorities. For example, the Surface Water Quality Objective defined by the EU Directive 88/347 for HCB is 0.03 µg/l, another proposed EEC Water Quality Objective for HCB is 0.01 µg/l (detection limit) due to persistence and bioaccumulation properties (CSTEE, 1994), while the WHO developed a drinking-water guideline of 1 µg/l based on an evaluation of animal carcinogenicity data.

7. EFFECT ASSESSMENT

In order to assess the risk posed by HCB in the marine environment 4 scenarios have been considered:

1. Assessment of risk for aquatic organisms
2. Assessment of risk to fish species by bioconcentration
3. Assessment of risk to organisms living in sediments
4. Assessment of risk to fish-eating predators (biomagnification)

To assess the risk HCB poses to the marine environment, it is necessary to assess the potential effects. To this end, the available toxicological information was examined to determine a Predicted No Effect Concentration for organisms living in the marine aquatic environment (i.e. $PNEC_{marine}$) for organisms living in sediment ($PNEC_{sediment}$) and for fish-eating predators ($PNEC_{oral/food}$).

7.1. Aquatic toxicity

As a first approach, this chapter only considers the following three trophic levels: aquatic plants, invertebrates and fish.

The evaluation of the data was conducted according to the quality criteria recommended by the European authorities (Commission Regulation 1488/94/EEC). The evaluation criteria are given in *Appendix 1*.

A summary of all data is given in *Appendix 3*. In total 20 data for fish, 27 data for invertebrates and 13 data for algae are given. Respectively 6, 12 and 2 data were considered valid for risk assessment purposes. For the respective taxonomic groups 5, 6 and 2 should be considered with care, and 8, 8 and 9 data, respectively, were judged as not valid for the risk assessment. The validity of 1 data point for fish and 1 data point for invertebrates could not be assigned.

It is necessary to distinguish the acute studies (LC50/EC50) from chronic studies (NOEC/EC10). In the tables presented in *Appendix 3*, the data are ranked based on class (fish, invertebrates, algae), criterion (LC50/EC50, NOEC/EC10), environment (freshwater/ saltwater) and validity (1-4).

Due to the low solubility of hexachlorobenzene, a large number of studies report that the selected acute or chronic effect did not occur at the maximum concentration that could be tested. However, a number of these studies are considered valid (Category 1) if the design and conditions of the experiment were judged to be reliable, even though the result (e.g. LC50 or NOEC) only represents a minimum ("greater than") value. The three trophic levels are reviewed hereafter.

7.1.1. Marine fish

Two acute toxicity studies are reported by Parrish *et al.* (1975) for 2 marine fish species, *Cyprinodon variegatus* and *Lagodon rhomboides*. Both were conducted in flow-through test systems with analysis of the test solutions and were considered valid. No mortalities were obtained after 96 hours at the maximum measured concentrations that could be tested, which were 0.0133 mg/l for *C. variegatus* and 0.0084 mg/l for *L. rhomboides*. Therefore, no acute effects on marine fish are reported at concentrations up to and exceeding the water solubility of hexachlorobenzene (0.005 mg/l). For risk assessment purposes, the 96h LC50s are all considered to be greater than 0.0084 mg/l based on *L. rhomboides* (Parrish *et al.*, 1975).

One longer term study with marine fish is available. This was a 10-day exposure of Gulf killifish (*Fundulus grandis*) under flow-through conditions with analysis of the solutions. The maximum concentration tested of 0.0057 mg/l (approximately equal to the water solubility) had no significant effect on survival, haematocrit and plasma cortisol levels. Because of the relatively short duration and the non-standard endpoints other than survival, the result should be used with care (category 2) (Laska *et al.*, 1978).

Therefore, no chronic toxicity to marine fish is reported at concentrations up to and including the water solubility of hexachlorobenzene (0.005 mg/l). For risk assessment purposes, the NOEC for marine fish is equal to or greater than 0.0057 mg/l (based on *F. grandis*, Laska *et al.*, 1978).

7.1.2. Freshwater fish

Twelve acute toxicity studies are reported for 11 freshwater fish species. Eight studies were considered not valid since they were carried out under static or semi-static conditions, without precautions to prevent volatile losses and without analysis of the test solutions. Seven of these (CITI, 1992; Johnson & Finley 1980; Könemann, 1981) employed concentrations well above the solubility of hexachlorobenzene. The remaining static study with *Leuciscus idus* (Von Knie *et al.*, 1983) reported a 48h LC50 of 0.007 mg/l based on nominal concentrations. Because there was no analysis of the solutions, and no other fish study found any evidence of acute toxicity, except those that tested above 10 mg/l, the result is not considered reliable.

Two studies (Calamari *et al.*, 1983) were conducted with *Onchorhynchus mykiss* and *Brachydanio rerio* under static conditions, but in closed systems to prevent volatility and with analysis of the test solutions. The LC50 values (> 0.03 mg/l for both species) refer to 24 h exposure (confirmed, Calamari, pers-comm. 2001, checked because of inconsistency in paper between text and table) and can be considered valid. The remaining acute studies were carried out under flow-through test conditions, with analysis, and are considered valid. None of these studies found any mortality of the fish at the maximum measured concentrations that could be tested, which exceeded the water solubility of hexachlorobenzene. The 96h LC50 values for *O. mykiss* and *Lepomis macrochirus* were >0.081 and >0.078 mg/l, respectively (Call *et al.*, 1983).

Therefore, in valid studies, no acute effects on freshwater fish are reported, at concentrations up to and exceeding the water solubility of hexachlorobenzene (0.005 mg/l). For risk assessment purposes, the 96h LC50s are all considered to be greater than 0.078 mg/l (based on *L. macrochirus*, Call *et al.*., 1983).

Five long-term results are reported for 3 fish species, all of which were performed in flow-through systems with analysis. The original source of a study with *B. rerio* (Korte *et al.*, 1981) could not be located (category 4) but the NOEC (0.005 mg/l) is not the lowest reported. A 90-day NOEC (0.0037 mg/l) for survival and growth of early life stages of *O. mykiss* is reported by US EPA (1988). Details of the study are not given in the original paper, although the investigator has confirmed the test conditions given in *Appendix 3*, and that the NOEC was the highest measured concentration tested (Spehar R.L., personal communication, 2000). So, category 2 was assigned to this study. The three remaining studies all showed no toxic effects at the maximum concentrations that were tested. A 10-day NOEC of 0.0258 mg/l was obtained for survival, haematocrit and observable symptoms in *Micropterus salmoides* (Laska *et al.*, 1978).An earlier report (Laseter *et al.*., 1976), which appears to describe the same study, observed changes in the kidney, liver and gall-bladder histology at 0.025 mg/l, but with no quantitative data on frequency or severity. A 28-day NOEC of 0.0038 mg/l was determined for survival and a qualitative assessment of growth of *Pimephales promelas* (Nebeker *et al.*, 1989). Both the Laska *et al.* and Nebeker *et al.* studies

should be used with care, because neither included a quantitative assessment of growth. However, a valid study is available that determined hatch, survival and growth of embryos and larvae of *P. promelas* in a flow-through system with analysis, which showed no effects after 28 days at 0.0048 mg/l, the maximum (measured) concentration tested (Carlson *et al.*, 1987; Ahmad *et al.*, 1984).

Therefore, no chronic toxicity to freshwater fish is reported at concentrations up to and including the water solubility of hexachlorobenzene. For risk assessment purposes, the NOEC for freshwater fish is equal to or greater than 0.0048 mg/l (based on *P. promelas*, Carlson & Mosian, 1987; Ahmad *et al.*, 1984). When taken into account, a 90-day NOEC for survival and growth of early life stages of *O. mykiss* (Spehar/EPA, 1988), the NOEC for freshwater fish is equal to 0.0037 mg/l.

7.1.3. Marine invertebrates

Six acute toxicity studies are reported for 6 marine invertebrates species. All reported LC50 values which were greater than the maximum concentration tested and were close to, or in excess of, the water solubility. Three were conducted under static conditions with no analysis and are considered invalid. Two studies were conducted in flow-through test systems with analysis (Parrish *et al.*, 1975) using *Palaemonetes pugio* and *Penaeus duorarum* which were judged to be valid, and provided LC50 values of > 0.017 and > 0.025 mg/l, respectively. A semi-static study with analysis was also considered valid. This used *Crangon septemspinosa* and gave a 96 hour LC50 of >0.0072 mg /l (McLeese *et al.*., 1980); thus for risk assessment purposes all valid LC/EC50s for marine invertebrates are greater than 0.0072 mg/l.

No long-term toxicity study is reported for marine invertebrates.

7.1.4. Freshwater invertebrates

Nine acute toxicity values are reported for 6 species of freshwater invertebrate (including a protozoan). Three of these were based upon nominal concentrations in static tests and are therefore considered as non-valid. The remaining values are reported as measured concentrations, and all are reported as 'greater than' values ranging from 0.0033 to 0.058 mg/l, being the maximum concentrations that were tested, due to solubility limitations. Four of these were carried out under flow-through conditions (Laska *et al.*, 1978; Nebeker *et al.*, 1989) as part of longer-term lethal studies (durations given in *Appendix 3*). Laseter *et al.*. (1976) appear to report the same study as Laska *et al.* (1978) using *Procambarus clarki*, but describe changes in the histology of the hepatopancreas at 0.0036 mg/l, but with little information on the exposure period or the frequency and severity of the effect.

Therefore, no acute effects on freshwater invertebrates are reported, at concentrations up to and exceeding the water solubility of hexachlorobenzene. For risk assessment purposes, the LC/EC50s are all greater than 0.0033 mg/l (based on *Gammarus lacustris*, Nebeker *et al.*., 1989).

Twelve long-term toxicity results are reported for freshwater invertebrates, including 4 which are also described above to determine acute toxicity (Laska *et al.*, 1978; Nebeker *et al.*, 1989) and one protozoan test (Yoshioka *et al.*, 1985).

For *Daphnia magna*, a "calculated" NOEC for reproduction of 0.00004 mg/l is reported (Scheubel, 1984). The original paper could not be obtained to validate the results (category 4), so data was abstracted from IUCLID, which also indicates 25% inhibition of reproduction at 0.00013 mg/l. However, the NOEC is several orders of magnitude lower than the other NOECs reported for *D.* and other invertebrates, and is probably not reliable. A static study with a protozoan (Geike & Parasher, 1976) employed a very high acetone level (5 ml/l) and was not considered valid. A 7-day NOEC (0.007 mg/l) for survival and reproduction of *Ceriodaphnia dubia* is reported by US EPA (1988). Details of the study are not given in the paper, although the investigator confirmed the test conditions given in *Appendix 3*, and that the NOEC was the highest measured concentration tested (Spehar R.L., personal communication, 2000). Therefore category 2 was assigned to this study.

Five of the remaining studies report measured concentrations in semi-static or flow-through systems but should be used with care (category 2). One employed freshwater oligochaetes (*Lumbriculus variegatus*) in a sand substrate (Nebeker *et al.*, 1989); one with *D.* only reported a 7-day NOEC for mortality (Nebeker *et al.*, 1989) and another only reported a LOEC of 0.023 mg/l (Calamari *et al.*, 1983). A study with *G. lacustris* provided the lowest invertebrate NOEC (0.0018 mg/l) but was not considered fully valid because significant mortality at the next higher concentration (0.0033 mg/l which was the highest concentration tested) was not attributed by the authors to the presence of hexachlorobenzene (Nebeker *et al.*, 1989).

A long-term mesocosm study using the freshwater snail, *Lymnaea palustris*, is reported by Baturo *et al.*. (1995). The snails were caged for 10 to 12 weeks in outdoor artificial pools (12 m^3) into which sediment, plants, invertebrates and fish had been introduced. Pools were treated with hexachlorobenzene by spraying to give nominal concentrations of 0.0005, 0.00125 and 0.005 mg/l, without replication, and compared with triplicate control pools. There was no effect on snail mortality at any concentration. Growth of juveniles from untreated mesocosms was not affected at any concentration when caged in the treated mesocosms; growth of adults was significantly lower in the lowest and highest concentrations of hexachlorobenzene, but not at the intermediate level. Fecundity and the utilisation of glycogen and polysaccharides were increased compared with controls, at all concentrations. Based on the absence of analytical monitoring to define the exposure, the absence of replicates of the treatments and the lack to establish a dose-effect relationship, the study was considered not reliable for risk assessment purposes (category 3).

Three long-term studies were considered valid and were based on measured concentrations. The lowest of the reported NOECs was 0.0047 mg/l for the growth, survival and reproduction of *Hyalella azteca* (Nebeker *et al.*, 1989). However, this was the maximum concentration tested, whereas a study with *D. magna* (Caspers *et al.*, 1993) provides a "true" NOEC for reproduction (0.017 mg/l), the next higher concentration causing a significant effect (15% inhibition of reproduction) and was the NOEC for survival (0.045 mg/l). At the NOEC level for reproduction, the authors

report that the nominal concentration was 0.0316 mg/l. The measured concentrations for the new solutions were 0.0216 to 0.0296 mg/l and the old solutions measured 0.0067 to 0.0082 mg/l; the NOEC is expressed here as the mean of these 4 values (0.017 mg/l) although the authors identify the lowest value. Although the nominal and measured concentrations are in excess of the water solubility of hexachlorobenzene, the result is in reasonable agreement with Calamari *et al.*. (1983) who reported a 14-day LOEC for *D. magna* of 0.023 mg/l (80% inhibition of reproduction; EC50 0.016 mg/l; NOEC not given).

Therefore, the most reliable, valid NOEC for freshwater invertebrates was 0.017 mg/l for the 21-day reproduction of *Daphnia magna* (Caspers *et al.*, 1993). If we take into account a 7-day study on survival and reproduction for *Ceriodaphnia dubia* (US EPA, 1988), the lowest NOEC would be 0.007 mg/l.

7.1.5. Marine algae

One study using two species of marine algae, *Thalassiosira pseudonana* and *Dunaliella tertiolecta*, tested together as a mixed culture, is reported (Biggs *et al.*., 1979). The test was static, with no analysis of the solutions, and was therefore not considered valid. However, no effects were observed on algal growth or size at the maximum concentration tested of 0.1 mg/l. Therefore, the result suggests that these marine species are not sensitive to hexachlorobenzene at or above the water solubility level.

7.1.6. Freshwater algae

Seven acute (EC50) values are reported for 5 freshwater algal species. Five were not considered valid because they were carried out without chemical analysis in static systems, with no precautions to prevent losses of hexachlorobenzene by volatility.

Two studies by the same authors (Calamari *et al.*, 1983) provide data for *Selenastrum capricornutum* in closed systems with analysis, measuring photosynthesis (^{14}C-fixation) after 3 hours exposure and growth over 96 hours. The former provided an approximate EC50 but should be used with care because only 2 concentrations were tested that showed any effect, and because of the non-standard endpoint. The growth study was considered valid, although the EC50 was greater than the highest concentration tested, at which there was minimal (12%) effect (see below). Thus, for risk assessment purposes, EC50 values for freshwater algae are greater than 0.03 mg/l (based on *S. capricornutum*, Calamari *et al.*., 1983).

Four of these studies provide NOEC or equivalent values. For the reasons given above, only the 96-hour growth study with *S. capricornutum* (Calamari *et al.*, 1983) was considered valid without restriction. Although a NOEC was not reported, the maximum concentration tested, 0.03 mg/l, was in excess of the water solubility and was stated to have caused 12% inhibition of growth. Since EC10 values for algae are generally accepted to be an alternative to a NOEC, this 'EC12' value is considered to be a reasonable estimate of the no effect level. Thus, the lowest, valid, $NOEC_{equivalent}$ for freshwater algae is 0.03 mg/l for *S. capricornutum* (Calamari *et al.*, 1983).

7.1.7. PNEC for marine environment

From an evaluation of the available toxicity data for aquatic organisms, few studies were able to obtain any acute or chronic effects at or below the limit of solubility, but it is reasonable to conclude that the sensitivity of both marine and freshwater organisms to hexachlorobenzene is quite similar.

A summary of the valid data selected for the derivation of PNEC values at different levels is given in Table 4. This table summarises the PNEC values derived from acute, chronic studies. When these studies are available, it is generally acknowledged that the latter are closer to real world than the former. As far as the North Sea is concerned, acute exposure is not relevant because of the absence of local sources.

The final PNEC calculated for the risk assessment of hexachlorobenzene is 0.37 µg/l.

Table 4: Summary of ecotoxicity data selected for the PNEC derivation of HCB, with the appropriate assessment factors for

Available valid data	Assigned assessment factor	Lowest toxicity values
At least 1 short-term LC50 from three trophic levels (fish, invertebrates, algae)	1000 None of these acute studies observed any toxicity at the maximum concentration tested due to solubility limitations. Therefore, a valid PNEC cannot be calculated.	- *L.s macrochirus*, LC50, 96h >0.078 mg/l, (Call *et al.*, 1983) -*G. lacustris*, LC50, 96h >0.0033 mg/l, (Nebeker *et al.,* 1989) - *S. capricornutum*, EC50, 96h >0.03 mg/l, (Calamari *et al.,* 1983)
Long-term NOEC from 3 species representing three trophic level (fish, invertebrates, algae)	10 **PNEC = 0.37 µg/l**	- *P. promelas*, NOEC*, 28d = 0.0048 mg/l, (Carlson & Kosian, 1987) - *O. mykiss*, NOEC, 90d =0.0037 mg/l (US EPA, 1988) - *D. magna*, NOEC, 21d = 0.017 mg/l, (Caspers *et al.,* 1993 - *C. dubia*, NOEC*, 7d=0.007 mg/l (US EPA, 1988) - *S. capricornutum*, EC12, 96h = 0.03 mg/l, (Calamari *et al.,* 1983)

* Maximum concentration tested, therefore represents a conservative estimate of the chronic NOEC for the trophic level.

7.2. Toxicity in sediments

7.2.1 Toxicological information on benthic invertebrates

As HCB is relatively insoluble in water and partitions strongly towards sediment, it is necessary to consider its toxicity to organisms living in the sediment. Three studies provide data on the effects of treated sediments for four species of sediment-dwelling invertebrates.

The available data on the effects on benthic organisms of HCB in sediment is summarised in *Appendix 7*. McLeese & Metcalfe, (1980) reported no mortality in a marine shrimp, *C. septemspinosa*, exposed to a measured sediment concentration of 0.3 mg HCB/kg dry weight, in a 96-hour sediment toxicity test, which was the maximum concentration tested. However, the HCB was added to the glass vessels, by evaporation of the solvent, and allowed to partition to the sediment and overlying water during the test. The overlying water concentrations were not reported (but the paper reported an aqueous 96h-LC50 of >0.0072 mg/l) and partition may not have been complete when the animals were added; the result should be used with care. The sediment was sand with a low organic carbon (OC) content (0.28%). If normalised to an OC content of 2%, the LC50 would be higher than 2.1 mg/kg dry weight.

The effects of HCB-spiked sediment, after 14 days exposure, on the survival and growth of *H. azteca* and *Chironomus tentans* (freshwater amphipod and freshwater midge larvae, respectively) were investigated by Barber *et al*. (1997). The tests were carried out according to ASTM standard methods, with analysis of the sediment concentrations, and were considered valid. There were no effects on either species at the maximum (measured) sediment concentration tested which was 84 mg/kg dry weight (normalised for 2% OC). The authors concluded that this was consistent with the absence of toxicity at the solubility limit in aqueous toxicity tests and that there was no evidence of toxicity as a result of sediment ingestion.

Fuchsman *et al.* (1998) investigated the effects of HCB-spiked sediments on the survival and growth of *C. tentans* (freshwater), the estuarine amphipod *Leptocheirus plumulosus* (at a salinity of 10‰) and *H. azteca* (under both freshwater and estuarine conditions). The 10-day tests were according to ASTM methods. Although the sediment used for spiking was known to contain a number of contaminants, including HCB, these were below the level causing significant effects. No significant incremental effects of the spiked HCB were detected for any of the species at the maximum measured concentration of 240 mg/kg dry weight, which was equivalent to 120 mg/kg dry weight when normalised to 2 % OC.

This absence of effects at 84 (Barber *et al.*., 1997) and 120 mg/kg dry weight (Fuchsman *et al.*, 1998) is in agreement with the predicted sediment quality criterion of 111.4 mg/kg dry weight (also normalised to 2 % OC) calculated by the New York Department of Environmental Conservation (1993) using the equilibrium partitioning method, but the parameters used for the prediction are unknown.

Using a predictive model described in the EU Technical Guidance Document for Risk Assessment (EC, 1996) would lead to a NOEC of 2.7 mg/kg dry weight (at 2% OC) (see *Appendix 7*).

It should be noted that the $NOEC_{water}$ was based on a study on fish, in which no toxicity was observed at the maximum concentration tested (Carlson & Kosian, 1987). Therefore, the estimated $NOEC_{sediment}$ also represents a minimum value. Because the $NOEC_{water}$ (0.0037 mg/l) was close to the aqueous solubility of HCB (0.005 mg/l), this predicted $NOEC_{sediment}$ is also approximately equal to the sediment concentration at which the porewater concentration reaches the solubility limit. Therefore, at higher sediment concentrations, the porewater concentration would not increase and any toxicity could be attributed to additional exposure resulting from sediment ingestion (or direct contact). Thus, the NOECs of 84 and 120 mg/kg dry weight demonstrate that any such additional exposure was insufficient to cause an effect.

Van Leeuwen *et al.* (1992) used a toxicity QSAR approach, employing only $\log_{Kow}$ (value used 5.73) and molecular weight, to estimate the sediment HCB level at which 95% of species in the freshwater community are unlikely to be affected. The QSAR - derived level for HCB for benthic organisms was estimated to be 2.32 mg/kg dry weight (normalised to 2 % total OC content). The corresponding aquatic (dissolved) concentration was 0.38 µg/l.

The laboratory studies and the predictions described above contrast markedly with predictions based on field data. Persaud *et al.* (1991) estimated a lowest-effect level for HCB of 0.04 mg/kg sediment (dry weight, normalised to 2% total OC content) using co-occurrence data for sediment concentrations and benthic species in the Great Lakes. The authors also estimated that benthic communities would be seriously impacted at sediment concentrations at or above 0.24 mg/kg HCB dry weight. For marine sediments, a similar approach, known as the Apparent Effects Threshold (AET) approach, was used to estimate the sediment concentration of HCB above which significant effects to benthic community composition were expected (Tetra Tech Inc., 1986). Using this approach, the effects threshold for HCB in marine sediment was predicted to be 0.0076 mg/kg dry weight (normalised to 2% total OC content). IPCS (1997) and Barber *et al.*. (1997) point out that these results illustrate the limitations of these field techniques, that they are unable to attribute effects to any one contaminant, impacted areas being invariably contaminated with a variety of chemicals.

7.2.2 Calculation of a $PNEC_{sediment}$

The calculated worst-case approach according to the TGD results in a NOEC of 2.7 mg/kg dry weight (*Appendix 7*). However, it should be noted that no effects are observed for a wide range of benthic organisms at sediment concentrations at or above 84 mg/kg dry weight (normalised to 2% OC) as shown from the available experimental data. Generally, it would be appropriate to apply an assessment factor of 10 because of the number of test data. However, since the data is restricted to survival and growth, and the maximum duration of these studies was 14 days, it is precautionary to apply a factor of 100 for a bioaccumulative substance such as HCB.

Thus, a factor of 100 applied to the NOEC concentration of 84 mg/kg gives a **$PNEC_{sediment}$ of 0.84 mg/kg dry weight (840 µg/kg)**, normalised to 2% organic carbon.

7.3 Secondary poisoning effects assessment

As food can be a significant source of exposure for a substance such as HCB which has a low water solubility and high lipid solubility, this risk assessment also addresses whether or not HCB present in the marine environment contributes to adverse effects in predatory animals feeding on marine fish.
To estimate the risk posed by HCB via uptake through the food chain (biomagnification) it is necessary to have information on the $PNEC_{oral/food}$. This represents the level of HCB present in food (in this case fish) which can be consumed by predatory species without producing adverse effects.

7.3.1 Estimation of $PNEC_{oral/food}$ for chronic toxicity

Two exposure limits for hexachlorobenzene have been proposed by the US EPA for non-cancer and cancer endpoints. For non-cancer the US EPA has proposed a HCB exposure limit of 0.8 µg/kg body wt/day. This limit was calculated by applying a 100-fold safety factor (10 for interspecies extrapolation and 10 to account for sensitive members of the human population) to the NOEC of 0.08 mg/kg body wt/day for liver toxicity from a 130-week rat study (Arnold *et al.* 1985). The exposure limit for cancer set at 6.25×10^{-3} µg/kg body wt/day was derived through the application of the linearized multi-stage mathematical dose-response extrapolation model to the hepatocellular carcinoma incidence data obtained from rats in a study conducted by Ertürk *et al.* (1986). However as genetic toxicology studies have indicated that HCB is not a genotoxic agent and the liver cancer observed in the experimental rodents apparently resulted from liver toxicity with resulting regenerative hyperplasia the use of this model to assess risk is inappropriate.

Based on these considerations the NOEC of 0.08 mg/kg body wt/day for liver toxicity is considered suitable for deriving a $PNEC_{oral/food}$. The Technical Guidance Document (1996) suggests that when chronic studies are available an assessment factor of 10 may be used. Thus applying an assessment factor of 10 to the NOEC of 0.08 mg/kg body wt/day for liver toxicity gives a $PNEC_{oral/food}$ value for chronic toxicity of 8 µg/kg body wt/day.

7.3.2 Estimation of $PNEC_{oral/food}$ for reproductive toxicity

In addition to information on chronic toxicity there are also data available on reproductive toxicology which can be used to determine a $PNEC_{oral/food}$ for reproductive effects. For example (Rush *et al.*, 1983). reported fetal and postnatal toxicity in the offspring of pregnant mink fed a diet containing HCB at concentrations of 1 or 5 ppm; the mortality rates of the weanlings was 8.2, 44.1 and 77.4 % in the 0, 1 and 5 ppm treatment groups, respectively. Bleavins *et al.*, (1984) also reported that the mink kittens born to dams fed a diet containing 1mg of HCB per kg of diet [or 0.04

mg/kg body wt (as converted by Hesse *et al.*, 1991)] were smaller than the kittens born to control animals. A NOEC was not found.

By contrast Grant *et al.* (1977) reported no evidence of reproductive toxicity when pregnant rats were fed diets containing 20 ppm of HCB, (i.e. equivalent to a dose of about 1 mg/kg bw per day). Such data support the proposition that the mink is especially sensitive to reproductive toxicants (Aulerich et al, 1977) and that the rat NOEC can not be considered to be protective of mustelid species.

Although mink feed on sea fish, the reproductive toxicology data on the mink is used to estimate the no effect concentration. Thus using the reproductive data from the mink studies (as representing the most sensitive species) and applying a 100-fold assessment factor, (i.e. includes an assessment factor of 10 since the NOEC was not determined and a second factor of 10 for reproductive affects as recommended in the Technical Guidance Document), to the lowest observed affect concentration (LOEC) of 0.04 mg/kg bw gives a $PNEC_{oral/food}$ value, for reproductive toxicology, of 0.4 µg /kg body wt.

7.3.3 Estimation of $PNEC_{oral/food}$ for birds

The above data relates to mammalian species but in considering the risk posed to fish eating birds it is also important to derive a $PNEC_{oral/food}$ for avian species. A summary of the toxicological data on Japanese quail and Eurasian Kestrel is reported in the WHO IPCS Environmental Health Criteria Report on Hexachlorobenzene (1997). In a study to examine the toxicity of HCB in birds Japanese quail were maintained for 90 days on a diet containing HCB at concentrations of 80, 20, 5, 1 or 0 µg HCB/g diet respectively (Vos, 1971). The birds in the high dose group showed tremors, liver toxicity and mortality; mean egg production was decreased in the 80 and 20 µg HCB/g diet dose groups while hepatic toxicity characterised by increase in liver weight was observed in the 5 µg HCB/g diet dose group. The no-effect level was determined to be the 1 µg HCB/g diet dose group.

Eurasian kestrels fed for 65 days with mice containing 200 µg HCB/g fresh bw showed signs of toxic effects; again a NOEC was not determined.
Using the data from the 90 day study in Japanese quail the $PNEC_{oral/food}$ for sub-chronic effects in avian species is calculated to be 10 µg/kg bw/day. The calculation assumes that a bird eats about 10% of its body weight per day (i.e. 100 grams of food for a bird weighing 1 kg) thus intake from a diet containing 1 µg HCB/g diet is about 100 µg HCB/kg bw. Applying an assessment factor of 10 for data from a 90 day study gives a $PNEC_{oral/food}$ for subchronic avian toxicity of 10 µg/kg body wt/day. As the Japanese quail is considered to be more sensitive to toxic effects than many environmentally relevant species such as gulls (Cowan *et al.*, 1995) an additional safety factor for sensitive species is not required.

7.3.4 Summary of $PNEC_{oral/food}$

In summary using the data from laboratory rodents it is possible to determine a $PNEC_{oral/food}$ for chronic toxic effects, i.e. 8 µg/kg body wt/day. A PNEC (oral/food) for reproductive toxicology was estimated using the reproductive toxicity data from the

mink which is reported to be exquisitely sensitive to reproductive toxicants (Aulerich *et al.*, 1977), i.e. 0.4 µg/kg body wt/day. Finally a $PNEC_{oral/food}$ for avian species was determined using the data on Japanese quail a species considered to be more sensitive to toxic effects of chemicals than many environmentally relevant species such as gulls (Cowan *et al.*, 1995), i.e. 10 µg/kg body wt/day.

7.4. Persistence

HCB is distributed widely in the environment and has been detected in water, sediment and various biota (see section 8). It is persistent; estimated half lives in soil from aerobic and anaerobic degradation range from 2.7 to about 22 years. MacKay *et al.* (1992) suggested the half life of HCB in water was greater than 6 years while Howard *et al.* (1991) predicted a half life in the range from 2.7 to 5.7 years. The half-life for evaporation from water was measured as ca 8 hours under laboratory conditions (Howard, 1989).

The reaction rate of HCB in air with OH radicals was measured by Hites *et al.* (1997, in Bailey, 1998) who suggested an average reaction rate of 2.5 10^{-14} cm^3 mol^{-1} sec^{-1} . Assuming a OH concentration of $5x10^5$ radicals per cm^3 the corresponding half life of HCB in air is 1.76 years. Prinn *et al.* (1995) suggested a half life of about 1 year.

7.5. Bioaccumulation

Its persistence and high octanol/water partition coefficient (log_{Kow} = 5.5) provides the combination of properties for HCB to bioconcentrate in organisms. Bioconcentration factors (BCFs) have been measured in a variety of biota and range from about 300 to higher than 35,000 l/kg (WHO IPCS, 1997). Based on the analyses of a large number of studies Meylan *et al.*, (1999) suggested a representative BCF of 18621 l/kg (Log BCF 4.27).

Higher species such as birds and mammals which can metabolise and excrete HCB, show little evidence of bioaccumulation. For example Braune and Norstrom (1989) in a field study on HCB body burden in herring gulls calculated a biomagnification factor (whole body, wet weight) of 31. It has been suggested that for continuous low exposure the BCF for warm-blooded animals is probably no more than 1-10 fold and may, in many cases, probably be less than 1.
Thus as indicated in this report and in a Swedish EPA report on POPs (1998) and Muir *et al.* (1992), birds and mammals do not appear to accumulate HCB to any great extent provided that exposures are relatively low. Based on this information it appears unlikely that birds and mammals are at risk of toxic effects via bioaccumulation.
For more detailed information on BCF values see *Appendix 6.*

8. EXPOSURE ASSESSMENT

The distribution of the concentrations in the river waters or sediments have been mainly obtained from the COMMPS database and were statistically analysed. This analysis (Govaerts *et al*., 2001) first disregards the outliers, then estimates the parameters of a log-normal distribution at each location (by applying the maximum likelihood approach) and finally aggregates all the local distributions into a regional one. The curves provided are the aggregated ones. They also include some heavily polluted areas which are not representative of the regional mean. The 90-percentile of the distribution is clearly the worst case for the marine environment which does not take into account further dilution into the sea.
In the COMMPS database, the concentrations corresponding to the locations situated at estuaries or in coastal areas have been identified and reported in *Appendix 4a* and *b* to illustrate the exposure in the marine environment more specifically.

8.1. Concentration of HCB measured in water

The exposure assessment is based on exposure data from analytical monitoring programs. HCB has been measured in a number of water systems including the marine environment and river waters.

8.1.1. Marine waters

A summary of the available monitoring data of HCB levels in the marine environment is shown in *Appendix 4a*. These data indicate that in coastal waters and estuaries concentrations of HCB range from less than 0.001 ng/l to 196 ng/l (sample from Forth Estuary in Scotland in 1987). Typical more recent data (1994-1996) suggests that marine and estuarine concentrations of HCB are mostly below 1 ng/l, which is the detection limit. Worst case concentrations could be up to 4-8 ng/l.

8.1.2 River waters

A compilation of monitoring data collected by the Water Research Council in the UK (WRc, 1998) indicates that 87% of the measurements have a mean HCB concentration of 0.005 μg/L (5 ng/l) or less. This value is supported by the statistical analysis of the COMMPS database, which contains monitoring data from rivers of six European countries (B,D,DK,F,UK,NL) (EU COMMPS, 1998). In this latter case the statistical analysis showed a 90-percentile distribution at 8 ng/l, the distribution of concentrations being illustrated in Fig. 1.

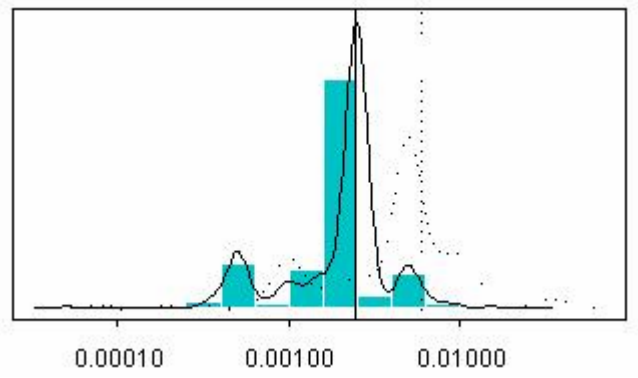


Fig. 1: Distribution of HCB concentrations in European surface waters in µg/l.

8.1.3 Calculation of a predicted environmental concentration of HCB in the marine environment (i.e. PEC_{marine})

The calculation of PEC_{marine} is based solely on monitoring data which indicate that the levels of HCB in marine waters are usually below 1 ng/l, but with some values measured in the range from 4 to 8 ng/l. As this latter value corresponds to the 90 percentile of the distribution of concentrations observed in river water, the value of 8 ng/l could be considered as a conservative worst-case PEC.

8.2. Concentrations of HCB measured in sediments

A consolidation of HCB measurements in river sediments has been presented in the EU COMMPS report from the Fraunhofer Institute (1998). The results of the statistical analysis of the corresponding database according to Govaerts *et al*. (2001) indicate that the 90-percentile value for HCB concentrations in river sediments in Europe is 50 µg/kg dry weight. The distribution of concentrations is illustrated in Fig. 2. This figure shows that there are several high local concentrations in European rivers but the levels of HCB in main estuarine sediments, as reported in *Appendix 4b*, do not exceed 24 µg/kg, with a mean value varying from 1 to 8 µg/kg dw.

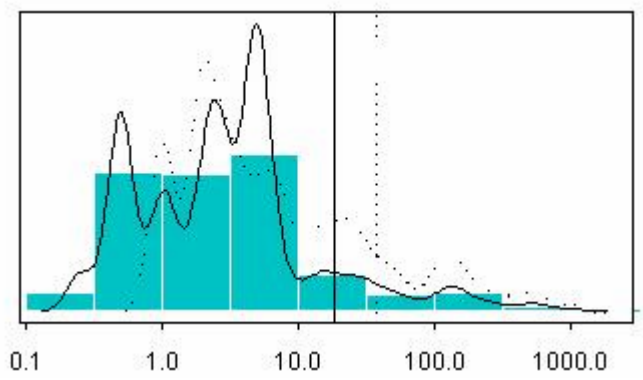


Fig.2: Distribution of HCB concentrations in sediments of European rivers, in µg/kg dry weight

8.2.1 Calculation of a predicted environmental concentration of HCB in sediment (i.e. $PEC_{sediment}$)

For the purpose of the risk assessment for sediment a worst case $PEC_{sediment}$ of 50 µg/kg dry weight has been used in the calculations. This value corresponds to the 90-percentile of the distribution of concentrations in sediment. However, typical values are generally below 24 µg/kg dry weight.

8.3. Concentrations of HCB measured in marine fish

A compilation of data describing the concentrations of HCB measured in the hepatic tissue from marine and estuarine fish has been prepared by the Water Research Center in the UK (WRc, 1998). The data indicate that the concentrations of HCB in the livers of marine fish range from 4 to 570 ng/g liver ww. Of the 72 measurements reported four analyses recorded values higher than100 ng/g liver ww, four were in the range of 50 to 100 ng/g, 28 were in the range of 10 to 50 ng/g and 36 of the measurements were less than 10 ng/g liver ww.

Measurements of HCB concentrations in the edible parts of marine fish are lower than those found in the liver. For example Ernst (1986) reported that HCB levels in muscle tissues from fish collected from the North Sea (species not reported) averaged 0.3-0.4 ng/g ww, with a maximum of 0.8 ng/g. Levels of HCB were below the determination limit (DL) in herring muscle (DL = 1 ng/g) of fish from the Clyde Sea near Scotland (Kelly and Campbell, 1994) while Kelly and Campbell (1994) reported that HCB concentrations in herring muscle, in fish collected from the Firth of Forth and the North Sea were 2.0 and 2.3 ng/g ww, respectively. Levels of HCB in muscle tissues of herring *(Clupea harengus)* from the Baltic Sea ranged from < 1 to 39 ng/g (Hansen *et al.*, 1985); concentrations in whitefish *(Coregonus lavaretus)* and trout *(Salmo trutta)* ranged from < 1 to 9 ng/g fresh weight in a 1992 survey (Atuma *et al.*, 1993). Nendza *et al.*. (1997) found a geometric mean value of 1.7 ng/g ww for marine fish.

A comprehensive study (Bignert *et al.* 1998) reports temporal trends of HCB concentrations measured in marine (herring, cod) and fresh-water fish species (pike, char) from Swedish coastal areas and lakes. The main results are given in Fig. 3-5. Statistically significant decreases of about 5 to 8% per year are observed over the last decade, if the concentrations are normalised to take into account the lipid content of the fish.
The 1995 values are all in the range of 10 to 25 ng/g lipid weight. There are no obvious spatial variations in concentrations and similar rates of decrease are observed in marine and fresh water species.

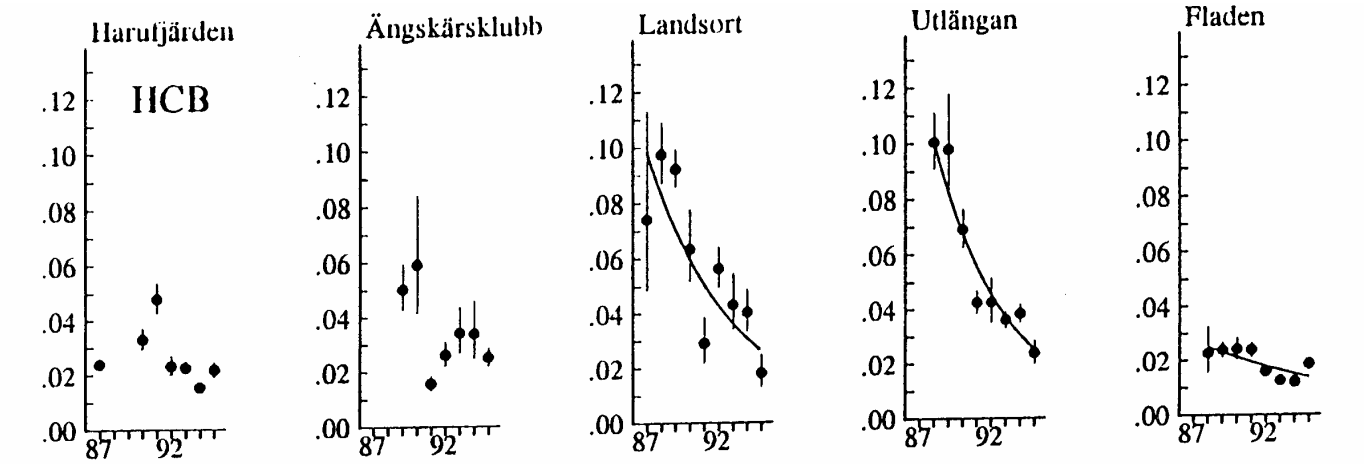


Figure 3: Concentrations of HCB in muscle tissue of herring (µg / g lipid weight) at various locations along the Swedish coast. The herrings were collected in autumn. The calculated log-linear regression line is shown if change over time is significant.

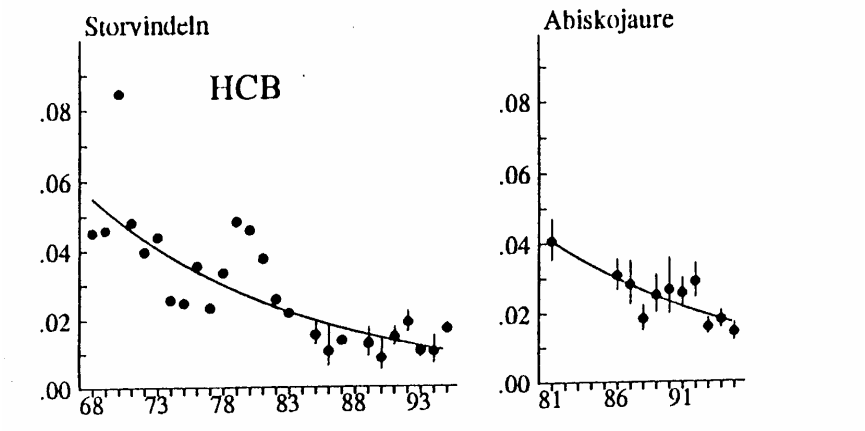


Figure 4: Concentrations of HCB (µg / g lipid weight) in pike from lake Storvindeln and in char from lake Abiskojaure in the Arctic part of Sweden. The calculated log-linear regression line is shown if change over time is significant.

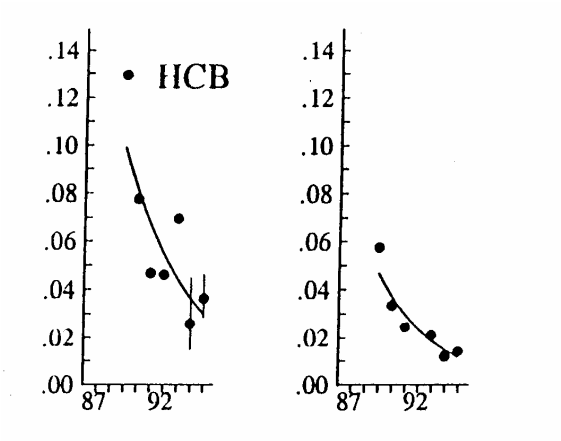


Figure 5: Concentrations of HCB (µg / g lipid weight) in cod liver in the Baltic Proper and the Swedish West coast. The calculated log-linear regression line is shown if change over time is significant.

In the 1997 issue of the "Umweltprobenbank des Bundes" report, HCB concentrations in marine and fresh water organisms are given. The values, normalised to take into

account the fat content, are reported to range from 15 to 45 µg/kg lipid weight both for mussels (M*ytilus edulis*) and eel (*Zoarces viviparus*) in the coastal area near Hamburg for the years 1992-1994.
In the Elbe estuary, the HCB concentration measured in bream (A*bramis brama*) varies between 0.8 to 1.5 µg/g lipid weight, demonstrating that the load in fresh water organisms is about 30 times higher than in the marine organisms mainly due to the dilution of local pollution in the estuary.

8.3.1 Calculation of a Predicted Environmental Concentration of HCB in fish (i.e. PEC_{fish})

Monitoring data of HCB in marine fish indicate that a typical level of HCB in muscle is about 1 ng/g while the mean concentration of HCB in liver tissue is 29.2 ng/g. Assuming fatty tissue represents 10% of the fish weight and the liver represents concentrations of HCB in "fatty tissue" a worst case assumption is that for the prey (marine fish) the PEC_{fish} is about 3 ng/g.

8.4 Concentrations of HCB measured in eggs

The mean level of HCB in herring gull eggs from Norwegian coastal waters in 1981 was 120 ng/g ww (Moksnes & Norheim, 1986). In a study from the Netherlands, mean levels in eggs of common terns collected in 1987 were 30 ng/g ww and in those of black-headed gulls collected in 1988 were 93 µg/g fat (Stronkhorst *et al.*, 1993). Levels of HCB found in eggs of sea-bird species *(Haemalopus ostralegus, Larus ridibundus, Larus argentatus* and *Sterna hirundo)* from the banks of a river near an organochlorine chemical plant in Germany were $\leq$ 500 ng/g ww (Heidmann, 1986).

Studies on the concentrations of HCB in the North American and European environment indicate that levels from 1940 and later have apparently dropped substantially in the past 30 years (PTI, 1997). For example, the concentration of HCB in herring gull eggs dropped markedly in the late 1970s with a slower decline since then (PTI, 1997). Studies on fish and other wildlife show a less dramatic decline, perhaps partly due to a shorter time span for the studies (PTI, 1997). The concentration of HCB in human blood plasma is also reported to have dropped from about 2.5 ppm in 1986 to 0.5 ppm in 1993 and in cow's milk from 6 ppb in 1973 to less than 1 ppb in 1983 (PTI, 1997).

9. RISK ASSESSMENT

The marine risk assessment for HCB described in this report is largely based the methodology laid down in the EU Risk Assessment Regulation (1488/94) and the Guidance Documents of the EU Existing Substances Regulation (793/93). The basic approach has been to use available ecotoxicological data to derive "predicted no effect concentrations" (PNEC) which is then compared with the "predicted environmental concentrations" (PEC) of HCB. If the PEC is less than the PNEC (i.e., a ratio less than one), then the prediction is that the risks are of no concern. If the PEC exceeds the

PNEC, then further refinement of the risk assessment may be necessary and eventually risk reduction may be needed.

In addition to determining the risk of HCB to marine organisms in the aquatic phase, the physico-chemical properties of HCB (e.g. low water solubility, high lipophility) necessitate to consider the risks to other compartments as well.
HCB will readily partition into sediments and has the potential to accumulate in organisms. Therefore the risk to sediment organisms and the risk of secondary poisoning through the marine food chain are also considered.

The potential risks posed by HCB to the marine environment were considered by following several approaches.

9.1 Assessment of risk for aquatic organisms

The risk posed by HCB to organisms living in the marine environment was assessed by comparing the predicted no effect concentration (PNEC) from toxicological studies with representative aquatic organisms with the predicted environmental concentrations (PEC) of HCB in marine water. A PNEC value of 0.37 µg/l was derived from the results of toxicological studies with organisms representing three different trophic levels, i.e. aquatic plants, invertebrates and fish.

The PEC value was determined from monitoring data for rivers discharging into the North Sea and from measurements in North Sea coastal and estuarine waters. Overall the data indicate that the typical PEC for HCB in both estuarine and marine waters is less than 0.001 µg/l (below 1 ng/l). HCB concentrations measured in the river water indicate a 90-percentile of the distribution of measurements of 0.008 µg/l (8 ng/l). These concentrations will be diluted when reaching the marine environment. This is supported by the measurements for sea water that rarely exceed 0.001 µg/l (i.e. 1 ng/l). From the observed distribution of concentrations, it can be seen that 0.008 µg/l (8 ng/l) should be considered as a worst case.

Based on the available toxicological and monitoring data, the calculated PEC/PNEC ratios for surface water are 0.003 and 0.02 for the typical and worst-case situation, respectively. These results indicate that the present levels of HCB in surface waters are unlikely to represent a risk to the marine environment in the North Sea region.

9.2 Assessment of risk to fish species as evaluated by bioconcentration and monitoring data

A question surrounding laboratory based toxicology studies, with chemicals which have a high potential to bioconcentrate from water, is whether the duration of laboratory based toxicity studies is sufficient to fully identify potential effects on an organism as a result of continuous exposure and uptake of the chemical from the environment. To address this question it is appropriate to consider both the bioconcentration factor (BCF) and the no effect concentration (NOEC), to calculate a critical body burden

(CBB), which predicts the level of HCB within the tissues of the organism which, if exceeded, might produce a toxic effect (Nendza *et al.*., 1997). If the CBB can be estimated reliably, it is possible to compare this with monitored concentrations in relevant field biota, to determine whether long-term, field exposure (and uptake of the chemical by any route) has resulted in body burdens approaching or exceeding the critical value. The approach assumes that the CBB resulting from such "lifetime" exposure will be the same as that which is achieved after a substantial, but shorter, exposure (to a potentially higher level) in the toxicity test, and therefore does not require that the NOEC test was sufficiently long that the body burden of the test organism would have reached steady state. However, it does assume that a critical concentration is relevant for the whole organism, or the particular tissues which have been analyzed during the bioconcentration study and the field monitoring. Similarly, the bioconcentration data need not be derived from a study in which steady state was achieved, but should be of (at least) the same duration as the NOEC study. Thus:

$$CBB = NOEC \times BCF$$

For HCB, the NOEC of 3.7 µg/l was derived from a 90 day toxicity study in fish (US EPA, 1988). For studies of similar or greater duration, the BCFs for fish range from 2,040 to approximately 45,000 (*Appendix 6*). For these purposes, it is not appropriate to adopt the highest BCF, since this might overestimate the CBB; therefore, as a conservative estimate, the BCF of 2040 for *Gambusia affinis* (Isensee *et al.*., 1976) is used. Using these values:

$$\begin{aligned} CBB &= 3.7\ \mu g/l \times 2040 \\ &= 7548\ (\mu g/kg) \\ &= 7.5\ \mu g/g\ (ww) \end{aligned}$$

The risk posed by HCB due to bioconcentration was assessed by comparing the calculated CBB of 7.5 µg/g, with the concentrations of HCB measured in marine fish which indicates about 1-3 ng/g ww for edible flesh and liver respectively (see section 8.3.1.). This comparison showed that the actual concentration of HCB in marine fish is about 2,000 to 7,500 fold lower, based on liver and edible tissue concentrations respectively, than the critical body burden associated with toxic effects. Such data indicate that toxicity due to the observed bioconcentration of HCB in fish is unlikely.

9.3 Assessment of risk posed to organisms living in sediments

The measured concentrations in estuarine sediments show a range of mean values from 1 to 8 µg/kg dw. For this risk assessment 8 µg/kg dw is used as the typical mean values for $PEC_{sediment}$

A worst case $PEC_{sediment}$ of 50 µg/kg was derived from the consolidated information on measurements of HCB in sediments prepared by the Fraunhofer Institute Umweltchemie and Ökotoxikologie (EU COMMPS report, 1998) and a $PNEC_{sediment}$ of 840 µg/kg was calculated from available toxicological information (see section 7.2.2.).

The PEC/PNEC ratio under typical conditions is 0.01, representing a safety margin of 105.

The PEC/PNEC ratio under worst case conditions is 0.06 (safety margin of 17), indicating that HCB in sediment does not pose an unacceptable risk to organisms living in the sediment.

9.4 Assessment of risk to fish-eating predators (biomagnification)

As food can be a significant source of exposure for a substance such as HCB which has a low water solubility and high lipid solubility any risk assessment also addresses whether or not HCB present in the marine environment contributes to adverse effects in predatory animals higher up the food chain which feed on marine fish.
To estimate the risk posed by HCB via uptake through the food chain it is necessary to have information on 4 parameters:

1. the $PNEC_{oral/food}$; which represents the level of HCB present in food (in this case fish) which can be consumed by predatory species higher in the food chain without producing adverse effects,
2. the predicted environmental concentration PEC_{fish}, i.e. the predicted concentration of HCB in fish
3. the estimated daily intake of HCB for predators eating fish contaminated with HCB, i.e. EDI (food) .
4. the potential for the predator to bioaccumulate HCB via the food chain which helps refine the $PNEC_{oral/food}$.

Estimated Daily Intake (EDI) of HCB by predators eating fish contaminated with HCB

The Estimated Daily Intake (EDI) of HCB in predators eating fish contaminated with HCB can be calculated from the feeding rate (FR) of the predator and the PEC of HCB in the fish, i.e. PEC_{fish}. Thus:

$$EDI = FR \times PEC_{fish}$$

where FR is the estimated as the amount of food ingested per kg body weight of the predator and the PEC_{fish} is estimated from biomonitoring studies of HCB in marine fish (see 8.3).
Published information from the US EPA (1992) estimates that the feeding rates (FR) for the mink and eagle are 0.15 and 0.11 kg food/kg bw respectively.
Assuming the PEC_{fish} of HCB in fish is 1-3 µg/kg and using the feeding rates for 0.15 and 0.11 for the mink and eagle respectively, gives EDI values of 0.45 µg HCB/kg bw/day for the mink and 0.33 µg HCB/kg bw/day for the eagle.
The consolidated information suggests that the EDI of HCB for predators (i.e. eagle and mink) eating fish contaminated with HCB is in the range of 0.33 to 0.45 µg HCB/kg bw/day. Comparing the Estimated Daily Intake of HCB with the $PNEC_{oral/food}$ for mammalian chronic toxicity, (i.e. 8 µg/kg bw/day) and the $PNEC_{food/oral}$ for avian sub-chronic toxicity, (i.e. 5 µg/kg bw/day) indicates a lack of general toxicological risk for mammals or birds eating fish contaminated with HCB. For reproductive toxicology the $PNEC_{food/oral}$ for the mink (the most sensitive species for reproductive toxicants)

C. (1984): Aquatic toxicity tests to characterize the hazard of volatile organic

was estimated to be about 0.4 µg/kg bw/day which is close to the EDI of 0.45 µg HCB/kg bw/day calculated for mink eating contaminated fish. It cannot be excluded that adverse reproductive effects may be occurring in highly sensitive species such as mink and ferret following ingestion of fish contaminated with HCB.

To estimate the risk posed to developing herring gull embryos the environmental concentrations of HCB measured in eggs, (PEC) of 30 to 120 ng/g ww was compared with the dose of HCB reported to reduce embryo weights, i.e. 1,500 ng/g ww (WHO IPCS, 1997). While the available information on embryo toxicity is limited, the results do indicate that the PEC of HCB in gull eggs is about 50 to 12-fold lower than the concentration of HCB in eggs reported to produce embryo toxicity. However, as a NOEC and resulting PNEC for chick embryo toxicity has not been determined it is not possible to fully exclude the possibility that concentrations of HCB in the eggs of fish-eating birds may cause toxic effects to the embryo's.

The above discussion on the assessment of risk posed to predators eating fish contaminated with HCB has, up to now, not addressed the concern that HCB may bioaccumulate resulting in dietary sources fo HCB contributing substantially to exposures higher in the food chain. An examination of available information suggests however that bioaccumulation in top predators is not an important factor in risk assessment. Experimental studies indicate that the concentration factors in warm blooded animals relative to their dietary intake is not large. Quoted figures of 3 fold in liver or up to 150 fold may in fact represent a decrease in overall body burden concentrations relative to that in food. At low exposure rates, the bioaccumuation factor for warm blooded animals is probably no more than 1-10 fold and may well be less than 1 in many cases (WHO IPCS Environmental Health Criteria Hexachlorobenzene, 1997; Swedish EPA, 1998; Muir *et al*., 1992) is indicating that the risk of bioaccumulation and secondary poisoning in such specis is low.

10. CONCLUSION

The calculated PEC/PNEC ratios for HCB for the various scenarios are summarised in the table below. It can be concluded that the present levels of HCB in surface water are unlikely to represent a risk to aquatic organisms in the North Sea region. This conclusion is supported by considering the bioconcentration in fish, which demonstrates that the exposures as determined by monitoring data are far below the critical body burden. Furthermore, the current levels of HCB in sediment are unlikely to pose unacceptable risks to organisms living in sediments.

The data also indicate that there is little risk of general toxicity occurring in fish eating mammals or birds. However, it cannot be excluded that adverse reproductive effects may occur in highly sensitive species such as mink, ferret, or other fish-eating mammals, since their dietary effect levels are only a few times higher than concentrations of HCB measured in various species of fish. To this end it must be noted that environmental concentrations of HCB continue to show a decreasing trend with time (Bailey, 2001), so there will be a corresponding further reduction in the risk of adverse effects in marine wild life.

Summary table for PEC/PNEC ratios for hexachlorobenzene in various environmental compartments based on worst-case scenarios

Compartment	**PEC**	**PNEC**	**PEC/PNEC**
Aquatic			
typical	0.001 µg/l	0.37 µg/l	0.003
worst case	0.008 µg/l	0.37 µg/l	0.02
Fish (CBB approach)	1-3 ng/g ww	7.5 µg/g ww	0.0001-0.0004
Sediment			
Typical	8 µg/kg dw	840 µg/kg dw	0.01
worst case	50 µg/kg	840 µg/kg dw	0.06
	EDI	**PNEC**	**EDI/PNEC**
Predators			
- Rodent (chronic toxicity)	0.33-0.45 µg/kg bw	8 µg/kg bw/day	0.041-0.056
- Quail (sub chronic toxicity)	(for eagle-mink)	10 µg/kg bw/day	0.033-0.045
- Mink (reproductive toxicity)		0.4 µg/kg bw/day	0.825-1.13

11. REFERENCES

Abernethy S., Bobra A. M., Shiu W. Y., Wells P. G. and Mackay D. (1986): Acute lethal toxicity of hydrocarbons and chlorinated hydrocarbons to 2 planktonic crustaceans: The key role of organism-water partitioning; Aquatic Tox. **8** 163-174.

Ahmad N., Benoit D., Brooke L., Call D., Carlson A., DeFoe D., Huot J., Moriarty A., Richter J., Shubat P., Veith G. and Wallbridge chemicals in water: A toxicity data summary - Parts I and II; EPA-600/3-84-009, US Environmental Protection Agency, Duluth, MN.

Arnold, D.L., Moodie, C.A., Charbonneau, S.M., Grice, H.C., McGuire, P.F., Brice, F.R., Collins, B.T., Zawidzka, Z.Z., Krewski, D.R., Nera, E.A. & Munro, I.C. (1985): Long-term toxicity of hexachlorobenzene in the rat and the effect of dietary vitamin A. Food Chem. Toxicol. 23, 779-793

Atuma, S.S., Andersson, O., Linder, C-E and Hansson, L. (1993): Levels of some organochlorine compounds in sea trout (*Salmo trutta*) and whitefish (*Caregonus lavaretus*) from the Gulf of Bothnia. Aqua. Fenn, 23: 221-226

Aulerich, R. J. and Ringer, R.K. (1977): Current status of PCB toxicity to mink and effects on their reproduction. Arch. Environ. Contam. Toxicol. 6: 279-292.

Avrahami, M. and Steele, R. T. (1972): Hexachlorobenzene II Residues in laying pullets fed HCB in their diets and effects on egg production hatchability and on chickens; N Z J of Agric Res 15 482-8

Avrahami, M. (1975): Hexachlorobenzene IV: Accumulation and elimination of HCB in pigs after oral dosing; N Z J Emptl Agric 3, 285-7

Bailey, R.E. (1998): Global Hexachlorobenzene Emissions; Report to the Chlorine Chemistry Council of the Chemical Manufacturers Association

Bailey, R.E. (2001): Global hexachlorobenzene emissions; Chemosphere, 43, 167-182

Barber, T. R., Fuchsman, P.C., Chappie, D.C., Sferra, J.C. and Sheehan, P.J. (1997): Toxicity of Hexachlorobenzene to *Hyalella azteca and Chironomus tentans* in spiked sediment bioassays. Environ. Toxicol. Chem. 16:1716-1720

Baturo, W., Lagadic, L. and Caquet, T. (1995): Growth, fecundity and glycogen utilisation in Lymnaea palustris exposed to atrazine and hexachlorobenzene in freshwater mesocosms. Env. Toxicol. Chem. 14, 503-511.

Berdowski, J.J.M., Baas, J., Bloos, J.P.J., Visschedijk, A.J.H., Zandveldt, P.Y.J. (1997): The European atmospheric emissions inventory of heavy metals and persistent organic pollutants; TNO Institute of Environmental Sciences, Energy Research and Process Innovation, The Netherlands

Biggs, D.C., Rowland, R.G. and Wurster, C.F. (1979): Effects of trichroethylene, hexachlorobenzene and polychlorinated biphenyls on the growth and cell size of marine phytoplankton. Bull. Environm. Contam. Toxicol. **21**, 196-201.

Bignert A., Olsson M., Persson W., Jensen S., Zakrisson S., Litzén K., Eriksson U., Häggberg L., Alsberg T.(1998): Temporal trends of organochlorines in Northern Europe, 1967-1995. Relation to global fractionation, leakage from sediments and international measures; Environmental Pollution 99, 177-198

Bleavins, M.R., Aulerich, R.J., and Ringer, R.K. (1984): Effects of chronic dietary hexachlorobenzene exposure on the reproductive performance and survivability of mink and European ferrets. Arch. Environ. Contam. Toxicol. 13, 357-365

Braune, B. M.and Norstrom, R. J. (1989): Dynamics of organochlorine compounds in herring gulls: III Tissue distribution and bioaccumulation in Lake Ontario gulls. Environ. Toxicol. Chem., 8:957-968

Calamari, D., Galassi, S., Setti, F. and Vighi, M. (1983): Toxicity of selected chlorobenzenes to aquatic organisms. Chemosphere 12: 253-262.

Call, D. J., Brooke L.T., Ahmad N. and Richter J.E. (1983): Toxicity and Metabolism Studies with EPA Priority Pollutants and Related Chemicals in Freshwater Organisms; EPA 600-3-83-095, PB 83-263-665.

Carlson, A.R. and Kosian P.A. (1987): Toxicity of chlorinated benzenes to fathead minnows (*Pimephales promelas*); Arch. Environ. Contam. Toxicol. **16** 129-135.

Caspers, N., Hartmann, P., Kanne, R. and Knoop G. (1993): UWSF- Z. Umweltchem. Oekotox. **5** (5) 265-270

CITI (1992): Biodegradation and Bioaccumulation: Data of Existing Chemicals Based on the CSCL Japan; Chemicals Inspection and Testing Institute, Japan .

Cowan, C.E., Versteeg, D.J., Larson, R.J. and Kloepper-Sams, P.J. (1995): Integrated approach for environment assessment of new and existing substances. Regulatory Toxicology and Pharmacology. 21, 3-31.

Craig, N.C.D. (1978): A summary of the toxicity of various materials to aquatic life; Vol IV Hexachlorobenzene; ICI Report BL/A/1867

CSTEE (1994): EEC water quality objectives for chemicals dangerous to aquatic environment (List 1); The views of the Scientific Advisory Committee on Toxicity and Ecotoxicity of Chemicals, DGXI; Environmental Contamination and Toxicology, 137, 83-110

de Vos, R.H., Bauwman, J. and Engel, A.B. (1972): Residues of organochlorine pesticides in broilers from feed fortified with known levels of these compounds; Pestic Sci 3, 421-32

de Walle F.B. (1995): Environmental presence of halogenated substances in Europe, TNO report prepared for Euro Chlor

ECVM (1998): European Council of Vinyl Manufacturers – ECVM Industry Charter for the production of Emulsion PVC based on "The environmental impact of the manufacture of PVC – A description of Best Available techniques – 1994"

Eder, G. (1984): Organische Umweltchemikalien in marinen Sedimenten. Veroeff. Inst. Meeresforsch. Bremerhaven 20, 41-48

El-Dib, M.A. and Badawy, M.I. (1985): Organochlorine insecticides and PCBs in waste sediment and fish from the Mediterranean Sea; Bull. Environ. Contam. Toxicol. 34: 216-217

Ernst, W. (1986): Hexachlorobenzene in the marine environment, distribution, fate and ecotoxicological aspects. In Morris C.R. & Cabral, J.P.R. (editors) Hexachlorobenzene proceedings of International Symposium Lyon International Agency for Research on Cancer. 212-222, IARc Scientific Publication N° 77

Ertürk, E., Lambrecht, R.W., Peters, H.A., Cripps, D.J., Gocmen, A., Morris, C.R. and Bryan, G.T. (1986) in Hexachlorobenzene: Proc. Int. Symp., IARC Sci. Publ., Hrsg. Morris, C.R. & Cabral, J.R.P.: Oncogenicity of hexachlorobenzene, Bd. 77, S. 417-423, IARC, Lyon

EU COMMPS Database (1998): Fraunhofer Institute, Umweltchemie und Ökotoxicologie, Report 97/723/3040/DEB/EI prepared for the European Commission DGXI – Proposal for a list of priority substances in the context of the draft water framework directive COM(97)49 FIN

Euro Chlor, 5th Technical Seminar – 8/9 February 2001, Barcelona: TSEM 01/265 – Organochlorine emissions and exposure levels – Prof. A LecIouxFigueroa I C and Simmons M S (1991); Structure-activity relationships of chlorobenzenes using dna measurement as a toxicity parameter in algae; Environ. Toxicol. and Chem. **10** 323-329.

Figueroa, I. and Simmons, M.S. (1991): Structure Activity Relationships of Chlorobenzenes Using DNA Measurement as a Toxicity Parameter in Algae. Environ. Toxicol. and Chem. 10:323-329

Fuchsman, P.C., Barber, T.R. and Sheehan, P.J. (1998): Sediment Toxicity Evaluation for Hexachlorobenzene; Spiked Sediment Tests with *Leptocheirus plumulosus, Hyalella azteca and Chironomus tentans*. Arch. Environ. Contam. Toxicol.35: 573-579

Geike, F. and C.V. Parasher, (1976a.): Effect of hexachlorobenzene on some growth parameters of *Chlorella pyrenoidosa*. Bull. Environ. Contam. Toxicol. 15: 670-677.

Geike, F. and C.D. Parasher (1976b): Effect of hexachlorobenzene (HCB) on growth of *Tetrahymena pyriformis*. Bull. Environ. Contam. Toxicol. 16: 347-354.

Geyer, H., Scheunert, I. and Korte, F. (1985): The effects of organic environmental chemicals on the growth of the alga *Scenedesmus subspicatus*: A contribution to environmental biology. Chemosphere 14(9) 1355-1369

Govaerts, B., Beck, B., Lecoutre, E., Le Bailly, C and Vanden Eeckaut (2001): From monitoring data to regional distributions: a practical methodology applied to water risk assessment. Paper Institut de Statistique, Université Catholique de Louvain, Louvain-la-Neuve, Belgium (also submitted to "Environmental Metnics")

Grant, D.L., Phillips, W.E.J. and Hatina, G.V. (1977): Effect of hexachlorobenzene on reproduction in the rat. Arch. Environ. Contam. Toxicol., 14: 422-425

Hansen, P.D., von Westernhaten, H. and Rosenthal, H. (1985): Chlorinated hydrocarbons and hatching success in Baltic herring spring spawners; Mar. Environ. Res. 15: 59-76.

Harper, D.J., Ridgeway, I.M. and Leatherland, T.M. (1992): Concentrations of hexachlorobenzene, trichlorobenzenes and chloroform in the waters of the Forth Estuary, Scotland. Mar. Pollut. Bull. 24 (5): 244-249

Heidmann, W.A. (1986): Hexachlorobenzene residues in selected species of land and sea birds in northern Germany. In: Morris, C.R. and J.R.P. Cabral. eds. Hexachlorobenzene: Proceedings of an International Symposium, held at IARC, Lyon, France, 24-28 June 1985. International Agency for Research of Cancer, IARC Scientific Publications No. 77, Lyon, France, pp. 223-228.

Hesse, J.B., Speijers, G.J.A. and Taalman, R.D.R.M. (1991): Integrated Criteria Document: Chlorobenzenes Effects. Appendix. NTIS PB92-133057.

Hites, R. A. Indiana University, reported by Bailey (1997)

Howard, P.H. (1989): Handbook of Environmental fate and exposure data for organic chemicals, Lewis Publishers, Chelsea, Michigan (USA)

Howard, P.H. (1991): Handbook of Environmental Degradation Rates; Lewis publ.

Isensee, A.R., Holden, E.R., Woolson, E.A. and Jones, G.E.: Soil persistence and aquatic bioaccumulation potential of hexachlorobenzene (HCB). J. Agric. Food Chem. 24, 1210-1214Jacobs, A, Blangetti, M & Hellmund, E (1974): Speicherung chlorierter Rheinwasserschadstoffe in Fettgewebe von Ratten; Vom Vasser 43; 259-274

IUCLID (2000): Public data on high volume chemicals. European Commission, Joint Research Centre, Institute for Health and Consumer Protection, European Chemicals Bureau. ISDN 92-828-8647-7.

Jacobs, A., Blangetti, M. and Heldmund, E. (1974): Accumulation of chlorinated contaminants of the Rhine in fat tissue of rats; Vom Wasser, 43:259-274

Johnson, W.W. and Finley, M.T. (1980): Handbook of acute toxicity of chemicals to fish and aquatic invertebrates; US Dept. of the Interior: Fish and Wildlife Service. Resource Publication 137, Washington DC.

Kelly, A.G. and Campbell, A. (1994): Organochlorine contaminants in liver of cod (*Gadus morhua*) and muscle of herring (C*lupea harengus*) from Scottish waters. Mar. Pollut. Bull., 28(2): 103-108

Kenega, E.E. (1975): Partitioning and uptake of pesticides in biological systems; In: Environmental dynamics of pesticides; Eds Haque, R and Freed; V H Publ Plenum press; pp 216-273

Knie, J., Hälke, A., Juhnke, I., Schiller, W. (1983): Ergebnisse der Untersuschungen von chemischen Stoffen mit vier Biotests (results of studies on chemical substances with four biotests); Dtsch. Gewaesserkundliche Mitteilungen: 27(3): 77-79

Könemann, H. (1981): Quantitative structure activity relationships in fish toxicity studies I: Relationship for 50 industrial pollutants; Toxicology 19 (3) 209-221.

Korte, F. *et al*. (1981): Ueberpruefung der Durchfuehrbarkeit von Pruefungsvorschriften und der Aussagekraft der Grundpruefung des E. Chem G. Forschungsbericht 106 04 006/01; Umweltbundesamt, Berlin

Laseter, J.L., Bartell, C.K., Laska, A.L., Holmquist, D.G., Condie, D.B., Brown, J.W. and Evans, R.L. (1976): An ecological study of hexachlorobenzene (HCB). Department of Biological Sciences, University of New Orleans, prepared for Office of Toxic Substances, Washington, D.C., U.S. Environmental Protection Agency (EPA 560/6-76-009).

Laska, A., Bartell, C., Condie, D., Brown, J., Evans, R. and Laseter J. (1978): Acute and chronic effects of hexachlorobenzene and hexachlorobutadiene in Red Swamp Crayfish *(Procambarus clarki)* and selected fish species; Toxicol. and Appl. Pharmacol. **43** 1-12.

Mackay, D., Patterson, S. (1990): Fugacity models, in: Karcher, W. Devillers, J. (Eds); Practical applications of quantitative activity relations in environmental chemistry and toxicology. 433-460

Mackay, D., Shiu, W.Y. and Ma, K.C. (1992): Illustrated handbook of physical-chemical properties and environmental fate for organic chemicals. Volume IV: oxygen, nitrogen and sulfur containing compounds. Lewis Publishers, Chelsea, Michigan, USA.

McLeese, D.W. and Metcalfe, C.D. (1980): Toxicities of eight organochlorine compounds in sediment and seawater to *Crangon septemspinosa*. Bull. Environ. Contam. Toxicol. 25: 921-928.

Metcalf, R. L., Kappor, I. P., Lu, P.-Y., Schuth, C.K. and Sherman, P. (1973): Model ecosystem studies of the environmental fate of 6 organochlorine pesticides; Env Health perspectives 4, 35-44

Meylan, W.M., Howard, P.H., Boethling, R.S., Areonson, D., Printup, H., Gouchiet, S. (1998): Improved method for estimating bioconcentration/bioaccumulation factor from octanol/water partition coefficient; Environ. Toxicol. Chem.

Mohnke, M., Rohde, K.-H., Bruegmann, L. and Franz, P. (1986): Trace analysis of some chlorinated hydrocarbons in waters by gas-liquid chromatography. J. Chromatogr. 364, 323-337

Moksnes, M.T. and Norheim, G. (1986): Levels of chlorinated hydrocarbons and composition of PCB in Herring Gull *Larus argentatus* eggs collected in Norway in 1969 compared to 1979-81. Environ. Pollut. 11: 109-116.

Monitor 16, Persistent Organic Pollutants, A Swedish View of an International Problem, Swedish EPA, 1998

Muir, D.C.G., Wagemann, R., Grift, N.P., Norstrom, R.J., Simon, M. and Lien, J. (1988): Organochlorine chemical and heavy metal contaminants in white-beaked dolphins (*Lagenorhynchus albirostris*) and pilot whales (*Globicephala melaena*) from the coast of Newfoundland, Canada. Arch. Environ. Contam. Toxicol. 17: 613-629.

Muir, D.C.G., Wagemann, R., Hargraven, B.T., Thomas, D.J., Peakall, D.B. and Norstrom, R.J. (1992): Arctic Marine ecosystem contamination, Sci. Tot. Env. 122, 75-134

Nebecker, A.V., Griffis, W.L., Wise, C.M., Hopkins, E. and Barbitta, J.A. (1989): Survival, reproduction and bioconcentration in invertebrates and fish exposed to hexachlorobenzene. Environ. Toxicol. Chem. 8: 601-611.

Nendza, M., Herbst, T., Kussatz C. and Gres, A.(1997): Potential for Secondary Poisoning and Magnification in Marine Organisms. Chemosphere, 35, 1875 - 1885 (1997).

New York Department of Environmental Conservation (1993): Technical guidance for screening contaminated sediments. Division of Fish and Wildlife and Division of Marine Resources, Albany, NY, USA.Parasher C D, Ozel M and Geike F (1978); Effect of chlorobenzene and acetone on algal growth: physiology and ultrastructure; Chem. Biol. Interact. **20** (1) 89-95.

Parasher C.D., Ozel, M. and Geike, F. (1978): Effect of chlorobenzene and acetone on algal growth: physiology and ultrastructure; Chem. Biol. Interact. 20 (1) 89-95.

Parker, J.G. (1984): The effects of selected chemicals and water quality on the marine polychaete *Ophryotrocha diadema*; Water Res. **18** 865-868.

Parrish, P.R., Cook, G.H. and Patrick, J.H. (1974): Hexachlorobenzene: Effects on several estuarine animals; Proc 28th Ann Conf SE Assoc Gamefish Commissioners; pp 179-87

Parrish, P.R., Cook, G.H. and Patrick, J.M. (1975): Hexachlorobenzene: Effects on several estuarine animals; Proc. 28th Ann. Conf. S.E. Assoc. Game Fish Commissioners, 179-186.

Pedersen, F., Tyle, H., Niemelä, J.R., Guttmann, B., Lander, L., Wedebrand, A. (1994): Environmental Hazard Classification – Data collection and interpretation guide; TemNord 1994:589

Persaud, D., Jaagumagi, R. and Hayton, A. (1991) draft: The provincial sediment quality guidelines. Ontario Ministry of the Environment, Toronto.

Prinn, R.G., Weiss, R.F., Miller, B.R., Huang, J., Alyea, F.N., Cunnold, D.M., Fraser, P.J., Hartley, D.E., Simmonds, P.G. (1995): Atmospheric trends and lifetime of CH3CCl3 and global OH concentrations; Science 269 14 july, p. 187-192

PTI Environmental Services: Persistent Chlorinated Compounds in Wildlife: Trends in North America and Europe. Chlorine Chemistry Council Publication. Technical Bulletin 97-1 (1997)

RIWA (1993) Samenwerkende Rijn- en Maas Waterleidingbedrijven - Samenstelling van het Rijnwater in 1990-91, RIWA report, The Netherlands.

Rogers, H.R., Crathorne, B. and Leatherland, T.M. (1989): Occurrence of chlorobenzene isomers in the water column of a UK estuary; Marine Pollution Bulletin, 20: 276-281

Rush, G.F., Smith, J.H., Maita, K., Bleavins, M., Aulerich, R.J., Ringer, R.K. and Hook, J.B. (1983): Perinatal hexachlorobenzene toxicity in the mink. Environ. Res., 31: 116-124 Scheubel J.B. (1984); Ueberpruefung der Durchfuehrbarkeit von Pruefungsvorschriften und der Aussagekraft der Stufe I und II des Chemikaliengesetzes.; Forschungsbericht 106 04 011/5; Umweltbundesamt, Berlin.

Scheubel J. B. (1984): Ueberpruefung der Durchfuehrbarkeit von Pruefungsvorschriften und der Aussagekraft der Stufe I und II des Chemikaliengesetzes; Forschungsbericht 106 04 011/5; Umweltbundesamt, Berlin

Spehar, R. – Personal communication – e-mail: spehar.robert@epamail.epa.gov – February 2000

Stronkhorst, J., Ysebaert, T.J., Smedes, F., Meininger, P.L., Dirksen, S. and Boudewijn, T.J. (1993): Contaminants in eggs of some waterbird species from the Scheldt, Estuary, SW Netherlands. Mar. Pollut. Bull., 26(10): 572-578

Swedish Environmental Protection Agency (1998): Persistent Organic Pollutants, a Swedish view of an international problem. Monitor 16, Swedish EPA, Stockholm, Sweden

Tetra Tech Inc. (1986): Development of sediment quality values for Puget Sound. Vol. 1. Prepared for Puget Sound Dredged Disposal Analysis and Puget Sound Estuary Program, Bellevue, Washington.

TGD (1996): Technical Guidance Document in support of the Commission Directive 93/67/EEC on risk assessment for new notified substances and the Commission Regulation (EC) 94/1488/EEC on risk assessment for existing substances (Parts I, II, II and IV) EC Catalogue numbers CR-48-96-001-EN-C, CR-48-96-002-EN-C, CR-48-96-003-EN-C, CR-48-96-004-EN-C

US EPA (1987): Acute toxicity handbook of chemicals to estuarine organisms; EPA/600/8-87/017 Environmental Research Laboratory, Gulf Breeze, FL.US EPA (1988):Ambient aquatic life water quality criteria for hexachlorobenzene. Duluth, Minnesota, US Environmental Protection Agency, Office of Research and Development

US EPA (1992): FLUSH User's manual exposure evaluation division. Office of Pollution Prevention and Toxics, 401 Main Street, Washington D.C.

Van Leeuwen, C.J., van der Zandt, P.T.J., Aldenberg, T., Verhaar, H.J.M. and Hermans, J.L.M. (1992): Application of QSARs, extrapolation and equilibrium partitioning in aquatic effects assessment. I. Narcotic industrial pollutants. Environ. Toxicol. Chem. 11: 267-282.

Vos, J.G., Breeman, H.A. and Benschop, H. (1968): The occurrence of the fungicide hexachlorobenzene in wild birds and its toxicological importance: A preliminary examination; Meded. Fac. Landbouwwet. Rijksuniv. Gent; 33 (3); 1263-69

Vos, J.G., van der Maas, H.L., Musch, A. and Ram, E. (1971): Toxicity of hexachlorobenzene in Japanese quail with special reference to porphyria, liver damage, reproduction, and tissue residues. Toxicol. Appl. Pharmacol. 18, 944-957

WHO IPCS (1997): Environmental Health Criteria 195 – Hexachlorobenzene; ISBN 92 4 157195. ISSN 0250-863X

WRc (1998): Collation and evaluation of European monitoring data on mercury and chlorinated organic compounds; report prepared for Euro Chlor – WRc – Medmenham – SL7 2HD, UK

Yoshioka, Y., Ose, Y. and Sato, T. (1985): Testing for the toxicity of chemicals with *Tetrahymena pyriformis*; The Science of the Total Environment 43 149-157.

APPENDIX 1

Environmental quality criteria for assessment of ecotoxicity data

The principal quality criteria for acceptance of data are that the test procedure should be well described (with Reference to an official guideline) and that the toxicant concentrations must be measured with an adequate analytical method.

Four cases can be distinguished and are summarised in the following table according to criteria defined in IUCLID system).

Table: Quality criteria for acceptance of ecotoxicity data

Case	Detailed description of the test	Accordance with scientific guidelines	Measured concentration	Conclusion: reliability level
I	+	+	+	[1] : valid without restriction
II	±	±	±	[2] : valid with restrictions; to be considered with care
III	insufficient or -	-	-	[3] : invalid
IV	the information to give an adequate opinion is not available			[4] : not assignable

The selected validated data LC50, EC50 or NOEC are divided by an assessment factor to determine a PNEC (Predicted No Effect Concentration) for the aquatic environment.

This assessment factor takes into account the confidence with which a PNEC can be derived from the available data: interspecies- and interlaboratory variabilities, extrapolation from acute to chronic effects.

Assessment factors will decrease as the available data are more relevant and refer to various trophic levels.

Ultimate distribution in the environment according to Mackay level 1 model (details of calculation)

Fugacity Level I calculation

Chemical: Hexachlorobenzene

Temperature (C)	20
Molecular weight (g/mol)	284.78
Vapor pressure (Pa)	.23000000E-2
Solubility (g/m3)	0.01
Solubility (nol/m3)	.17557412E-4
Henry's law constant (PA.m3/mol)	131.00
Log octanol water part. coefficient	5.50
Octanol water part. coefficient	316227.77
Organic C-water part. coefficient	129653.38
Air-water partition coefficient	0.05
Soil-water partition coefficient	3889.60
Sediment-water partition coefficient	7779.20
Amount of chemical (moles)	1
Fugacity (Pa)	.19613913E-6
Total VZ products	5098421.61

Phase properties and compositions:

Phase:	Air	Water	soil	Sediment
Volume (m3):	.6000E+10	.70000E+7	.45000E+5	.21000E+5
Density(kgm3):	.12056317E+2	.10000E+4	.15000E+4	.15000E+4
Frn org carb.:	.00000E+0	.00000E+0	.20000000E-1	.40000000E-1
Z mol/m3.Pa	.41029864E-3	.76336577E-2	.29691886E+2	.59383773E+2
VZ mol/Pa	.24617918E+7	.53435603E+5	.13361348E+7	.12470592E+7
Fugacity	.19613913E-6	.19613913E-6	.19613913E-6	.19613913E-6
Conc mol/m3	.8047562E-10	.14972590E-8	.58237409E-5	.11647481E-4
Conc g/m3	.22917847E-7	.42638942E-6	.16584849E-2	.33169698E-2
Conc ug/g	.19008993E-5	.42638942E-6	.11056566E-2	.22113132E-2
Amount mol	.48285372E+0	.10480813E-1	.26206834E+0	.24459711E+0
Amount %	48.29	1.05	26.21	24.46

APPENDIX 3

SUMMARY TABLE OF ECOTOXICITY DATA ON HEXACHLOROBENZENE

1. FISH

Species	Duration h (hours)/ d (days)	Type of Study	Criterion (LC50/EC50 NOEC/LOEC)	Concentration (mg/l)	Validity	Comments	Reference
EC50/LC50 STUDIES							
1. Freshwater							
Lepomis macrochirus	96h	AF-T	LC50	>0.078	1	No mortality	Call *et al.*, 1983
Oncorhynchus mykiss	96h	AF-T	LC50	>0.081	1	No mortality or other symptoms	Call *et al.*, 1983; Ahmad *et al.*, 1984
Oncorhynchus mykiss	48h	ASC	LC50	>0.03	1	No mortality	Calamari *et al.*, 1983
Brachydanio rerio	48h	ASC	LC50	>0.03	1	No mortality	Calamari *et al.*, 1983
Leuciscus idus	48h	NS	LC50	0.007	3		Knie *et al.*, 1983
Poecilia reticulata	14d	NSS	LC50	>0.285	3	No mortality. Also no mortality after 14days.	Könemann, 1981
Oryzias latipes	48h	NS	LC50	>5	3		CITI, 1992
Oncorhynchus kisutch	96h	NS	LC50	>50	3		Johnson and Finley, 1980
Ictalurus punctatus	96h	NS	LC50	14	3	Greatly above solubility.	Johnson and Finley, 1980
Lepomis macrochirus	96h	NS	LC50	12	3	Greatly above solubility.	Johnson and Finley, 1980
Micropterus salmoides	96h	NS	LC50	12	3	Greatly above solubility.	Johnson and Finley, 1980
Pimephales promelas	96h	NS	LC50	22	3	Greatly above solubility.	Johnson and Finley, 1980
2. Saltwater							
Lagodon rhomboides	96h	AF-T	LC50	>0.0084	1	No mortalities	Parrish *et al.*, 1975

APPENDIX 3

SUMMARY TABLE OF ECOTOXICITY DATA ON HEXACHLOROBENZENE

Cyprinodon variegatus	96h	AF-T	LC50	>0.0133	1	No mortalities	Parrish *et al.*, 1975

APPENDIX 3

SUMMARY TABLE OF ECOTOXICITY DATA ON HEXACHLOROBENZENE

Species	Duration h(hours)/ d(days)	Type of Study	Criterion (LC50/EC50 NOEC/LOEC)	Concentration (mg/l)	Validity	Comments	Reference
NOEC/LOEC STUDIES							
1. Freshwater							
Pimephales promelas	32d	AF-T	NOEC	0.0048	1	Hatch, survival and growth. Maximum concn. tested.	Carlson and Kosian, 1987; Ahmad *et al.*, 1984
Pimephales promelas	28d	AF-T	NOEC	0.0038	2	Survival. Growth normal but not analysed statistically. Maximum concn. tested.	Nebeker *et al.*, 1989
Micropterus salmoides	10d	AF-T	NOEC	0.0258	2	Survival, hematocrit and observable symptoms. Maximum concn. tested. Salinity ‰	Laska *et al.*, 1978
Oncorhynchus mykiss	90d	AF-T	NOEC	0.0037	2	Growth and survival. Maximum concn. tested.	US EPA (1987) Spehar (2000)
Brachydanio rerio	14d	AF-T	NOEC	0.005	4	Original data not located	Korte *et al.*, 1981
2. Saltwater							
Fundulus grandis	10d	AF-T	NOEC	0.0057	2	Survival, hematocrit and plasma cortisol levels. Maximum concn. tested. Salinity 4 -5 ‰.	Laska *et al.*, 1978

APPENDIX 3

SUMMARY TABLE OF ECOTOXICITY DATA ON HEXACHLOROBENZENE

2. INVERTEBRATES

Species	Duration h (hours)/d (days)	Type of Study	Criterion (LC50/EC50 NOEC/LOEC)	Concentration (mg/l)	Validity	Comments	Reference
EC50/LC50 STUDIES							
1. Freshwater							
Gammarus lacustris	96h	AF-T	LC50	>0.0033	1	28d LC50 also >0.0033 mg/l, but significant mortality. No significant mortality at 0.0018 mg/l.	Nebeker *et al.*, 1989
Hyalella azteca	96h	AF-T	LC50	>0.0047	1	30d LC50 also >0.0047 mg/l. No significant mortality at 0.0047 mg/l.	Nebeker *et al.*, 1989
Daphnia magna	48h	AF-T	LC50	>0.005	1	Solubility limit. Also no mortality after 7 days.	Nebeker *et al.*, 1989
Procambarus clarki	96h	AF-T	LC50	>0.027	1	10d LC50 also >0.027 mg/l. No significant mortality at 0.027 mg/l.	Laska *et al.*, 1978
Daphnia magna	24h	ASC	EC50	>0.03	1		Calamari *et al.*, 1986
Tanytarsus dissimilis	48h	AS	LC50	>0.058	1	(Midge larvae)	Call *et al.*, 1983
Daphnia magna	48h	NSC	LC50	>0.0047	3	Non-standard age, temperature and distilled water as diluent.	Abernethy *et al.*, 1986
Daphnia magna	24h	NS	EC50	>0.1	3		Knie *et al.*, 1983
Tetrahymena pyriformis (Protozoan)	24h	NS	EC50	>50	3	Extrapolated (estimated) value, greatly above solubility.	Yoshioka *et al.*, 1985

APPENDIX 3

SUMMARY TABLE OF ECOTOXICITY DATA ON HEXACHLOROBENZENE

Species	Duration h (hours)/d (days)	Type of Study	Criterion (LC50/EC50 NOEC/LOEC)	Concentration (mg/l)	Validity	Comments	Reference
2. Saltwater							
Crangon septemspinosa	96h	ASS	LC50	>0.0072	1	No mortalities. Renewal after 48h.	McLeese and Metcalfe., 1980
Palaemonetes pugio	96h	AF-T	LC50	>0.017	1		Parrish *et al.*, 1975
Penaeus duorarum	96h	AF-T	LC50	>0.025	1	33% mortality at this concn.	Parrish *et al.*, 1975
Artemia	24h	NSC	LC50	>0.0033	3		Abernethy *et al.*, 1986
Ophryotrocha diadema	48h	NS	LC50	>10	3	Greatly above solubility.	Parker, 1984
Crassostrea virginica	48h	NS	EC50	>1	3	Embryo-larval development	US EPA, 1987

APPENDIX 3

SUMMARY TABLE OF ECOTOXICITY DATA ON HEXACHLOROBENZENE

Species	Duration h(hours)/d(days)	Type of Study	Criterion (LC50/EC50 NOEC/LOEC)	Concentration (mg/l)	Validity	Comments	Reference
NOEC/LOEC STUDIES							
1. Freshwater							
Hyalella azteca	30d	AF-T	NOEC	0.0047	1	Growth reproduction and survival. Maximum concn. tested	Nebeker *et al.*, 1989
Daphnia magna	21d	ASS	NOEC	0.017	1	NOEC for reproduction which was inhibited 15% at 0.045 mg/l	Caspers *et al.*, 1993
Daphnia magna	21d	ASS	NOEC	0.045	1	NOEC for mortality (zero effect). Maximum concn. tested.	Caspers *et al.*, 1993
Gammarus lacustris	28d	AF-T	NOEC	0.0018	2	Parameter: survival. Significant mortality 0.0033 mg/l, but not attributed to HCB.	Nebeker *et al.*, 1989
Lumbriculus variegatus	49d	AF-T	NOEC	0.0047	2	Survival, growth and asexual reproduction. Worms held in quartz sand.	Nebeker *et al.*, 1989
Daphnia magna	7d	AF-T	NOEC	0.005	2	NOEC for mortality.	Nebeker *et al.*, 1989
Daphnia magna	14d	ASS	LOEC	0.023	2	80% inhibition of reproduction at 0.023 mg/l. NOEC not reported.	Calamari *et al.*, 1983
Procambarus clarki	10d	AF-T	NOEC	0.027	2	NOEC for mortality.	Laska *et al.*, 1978
Species	**Duration h(hours)/d(days)**	**Type of Study**	**Criterion (LC50/EC50 NOEC/LOEC)**	**Concentration (mg/l)**	**Validity**	**Comments**	**Reference**

APPENDIX 3

SUMMARY TABLE OF ECOTOXICITY DATA ON HEXACHLOROBENZENE

Ceriodaphnia dubia	7d	ASS	NOEC	0.007	2	Survival and reproduction. Maximum concn. tested	US EPA, 1988 Spehar (personal com.)
Lymnaea palustris	70 to 84 h	NS	NOEC	0.005 (?)	3	Mesocom study. No replication of treatments. No effect on survival. Growth and fecundity generally enhanced.	Baturo *et al.,* 1995
Tetrahymena pyriformis (Protozoan)	10d	NS	NOEC	<0.001	3	Acetone at 5 ml/l. Effects on dry matter, total-N, etc. No statistical analysis.	Geike and Parasher, 1976a
Daphnia magna	21d	?SS	NOEC	0.00004	4	Calculated NOEC reference could not be obtained, data abstracted from IUCLID. Test parameter: reproduction.	Scheubel, 1984
2. Saltwater							
No data available.							

APPENDIX 3

SUMMARY TABLE OF ECOTOXICITY DATA ON HEXACHLOROBENZENE

3. AQUATIC PLANTS

Species	Duration h(hours)/ d(days)	Type of Study	Criterion (LC50/EC50 NOEC/LOEC)	Concentration (mg/l)	Validity	Comments	Reference
EC50/LC50 STUDIES							
1. Freshwater							
Selenastrum capricornutum	96h	ASC	EC50	>0.03	1	Growth. 12% inhibition at this concn.	Calamari *et al.*, 1983
Selenastrum capricornutum	3h	ASC	EC50	0.03	2	Photosynthesis inhibition. Approx. value based on 2 concentrations.	Calamari *et al.*, 1983
Cyclotella meneghiniana	48h	NS	EC50	0.002	3	DNA reduction.	Figueroa and Simmons, 1991
Chlorella pyrenoidosa	76h	NS	EC50	>10	3	<50% effect on chlorophyll, dry matter, carbohydrates and total-N. Acetone as solvent at 0.33%, with aeration. Solvent control showed effects.	Parasher and Geike, 1978
Chlorella pyrenoidosa	46h	NS	EC50	>10	3	<50% effect on chlorophyll, dry matter, carbohydrates and total-N. Acetone at 0.33% with aeration. Tested at 30ºC.	Geike and Parasher, 1976b
Scenedesmus subspicatus	96h	NS	EC50	> 0.01	3		Geyer *et al.*, 1985
Haematococcus pluvialis	4h	NS	EC50	>0.04	3	Oxygen production	Knie *et al.*, 1983
2. Saltwater							
Thalassiosira pseudonana and *Dunaliella tertiolecta* (mixed)	72h	NS	EC50	>0.1	3	Growth and cell size.	Biggs *et al.,* 1979

APPENDIX 3

SUMMARY TABLE OF ECOTOXICITY DATA ON HEXACHLOROBENZENE

Species	Duration h(hours)/ d(days)	Type of Study	Criterion (LC50/EC50 NOEC/LOEC)	Concentration (mg/l)	Validity	Comments	Reference
NOEC/LOEC STUDIES							
1. Freshwater							
Selenastrum capricornutum	96h	ASC	EC12	0.03	1	12% inhibition of growth. Approx equivalent of NOEC.	Calamari *et al.*, 1983
Selenastrum capricornutum	3h	ASC	NOEC	0.018	2	Photosynthesis inhibition.	Calamari *et al.*, 1983
Scenedesmus subspicatus	96h	NS	EC10	> 0.01	3		Geyer *et al.*, 1985
Haematococcus pluvialis	4h	NS	EC10	>0.04	3	Oxygen production	Knie *et al.*, 1983
2. Saltwater							
Thalassiosira pseudonana and *Dunaliella tertiolecta* (mixed)	72h	NS	NOEC	>0.1	3	Growth and cell size. Maximum concn. tested.	Biggs *et al.,* 1979

APPENDIX 3

SUMMARY TABLE OF ECOTOXICITY DATA ON HEXACHLOROBENZENE

LIST OF ABBREVIATIONS USED IN TABLES

A = Analysis

C = Closed system or controlled evaporation

h = hour(s)

d = day(s)

N = nominal concentration

S = static

SS = semistatic

F-T = flowthrough

Validity column:
1 = valid without restriction
2 = valid with restrictions: to be considered with care
3 = invalid
4 = not assignable

APPENDIX 4a

Levels of Hexachlorobenzene (ng/l) in Sea Water and Estuaries

Region	Year of Measurement	Concentration (ng/l)	Reference
Belgian coast			
Nieuwport	1996	2 - 5	COMMPS
Knokke-Heist	1997	2 - 5	
Diksmuide	1996	2 - 5	
Blankenberge	1997	2 - 5	
Oostende	1997	3 - 8	
Schelde estuary (B)			
Berlare/Antwerpen	1996 – 1997	2 - 12	COMMPS
SchaarvanOuden	1996 – 1997	< 1 - 2	
Elbe estuary (D)			
Cuxhaven	1994 - 1995	< 0.1 - 4	COMMPS
Weser estuary (D)			
Bumen	1994	1	COMMPS
Rhine-Meuse (NL)			
Haringvlietsluizen	1996 - 1997	< 0.1 - 1	COMMPS
Seine estuary	1995 - 1996	1 - 10	COMMPS
UK			
Waver estuary	1994 – 1996	< 5	COMMPS
Wyre estuary	1994 – 1996	< 5 (up to 20)	
Lune estuary	1994 – 1996	< 5	
Ribble estuary	1994 – 1996	< 5 (up to 50)	
Kent estuary	1994 – 1996	< 5	
Mersey estuary	1994 – 1996	< 5 (up to 20)	COMMPS
North Atlantic Ocean		0.001 - 0.036	De Walle (1995)
North Sea Norwegian region		< 0.03	De Walle (1995)
Ligurian Sea		0.4 - 2.0	De Walle (1995)
Mediterranean Sea	1982-83	mean 2.13 (range ND - 12.6)	El-Dib and Badawy (1985)
North Sea Netherlands/Belgium	1993	< 10 (Detection limit 10)	RIWA (1993)
North Sea (coastal waters and estuaries)	1979-80	mean 2.7 (range 0.03 - 15)	Ernst (1986)
Scotland (Forth Estuary)	1987 1990	< 0.01 – 196 0.7 - 8.0	Rogers *et al.* (1984) Harper *et al.* (1992)
North Sea (Helgoland)		1.28 (0.04 - 4.12)	Eder (1984)
Baltic Sea		0.07 - 0.12	Mohnke (1986)

COMMPS refers to the database developed in the Fraunhofer Institute study (1998)

APPENDIX 4b

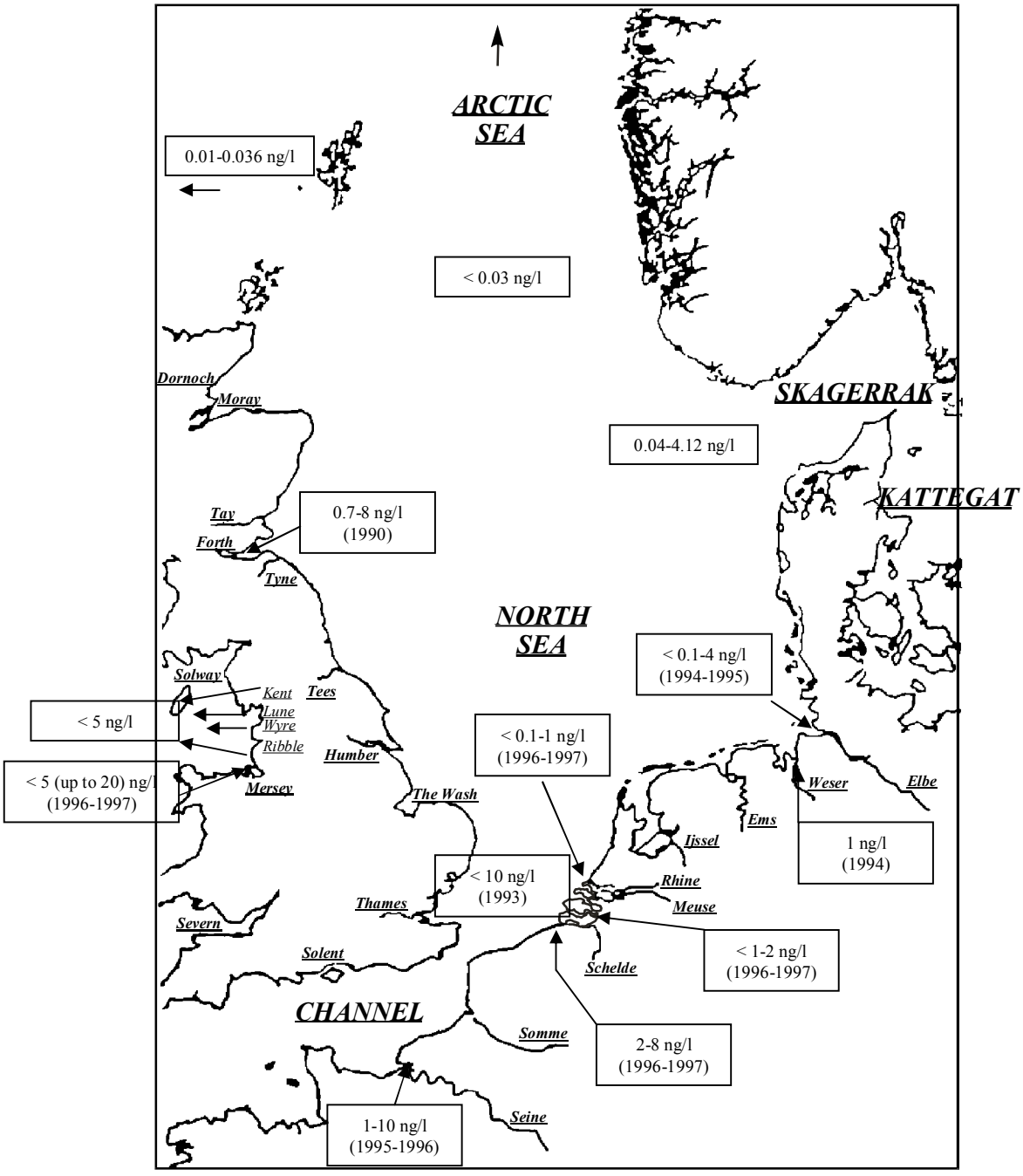
Levels of Hexachlorobenzene (µg/kg d.w.) in Sediment

Region	Year of Measurement	Concentration (µg/kg d.w.)	Reference
Elbe estuary (D) (Cuxhaven)	 1994 - 1995	 0.5 – 6.3	 COMMPS
Weser estuary (Bremen) (D)	1994 - 1995	2.3 – 11	COMMPS
Tay estuary (GB)	1996	0.2	COMMPS
Seine estuary (F)	1995 - 1996	1 - 5	COMMPS
Belgian Coast Heist op den Berg	 1994 - 1995	 10 - 16	COMMPS
Schelde estuary (B) (Pecq) Schaarvandenouden	 1994 – 1995 1996 - 1997	 < 1 – 2.4 < 1 - 4	 COMMPS
Rhine-Meuse (NL) Haringvlietsluizen	 1996 - 1997	 4 – 9	 COMMPS
Nordzee Kanaal (Ijmuiden) (NL)	 1996 - 1997	 1 – 10	 COMMPS
Rhine (D/Nl Border) Lobith Maasshuis	 1994 – 1995 1994 – 1995	 7 – 22 4 - 24	 COMMPS

COMMPS refers to the database developed in the Fraunhofer Institute study (1998)

APPENDIX 5

NORTH SEA MONITORING DATA ON HEXACHLOROBENZENE



APPENDIX 6

Bioconcentration Factors (BCF) for Hexachlorobenzene From Fresh Water by Various Fresh Water Species

Type of organism/ species	Concentration exposed	Period of exposure (d)	BCF	References
Algae				
Oedogonium cardiacum	11.5 µg/l	7	623	Laseter *et al.*, 1976
Oedogonium cardiacum	1.7 µg/l	31	90	Laseter *et al.*, 1976
Invertebrates				
Culex pipeus	0.85 µg/l	3	16	Metcalf *et al.*, 1973
Daphnia magna	0.85 µg/l	3	236	Metcalf *et al.*, 1973
Daphnia magna	1.7 µg/l	31	940	Isensee *et al.*, 1976
Crayfish - Procambarus clarki	31.7 µg/l	10	141 male 162 female	Laseter *et al.*, 1976
Molusc – Hellisoma sp	1.7 µg/l	31	1630	Insensee *et al.*, 1976
Sailfin mollies – Poecilia latipinna	62.2 µg/l	10	2397	Laseter *et al.*, 1976
Sailfin mollies – Poecilia latipinna	7.9 µg/l	10	2241	Laseter *et al.*, 1976
Fish				
Bass – Micropterus salmoides	10 µg/l	15	1100 in muscle	Laseter *et al.*, 1976
Mosquito fish – Gambusia affinis	0.85 µg/l	3	93	Metcalf *et al.*, 1973
Channel catfish – Ictalurus Punctatus	1.7 µg/l	31	15840	Isensee *et al.*, 1976
Rainbow trout – Salmo gairdneri	1 µg/l	31	8500	Kenega, 1975
Salmon – Salmo salar	2 µg/l	2	4400	Craig, 1978
Mosquito fish – Gambusia affinis	2.2 µg/l	-	93	Laseter *et al.*, 1976
Mosquito fish – Gambusia affinis	1.7 µg/l	31	2040	Isensee *et al.*, 1976

Note that several studies used an exposure concentration above the solubility level of 5 µg/l. The BCF derived from such studies may be considered as suspect.

APPENDIX 6

Bioconcentration Factors (BCF) for Hexachlorobenzene From Marine Water by Various Marine Species

Type of organism/ species	Concentration exposed	Period of exposure	BCF	References
Invertebrates				
Grass shrimp – Palaemonetes pugio	17 µg/l	4 days	1585	Parish *et al.*, 1974
Pink shrimp – Pannaeus duorarum	25 µg/l	4 days	840	Parish *et al.*, 1974
Fish				
Sheepshead minnow – Cyprinodon variegatus	13 µg/l	4 days	6690	Parish *et al.*, 1974
Pinfish – Lagodon rhomboides	8 µg/l	4 days	9405	Parish *et al.*, 1974
Pinfish – Lagodon rhomboides	5 µg/l	42 days	43000 liver 27000 muscle 48500 remainder	Parish *et al.*, 1974
Birds				
Japonese quail – Coturnix coturnix japonica	500 µg/l	4 weeks	3 in liver	Vos *et al.*, 1968
Chickens	100 µg/l	26 weeks	21 in fat	Avrahami & Steele, 1972
Chickens	0.3 µg/l	7 weeks	10 relative to food	de Vos *et al.*, 1972
Mammals				
Albino rats	2 mg/kg b.w.	12 weeks	150 in fat	Jacobs *et al.*, 1974
Pigs	10 µg/g	16 weeks	5.6 in fat	Avrahami, 1975

Bioconcentration Factors (BCF) for Hexachlorobenzene From Marine Water by Other Species

Type of organism/ species	Concentration exposed	Period of exposure	BCF	References
Birds	500 µg/l	4 weeks	3 in liver	Vos *et al.*, 1968
Japonese quail – Coturnix coturnix japonica	100 µg/l	26 weeks	21 in fat	Avrahami & Steele, 1972
Chickens	0.3 µg/l	7 weeks	10 relative to food	de Vos *et al.*, 1972
Chickens				
Mammals	2 mg/kg b.w.	12 weeks	150 in fat	Jacobs *et al.*, 1974
Albino rats	10 µg/g	16 weeks	5.6 in fat	Avrahami, 1975
Pigs				

APPENDIX 7

TOXICOLOGICAL INFORMATION ON BENTHIC INVERTEBRATES

The PNEC for sediment can be calculated from the aquatic toxicity data ($PNEC_{water}$), according to the method described in the EU TGD, based on equilibrium partitioning theory (Di Toro *et al.*, 1991). This calculation requires knowledge of the organic carbon:water partition coefficient (K_{oc}) and the characteristics of the sediment must also be defined, in particular the weight fraction of organic carbon in the sediment (F_{oc}). The TGD default for freshwater sediment is F_{oc} = 0.05 (5% organic carbon). Although this level, or higher, is typical of estuaries, particularly in the upper, silt-rich area near to the riverine input, the F_{oc} tends to decrease towards the mouth of the estuary and the coastal sea, declining to 1% or less in coarse, sandy offshore sediments. Therefore, for these purposes, a value of 2% (F_{oc} = 0.02) is selected, as a "reasonable worst-case" average for estuarine and coastal areas, since it is likely that the majority of the monitoring data (and the highest levels of contaminants) are found in these regions. It should be noted that the affinity of hydrophobic chemicals for organic carbon will result in a general positive correlation between organic matter content and contaminant concentration. Thus, although the calculated $PNEC_{sediment}$ would be lower if the F_{oc} was lower than 2%, the exposure level (PEC) in such sediment is also likely to be lower.

For substances with a K_{oc} value of 2000 or above, the $PNEC_{sediment}$ is directly proportional to F_{oc}. Therefore, if the available monitoring data specifies the organic carbon level of the sediment, the PNEC can be simply corrected to the same carbon level. (For K_{oc} <2000, the the proportionality is not exact, due to the TGD method of calculation, but is a sufficiently good approximation for these purposes).

However, a prediction using equilibrium partitioning can also be carried out based on the EU Technical Guidance Document for Risk Assessment (EC, 1996). Using the quantitative structure-activity relationship (QSAR) for calculating K_{oc} from K_{ow} for non-polar hydrophobic organics ($\log K_{oc} = 0.81 \cdot \log K_{ow} + 0.1$), $\log K_{oc}$ for HCB ($\log K_{ow}$ of 5.5) is estimated to be 4.56. The NOEC for sediment is predicted by estimating the sediment concentration that would result in a porewater concentration equal to the aquatic NOEC, as follows:

$NOEC_{sediment}$ (mg/kg dry weight) = $NOEC_{water}$ (mg/l) x K_{oc} x F_{oc}

Where: F_{oc} is the organic carbon fraction (0.02, for 2% OC):

$NOEC_{sediment}$ for HCB = 0.0037 x 36,308 x 0.02
= **2.7 mg/kg dry weight, at 2% OC.**

The EU TGD calculation is identical, except that $PNEC_{sediment}$ is calculated from $PNEC_{water}$, and the basic formula expresses the result on a wet sediment basis, as follows:

$$PNEC_{sediment} = \frac{K_{sed\text{-}water} \times PNEC_{water} \times 1000 \text{ mg/kg ww}}{RHOsed}$$

Where:
$PNEC_{water}$ is the predicted no effect concentration in water (mg/l)

APPENDIX 7

RHO_{sed} is the bulk density of wet sediment (kg/m^3), set at 1300
$K_{sed\text{-}water} = F_{solid} \times F_{oc} \times K_{oc} \times RHO_{solid} \times 10^{-3}$

Where: Fsolid is the volume fraction of solid in sediment (m^3/m^3), set at 0.2
RHO_{solid} is the density of the solid phase (kg/m^3) set at 2500
Thus, for K_{oc} = 36,308 and F_{oc} = 0.02, the $K_{sed\text{-}water}$ is 363.1

Thus, substituting NOEC for PNEC:

$NOEC_{sediment}$ = (363.1 x 0.0037 x 1,000)/1300
= 1.03 mg/kg ww

The fixed sediment characteristics define a sediment wet/dry ratio of 2.6. Therefore:

$NOEC_{sediment}$ = 1.03 x 2.6
= **2.7 mg/kg dry weight**

Toxicity Data of HCB in Sediment Dwelling Organisms

Species	**Treatment**	**Validity**	**Reference**
Crangon septemspinosa	No evidence of mortality in *Crangon septemspinosa* treated for 96 hours at a concentration of 2.1 mg/kg (normalized to 2% OC).	2	McLeese & Metcalfe, (1980)
Chironomus tentans	No significant mortality or reduction in growth following a 14-day exposure to sediments spiked at a measured concentration of 84 mg/kg (2% OC).	1	Barber *et al* (1997)
Hyella azteca	No significant mortality or reduction in growth following a 14-day exposure to sediments spiked at a measured concentration of 84 mg/kg (2% OC).	1	Barber *et al* (1997)
Leptocheirus plumulosus	No significant mortality or reduction in growth following a 10-day exposure to sediments spiked at a concentration of 120 mg/kg (2% OC).	1	Fuchsman *et al* (1998)
Hyella azteca	No significant mortality or reduction in growth following a 10-day exposure to sediments spiked at a concentration of 120 mg/kg (2% OC) in freshwater and at a salinity of 10‰ .	1	Fuchsman *et al* (1998)
Chironomus tentans)	No significant mortality or reduction in growth following a 10-day exposure to sediments spiked at a concentration of 120 mg/kg (2% OC).	1	Fuchsman *et al* (1998)