RIVM report 601501021/2004

Environmental Risk Limits for Mineral Oil (Total Petroleum Hydrocarbons)

E.M.J. Verbruggen

This investigation has been performed for the account Directorate-General for Environmental Protection, Directorate for Chemicals, Waste and Radiation, in the context of the project "Setting Integrated Environmental Quality Standards", RIVM-project no. 601501.

National Institute for Public Health and the Environment, PO Box 1, 3720 BA Bilthoven, The Netherlands.
Tel. 31-30-2749111, fax. 31-30-2742971

Abstract

In this report maximum permissible concentrations and serious risk concentrations for ecosystems are derived for mineral oil (total petroleum hydrocarbons). The used method is based on a fraction analysis approach, in which aliphatic and aromatic compounds are regarded separately and are both further divided into different fractions. For each fraction or block separate risk limits are derived. The toxic unit approach must be applied to these blocks to calculate the environmental risk limits for the total (sum) toxicity of a specific oil type.

Preface

Thanks are due drs. E.M. Maas and ing. M. Adams, who were contact persons at the ministry of Housing, Spatial Planning and the Environment (VROM-DGM/SAS). Dr. M.P.M. Janssen and drs. T.P. Traas who are involved in the RIVM-project 601501, in which the work was performed, are acknowledged for their contributions.

The results as presented in this report have been discussed by the members of the "Setting Integrated Environmental Quality Standards Advisory Group" (OZBG-eco), who are acknowledged for their contribution. The advisory group provides a non-binding scientific comment on the final draft of a report in order to advise the steering committee of the project Setting Integrated Environmental Quality Standards (INS) on the scientific merits of the report.

Further, to have scientific agreement on the risk limits for oil, a steering group has been founded in which the derivation of the risk limits has been discussed during the entire process. The regular participants of this group are Margriet Beek (RIZA), Jos Brils (TNO-MEP), Mike Comber (CONCAWE), Dennis Kalf (RIZA), Tom Parkerton (CONCAWE) Janny Pijnenburg (RIKZ), and Theo Traas (RIVM). They are acknowledged for their fruitful discussions, feedback and contribution to this report. Further, the following people who attended one or more meetings are also acknowledged for their valuable contribution: Joop Harmsen (WUR-Alterra), Heather Leslie (UU/UvA), Janneke Lourens (RIKZ), Leonard Osté (RIZA), Jaap Postma (AquaSense), Serge Rotteveel (RIZA), and Martin Scholten (TNO-MEP).

For the feasibility of the analytical method that underlies the fraction approach a conference call was organised. John Flaherty (Exygen Research, PA, USA), Ileana Rhodes (Shell/Equilon, TX, USA), Ruud Peters (TNO-MEP, The Netherlands), Dan Letinski (Exxon Mobil Biomedical Sciences, NJ, USA) are thanked for their participation and Tom Parkerton for helping in organising this call.

Further, all people who contributed to the oil experiments before the onset of this project in October 2001 are acknowledged. Of these people especially Leon de Poorter, Martin Scholten and Wiegert Dulfer are acknowledged for initiating the fresh water sediment program. Bert van Hattum is acknowledged for his contribution to the block method in the marine sediment program.

Contents

Samenvatting

In dit rapport zijn maximaal toelaatbare risiconiveaus (MTR's) en "serious risk concentrations" voor ecosystemen (SRC's$_{eco}$) afgeleid voor minerale olie, ook wel aangeduid als "total petroleum hydrocarbons" (TPH). Deze milieurisicogrenzen worden afgeleid op basis van ecotoxicologische en milieuchemische data, en representeren het potentiële risico van stoffen voor een ecosysteem. Ze vormen de wetenschappelijke basis voor milieukwaliteitsnormen die worden vastgesteld door het ministerie van VROM.
Minerale olie is te beschouwen als een complex mengsel van verschillende alifatische en aromatische koolwaterstoffen. De samenstelling van minerale olie in het milieu kan sterk variëren. De huidige norm voor olie houdt geen rekening met deze variabiliteit en is dan ook niet direct te relateren aan de toxiciteit van minerale olie. Omdat verschillende oliecomponenten verschillend accumulatiegedrag bezitten, werd minerale olie opgedeeld in verscheidene fracties, waarbij de scheiding tussen alifatische en aromatische stoffen de belangrijkste is. Het milieuchemisch gedrag (log K_{ow}, log K_{oc}, oplosbaarheid en BCF) van de stoffen in deze afzonderlijke fracties is relatief homogeen. Tussen de fracties onderling bestaan wel grote verschillen in deze parameters. In dit rapport worden over fracties verdeelde milieurisicogrenzen voorgesteld die wel rekening houden met de verschillen in toxiciteit van verschillende oliecomponenten. De achtergrond hiervan en de methodes die zijn gehanteerd zijn hieronder kort samengevat.
Er is vanuit gegaan dat de toxiciteit van minerale olie voornamelijk wordt veroorzaakt door narcose, waarvoor de totale concentratie aan stoffen in de celmembraan de verklarende parameter is. De toxiciteit van narcotische stoffen is verschillend als deze wordt gerelateerd aan de externe concentratie, als gevolg van verschillend accumulatiegedrag. Echter, verschillende stoffen veroorzaken bij op molaire basis gelijke concentraties in de celmembraan een vergelijkbaar narcotisch effect. De gesommeerde interne concentratie van verschillende stoffen leidt tot hetzelfde narcotische effect als dat van een zelfde concentratie van een afzonderlijke stof. Dit laatste verschijnsel wordt ook wel concentratie-additie genoemd. Vanwege deze eigenschappen van narcose, werden voor het afleiden van de milieurisicogrenzen van minerale olie interne concentraties van de verschillende fracties berekend.
Om de totale interne membraanconcentraties te berekenen, werd eerst de opgeloste concentratie van de verschillende fracties in water berekend, door de partitie tussen organisch koolstof en water en de wateroplosbaarheid van stoffen aanwezig als mengsel te beschouwen. Hieruit werd voor iedere fractie de concentratie in het membraan berekend met behulp van een partitiecoëfficiënt tussen de celmembraan en water. Vervolgens werden de molaire concentraties van alle fracties gesommeerd.
De respons uit de toxiciteitsstudies werd daarna uitgezet tegen de al of niet log-getransformeerde concentraties. Uit de dosis respons vergelijking werden een EC10 en EC50 berekend. Verder werd door middel van een statistische analyse van de variantie een NOEC afgeleid.
De gehanteerde methodiek wijkt op enkele punten af van de gangbare afleiding van milieurisicogrenzen. Een afwijking van de gebruikelijke methodiek is het feit dat de toxiciteit, en dus ook de risiconiveaus, niet direct aan de externe concentraties worden gerelateerd. Ook het feit dat milieurisicogrenzen in samenhang worden afgeleid voor verschillende onderdelen van een mengsel is afwijkend.
Omdat er voor het op deze wijze van afleiden van milieurisicogrenzen relatief weinig gegevens beschikbaar waren, zijn er door het Rijksinstituut voor Kust en Zee (RIKZ) en het Rijksinstituut voor Integraal Zoetwaterbeheer en Afvalwaterbehandeling (RIZA) aanvullende

toxiciteitsexperimenten verricht met twee typen olieproducten (DMA en HV46) en zeven soorten sedimentorganismen. Behalve de toxiciteit werden ook de concentraties van de verschillende fracties in sediment bepaald. Deze concentraties werden met behulp van evenwichtspartitie omgerekend naar interne concentraties.
De toxiciteit werd zowel uitgedrukt op basis van de sedimentconcentratie (conventionele methode; ter vergelijking) als op basis van interne concentraties (totale concentratie in celmembranen). Voor zes soorten werden significante dosis-effect relaties gevonden. Voor één soort kon er geen significante dosis-effect relatie worden gevonden.
De milieurisicogrenzen zijn gebaseerd op de toxiciteitsgegevens voor de dieselolie DMA. Voor dit product zijn de resultaten van de hier toegepaste methode in goede overeenstemming met elders gepubliceerde toxiciteitsstudies met minerale olie en niet gesubstitueerde aromatische en alifatische verbindingen. Verder bleek de soortgevoeligheidsverdeling van letale interne concentraties voor DMA goed overeen te komen met die voor PAK's uit de literatuur.
Voor het zwaardere type olie, het smeermiddel HV46, was het waarschijnlijk, dat naast narcose fysische besmeuring van de organismen eveneens verantwoordelijk was voor de toxiciteit. Uit de beperkt beschikbare gegevens kan worden geconcludeerd dat de afgeleide milieurisicogrenzen, die zijn gebaseerd op de narcotische werking van olie, waarschijnlijk ook bescherming bieden tegen de nadelige effecten van fysische besmeuring.
Op basis van de eindpunten uit de toxiciteitsexperimenten voor DMA met zes soorten werd een soortgevoeligheidsverdeling berekend en werden de HC5 en de HC50 afgeleid. Deze waarden, die zijn gebaseerd op interne concentraties, werden met behulp van evenwichts-partitiecoëfficiënten tussen de celmembraan en water en tussen bodem/sediment en water teruggerekend naar milieurisicogrenzen in water, bodem en sediment voor iedere fractie afzonderlijk. Hierbij werd de interne concentratie corresponderend met de HC5 en HC50 steeds aan iedere fractie afzonderlijk toegekend. In Tabel 1 is weergegeven welke milieurisicogrenzen voor minerale olie zijn afgeleid.

Tabel 1: Overzicht van de milieurisicogrenzen per compartiment

Compartiment		Milieurisicogrenzen	Methode
Oppervlaktewater	Opgelost	MPC/SRC_{eco}	EP
	Totaal*	MPC/SRC_{eco}	
Grondwater	Opgelost	MPC/SRC_{eco}	EP
Bodem	Standaard bodem **	MPC/SRC_{eco}	EP
Sediment	Standaard sediment **	MPC/SRC_{eco}	Statistische extrapolatie op interne concentraties afgeleid met EP

* Gebaseerd op 30 mg/L gesuspendeerd materiaal met 20% organisch stof
** Met 10 % organisch stof en 25 % lutum

De berekende milieurisicogrenzen voor de afzonderlijke alifatische en aromatische fracties zijn samengevat in Tabel 2 en 3. De nieuwe milieurisicogrenzen voor bodem en sediment zijn voor de kleinere alifaten (≤ C17) en aromaten (≤ C35) strenger dan de huidige normen (MTR: 1000 mg/kg_{dw}; SRC_{eco}: 5000 mg/kg_{dw}) en voor de hogere fracties minder streng. Milieurisicogrenzen voor olie in water zijn niet eerder afgeleid. De waarden voor de SRC_{eco} zijn lager dan de humaan-toxicologische waarden (Franken et al., 1999), die afgeleid zijn op basis van dezelfde indeling in fracties.
Het toekennen van milieurisicogrenzen aan afzonderlijke fracties maakt het mogelijk olie van verschillende samenstelling te toetsen aan de norm. Voor toetsing van metingen in het veld, moeten de metingen gerelateerd worden aan de milieurisicogrenzen voor de afzonderlijke fracties ($meting_i$/$milieurisicogrens_i$) en deze quotiënten moeten gesommeerd voor het gehele

oliemengsel kleiner zijn dan 1. Deze benadering wordt ook wel de “toxic unit” (TU) benadering genoemd.

Tabel 2: Afgeleide milieurisicogrenzen voor minerale olie in standaard bodem en sediment (10% organisch stof) ingedeeld in overeenstemming met de TPHCWG methode (Gustafson et al., 1997). Vanwege additiviteit moet een “toxic unit” (TU) benadering worden toegepast.

Stoffen	EC fractie	MTR [mg/kg_{dw}]	Max. TU	SRC_{eco} [mg/kg_{dw}]	Max. TU
Alifatisch	>5-6	0,55	> 1	16	> 1
	>6-8	0,54	> 1	15	> 1
	>8-10	0,49	> 1	14	> 1
	>10-12	0,91	> 1	26	> 1
	>12-16	9,9	> 1	280 [a]	0,29
	>16-21	- [a]	0,00	- [a]	0,00
Aromatisch	>5-7 (benzeen)	1,3	> 1	39	> 1
	>7-8 (tolueen)	1,5	> 1	44	> 1
	>8-10	1,7	> 1	49	> 1
	>10-12	2,0	> 1	56	> 1
	>12-16	2,4	> 1	68	> 1
	>16-21	3,1	> 1	88	> 1
	>21-35	7,0	> 1	200 [a]	0,49

[a] Als er geen stoffen uit een fractie met een lagere milieurisicogrens aanwezig zijn, mogen deze waarden achterwege worden gelaten, omdat de oplosbaarheid zo laag is dat de interne concentratie corresponderend met de milieurisicogrens niet zal worden bereikt. In de “toxic unit” benadering kan de bijdrage van deze fractie de maximale TU waarde niet overschrijden. Als de maximale TU waarde nul is, is de bijdrage aan de toxiciteit verwaarloosbaar.

Tabel 3: Afgeleide milieurisicogrenzen voor minerale olie in water ingedeeld in overeenstemming met de TPHCWG methode (Gustafson et al., 1997). Vanwege additiviteit moet een “toxic unit” (TU) benadering worden toegepast.

Stoffen	EC fractie	MTR [µg/L]	MTR_{totaal} [µg/L] [b]	Max. TU	SRC_{eco} [µg/L]	$SRC_{eco,totaal}$ [µg/L] [b]	Max. TU
Alifatisch	>5-6	12	15	> 1	330	420	> 1
	>6-8	2,6	5,8	> 1	74	170	> 1
	>8-10	0,33	3,3	> 1	9,4	94	> 1
	>10-12	0,084	5,6	> 1	2,4	160	> 1
	>12-16	0,047	59	> 1	1,3 [a]	1700 [a]	0,29
	>16-21	- [a]	- [a]	0,00	- [a]	- [a]	0,00
Aromatisch	>5-7 (benzeen)	81	89	> 1	2300	2600	> 1
	>7-8 (tolueen)	55	64	> 1	1600	1800	> 1
	>8-10	36	47	> 1	1000	1300	> 1
	>10-12	21	33	> 1	600	940	> 1
	>12-16	9,0	23	> 1	260	670	> 1
	>16-21	2,5	21	> 1	71	600	> 1
	>21-35	0,21	42	> 1	6,1 [a]	1200 [a]	0,49

[a] Als er geen stoffen uit een fractie met een lagere milieurisicogrens aanwezig zijn, mogen deze waarden achterwege worden gelaten, omdat de oplosbaarheid zo laag is dat de interne concentratie corresponderend met de milieurisicogrens niet zal worden bereikt. In de “toxic unit” benadering kan de bijdrage van deze fractie de maximale TU waarde niet overschrijden. Als de maximale TU waarde nul is, is de bijdrage aan de toxiciteit verwaarloosbaar.
[b] De totale concentratie betreft de som van de concentraties in de opgeloste fase en in zwevend stof.

Informatie in de openbare literatuur over het voorkomen van minerale olie in het milieu in Nederland is moeilijk te vergelijken met de afgeleide milieurisicogrenzen. De beschikbare gegevens refereren meestal aan totaal minerale olie zonder opsplitsing in alifatische en aromatische stoffen. In algemene zin is olie wijd verspreid in het milieu aanwezig.

Achtergrondgehalten, die voornamelijk een natuurlijke oorsprong hebben, kunnen oplopen tot 100 mg/kg_{dw}. Concentraties van vervuilde grond en sediment kunnen meer dan 1000 mg/kg_{dw} bedragen. Het is waarschijnlijk dat deze concentraties, hoewel niet volledig gesplitst in fracties, tenminste hoger dan de MTR zijn.
De verwachting is dat met name het voorkomen van aromaten en de kortketenige alifaten van belang is zijn voor het bepalen van de toxiciteit. Dit strookt met het feit dat de hogere alifatische componenten, door hun geringe oplosbaarheid en bioaccumulatie, een betrekkelijk kleine bijdrage leveren aan de interne concentratie. Om na te gaan wat de implicaties van de afgeleide milieurisicogrenzen zijn, wordt geadviseerd om de analyse van fracties van minerale olie toe te passen op een aantal schone referentiesedimenten en verontreinigde veldmonsters, bij voorkeur in combinatie met andere technieken (zoals SPME) die informatie geven over het bioaccumulerend vermogen van een veldmonster en met bioassays die direct informatie verschaffen over de toxiciteit van het monster.

Summary

In this report maximum permissible concentrations (MPCs) and serious risk concentrations for the ecosystem ($SRCs_{eco}$) are derived for mineral oil or total petroleum hydrocarbons (TPH). These environmental risk limits (ERLs) are derived using data on ecotoxicology and environmental chemistry, and represent the potential risk of substances to the ecosystem. They are the scientific basis for environmental quality standards (EQSs) set by the Ministry of VROM.
Mineral oil can be considered as a complex mixture of aromatic and aliphatic hydrocarbons. The composition of mineral oil in the environment may vary strongly. The present standard for mineral oil does not take into account this variability and, consequently, it can not be directly related to the toxicity of mineral oil. Because different components of mineral oil exhibit different accumulation potential, mineral oil is divided over several fractions, for which the separation between aliphatic and aromatic compounds is the most important. The environmental chemistry (log K_{ow}, log K_{oc}, solubility and BCF) of compounds within these individual fractions is relatively homogeneous. Among the different fractions there are large differences in these parameters. In this report, environmental risk limits that are split up in different fractions are proposed that allow for differences in toxicity of different components of oil. The background of this and the used methods are briefly summarised below.
It is assumed that toxicity of mineral oil is mainly caused by narcosis, for which the total concentration of compounds in the cell membrane is the key parameter. The toxicity of compounds that act by narcosis is different, if it is related to the external concentration, as a consequence of different accumulation potential. However, different compounds cause a similar narcotic effect at concentrations in the cell membrane that are equal on molar basis. The sum of the internal concentrations of different compounds gives rise to the same narcotic effect as that of a similar concentration of an individual compound. This latter phenomenon is called concentration additivity. Because of these properties of narcosis, internal concentrations of the different fractions were calculated to derive the environmental risk limits for mineral oil.
To calculate the total internal membrane concentrations, the dissolved aqueous concentrations were calculated first, by considering partitioning between organic carbon and water and the aqueous solubility of compounds present as a mixture. From this, the concentration in the cell membrane was estimated for each fraction separately using a partition coefficient between the membrane and water. Subsequently, the molar concentrations of all fractions were summed. The response from the toxicity studies was plotted against the concentrations, whether or not log-transformed. From the dose-response relationship an EC10 and EC50 were calculated. Further, a NOEC was derived by means of statistical analysis of the variance.
The used methodology deviates at some points from the normal procedure to derive environmental risk limits. A deviation from the common methodology is the fact that toxicity, and consequently ERLs, are not directly related to external concentrations. Also the fact that risk limits are derived connectedly for the different parts of a mixture is deviating.
A limited amount of data was available to derive environmental risk limits for mineral oil using this concept. Therefore, the National Institute for Coastal and Marine Management (RIKZ) and the Institute for Inland Water Management and Waste Water Treatment (RIZA) initiated toxicity studies with two types of oil products (DMA and HV46) and seven benthic species. Next to the toxicity also the concentrations of the different fractions in sediment were analysed. These concentrations were transferred to internal concentrations using equilibrium partitioning.

The toxicity was expressed in sediment concentrations (conventional method; for comparison), as well as in internal concentrations (total cell membrane concentrations). Six species yielded significant dose-response relationships. For one species no significant dose-response relationship could be established.
The environmental risk limits are based on the toxicity data for the gas oil DMA. For this product, the results of the method used here are in good agreement with single species toxicity studies for mineral oil and non-substituted aromatic and aliphatic compounds published elsewhere. Further, the species sensitivity distribution (SSD) of the lethal internal concentrations appeared to be very similar to the SSD for PAHs retrieved from literature.
For the heavier oil type, the lubricant HV46, it was likely that besides narcosis physical soiling of the organisms was responsible for the toxicity too. From the limited available data it can be concluded that the derived risk limits, which are based on the narcotic effects of oil, are likely to be protective against the adverse effects of physical soiling as well.
On basis of the endpoints from the toxicity studies with DMA for six species, a species sensitivity distribution (SSD) was calculated and the HC5 and HC50 were derived. These values, which were based on internal concentrations, were transferred to the environmental risk limits for water, soil and sediment for each fraction separately by means of the equilibrium partition coefficients between membranes and water and between soil/sediment and water. To do this, the internal concentration corresponding to the HC5 and HC50 was repeatedly assigned to each fraction separately. In Table 1 an overview is given of which risk limits for mineral oil are derived.

Table 1: Overview of ERLs by compartment.

Compartment		Reported ERLs	Method
Surface water	Dissolved	MPC/SRC_{eco}	EqP
	Total*	MPC/SRC_{eco}	
Groundwater	Dissolved	MPC/SRC_{eco}	EqP
Soil	Standard soil **	MPC/SRC_{eco}	EqP
Sediment	Standard sediment **	MPC/SRC_{eco}	Statistical extrapolation on internal concentrations derived by EqP

* Based on 30 mg/L suspended matter with 20% organic matter
** With 10 % organic matter and 25 % clay

The calculated environmental risk limits, assigned to the individual aliphatic and aromatic fractions are summarised in Tables 2 and 3. The assignment of environmental risk limits to individual fractions enables the assessment of oil types of different composition. For comparing field measurements with the ERLs for mineral oil, the measurements of the individual fractions should be divided by the individual ERLs ($measurement_i/ERL_i$) and the sum of these ratios should be smaller than one. This approach is known as the “toxic unit” (TU) approach. The ERLs for soil and sediment are more strict than the present ERLs for mineral oil (MPC: 1000 mg/kg_{dw}; SRC_{eco}: 5000 mg/kg_{dw}) for the small aliphatic (≤ 17) and aromatic compounds (≤ 35), but are less strict for the higher fractions. ERLs for water have not been established as yet. The values for the SRC_{eco} are lower than the human-toxicological values (Franken et al., 1999), which are derived on basis of the same classification of fractions.
Information in the open literature on the environmental occurrence of mineral oil in the Netherlands is difficult to compare with the derived risk limits. The available data refer mostly to TPH without separation into aliphatic and aromatic compounds. In general, mineral oil is widely distributed in the environment. Background concentrations, which mainly have a natural origin, can be up to 100 mg/kg_{dw}. Concentrations of polluted soil and sediment can be

over 1000 mg/kg_{dw}. It is likely that these measured concentrations, although not fully separated into fractions, are at least above the derived MPC.

Table 2: Derived risk limits for mineral oil in standard soil and sediment (10% organic matter) categorised according to the TPHCWG method (Gustafson et al., 1997). Because of additivity a toxic unit (TU) approach should be used.

Compounds	EC fraction	MPC [mg/kg_{dw}]	Max. TU	SRC_{eco} [mg/kg_{dw}]	Max. TU
Aliphatic	>5-6	0.55	> 1	16	> 1
	>6-8	0.54	> 1	15	> 1
	>8-10	0.49	> 1	14	> 1
	>10-12	0.91	> 1	26	> 1
	>12-16	9.9	> 1	280 [a]	0.29
	>16-21	- [a]	0.00	- [a]	0.00
Aromatic	>5-7 (benzene)	1.3	> 1	39	> 1
	>7-8 (toluene)	1.5	> 1	44	> 1
	>8-10	1.7	> 1	49	> 1
	>10-12	2.0	> 1	56	> 1
	>12-16	2.4	> 1	68	> 1
	>16-21	3.1	> 1	88	> 1
	>21-35	7.0	> 1	200 [a]	0.49

[a] If no block of compounds with a lower ERL are present, these values may be neglected, because solubility is so low that the internal concentration corresponding to the ERL will not be reached. In the toxic unit approach, the contribution of this fraction can not exceed the maximum toxic unit value. If the maximum toxic unit value is zero, the contribution to toxicity is negligible.

Table 3: Derived risk limits for mineral oil in water categorised according to the TPHCWG method (Gustafson et al., 1997). Because of additivity a toxic unit (TU) approach should be used.

Compounds	EC fraction	MPC [µg/L]	MPC_{total} [µg/L] [b]	Max. TU	SRC_{eco} [µg/L]	$SRC_{eco,total}$ [µg/L] [b]	Max. TU
Aliphatic	>5-6	12	15	> 1	330	420	> 1
	>6-8	2.6	5.8	> 1	74	170	> 1
	>8-10	0.33	3.3	> 1	9.4	94	> 1
	>10-12	0.084	5.6	> 1	2.4	160	> 1
	>12-16	0.047	59	> 1	1.3 [a]	1700 [a]	0.29
	>16-21	- [a]	- [a]	0.00	- [a]	- [a]	0.00
Aromatic	>5-7 (benzene)	81	89	> 1	2300	2600	> 1
	>7-8 (toluene)	55	64	> 1	1600	1800	> 1
	>8-10	36	47	> 1	1000	1300	> 1
	>10-12	21	33	> 1	600	940	> 1
	>12-16	9.0	23	> 1	260	670	> 1
	>16-21	2.5	21	> 1	71	600	> 1
	>21-35	0.21	42	> 1	6.1 [a]	1200 [a]	0.49

[a] If no block of compounds with a lower ERL are present, these values may be neglected, because solubility is so low that the internal concentration corresponding to the ERL will not be reached. In the toxic unit approach, the contribution of this fraction can not exceed the maximum toxic unit value. If the maximum toxic unit value is zero, the contribution to toxicity is negligible.
[b] The total concentration refers to the sum of the concentrations in the dissolved phase and in particulate matter.

It is expected that the presence of aromatic and short-chained aliphatic compounds is important in determining the toxicity of mineral oil. This complies with the fact that the long-chained aliphatic compounds contribute to a minor extent to the internal concentrations, because of their limited solubility and bioaccumulation potential. To investigate the implications of the derived risk limits it is recommended that the fraction analysis of mineral

oil be applied to a set of clean reference sediments and field polluted sediments, preferably in combination with other techniques that give information on the bioaccumulation potential of a field sample (such as SPME) and with bioassays that provide direct information on the toxicity of a field sample.

1. Introduction

In this report maximum permissible concentrations (MPCs) and serious risk concentrations for the ecosystem ($SRCs_{eco}$) are derived for mineral oil or total petroleum hydrocarbons (TPH). This report is a result in the project "Setting Integrated Environmental Quality Standards". The aim of the project is to derive environmental risk limits (ERLs) for substances in the environment for the compartments air, (ground)water, sediment and soil. Environmental risk limits (ERLs) serve as advisory values to set environmental quality standards (EQS) by the Ministry of VROM for various policy purposes. The term EQS is used to designate all legally and non-legally binding standards that are used in Dutch environmental policy and Table 4 shows the correspondence between ERLs and EQSs. The various ERLs are:

- the negligible concentration (NC) for water, soil, groundwater, sediment and air
- the maximum permissible concentration (MPC) for water, soil, groundwater, sediment and air
- the ecotoxicological serious risk concentration (SRC_{eco}) for water, soil, groundwater and sediment

Table 4: Environmental risk limits (ERLs) and the related environmental quality standards (EQS) that are set by the Dutch government in the Netherlands for the protection of ecosystems.

Description	ERL	EQS
The NC represents a value causing negligible effects to ecosystems. The NC is derived from the MPC by dividing it by 100. This factor is applied to take into account possible combined effects.	NC (for air, water, soil, groundwater and sediment)	Target value (for air, water, soil, groundwater and sediment)
The MPC is the concentration of a substance in air, water, soil or sediment that should protect all species in ecosystems from adverse effects of that substance. A cut-off value is set at the fifth percentile if a species sensitivity distribution of NOECs is used. This is the hazardous concentration for 5% of the species, the $HC5_{NOEC}$.	MPC (for air, water, soil, groundwater and sediment)	MPC (for air, water and sediment)
The SRC_{eco} is the concentration of a substance in the soil, sediment or groundwater at which functions in these compartments will be seriously affected or are threatened to be negatively affected. This is assumed to occur when 50% of the species and/or 50% of the microbial and enzymatic processes are possibly affected, the $HC50_{NOEC}$.	SRC_{eco} (for water, soil, groundwater and sediment)	Intervention value after comparison with SRC_{human} (for soil, sediment and groundwater)

The process of deriving integrated ERLs is shown schematically in Figure 1.1. ERLs for soil and sediment are calculated for a standardised soil. ERLs for water are reported for dissolved and total concentrations (including a standard amount of suspended matter) and if found significantly different, differentiated to fresh water and salt water. Each of the ERLs and its corresponding EQS represents a different level of protection, with increasing numerical values in the order NC < MPC[1] < SRC_{eco}. The EQS demand different actions when one of them is exceeded, explained elsewhere (VROM, 1994).

[1] A complicating factor is that the term MPC is used both as an ERL and as an EQS. For historical reasons, however, the same abbreviation is used.

Environmental quality standards for mineral oil were already presented in the early 1990s. These EQSs had a weak scientific background and comprised the total C10-C40 fraction of petroleum hydrocarbons. In 1999 new environmental quality standards for total petroleum hydrocarbons were presented, based on human toxicological risk assessment data (Franken et al., 1999). These environmental quality standards were based on the toxicity of different aliphatic and aromatic fractions. In addition, an update based on ecotoxicity data was proposed in 2000. In this report the results of this update are presented and compared with the standards based on human toxicological risk assessment.
The method used for the derivation of the ERLs for mineral oil deviates from the general methods in the project "Setting Integrated Environmental Quality Standards" (Traas, 2001). Generally, EQSs are directly related to environmental exposure concentrations. Here, EQSs are firstly related to internal effect concentrations, which are than transferred to environmental concentrations. Furthermore, different constituents of mineral oil are combined in order to derive EQSs for fractions of mineral oil, rather than deriving EQSs for each compound separately.
Mineral oil occurs as a complex mixture, of which the composition may vary widely. Moreover, in the environment its composition may change due to several physical, chemical, and biological processes (e.g. biodegradation, dissolution in water, and volatilisation), generally referred to as weathering of the oil (Potter and Simmons, 1998). Mineral oil is used for different purposes, with the use as fuel and greases being the most important.
Because TPH is a mixture of many compounds, it is first divided in blocks of aromatic and aliphatic compounds according to equivalent carbon numbers with equal properties regarding environmental chemistry and ecotoxicology (Gustafson et al., 1997; CONCAWE, 1996). It is assumed that the toxicity of oil is mainly caused by narcosis (CONCAWE, 1996; Peterson, 1994; Verbruggen and Hermens, 2001). The key parameter for narcosis is the total molar concentration of compounds in the cell membranes, for which the effects of different compounds or mixtures of compounds at the same concentration are expected to be equal (McCarty and Mackay, 1993; Van Wezel and Opperhuizen, 1995).
The contribution of the homogeneous blocks of compounds to this internal membrane concentration is first calculated from the sediment concentrations of the individual blocks. For this purpose, equilibrium partitioning equations are used for the equilibrium between oil and (pore) water, between organic matter and (pore) water, and between (pore) water and the cell membrane. Then the concentrations of the individual blocks are summed to obtain a total concentration in the cell membranes.
The response from the toxicity studies was expressed on basis of the total internal concentrations to obtain the effect parameters needed for further analysis (NOEC, EC10, and EC50). These effect parameters for the various species are than used as input for a species sensitivity distribution for mineral oil. The risk levels HC5 and HC50 are based on these total internal membrane concentrations. For the derivation of the final MPC and SRC_{eco}, the HC5 and HC50 values are recalculated to concentrations in water and sediment for each block using equilibrium partitioning equations.
For all blocks together a toxic unit (TU) approach should be applied, because the effects of the compounds in those blocks are additive. In this approach the concentration for each individual block (C_i) is compared with the corresponding risk limit for that block in an environmental compartment. The sum of toxic units (ΣTU) is calculated by summing the ratios for all these blocks:

$$\sum TU = \sum_{i=1 to n} \frac{C_i}{ERL_i} \quad (1)$$

The risk limit for a compartment is exceeded if the sum of toxic units (ΣTU) is larger than unity. For comparing field samples with the ERL it is necessary to measure the different blocks of aromatic and aliphatic compounds.
Summarising the different steps and assumptions:

1. Oil is a mixture with compounds of different characteristics. In this exercise mineral oil is divided in blocks of equivalent carbon numbers (EC) within the groups of aromatic and aliphatic compounds.
2. The environmental fate of these different blocks in water is well-defined (log K_{ow}, log K_{oc}, solubility, and BCF).
3. It is assumed that the oil constituents affect the target organism mainly by narcosis: The effect of the different blocks is comparable when expressed in concentration (in moles) present in the target lipids (of the membrane).
4. The water concentration of the different blocks can be estimated by equilibrium partitioning between three phases: the organic matter of the sediment, oil and water.
5. The target lipid concentrations of the different blocks can be estimated from the water concentration by the equilibrium partition coefficient between water and cell membranes.
6. The internal molar concentrations of the individual blocks are added up to a total internal concentration.
7. For each type of oil a dose effect curve is made for the different species using the total internal concentrations, whether or not log-transformed.
8. Endpoints are estimated from these dose-response curves (EC10 or EC50) or from a statistical analysis of variance (NOEC).
9. The endpoints for the different species (EC10s) are brought together in making a species sensitivity distribution from which the HC5 and HC50 are estimated.
10. The HC5 and HC50 values, still based on internal concentrations, are recalculated to concentrations in water and sediment for each block individually using equilibrium partitioning equations.
11. For comparing measurements with the ERL it is necessary to
    a. measure the different blocks of aromatic and aliphatic compounds
    b. add the quotients of the measured concentration and the ERL: $\Sigma(\text{measurement}_i/\text{ERL}_i)$
    c. conclude that the ERL is exceeded when the sum is greater than unity

In chapter 2 the physical-chemical characteristics of the different aliphatic and aromatic compounds will be described first. The relationships given here are necessary for transferring water concentrations to internal concentrations and vice versa. Chapter 3 provides information on the handling of the data, the division in blocks and the derivation of the ERLs, whereas chapter 4 focuses on the results of the various analyses and the estimation of the ERLs.

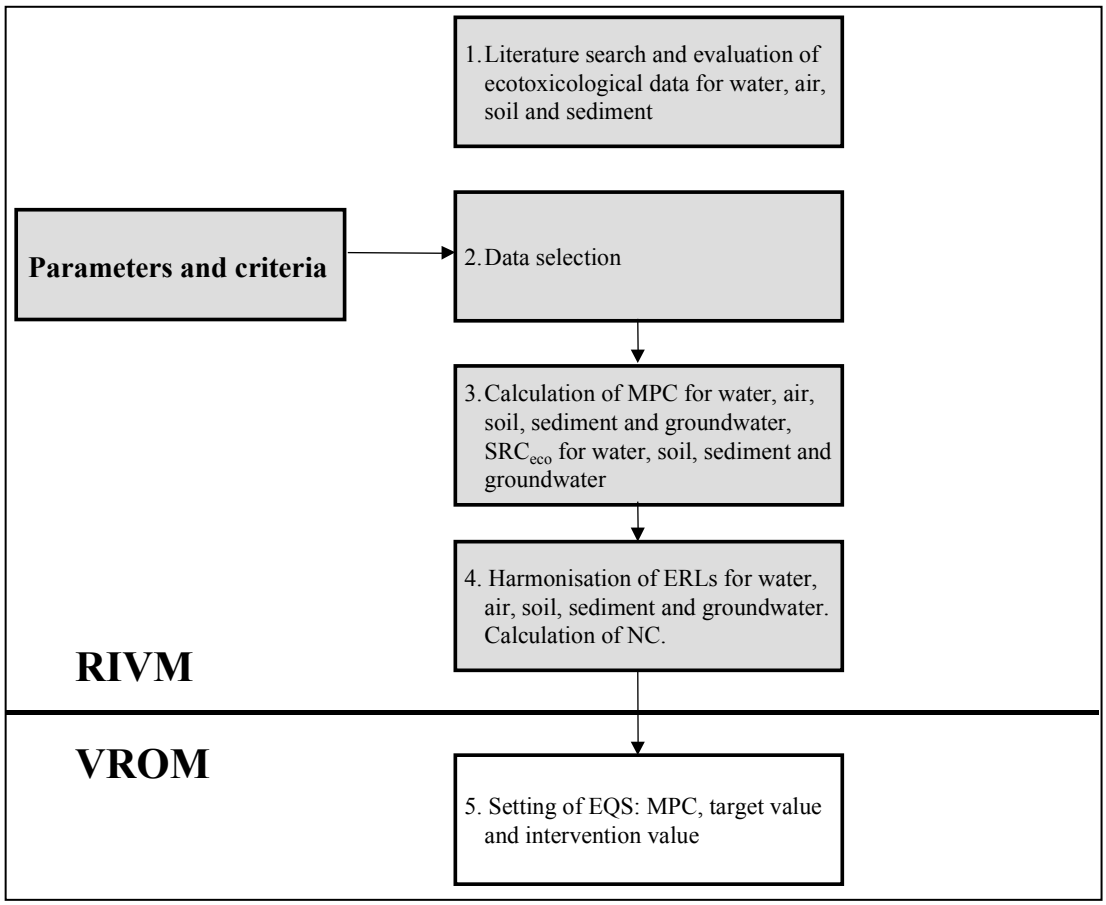

Figure 1.1: The process of deriving Integrated environmental risk limits. Above the line the method to derive ERLs is indicated, i.e. MPC, NC and SRC_{eco}. Below the line the MPC and target value is indicated, set by the Ministry of Housing, Spatial Planning and the Environment (VROM).

2. Substance properties and use

2.1 Composition and analysis

2.1.1 Hydrocarbons in the environment

2.1.1.1 Origin of hydrocarbons

Mineral oil is a complex mixture of aromatic and aliphatic hydrocarbons (Potter and Simmons, 1998). Aliphatic and aromatic compounds extracted from sediment may have different origins. These may be petroleum inputs (petrogenic), partial combustion of fuels and forest and grass fires (pyrolytic), biosynthesis by marine or terrestrial organisms and early diagenetic transformation of natural products to hydrocarbons (biogenic). Further, the residence time in the environment is important for the composition of the oil. Changes in composition occur in composition due to selective desorption, evaporation, chemical and photo-oxidation and biodegradation (Readman et al., 2002). The term weathering collectively denotes these processes.

The resolved saturated hydrocarbons can have both a petrogenic and a biogenic origin. The *n*-alkanes with chains of an even number of carbon atoms are more representative of petroleum spills, while the compounds with an odd number represent the biogenic compounds. Algae form the compounds near *n*-C17, while compounds near *n*-C_{29} (from *n*-C_{21}, especially *n*-C_{27}, *n*-C_{29}, and *n*-C_{31}) have its origin in waxes from terrestrial plants. An equal distribution of *n*-alkanes with an even and odd number of carbon atoms points at a petrogenic input (Bouloubassi et al., 2001; LeDréau et al., 1997; Gogou et al., 2000). However, also *n*-alkanes with an even number of carbon atoms may have a natural origin (Volkman et al., 1992).

The bulk of unresolved complex matter (UCM), i.e. the hump in the gas chromatogram, can be largely attributed to the aliphatic fraction. This UCM often forms a major part of this aliphatic fraction. The UCM is mostly ascribed to petrogenic sources. A high UCM indicates a degraded or weathered source of petroleum. Detritus possibly forms a secondary source of the UCM (Bouloubassi et al., 2001). This UCM is reported in the range of C16-C40[2] (Gogou et al., 2000) or C18-C35 (Readman et al., 2002), sometimes bimodal centred at C23 and C30, with the first hump possibly being of natural origin (Bouloubassi et al., 2001; Gogou et al., 2000). In sediments almost free from petrogenic input, a UCM is sometimes absent (Shetland Island: Webster et al., 2000; French Mediterranean: Mille et al., 1992; Great Barrier reef: Volkman et al., 1992) or relatively low with ratios of UCM versus *n*-alkanes below 4 (Lake Michigan: Doskey, 2001; Yangtze River estuary: Bouloubassi et al., 2001; Black Sea: Maldonado et al., 1999; Black Sea: Readman et al., 2002).

Two groups of cyclic aliphatic hydrocarbons that are commonly used to identify the source of crude oil and to assess the degree of alteration of the oil are the hopanes and steranes. These compounds are rather stable in the environment and crude oils have a specific pattern of these compounds (LeDréau et al., 1997; Bouloubassi et al., 2001; Webster et al., 2000). Pristane and phytane are two branched aliphatic hydrocarbons, which are reported frequently as constituents of petroleum, but which might have a biogenic origin as well (Volkman et al., 1992). In comparison with the corresponding *n*-alkanes they can be used to examine the degree of weathering (LeDréau et al., 1997).

Aromatic compounds may have three different origins. They can be petrogenic, biogenic as well as pyrolytic. The lower molecular weight and especially the alkylated PAHs (e.g. naphthalenes and phenanthrenes) are related to petroleum sources, while the higher,

[2] C-numbers refer to the equivalent carbon number, see § 2.2.1.

unsubstituted PAHs (e.g. benzo[*a*]pyrene) have its origin mostly in combustion of fossil fuels and other sources such as forest fires (Bouloubassi et al., 2001; Gogou et al., 2000; LeDréau et al., 1997; Readman et al., 2002; Webster et al., 2000). PAHs preferentially occur in fine particles (Webster et al., 2000; Bouloubassi et al., 2001).
The aromatic fraction may also contain biogenic compounds. Perylene and derivatives, retene and some tetrahydrochrysenes are mostly from natural origin (Bouloubassi et al., 2001).
Also the chromatograms of the aromatic fraction often show a UCM, which can be attributed to petroleum spills too (LeDréau et al., 1997; Potter and Simmons, 1998; Potter and Duval, 2001; Readman et al., 2002). However, sometimes it is reported that the UCM only occurs in the aliphatic fraction (LeDréau et al., 1997; Mille et al., 1992), which might be an extra indication that the aromatic UCM is petrogenic while the aliphatic UCM can also have a natural origin (Mille et al., 1992). In mussels from an unpolluted site, the accumulated UCM appeared to occur completely in the aliphatic fraction too. However, in mussels from oil polluted sediments, this was not the case with a significant contribution to the UCM from the aromatic fraction (Rowland et al., 2001). In the extract of a sediment from Chesapeake Bay (NIST SRM 1941a), a historically more polluted area, the UCM appears to be almost completely in the aliphatic fraction higher than pentadecane. For the analysed crude oil from the same study (NIST SRM 1582) the aromatic UCM is much more pronounced (Mazeas and Budzinski, 2002).

2.1.1.2 Background levels

Unpolluted intertidal and estuarine sediments may have a total hydrocarbon concentration of up to 10 mg/kg_{dw} and may be two to three times higher if there is a significant contribution from plant waxes (aliphatic). In organic-rich marine sediments the concentration of total aliphatic hydrocarbons may rise to 100 mg/kg_{dw}. However, above this value petroleum input is the most likely source (Volkman et al., 1992; Readman et al., 2002).

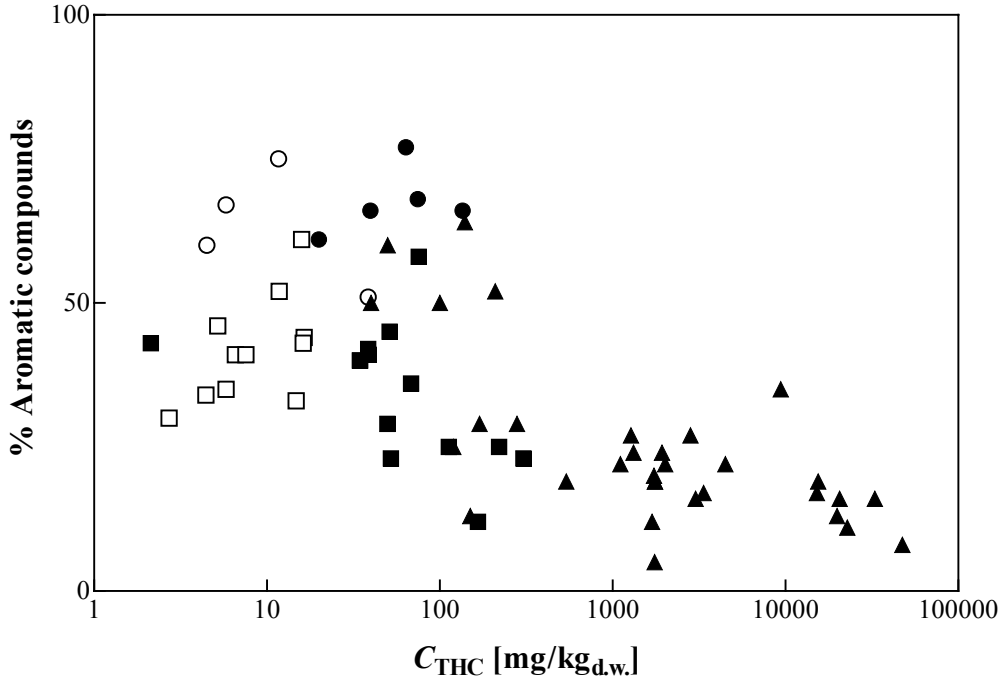

Figure 2.1: Percentage compounds in the aromatic fraction as a function of the concentration of total hydrocarbons (C_{THC}). ■ and □ Readman (2002), ▲ from Mille (1992), ● and ○ from LeDréau (1997). Open symbols denote low or absent UCM, indicating low petrogenic input.

To assess what part of the total hydrocarbon concentration of the background levels belongs to the aromatic and what part to the aliphatic fraction, the data in literature have been examined. Often a low content of aromatic hydrocarbons is reported in control sediments, lying in the range of 0 to 15% (Maldonado et al., 1999; Bouloubassi et al., 2001; Gogou et al., 2000; Webster et al., 2000). However, the reported concentration is rather the sum of the concentrations of all resolved PAHs and alkylated PAHs instead of all aromatic compounds. Often the used analytical method is GC-MS in which selective ion fragments of PAHs are quantified. As a result the unresolved aromatic compounds will mostly not be quantified. In the few studies (Readman et al., 2002; LeDréau et al., 1997; Mille et al., 1992) in which the total amount of both aliphatic and aromatic compounds are determined by weight, the percentage of aromatic compound is much higher (Figure 2.1). From these data it appears that about 50% of the background concentration is found in the aromatic fraction. From Figure 2.1 it appears that for petrogenic compounds (closed symbols to the right) this fraction is generally lower.

2.1.2 Analytical methods to determine mineral oil

The standard method to perform oil analyses in the Netherlands is the NEN 5733 method. According to this method the sample is extracted by shaking successively with acetone and petroleum ether and is purified with florisil (magnesium silicate). The purified extract is then concentrated with a Kuderna Danish evaporator before analysis with GC-FID, a gas chromatograph coupled to a flame ionisation detector (Harmsen and Zweers, 2000). In literature, GC-FID is most frequently used for the quantification of TPH. For identification purposes a mass spectrometer (MS) is also used often in research.
In the NEN 5733 method aliphatic and aromatic compounds are not separated, which is necessary to divide TPH into fractions of similar properties. To distinguish between the aliphatic and aromatic compounds a separation step is often carried out before GC-analysis. Because of the importance for the methodology used in this report, a conference call was organised to explore the feasibility of the fraction analysis [3].
Separation of TPH in aliphatic and aromatic compounds is possible by fractionation over an alumina or a silica column. There are several established methods to perform such fractionation. Examples of these methods are the methods of U.S. Environmental Protection Agency (EPA 3611B method, 1996a), the Massachusetts Department of Environmental Protection (MADEP EPH method, 1998), the Texas Natural Resource Conservation Commission (TNRCC 1006 method, 2000), and the Total Petroleum Hydrocarbon Working Group (TPHCWG Direct method, 2000; Weisman, 1998). The EPA 3611B method utilises an alumina column. The TPHCWG Direct method is a modification of this method or as alternative fractionation of the EPA 3630C method for silica gel clean-up (U.S. EPA, 1996b). The TNRCC 1006 method as well as the MADEP EPH method is based on the EPA 3630C method with fractionation over a silica gel column.
In recent literature, a silica gel column, sometimes after clean-up with alumina (Mazeas and Budzinski, 2002; Doskey, 2001; Gogou et al., 2000; Volkman et al., 1992), as well as an alumina column (Maldonado et al., 1999), or a combination of both (Readman et al., 2002; Rowland et al., 2001; Potter and Duval, 2001; LeDréau et al., 1997) are used for the fractionation of TPH in aliphatics and aromatics. A similar third method that is applied, is a separation over a normal-phase HPLC, sometimes preceded by a clean-up with silica (Bouloubassi et al., 2001; Webster et al., 2000).

[3] Conference call on analysis of mineral oil, date: February 26th 2002; participants: Flaherty J (Exygen Research, PA, USA), Rhodes IAL (Shell/Equilon, TX, USA), Peters RJB (TNO-MEP, The Netherlands), Letinski DJ (Exxon Mobil Biomedical Sciences, NJ, USA), Verbruggen EMJ (RIVM, The Netherlands).

In the conference call, it was generally agreed upon that silica is the best column material to use, because it has a better overall performance and is easier to use. 90% of the compounds ends up in the right fraction, with olefins and large cyclic compounds such as hopenes in the aliphatic fractions and sulphur in the aromatic fraction (source: conference call on analysis of mineral oil).
The limit of detection for total petroleum hydrocarbons (TPH) is in the order of 50 ppm. If fractionation is applied, the limit of detection per fraction is lower. The limit of detection is dependent on the shape of the chromatogram. If the chromatogram consists of only one peak, the limit of detection will of course be lower than if the chromatogram only contains a bulk of unresolved matter. Mentioned values are from 2 to 10 ppm or from 2 to 4 ppm. As a general value 5 ppm is given. However, if the sample is not volatile, it can be evaporated. The limit of detection can be reduced in this way (source: conference call on analysis of mineral oil).

2.2 Physicochemical properties

2.2.1 Equivalent carbon number

In the Netherlands, mineral oil is defined as the TPH fraction between equivalent carbon (EC) number 10 and 40, i.e. the part that falls between *n*-decane and *n*-tetracontane in the gas chromatogram. Physico-chemical properties are often related to the EC number, which is an index for the relative retention time on a gas chromatographic (GC) system normalised to *n*-alkanes, in which the carbon numbers are those of the *n*-alkanes (Gustafson et al., 1997). The values for the EC numbers used in this report were retrieved from the Total Petroleum Hydrocarbon Criteria Working Group (Gustafson et al., 1997), and data on gasolines performed for CONCAWE (Exxon Biomedical Sciences Inc., 1996). The relationship between the EC number and boiling point (from Lide, 1998) is shown in Figure 2.2 and is described by Equation 2. This equation is practically identical to the relationship presented in Gustafson et al. (1997).

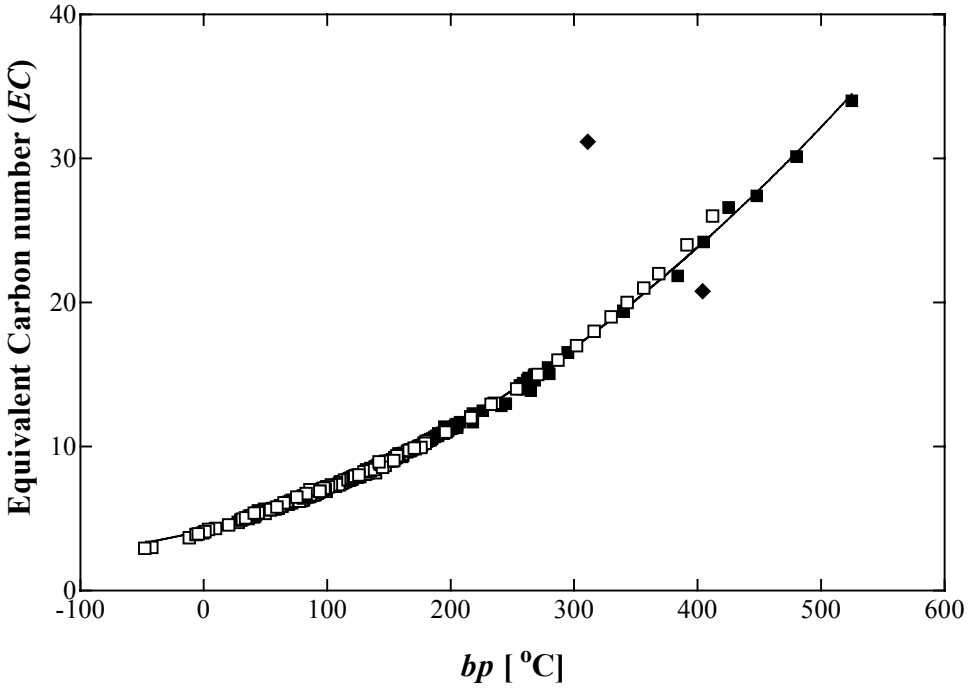

Figure 2.2: Relationship between equivalent carbon number and boiling point (bp). □: Aliphatic compounds; ■: aromatic compounds; ♦: pyrene and benzo[e]pyrene were considered outliers.

$$EC = 4.21 + 0.0217 \cdot bp + 6.85 \cdot 10^{-5} \cdot bp^2 \quad \left(n = 272,\ r^2 = 0.998\right) \tag{2}$$

A close relationship exists also between vapour pressure and EC number. For other physicochemical parameters, such as solubility and the *n*-octanol-water partition coefficient, the relationship with equivalent carbon number is not as clear. However, if aromatic and aliphatic compounds are taken separately, a good correlation exists between the equivalent carbon number and these physicochemical properties (Gustafson et al., 1997).

2.2.2 Molecular weight

For the assessment of narcotic effects, concentrations have to be expressed on a molar basis. To convert concentrations of mineral oil from grams into moles relationships between the molecular weight (*Mw*) and *EC* number are used (Figure 2.3). For the molecular weight the following equations have been used for aliphatic and aromatic compounds determined by regression from all the aliphatic compounds and all polyaromatic hydrocarbons (PAHs).

- Aliphatics:

$$Mw_{\mathrm{Al}} = 14.07 \cdot EC + 3.51 \quad \left(n = 210,\ r^2 = 0.985\right) \tag{3}$$

- Aromatics

$$Mw_{\mathrm{Ar}} = 6.36 \cdot EC + 60.86 \quad \left(n = 41,\ r^2 = 0.986\right) \tag{4}$$

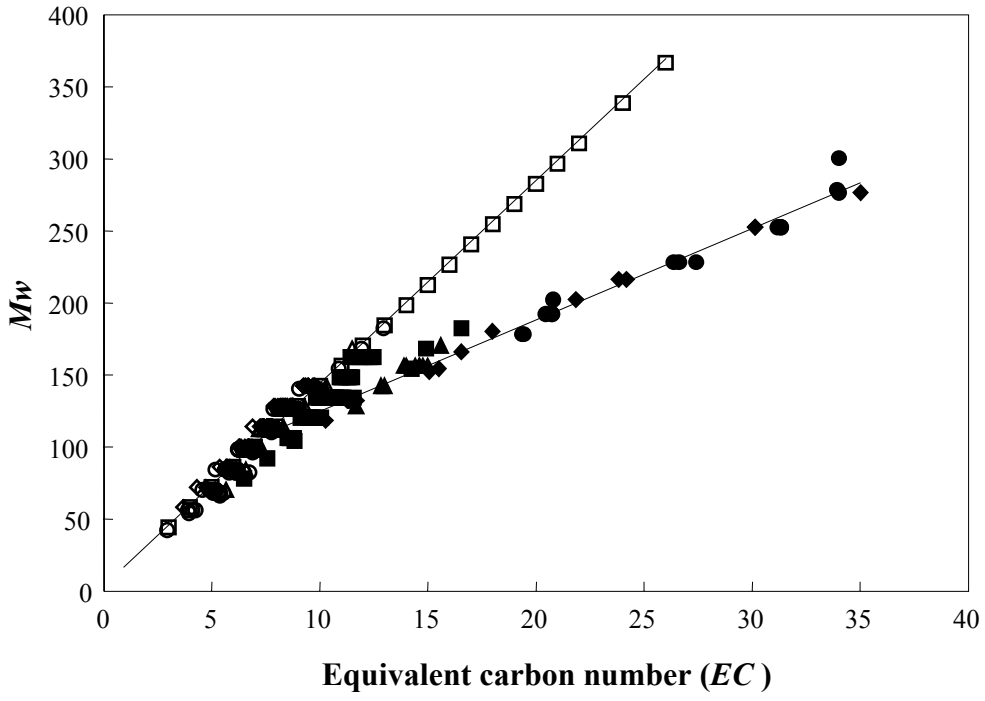

Figure 2.3: Relationship between molecular weight and equivalent carbon number. □: n-Alkanes; ◊: branched alkanes; Δ: cycloalkanes; ○: unsaturated aliphatics; ■: alkyl benzenes and biphenyls; ♦: naphtheno benzenes; ▲: alkyl naphthalenes; ●: polynuclear aromatics. Mw from Lide (1998), EC numbers see 2.2.1.

2.2.3 *n*-Octanol-water partition coefficient (log K_{ow})

A comparison was made between measured and estimated log K_{ow} values. It appeared that the relationship between calculated values by the estimation program ClogP (Daylight Chemical Information Systems, 2002) and experimental logPstar values from the MedChem database shows a nearly one-to-one relationship for over 100 aliphatic and aromatic hydrocarbons (Figure 2.4). The values estimated by the KOWWIN estimation program (U.S. EPA, 2000) are less accurate, regardless whether the experimental values from KOWWIN or the logPstar values are used. Therefore, ClogP is used as estimation program for the log K_{ow} values in the relationships used throughout this report. By taking the calculated log K_{ow} values, the compounds for which no experimental log K_{ow} values are available, are not omitted in the relationships between log K_{ow} and other parameters.

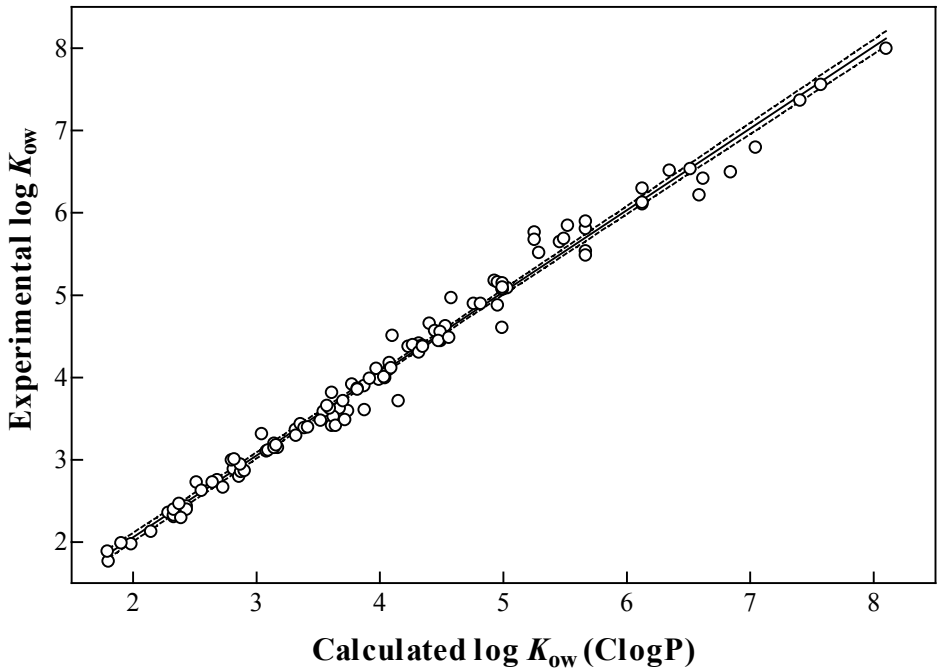

Figure 2.4: Relationship between the experimental data on log K_{ow} *of logPstar from the MedChem database and ClogP. Lines represent linear regression with 95% confidence interval:* $\log K_{ow}$*(exp.)=0.993(±0.011)·ClogP+0.071(±0.047),* r^2*=0.987, n=117.*

For the relationship between log K_{ow} and equivalent carbon number, the ClogP values are plotted versus *EC* number (Figure 2.5). For both the aliphatic and aromatic compounds a regression analysis was made.

First, for the aliphatic compounds, a linear regression between log K_{ow} and *EC* number was made separately for *n*-alkanes (paraffins), branched alkanes (iso-paraffins), cycloalkanes (cyclo-paraffins) and unsaturated aliphatics (olefins). The slope of these regressions for all the groups of aliphatic compounds appeared not significantly different from the slope of 0.53 for *n*-alkanes, however, the intercept varied from 0.13 to 0.71. Therefore, a weighted average of the intercept was used, where the compositional information on gasoline samples MRD-95-044 to MRD-95-049 (Exxon Biomedical Sciences Inc., 1996) was included for the relative contribution of paraffins, iso-paraffins, cyclo-paraffins and olefins. The resulting equation for aliphatic compounds is:

$$\log K_{ow} = 0.53 \cdot EC + 0.55 \qquad (5)$$

Second, for the aromatic compounds, a relationship between log K_{ow} and EC number is found with a slope of 0.15 for the non-substituted, completely aromatic compounds. However, the aromatic compounds also include alkylated benzenes and naphthalenes, and naphtheno benzenes. For these compounds, the log K_{ow} will be higher in comparison with pure aromatic compounds with the same equivalent carbon numbers, as can be clearly seen from the alkylated benzenes and alkylated naphthalenes in Figure 2.5. For the aromatic compounds the intercept has also been determined in a weighted analyses for the composition. Based on a fixed slope for aromatic compounds, the intercept of the relationship between log K_{ow} and *EC* number is calculated for each aromatic compound separately. The final intercept is calculated from the values for the individual compounds multiplied with their contribution to the composition of the aromatic fraction. The resulting equation for aromatic compounds is:

$$\log K_{ow} = 0.15 \cdot EC + 1.76 \qquad (6)$$

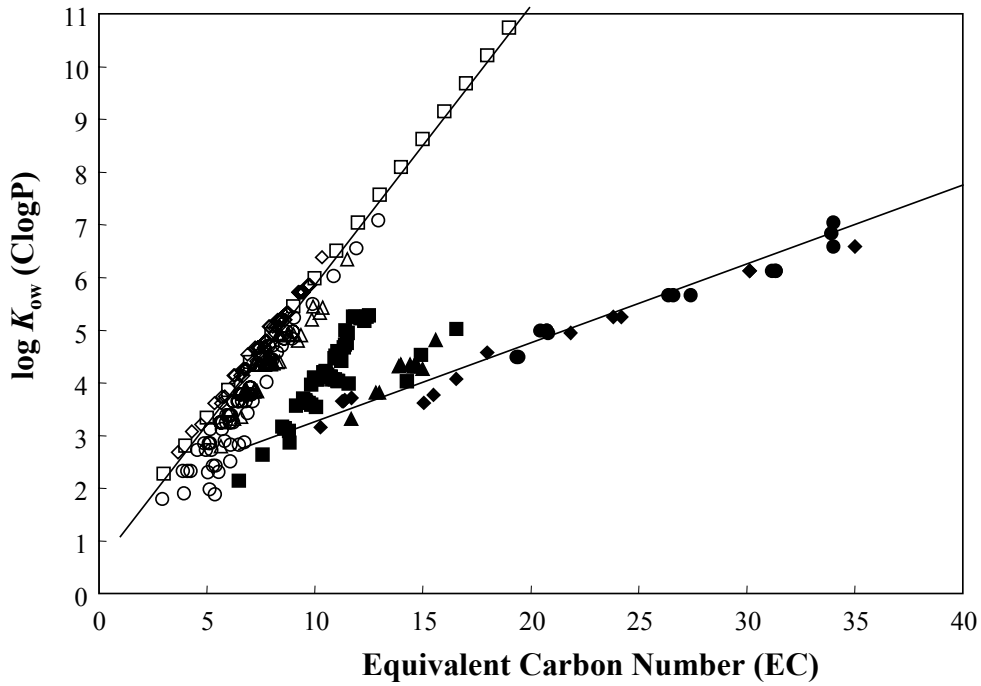

Figure 2.5: Relationship between log K_{ow} *and EC. Drawn lines are weighted regression lines for aliphatic and aromatic compounds respectively (see text).* □*: n-Alkanes;* ◊*: branched alkanes;* Δ*: cycloalkanes;* ○*: unsaturated aliphatics;* ■*: alkyl benzenes;* ♦*: naphtheno benzenes;* ▲*: alkyl naphthalenes;* ●*: polynuclear aromatics.*

The resulting equations thus have the slopes for *n*-alkanes and pure aromatic compounds, while the intercepts are corrected by 0.1-0.2 log unit (about a factor of 1.5) to correct for the contribution of unsaturated and cyclic compounds for the aliphatics and of alkylation and saturation in the case of aromatics. The data used for this weighted regression analysis are from gasoline samples only (Exxon Biomedical Sciences Inc., 1996). The six used gasolines, however, differ strongly in composition with respectively a cyclic, saturated, unsaturated and aromatic character, completed with two blends. Still, the resulting standard deviation in the intercepts is small: 0.13 for aliphatics and 0.06 for aromatics. A further advantage of the analyses of the gasolines is that the mixtures are characterised almost completely. In the TPHCWG series, compositional information is given for other types of oil samples as well, but the composition is not worked out in as much detail (Potter and Simmons, 1998).

2.2.4 Solubility

2.2.4.1 Pure compounds

A strong uniform relationship exists between the solubility of liquid aliphatic compounds and log K_{ow} (Verbruggen et al., 2000a):

$$\log S_L = -1.144 \cdot \log K_{ow} + 0.723 \quad (7)$$

For a good comparison with these data, the subcooled liquid solubility is used, if the compound is solid at room temperature (De Maagd et al., 1998b). A relationship that is almost equal to Equation 7 was found for the subcooled liquid solubility of polynuclear aromatic hydrocarbons (PAHs) (Schwarzenbach et al., 1993). The data for log K_{ow} and subcooled liquid solubility of PAHs, respectively determined by the accurate slow-stirring and generator-column technique, follow this line perfectly (De Maagd et al., 1998b), with equal slopes between these two parameters and molar volume as was found for *n*-alkanes (Verbruggen et al., 2000a).

For some higher aliphatic hydrocarbons the solubility has been recently determined by means of the slow-stirring method (Tolls et al., 2002). Good solubility data for PAHs were determined with the generator-column method (De Maagd et al., 1998b). Solubility data for lower aliphatic compounds (from Verbruggen et al., 2000a) and monoaromatic compounds (from Van Hattum et al., 2000) have been evaluated as well. It appears that for these four sets of compounds, there is a very good correlation between liquid solubility (S_L) and the calculated log K_{ow} from ClogP (see Figure 2.6).

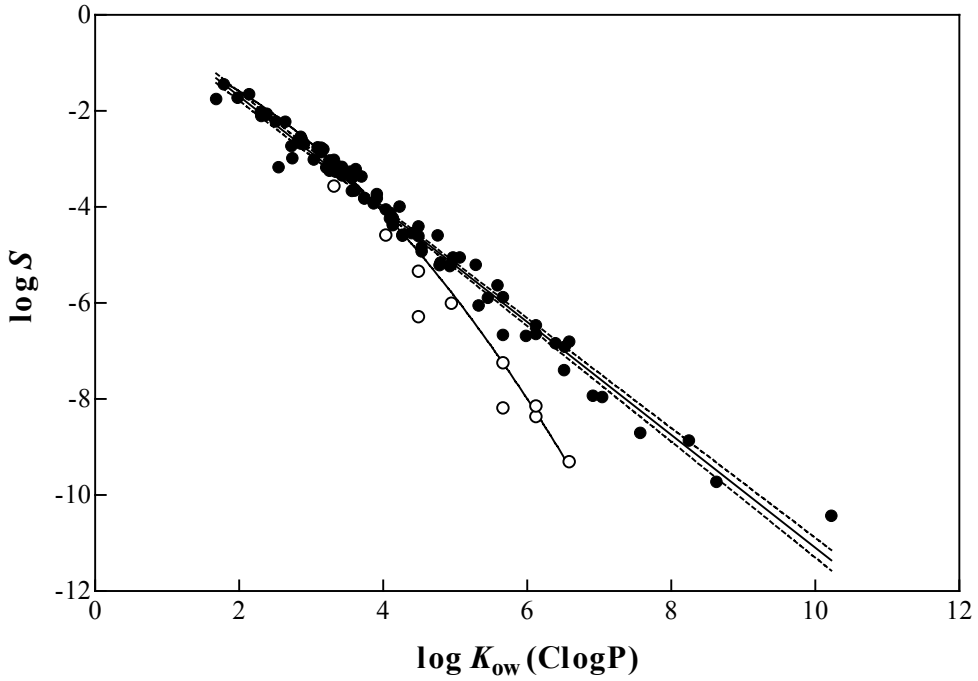

Figure 2.6: Relationship between liquid solubility (S_L) and log K_{ow} *for aliphatic and aromatic hydrocarbons (●) and solubility of solid aromatics compounds ($S_{solid,ar}$ ○).*

Because log K_{ow} serves as an intermediate between the equivalent carbon number and the used properties in this risk assessment, this relationship is also based on log K_{ow} values calculated by ClogP. The resulting equation is, as expected, only slightly different from the one based on experimental data:

$$\log S_L = -1.175 \cdot \log K_{ow} + 0.658 \quad \left(n = 88,\ r^2 = 0.982\right) \tag{8}$$

The solubility of solid PAHs starts to deviate from this line with decreasing solubility, because these compounds are solids at 25 ˚C and additional energy is needed to break up the crystals to dissolve the compounds. For the solid aromatics a linear relationship exists between solubility and log K_{ow}, which is clearly different from Equation 8 for the solubility of liquids:

$$\log S_{solid,ar} = -1.723 \cdot \log K_{ow} + 2.162 \quad \left(n = 10,\ r^2 = 0.959\right) \tag{9}$$

However, in the lower fractions both solid and liquid aromatic compounds occur. For this reason, the average solubility of aromatic compounds is not a linear relationship, but a polynomial one (curved line in Figure 2.6):

$$\log S_{aromatics} = -0.171 \cdot \log K_{ow}{}^2 - 0.231 \cdot \log K_{ow} - 0.436 \quad \left(n = 27,\ r^2 = 0.951\right) \tag{10}$$

2.2.4.2 Mixture solubility

The dissolution of compounds from oil into an aqueous phase can be well described by Raoult's law (Cline et al., 1991; Schluep et al., 2002; Verbruggen, 1999), also for more polar petrogenic compounds (Schmidt et al., 2002). With this equation the concentration at saturation in the aqueous phase (water, pore water) of each component (C_w) can be estimated from the mole fraction of that compound in the organic liquid oil phase (x) and the solubility of the pure (subcooled) liquid compound (S_L) (Banerjee, 1984):

$$C_w = x \cdot S_L \tag{11}$$

Although many pure compounds of mineral oil are solids at room temperature, mineral oil itself is mostly a (viscous) liquid, due to the heterogeneity of the compounds and thus Raoult's law can be applied. For solid compounds, the solubility of the subcooled liquid is used in Raoult's law instead of the solubility of the solid state.

2.2.5 Organic carbon-water partition coefficient

The partitioning of a compound between organic matter and water is described the organic carbon-water partition coefficient (K_{oc}), the ratio of the concentrations in both phases:

$$K_{oc} = \frac{C_w}{C_{oc}} \tag{12}$$

If not the fraction organic carbon, but the fraction organic matter is reported, the fraction organic carbon is calculated as the fraction organic matter divided by 1.7 (Traas, 2001). Many studies have been performed to determine the organic carbon-water partition coefficient (K_{ow}) of aromatic hydrocarbons, both monoaromatic and polycylic compounds. A well known relationship between K_{oc} and K_{ow} is the following equation of Karickhoff et al. (1979) based on experiments with 10 compounds of which 8 are non-halogenated aromatic compounds, mostly PAHs, in three sediments:

$$\log K_{oc} = \log K_{ow} - 0.21 \tag{13}$$

The data for monoaromatic compounds and PAHs for sediments (Karickhoff et al., 1979) but also for soils (Karickhoff, 1981) fit well to this equation. Similar results are presented for PAHs by other authors by means of the most appropriate techniques (De Maagd et al., 1998a). For non-substituted aliphatic compounds no data for log K_{oc} are available for soil and sediment. However, Poerschmann and Kopinke (2001) measured the partition coefficient of PAHs and *n*-alkanes to dissolved humic organic matter (HOM). When these partition coefficients are corrected for the percentage in organic carbon in organic matter (by the standard factor in INS of 1.7), the resulting log K_{oc} values for PAHs are in accordance with the other data for PAHs (Figure 2.7). The data for *n*-alkanes, however, are not in line with these data. The well-known relationship between log K_{oc} and log K_{ow} for predominantly hydrophobic compounds from Sabljić et al. (1995) seems to describe these data more accurate (Figure 2.7). Therefore, this equation is used for the aliphatic compounds:

$$\log K_{oc} = 0.81 \cdot \log K_{ow} + 0.1 \qquad (14)$$

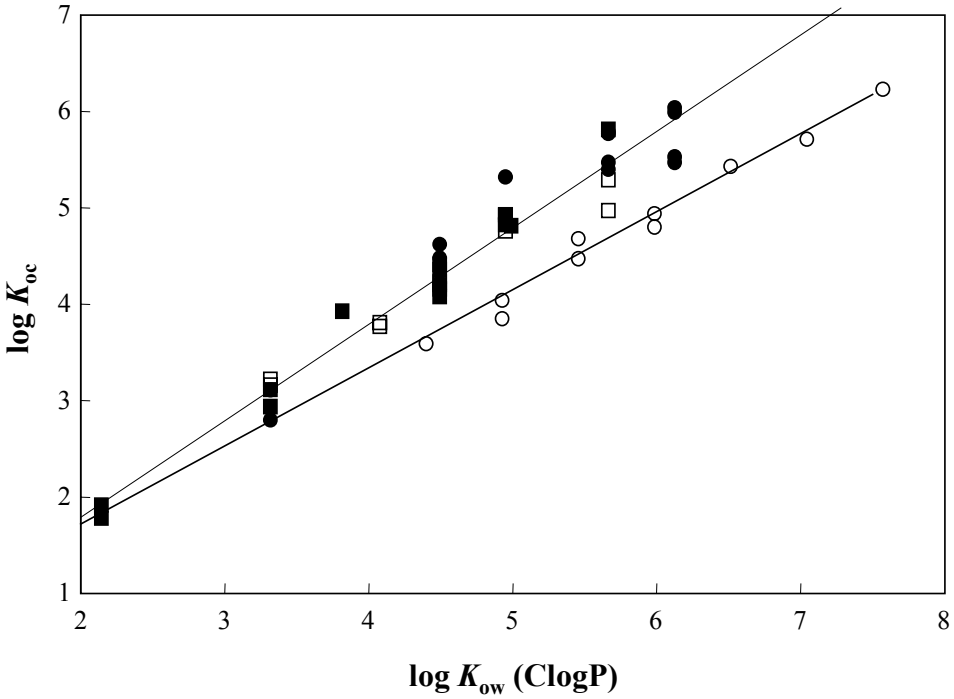

Figure 2.7: Organic carbon-water partition coefficients as a function of log K_{ow}*. Upper drawn line is the selected QSAR for the aromatic compounds (Karickhoff et al., 1979), lower drawn line is the selected QSAR for the aliphatic compounds (Sabljic et al., 1995). Data: ■: PAHs and benzene from (Karickhoff et al., 1979; Karickhoff, 1981) ●: PAHs from (De Maagd et al., 1998a); □: PAHs and ○: n-alkanes from (Poerschmann and Kopinke, 2001).*

2.3 Mode of toxic action

2.3.1 Narcosis and bioconcentration

The mode of toxic action of mineral oil is presumably narcosis or baseline toxicity (Peterson, 1994). This method is proposed in the risk assessment of petroleum derived products (CONCAWE, 1996). The research with marine benthic organisms preceding this report also showed that narcosis is the most likely mode of toxic action of mineral oil (Van Hattum et al., 2000).

Narcosis is an effect that is related to accumulation of xenobiotic organic chemicals in the cell membranes of organisms (Van Wezel and Opperhuizen, 1995). Narcosis has some important characteristics. First, the effects of different chemicals that act by narcosis are completely concentration additive. This effect has been shown in several studies with up to 50 chemicals for acute effects to fish (Könemann, 1981; Broderius and Kahl, 1985) and acute as well as chronic effects for daphnids (Hermens et al., 1984; Hermens et al., 1985). Further, the internal concentrations on a molar basis, at which narcotic effects occur, are equal for different compounds. All compounds, thus have the same, toxic potency for narcosis, for lethality mostly referred to as critical body residue (CBR) (McCarty and Mackay, 1993). It would therefore be useful to assess the accumulation of compounds from the environment to the cell membranes of the organisms, the target lipid for narcosis. The concentration in these membranes (C_m) can be estimated from the aqueous concentration (C_w) by the membrane-water partition coefficients (K_{mw}):

$$K_{mw} = \frac{C_m}{C_w} \tag{15}$$

From a large database of acute aquatic toxicity data (33 species and 145 chemicals, total 722 data points with log K_{ow} values ranging from –1.48 to 5.32), DiToro et al. (2000) derived species specific critical target lipid concentrations and a general equation for log K_{mw} as a function of log K_{ow}. The LC50 data were fitted against log K_{ow} with an equal slope but with different intercepts for both species and a few chemical classes. The LC50 data are related to the target lipid concentration for lethality for narcosis (C_m^*) by the membrane-water partition coefficients, as can be seen from rewriting Equation 15:

$$LC50 = \frac{C_m^*}{K_{mw}} \; or \; \log LC50 = \log C_m^* - \log K_{mw} \tag{16}$$

A linear relationship exists between log K_{mw} and log K_{ow}:

$$\log K_{mw} = a_0 + a_1 \cdot \log K_{ow} \tag{17}$$

By assuming that a_0 in this relationship is equal to zero, the y-intercept of the regression of log LC50 with log K_{ow} consists only of a target lipid concentration, that is species specific with a correction for certain chemicals classes. DiToro et al. (2000; 4th of April 2003) derived critical target lipid concentrations for narcosis for 33 aquatic species (lethality) and four algae species (growth), which range from 34.3 to 431 µmol/g lipid.
For the final chronic value (FCV), a parameter that is comparable with the MPC (HC_5)[4], a lipid concentration of 6.94 µmol/g_{lipid} has been derived for non-halogenated aliphatic hydrocarbons. Because there appeared no statistical difference between this group of compounds and ethers, alcohols and aromatics, all these chemical classes have the same critical target lipid concentration. For halogenated compounds, ketones and PAHs, correction factors are applied resulting in critical target lipid concentrations of 3.96, 3.95 and 3.79 µmol/g_{lipid}, respectively.
The regression also lead to the following equation for the membrane-water partitioning:

[4] The final chronic value (FCV) is used by the Environmental Protection Agency (EPA) in the US to set water, soil and sediment quality criteria (WQC and SQC, respectively). The value is derived from 5th percentile of the data set of acute lethal toxicity, which is called final acute value (FAV). To this FAV an acute to chronic ratio (ACR) is applied to derive the FCV.

$$\log K_{mw} = \tilde{} 0 + 0.945 \cdot \log K_{ow} \quad (18)$$

The choice that the intercept a_0 is equal to zero is based on the observation that predicted target lipid concentrations with omission of the factor a_0 are comparable with experimental body residues, but the variation in the ratio between observed and predicted internal concentrations for lethality is substantial. Nevertheless, this equation for partitioning to biological membrane lipids is very comparable with the following equation derived for partitioning of compounds to artificial membrane vesicles (phospholipid (DMPC) bilayers) in the range from log K_{ow} 1.76 to 5.18 (Verbruggen et al., 2000b):

$$\log K_{mw} = 0.33 + 0.92 \cdot \log K_{ow} \quad (19)$$

When more literature data for artificial membrane partitioning (DMPC) of 86 compounds are considered (Gobas et al., 1988; Dulfer and Govers, 1995; Vaes et al., 1997; Vaes et al., 1998), the partition coefficients derived from a polynomial fit are within a factor of 2 from Equation 18 for log K_{ow} values ranging from –1.5 to 6.5 (Figure 2.8). However, above and below these values a deviation from linearity can be observed:

$$\log K_{mw} = -0.076 + 0.857 \cdot \log K_{ow} + 0.082 \cdot \log K_{ow}^{\ 2} - 0.012 \cdot \log K_{ow}^{\ 3} \quad (n = 86,\ r^2 = 0.957) \quad (20)$$

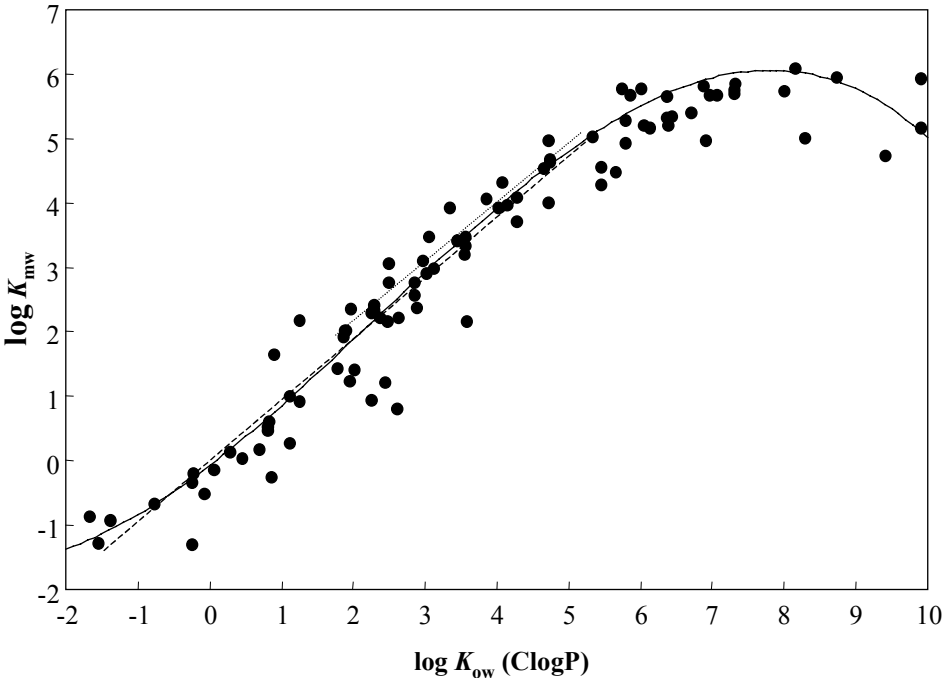

Figure 2.8: Partition coefficients to artificial DMPC membrane vesicles (K_{mw}) as a function of log K_{ow}. log K_{mw} *from (Gobas et al., 1988; Dulfer and Govers, 1995; Vaes et al., 1997; Vaes et al., 1998). ---- : Equation 18; ···· : Equation 19; — : Equation 20.*

On basis of the same studies, a linear relationship is claimed between log K_{mw} and log K_{ow} (Patel et al., 2002). However, in this regression a lot of the more hydrophobic compounds from the study of Gobas et al. (1988) are omitted without clarification. Moreover, if the slope of the relationship between log K_{mw} and log K_{ow} is considered, it changes from 0.761 to 0.971 if data above log K_{ow} of 4.5 are omitted (Patel et al., 2002). The latter value is more representative of the above-mentioned slope for partitioning to cell membranes. The change in

slope is therefore considered an indication of non-linearity and the claimed linearity by Patel et al. (2002) a result of the lack of enough data in the highly hydrophobic region. Further, in the individual studies (Gobas et al., 1988; Dulfer and Govers, 1995), a parabolic relationship between log K_{mw} and log K_{ow} was mentioned as well.
For PAHs the uptake by *Daphnia magna* seems to be linear with log K_{ow} up to the highest PAHs tested (Newsted and Giesy, 1987). The log K_{ow} value for all PAHs in the boiling point range to C35 is up to 7, where deviation from linearity is, at least, not substantial. For *n*-alkanes, however, this log K_{ow} is already exceeded at equivalent carbon numbers higher than 12. Further, the bioconcentration factors (BCF) of *n*-dodecane and 2,2,4,6,6-pentamethylheptane in fathead minnows appeared to be at least one order of magnitude lower, on a lipid weight basis than predicted from Figure 2.8. This can probably be attributed to biotransformation (Tolls and Van Dijk, 2002). If the very low solubility of these higher aliphatic compounds is considered together with the relatively low bioconcentration, it is evident that the contribution of these compounds to the internal concentration in the membrane is limited.
In this report, the linear Equation 18 is used for the fraction with log K_{ow} values up to 5.5. Above this value the polynomial Equation 20 is used.

2.3.2 Phototoxicity

For PAHs it is known that they can exert an enhanced toxicity due to a mechanism that is called phototoxicity or photoinduced toxicity (e.g. Mekenyan et al., 1994b; Newsted and Giesy, 1987). Ultraviolet (UV) light can activate PAH molecules so that they become in an excited state. The phototoxicity is related to the energy difference between the ground and excited state of the PAH molecules (Mekenyan et al., 1994a).
Bioaccumulated PAH molecules in excited state can lead to the formation of excited single-state oxygen, which is highly biologically reactive (photosensitization). Before uptake by the organisms, PAH molecules in excited state can also be transformed, to new compounds, which have different biological activities (photomodification), mostly by oxidation reactions (Krylov et al., 1997; Huang et al., 1997; El-Alawi et al., 2002a; El-Alawi et al., 2002a; El-Alawi et al., 2002b).
Phototoxicity is also observed for many different petroleum mixtures (Pelletier et al., 1997; Wernersson, 2003; Barron et al., 2003). In an experiment to distinguish between photosensitization and photomodification, it appeared that for herring larvae photosensitization was the major mechanism of toxicity. In this mechanism, bioaccumulated parent PAHs are activated by UV light (Barron et al., 2003). Therefore, bioaccumulation of the parent compound seems to be important for the photoinduced toxicity of oil. If this is the case, the risk limits based on internal concentrations implicitly take the effect of phototoxicity into account.

2.3.3 Physical soiling

Although physical soiling of aquatic, benthic and terrestrial organisms by undissolved mineral oil is possible, little is known about such an effect. However, it is recognised that a blanketing effect may have adverse effects on organisms (CONCAWE, 1996; Lourens et al., 2000). Effect levels have not yet been established. For this effect, the accumulated amount into the organisms is not an important parameter, because covering of the outside of organisms with oil causes this effect. Therefore, an assessment of the toxicity on basis of external total concentrations as well as internal concentrations should be made to cover the effects of physical soiling.

3. Methods

3.1 Data Search and selection

3.1.1 Toxicity data

Two sets of data have been used for the derivation of the risk limits. These data are the toxicity data performed with the gas oil DMA and the lubricant HV46 for marine (Brils et al., 2000; Kater and Schout, 2000) and fresh water (Rotteveel et al., 2002; Aquasense, 2002) sediment toxicity tests.
The marine species used were the amphipod *Corophium volutator* (mud shrimp), the luminescent bacterium *Vibrio fischeri* (Microtox) and the echinoderm *Echinocardium cordatum* (sea urchin). The exposure time for the mud shrimp was 10 days, with mortality as recorded endpoint. The Microtox solid phase test was exposed for 10 minutes, with bioluminescence inhibition as recorded endpoint. The exposure time for the sea urchin was 14 days, with mortality and reburial behaviour as the recorded endpoints. The sediment used was collected from Oesterput (Oosterschelde, The Netherlands), which was sieved with a 0.5 mm sieve for the tests with *Corophium* and *Vibrio* and a 0.5 cm sieve for the test with *Echinocardium* (Brils et al., 2000).
The fresh water species used were the insect *Chironomus riparius* (1^{st} instar midge larvae), the nematode *Plectus acuminatus*, the amphipod *Hyalella azteca* (juvenile, 7-14 days) and the insect *Ephoron virgo* (mayfly, juvenile, ≤ 2 days). For the midge larvae the exposure time was 10 days, with mortality, weight, and development to 2^{nd}, 3^{rd}, and 4^{th} instar larvae as recorded endpoints. The exposure time for the nematodes was 14 days, with mortality as recorded endpoint. For the amphipods and the mayflies the exposure time was 10 days and the recorded endpoints were mortality and weight. The sediment used was retrieved from lake Drontermeer (The Netherlands) and was sieved with a 0.5 mm sieve.
To compare the results of the used methods with other literature data, more toxicity data with oil were considered. These are taken from the PERF database for mortality to earthworms of field contaminated soil samples (Gas Research Institute/PERF, 2000), and acute toxicity data of DMA to *Daphnia magna* (water flea) (Verbruggen, 1999; Verbruggen and Hermens, 2001).

3.1.2 Chemical analyses

3.1.2.1 Analysis of total mineral oil

For all samples the total concentration of mineral oil (C10-C40) is determined by gas-chromatography with flame ionisation detection (GC-FID) (Rotteveel et al., 2002; Harmsen and Zweers, 2000). Concentrations were measured at the beginning of the experiments, except for three concentrations for both DMA and HV46 in the fresh water sediment that were also measured after 6 and 16 days. The total concentrations of mineral oil showed no significant decline in this period. This is in accordance with the experiments with DMA and HV46 in the marine sediment, where the total mineral oil concentration, measured again after 4 and 11 months, showed no significant decline either.
For the control sediments a significant analytical response was measured. The three control samples that were measured had a mineral oil content of 31, 107, and 34 mg/kg_{dw} (for the marine Oesterput sediments <0.5 mm and <0.5 cm, and the fresh water Drontermeer sediment, respectively).
The part of the oil added that is not recovered in the analysis, was disregarded. This seems to be justified, because this part was not extracted from the sediment with an organic solvent

extraction and will also not be bioavailable. However, this part may still be present in the sediment, because it was observed that the loss of TPH was compensated by an equivalent increase in organic carbon in the sediment as determined by ignition loss (Harmsen and Zweers, 2000).
What part of the analytical response is caused by the naturally occurring organic compounds and organic matter in the soil (background concentration, C_b) and what part can be attributed to the addition of the oil samples was estimated by fitting the total actual concentrations to the following formula:

$$\log C_{\text{actual}} = \log\left(recovery \cdot C_{\text{nominal}} + C_b\right) \tag{21}$$

With this equation, both high and low concentrations have equal influence on the estimated recovery. Then, for samples with a nominal concentration lower than 200 mg/kg$_{dw}$ the added part resulting from spiking with oil was calculated as:

$$C_{\text{added}} = recovery \cdot C_{\text{nominal}} \tag{22}$$

For samples with a nominal concentration above 200 mg/kg$_{dw}$ the added part resulting from spiking with oil was calculated as:

$$\log C_{\text{added}} = C_{\text{actual}} - C_b \tag{23}$$

Thus, at low concentrations the added part of the actual concentration is a fixed percentage of the nominal concentration, with the variable background being the rest of the actual concentration. At high concentrations, the added part of the actual concentration is the measured actual concentration minus a fixed background concentration. In doing so, the recovery does not become extremely high or low at the low concentrations and the background concentration does not become extremely high or low at the higher concentrations.

3.1.2.2 Fraction analysis

For the marine sediment samples, a distribution of the concentration per equivalent carbon number is given (Harmsen and Zweers, 2000). For two samples of both DMA and HV46 (high and low concentration) in fresh water sediments and for the products DMA and HV46 a complete fraction analysis was performed (Walraven and Peters, 2002) in analogy with the TPHCWG method (Potter and Simmons, 1998). In this method the sample is fractionated into an aliphatic and an aromatic fraction on a silica column. For each of these fractions the amount per equivalent carbon number was determined by GC-FID. Because no blank sediment, for which the actual concentration of total mineral oil can be substantial, was analysed for aliphatic and aromatic compounds, the estimation of the distribution for the lower concentrations and blank sediments is hampered.
The control sediments in the sediment bioassays had concentrations of 34 mg/kg$_{dw}$ for the Drontermeer sediment <0.5mm, 31 mg/kg$_{dw}$ for the Oesterput sediment <0.5 mm, and 107 mg/kg$_{dw}$ for the Oesterput sediment <0.5 cm. It can thus be stated that the sediments are rather close to the upper limit of what can be expected from only biogenic origin. For the sediments of lake Drontermeer and Oesterput sieved over 0.5 mm a ratio between aliphatic and aromatic compounds of one will be assumed (see 2.1.1).
The marine Oesterput sediment with particle size <0.5 cm is likely to contain relatively less aromatic compounds than that with a particle size <0.5 mm, because this sediment has less

fine particles, has the highest organic carbon content (1.9%) and a concentration of hydrocarbons of 107 mg/kg_{dw} (see 2.1.1). Because of the higher particle size an elevated level of debris from plants can be expected. The chromatogram of the extract of this sediment indeed shows a strong concentration at the higher boiling point ranges (90% higher than C21), the range in which the terrestrial plant waxes are found. However, it might as well be possible that this background concentration consists of UCM, which is related to a historic pollution with oil. In both cases, the background concentration is likely to contain more aliphatic compounds, but the extent of the enrichment is not known. Further, the higher background concentration might also be caused by soot particles. Therefore, it is assumed that the sediment with particle size <0.5 cm has also an equal amount of aromatic and aliphatic compounds, just as in the <0.5 mm sediments.
For fresh water lake Drontermeer sediment, the profiles of the low and high sediment concentrations were used together with the profile of the pure products DMA and HV46 to estimate the profile of the background concentration. The added part of the sediment concentration, estimated by Equation 22 (added concentrations below 200 mg/kg_{dw}) and 23 (added concentrations above 200 mg/kg_{dw}) were supposed to be similar in composition as the pure product. The difference (if positive) between the actual profile and this profile of the added part was assumed to be part of the background concentration. For the general background profile the relative profiles of the four concentrations were averaged, with equal amounts in the aliphatic and aromatic fraction.
With the profile for the background concentrations, the profiles for the added part at low and high nominal concentrations were estimated. From the resulting profiles for the added part of both the low and high sediment and the profile for the background concentrations, the rest of the profiles were estimated. The profiles of the intermediate concentrations were interpolated while profiles of the lowest and the highest concentrations were taken equal to the profiles of the low and the high concentration.
For the marine sediment, the boiling point profiles were available for all concentrations. The distributions of the added part over the aliphatic and aromatic fraction were assumed to be equal as in the fresh water sediments. Also in this case, the profiles of the low concentration was used for concentrations lying below this low concentration, while the profile of the high concentration was used for concentrations lying above this high concentration. Concentrations lying in between were interpolated.

3.2 Equilibrium partitioning and narcotic body burden

3.2.1 Outline of the method

Within the project “Setting Integrated Environmental Quality Standards” (Traas, 2001) ERLs for different compartments (water, soil, and sediment) are harmonised by applying the equilibrium-partitioning concept (DiToro et al., 1991), which assumes equilibrium between the concentration in organic carbon and (pore) water. In addition, it is assumed that toxicity is related to pore water concentrations, and that the sensitivity of aquatic organisms is comparable to that of organisms living in soil or sediment (Traas, 2001).
Here, the calculation of the narcotic body burden, i.e. the total molar concentration of compounds in the membrane of organisms, assumes equilibrium partitioning. The calculation of the membrane concentration is performed in two steps. First, the concentrations of the different fractions in the aqueous phase are calculated. This step involves a calculation of the different fraction of oil equilibrated over three phases simultaneously, instead over organic carbon and the aqueous phase only. These phase are organic carbon of the sediment or soil, an oil phase, which may be present in the form of droplets or a layer or coating on soil/sediment

particles, and an aqueous phase, which represents the pore water of the soil or sediment. In a second step, the accumulation in the cell membrane of compounds from each fraction is calculated from the aqueous concentration of that fraction. The narcotic body burden is the sum of the concentration in the cell membrane of all fractions together. These two steps will be described in more detail below.
This equilibrium partitioning method has some limitations. It does not take into account the time aspect of several processes (e.g. kinetic effects of uptake and desorption, biodegradation and biotransformation or ageing), or exposure routes other than via the aqueous phase (e.g. biomagnification).

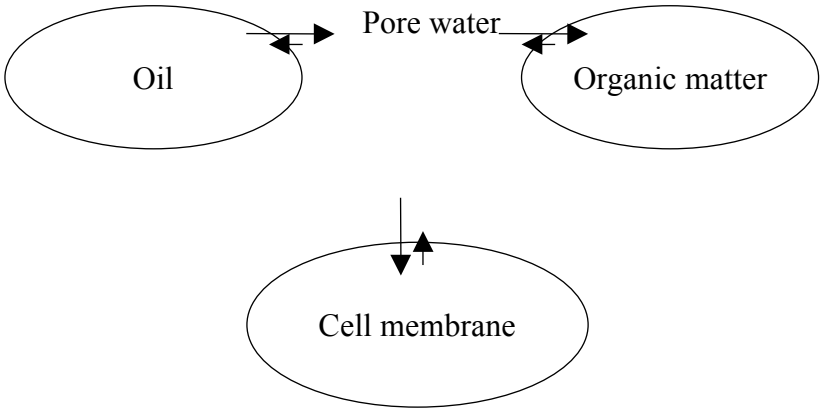

Figure 3.1: Diagram of the three phases that are used in the equilibrium partitioning method.

3.2.2 Boiling point ranges in the aliphatic and aromatic fraction

To calculate the total narcotic body burden from concentrations in sediment, values for molecular weight, solubility, organic carbon-water and membrane-water partition coefficients have to be assigned to each boiling point fraction in the aromatic and aliphatic fraction. Only the molecular weight is directly derived from the equivalent carbon number, the other properties are estimated from log K_{ow}, which is derived from the EC number by Equation 5 (aliphatic fraction) and Equation 6 (aromatic fraction).
For the calculation of the aqueous concentrations, the compositional information on boiling point ranges was used to redistribute the mixture over 19 fractions of 0.5 log K_{ow} unit. These fractions are shown in the table below, together with physicochemical properties.

3.2.3 Calculation of aqueous concentrations

The concentration in sediment was recalculated to a concentration in target lipids by calculating the concentration dissolved in pore water first. This was done by assuming that oil components can be present in three phases in the sediment, which are organic matter, oil droplets or coatings/layers and pore water (Figure 3.1). These phases are considered to be homogeneous. The concentrations of each component in organic matter and pore water and in the oil phase and pore water were assumed to be in equilibrium.
The aqueous concentration is calculated from the organic carbon-water partition coefficient by means of Equation 12. To estimate the values of K_{oc}, the equation presented by Karickhoff et al. (1979) (Equation 13) was used for the aromatic fractions and the equation from Sabljic et al. (1995) (Equation 14) was used for the aliphatic fractions.
A second route for partitioning to water is from the oil droplets or layers. To quantify this process, the aqueous concentration of an oil component is calculated from its mole fraction in

the organic oil phase and its solubility according to Raoult's law (Equation 11), with the solubility calculated from log K_{ow} by means of Equation 8.

Table 5: Used blocks in the calculations together with their properties

Compounds	Range *EC*	*Mw* [g/mol]	Log K_{ow}	log S_L [M]	log $S_{aromatics}$ [M]	log K_{oc}	log K_{mw}
Aliphatic	7-10	123	5.25	-5.51		4.35	4.96
	10-11	151	6.25	-6.69		5.16	5.64
	11-12	165	6.75	-7.27		5.57	5.86
	12-13	179	7.25	-7.86		5.97	6.01
	13-14	193	7.75	-8.45		6.38	6.06
	14-15	208	8.25	-9.04		6.78	6.03
	15-16	222	8.75	-9.62		7.19	5.89
	16-17	236	9.25	-10.21		7.59	5.63
	17-40	405	9.75	-10.80		8.00	5.26
Aromatic	7-12	125	3.25	-3.16		3.04	3.07
	12-15	147	3.75	-3.75		3.54	3.54
	15-18	166	4.25	-4.34	-5.16	4.04	4.02
	18-22	188	4.75	-4.92	-6.02	4.54	4.49
	22-25	210	5.25	-5.51	-6.88	5.04	4.96
	25-28	229	5.75	-6.10	-7.75	5.54	5.35
	28-32	252	6.25	-6.69	-8.61	6.04	5.64
	32-35	274	6.75	-7.27	-9.47	6.54	5.86
	35-38	293	7.25	-7.86	-10.33	7.04	6.01
	38-40	309	7.75	-8.45	-11.19	7.54	6.06

The molar aqueous concentration (C_w) for each fraction is calculated by numerically solving equations 11 and 12 for all 19 fractions from Table 5 simultaneously. The compositional information needed to perform the calculations is shown in the Appendix. Precision of the calculated concentrations was at least 1‰, but generally much better. Further, it was assumed that compounds in the higher aromatic fractions (especially PAHs that are solid at room temperature) couldn't be present in the aqueous phase at concentrations higher than the average solubility of the pure compounds of that fraction. When estimated concentrations by Raoult's law exceeded this solubility, the aqueous concentration was set equal to the average solubility of the pure compounds in that aromatic fraction (Equation 10).

3.2.4 Calculation of total membrane concentration

From the dissolved aqueous concentration, the concentration in the target lipids for narcosis (membranes) is calculated. For each fraction, the concentration in membranes was calculated from the concentration in pore water by the membrane-water partition coefficient according to Equation 15. For log K_{mw} in the range of log K_{ow} values below 5.5 Equation 18 was used. For the higher fractions, the polynomial relationship from Equation 20 was used. The total narcotic body burden is the sum of the membrane concentration of all aromatic and aliphatic fractions.

3.3 Derivation of ERLs

3.3.1 Endpoints used in the derivation of ERLs

Because the concentrations were recalculated to internal membrane concentrations and the endpoints originated from different toxicity studies, all NOEC, EC10 or EC50 values were recalculated. The method used to derive NOEC from the toxicity data was the Dunnett's multiple comparison test incorporated in GraphPad Prism (GraphPad Software Inc., 1996). It

should be noted that with the Dunnett's test all test concentrations are compared with the control, which in itself is not an ideal control, because it contains aliphatic and aromatic compounds from biogenic origin, and possibly some field contamination.
For most of the dose-response curves, the four parameter logistic equation incorporated in GraphPad Prism was fitted through the effect data versus the logarithms of the concentrations using nonlinear regression (GraphPad Software Inc., 1996):

$$Response = Bottom + \frac{Top - Bottom}{1 + 10^{(\log EC50 - \log C)\cdot Hillslope}} \tag{24}$$

In this equation log C is the logarithm of concentration, the response starts at the value of *Bottom* and goes to the value of *Top* with a sigmoidal shape. The parameter *Hillslope* describes the steepness of the curve. From this curve the $EC50$ (or $LC50$) is derived directly. The $EC10$ can also be calculated by this equation by replacing the parameter *Hillslope*:

$$\log EC10 = \log EC50 - \frac{0.954243}{Hillslope} \quad or \quad Hillslope = \frac{0.954243}{\log EC50 - \log EC10} \tag{25}$$

If in the dose-response curve the response is not increasing but decreasing with increasing concentration, the sign (+ or -) before the number 0.954243 (=log(0.9/0.1) should be reversed. For the endpoint mortality the maximum mortality (parameter *Top*) was held constant at 100% mortality. The minimum mortality was fitted and thus variable, provided that it was not negative. The percentage reburial of the sea urchin, and percentage development delay and dry weight of the midge larvae were treated in the same way.
Some parameters, such as dry weight of the amphipod *Hyalella* and the length of the mayfly, do not fit well with a log-logistic equation. For these parameters, the following dose-response curve for continuous endpoints was used (Slob, 2002):

$$Response = a \cdot \left(c - (c-1) \cdot e^{-\frac{C(concentration)}{b}} \right) \tag{26}$$

In this equation a, b, and c are variables. The EC50 and EC10 can be calculated as $-b\cdot\ln(0.5)$ and $-b\cdot\ln(0.9)$, respectively. For the nematode *Plectus*, the mortality data were also plotted with Equation 26, with the parameter c set to zero, to force the equation to 0% survival at high concentrations. However, the scatter of these data and control mortality (10-70%, on average 35%) are high, and thus the meaning of this analysis is limited.
For bioluminescence from the Microtox solid phase assay, the maximum and minimum values for luminescence are set to 100% and 0% because this is a scale relative to the control. The control sediment has thus 0% inhibition, and hence 100% luminescence. Through this equation, a log-logistic dose-response curve (Equation 25) can be fitted. However, in this case it is not known whether or not there is some inhibition of luminescence by the presence of the background concentration. Therefore, the data were also fitted to Equation 26, with the parameter c set to zero, to force the equation to go to 0% luminescence at very high concentrations. The results using both equations appeared to be rather similar. The Microtox data can also be fitted by a log-linear relationship between the logarithm of the parameter gamma (inhibited/remaining luminescence) and the concentration is used (Brils et al., 2000). Because the log-logistic dose-response curve yielded intermediate results in both cases this analysis was used.

3.3.2 Refined effect assessment

Environmental risk limits (ERLs) as derived in the project "Setting Integrated Environmental Quality Standards" are meant to protect the ecosystem at different levels. The aim of the MPC is to protect all species in the ecosystem. For statistical considerations the MPC is set equal to the concentration at which 95% of the species is protected, i.e. the HC5, assuming thereby to protect the whole ecosystem (VROM, 1989; Van Leeuwen et al., 1992). The SRC_{eco} serves as a trigger value for the purpose of soil remediation. The SRC_{eco} is set equal to the concentration at which 50% of the species is protected, i.e. the HC50 (Denneman and Van Gestel, 1990).
If at least 4 NOEC values of species from different taxonomic groups or different terrestrial processes are available, these levels are derived by means of a statistical extrapolation method. The derivation of ERLs in this manner is generally referred to as refined risk assessment (Traas, 2001). The refined risk assessment or statistical extrapolation method is based on the assumption that the sensitivities of species in an ecosystem can be described by a statistical frequency distribution. This statistical frequency distribution describes the relationship between the concentration of the substance in a compartment and a certain percentage of species unprotected. For a detailed overview of the theory and the statistical adjustments since its introduction, it is referred to the original literature (Kooijman, 1987; Van Straalen and Denneman, 1989; Wagner and Løkke, 1991; Aldenberg and Slob, 1993; Aldenberg and Jaworska, 2000).
The method described by Aldenberg and Jaworska (2000) is used in this report to evaluate the data. This method assumes that the sensitivities of species in an ecosystem can be described by a log-normal probability distribution. The HC50 and HC5, together with their upper and lower estimate of the two-sided 90% confidence interval, are derived from the geometric mean and the standard deviation multiplied by a extrapolation constant, which is dependent on the number of data and the protection level (HC5 or HC50).
The goodness of fit of the normal distribution is tested with the Kolmogorov-Smirnov $D \cdot \mathrm{sqrt}(n)$ test and the Anderson-Darling test (Aldenberg et al., 2002). The Kolmogorov-Smirnov test focuses in the middle of the distribution, while the latter highlights the differences between the tails of the fitted distribution and the data. The average, the standard deviation, and the number of the underlying data define this distribution.
Both the derivation of the HC5 and HC50 by means of the statistical extrapolation method and the goodness of fit tests were performed by the computer program ETX (Van Vlaardingen and Traas, 2002).

3.3.3 Derivation of negligible concentrations (NCs)

The negligible concentration (NC) is derived by multiplying the MPC with a factor 0.01. This factor is supposed to function as protection against mixture toxicity, since species are always exposed in the environment to mixtures of chemicals and complex mixtures of chemicals are generally best described as concentration-additive (Van Leeuwen et al., 1996; Deneer, 2000). It should be kept in mind that mineral oil in itself is already a very complex mixture, of which the compounds are assumed to act concentration additive.

4. Toxicity data and derivation of MPCs and $SRCs_{eco}$

4.1 Data and analysis

For 7 benthic species, the chronic toxicity data are shown in the table below. The results from the toxicity tests were expressed on measured actual mineral oil concentrations and on the calculated target lipid concentration by means of equilibrium partitioning. The results from the dose-effect curves and the ANOVAs are presented in the table below.
The dose-response curves for marine and fresh water sediment species based on actual concentration and estimated internal membrane concentrations are shown in Figures 4.1 to 4.4. In general the curves for the two petroleum products DMA and HV46 are more similar when expressed on basis of estimated membrane concentrations. However, for growth of *Chironomus riparius*, the dose-response relationship for the two types of oil is better predicted on basis of the actual concentration. The same was observed for development and mortality and for mortality of *Hyalella azteca* and *Ephoron virgo*.
Thus, in the tests with the fresh water sediment another mechanism for toxicity seems to be involved for those effects that occur at high actual sediment concentrations. Because actual oil concentrations seem to be the factor that is determining toxicity, physical soiling may cause toxicity in this case.
The high LC50 of the gas oil DMA for *Chironomus riparius* was also confirmed in an acute test (96 h). The toxicity experiment was also accompanied by a measurement of the bioavailable compounds by polyacrylate solid phase microextration (SPME) fibers (Leslie, 2003). Based on the same actual TPH concentrations as used above the LC50 is 3700 mg/kg_{dw}, corresponding to 210 mM based on internal concentrations. Expressed as average concentration on the fiber an LC50 of 350 mM could be derived. With the relationship for this type of fiber between membrane lipids and fiber concentration (Verbruggen et al., 2000b) an internal membrane concentration of 730 mM is estimated.

4.2 Comparison with other toxicity data

4.2.1 Toxicity of oil to terrestrial and aquatic organisms

For the comparison and validation, the used method was also applied to toxicity experiments with oil and terrestrial and aquatic organisms. The gas oil DMA was also tested in an acute test (48 hours) with *Daphnia magna*. With the fraction analysis for the pure product, the membrane concentration can be estimated at each test concentration with the concept of oil-water partitioning (3.2). When toxicity is expressed on basis of these estimated membrane concentrations (Figure 4.5), the EC50 for immobility is 68 mM (90% CI: 57-82 mM). This result is very similar to the EC50 of 78 mM derived on the basis of a hydrophobicity distribution profile, obtained with reversed phase-HPLC (Verbruggen, 1999; Verbruggen and Hermens, 2001). For *Daphnia magna* a lethal membrane burden of 111 mM was derived for narcotic aliphatic and monoaromatic compounds. For PAHs this level is 61 mM (DiToro et al., 2000). 87.5% of the internal concentration estimated for exposure to DMA at the EC50 is caused by the aromatic fraction, with probably a large contribution of the PAHs. The estimated EC50 corresponds thus very well to literature data for narcosis.

Table 6: Derived endpoints for the seven benthic bioassays

Species	Time	Endpoint	DMA								HV46							
			EC50	90% CI		EC10	90% CI		r^2	NOEC[a]	EC50	90% CI		EC10	90% CI		r^2	NOEC[a]
			Based on actual sediment concentrations [mg/kg_{dw}]															
C. volutator	10 d	Mortality	160	150	170	100	81	120	0.94	100	8600	7700	9600	3500	2700	4600	0.93	2700
V. fischeri	10 min	Luminescence	99	72	140	11	4.5	28	0.79	<40	3600	2000	6500	29	7.3	120	0.79	78
E. cordatum	14 d	Mortality	200	180	220	110	90	150	0.96	98	2500	910	6700	91	9.8	850	0.46	730
		Reburial	150	120	180	99	78	120	0.95	98	1500	700	3200	74	12	460	0.57	730
C. riparius	10 d	Mortality	3200	3200	3300	2200	2200	2300	1.00	710	3300	3200	3400	1400	1300	1600	1.00	1100
		Development	2400	2300	2500	1700	1500	2000	1.00	710	2600	2500	2700	1300	1100	1500	1.00	1100
		Dry weight	1900	1300	2900	1100	400	3000	0.97	710	1000	810	1300	280	150	510	0.99	210
P. acuminatus	14 d	Mortality	220[b]	40	400	33[b]	6.1	61	0.27	25	31000	0	70000	4800[b]	0	11000	0.08	>33000
H. azteca	10 d	Mortality	500	460	550	170	140	200	1.00	240	1800	850	4000	140	20	950	0.96	210
		Growth	67	0	180	10	0	27	0.86	25	130	19	240	19	2.9	36	0.95	84
E. virgo	10 d	Mortality	310	250	370	120	79	170	0.99	130	860	600	1200	530	220	1300	0.98	440
		Growth	160	0	370	25	0	56	0.87	<25	250	150	350	38	22	54	0.99	84
			Based on total target lipid concentrations [mM]															
C. volutator	10 d	Mortality	28	26	30	18	15	22	0.95	20	17	16	17	16	15	16	0.92	16
V. fischeri	10 min	Luminescence	16	12	21	1.6	0.73	3.6	0.85	<6.3	14	12	17	4.3	2.8	6.5	0.74	5.6
E. cordatum	14 d	Mortality	30	26	36	20	13	31	0.96	17	13[b]	11	16	7.1[b]	3.8	13	0.25	10
		Reburial	21	18	23	14	12	16	0.98	17	12[b]	10	14	6.9[b]	4.1	12	0.33	10
C. riparius	10 d	Mortality	200	200	210	190	190	190	1.00	120	12[c]	12	12	11[c]	11	11	1.00	9.3
		Development	190	190	190	180	180	190	1.00	120	12[c]	8.4	16	11[c]	2.7	46	1.00	9.3
		Dry weight	180	150	210	150	94	230	0.98	120	9.0[c]	8.3	9.8	6.5[c]	5.4	7.9	0.98	6.1[c]
P. acuminatus	14 d	Mortality	81[b]	33	130	12[b]	5.0	20	0.35	3.3	11[b]	3.8	18	1.6[b]	0.58	2.7	0.19	>13
H. azteca	10 d	Mortality	89	77	100	43	33	57	0.99	50	10	8.4	12	6.5	4.2	10	0.91	6.1
		Weight	4.4	0	9.3	0.67	0	1.4	0.93	3.3	5.0	0	10	0.76	0	1.5	0.98	3.4
E. virgo	10 d	Mortality	62	52	75	29	20	42	0.99	25	8.6	7.8	9.5	7.5	6.0	9.4	0.98	7.1
		Length	34	0	78	5.1	0	12	0.88	<3.3	18	13	22	2.7	2.0	3.4	0.94	3.4

[a]As determined with Dunnett's test. Note that the control is the blank sediment. This control value is therefore not equal to a zero concentration in sediment or internal membranes.

[b]r^2 of regression lower then 0.4 while other fits generally have r^2 higher than 0.8

[c]Difference between DMA and HV46 more than a factor of 10

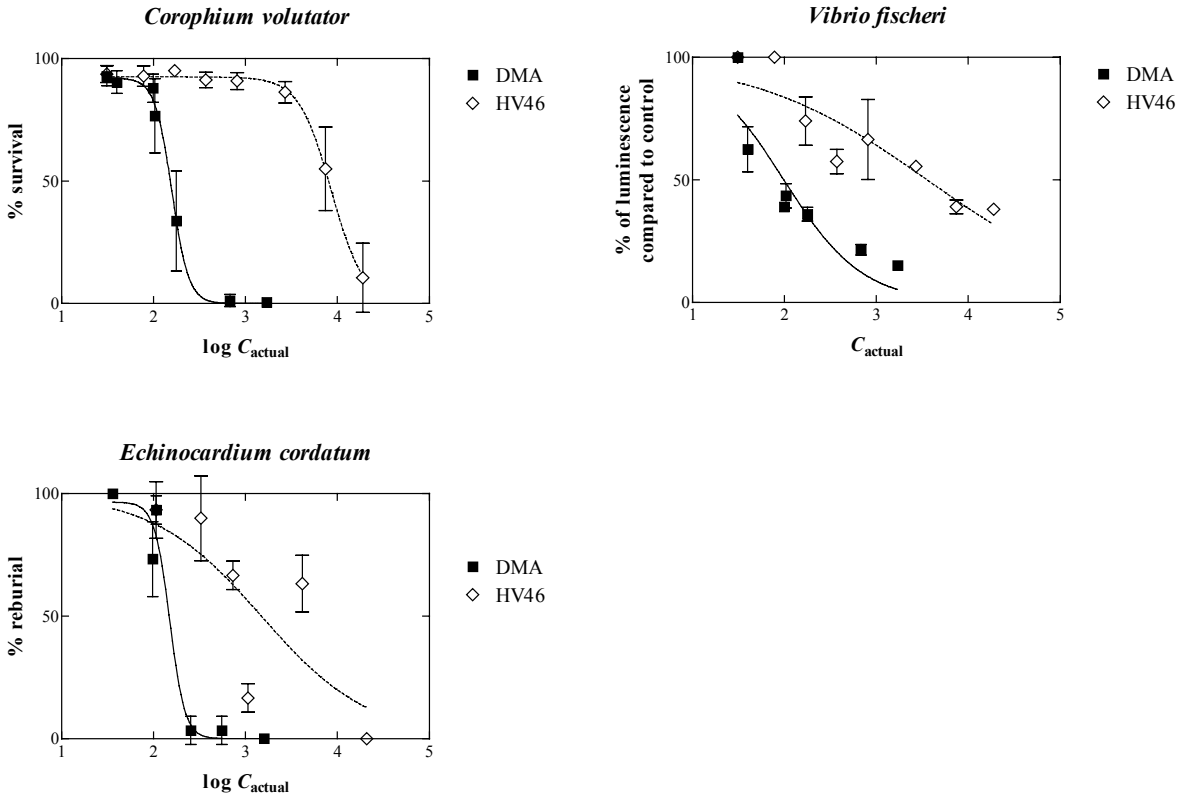

Figure 4.1: Toxicity data for marine sediment species (most sensitive tested endpoint shown on Y-axis) on basis of actual TPH concentrations in sediment (C_{actual} [mg/kg$_{dw}$]).

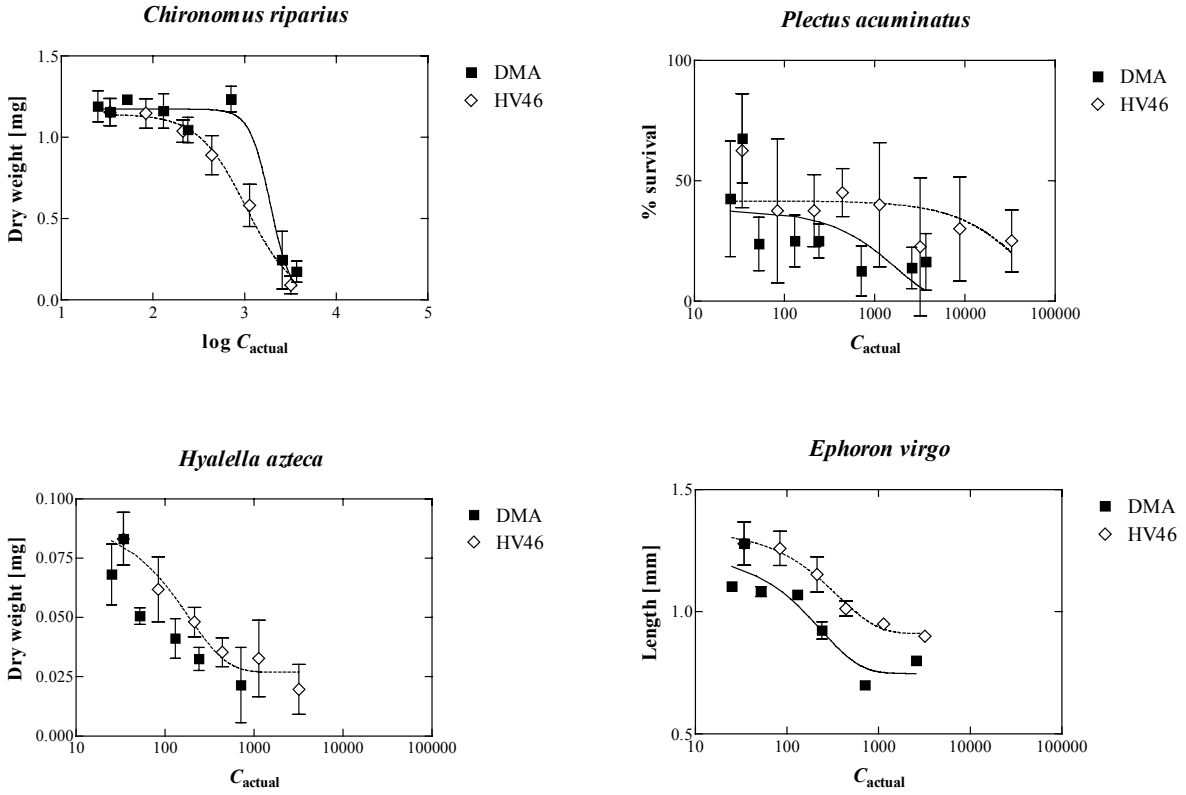

Figure 4.2: Toxicity data for fresh water sediment species (most sensitive tested endpoint shown on Y-axis) on basis of actual TPH concentrations in sediment (C_{actual} [mg/kg$_{dw}$]).

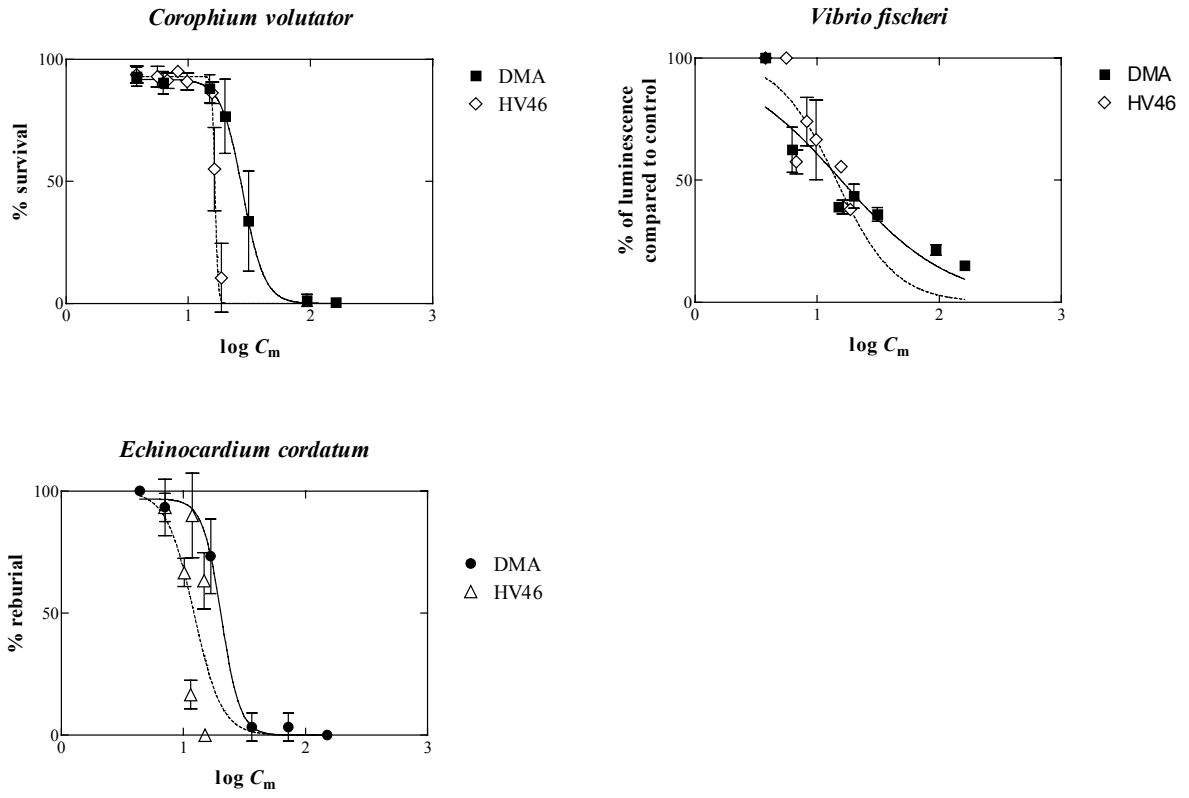

Figure 4.3: Toxicity data for marine sediment species (most sensitive tested endpoint shown on Y-axis) on basis of estimated internal membrane concentration (C_m [mM]).

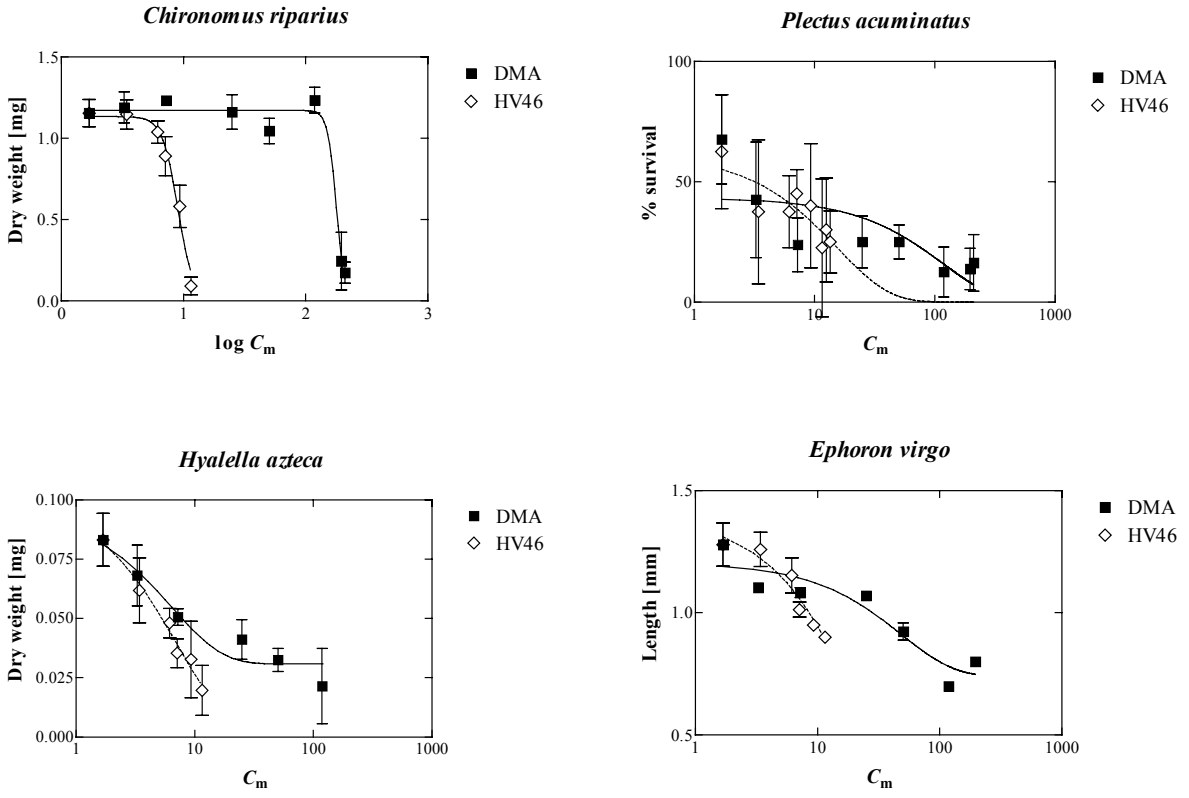

Figure 4.4: Toxicity data for fresh water sediment species (most sensitive tested endpoint shown on Y-axis) on basis of the estimated internal membrane concentration (C_m [mM]).

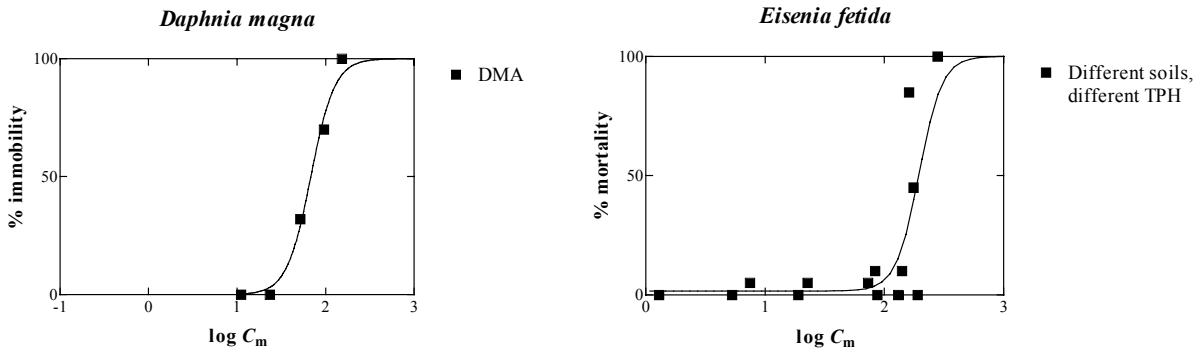

Figure 4.5: Toxicity of the gas oil DMA to water fleas (Daphnia magna) and toxicity of field samples with different types of oil and degree of weathering to earthworms (Eisenia fetida).

Toxicity of different contaminated soils to *Eisenia fetida* in a 14-d test (Gas Research Institute/PERF, 2000), was also expressed on basis of estimated internal concentrations. The method to calculate the internal concentrations was the same as for the sediment tests. Due to the very inhomogeneous character of both the soil types and the oil contamination, the scatter in the data is somewhat larger (Figure 4.5). However, a similar dose-response relationship as for *Daphnia magna* is observed. The LC50 for earthworms (*Eisenia fetida*) is 200 mM (90% CI: 150-250 mM). This value is in the same range as experimental body residues for earthworms (*Eisenia andrei*) with chlorobenzenes (Belfroid et al., 1993). Data for terrestrial plants, beetroot, rape, cucumber, onion, soybean, pea, corn, sorghum, wheat, and tomato, showed similar results with EC50s lying in the range of 79 to 200 mM, although the data are more scattered (Gas Research Institute/PERF, 2000).
Chronic toxicity experiments with *Daphnia* (OECD 211 reproduction test) have been carried with hydrocarbon solvents and petroleum products with a limited boiling point range and composition. For hydrocarbon fluids that consists of primarily isoalkanes with different boiling point ranges, the solubility cut-off point for chronic toxicity to *Daphnia magna* at a loading of 1 mg/L lies in the boiling range of 187-213 ºC (CEFIC, 2002). This corresponds to equivalent carbon numbers of 10.7 to 11.9 (Equation 2). For isoalkanes, the estimated membrane concentration is in this case somewhere between 22 and 75 mM. Data on some lubricant base oils with primarily isoalkane and cycloalkane constituents in the boiling point range above 300 ºC confirm this (data summarised in CONCAWE, 2001), although the presence of 4 to 10% aromatic compounds obscures the results of these studies. Residual aromatic extracts with boiling points higher than 500 ºC, which is equivalent to carbon numbers higher than 32 and log K_{ow} values higher than 6.6, do not show any chronic toxicity towards *Daphnia* either (CONCAWE, 2001). This is expected due to the very low solubility of this fraction (see 2.2.4.1 and Figure 2.6).
In a pilot experiment for the marine toxicity studies (Huwer et al., 1997; Scholten et al., 1997) toxicity experiments where performed with the mud shrimp *Corophium volutator* with various oil types in a marine sediment (Mokbaai, The Netherlands) with very low organic carbon content (0.22-0.25%). The LC50 of DMA was on average 196 mg/kg_{dw} (195, 140, 210, and 272 mg/kg_{dw}). Other gas oils showed similar LC50s: 180 mg/kg_{dw} for high density gas oil and 311 mg/kg_{dw} (438 and 221 mg/kg_{dw}) for low density gas oil. Fuel oil had a higher LC50 of more than 517 mg/kg_{dw}. The LC50s declined remarkably in the 28-d study in comparison with the 10-d study. The LC50 for DMA was 67 mg/kg_{dw} in the 28-d study and the LC10 17 mg/kg_{dw}. Taking the low organic carbon content of the pilot study into account, the oil-water partitioning will prevail at higher concentrations. Assuming the same composition as for DMA in the final study (Brils et al., 2000), this lower organic carbon

content would lead to higher estimated internal concentrations at similar sediment concentrations. Still, the results of this pilot study are rather comparable to the results of the final study, when expressed on basis of estimated internal concentrations. However, the results of the final study seem to show more resemblance to the 28-d study than to the 10-d study. In the 28-d toxicity study, the effect on growth was also examined. This parameter appeared to be less sensitive than mortality.
Hydrocarbon fluids that consists of primarily *n*-alkanes (C14-C16), isoalkanes (C10-C14) and cycloalkanes (C10-C16) were also tested (Huwer et al., 1997; Scholten et al., 1997). The 10-d LC50s for these mixtures were 242, 19, and 122 mg/kg_{dw}. The most toxic of these three, the isoalkane mixture Isopar-L appeared to be non-toxic as water soluble fraction (WSF) in the chronic toxicity study with water fleas, while a mixture with similar boiling point range, Shellsol TD, did show chronic toxicity (CEFIC, 2002). The internal membrane concentrations for these isoalkane mixtures are estimated to be in the range of 20 to 100 mM. The cycloalkane mixture is less toxic to mud shrimps, probably due to the lower hydrophobicity of this mixture. For the *n*-alkane mixture, which consists primarily of *n*-pentadecane the estimated membrane concentrations are so low (<1 mM) that toxicity is probably not caused by narcosis. A more likely explanation is physical soiling. For the gas oils and other hydrocarbon fluids, it is not clear how important physical soiling is for the observed toxicity. These effects can be explained by narcosis as well. For chronic aqueous test with the water soluble fraction (CEFIC, 2002), physical soiling can be excluded as explanation for the toxicity.

4.2.2 Comparison with toxicity of single compounds

For some of the species that were tested, experiments can be found in literature with single compounds that naturally occur in petroleum products. For the luminescent bacterium *Vibrio fischeri* several tests have been conducted with aromatic and some aliphatic compounds that occur in petroleum products (Arfsten et al., 1994; Johnson and Long, 1998; El-Alawi et al., 2001; El-Alawi et al., 2002a; Ren and Frymier, 2002). For these studies performed with the aqueous Microtox test the membrane concentrations at the EC50 can be calculated in the same manner for single compounds as was done for the experiments with petroleum products (Equations 18 and 20). For those compounds for which the aqueous concentration was below the water solubility, the geometric mean of the short-term EC50s (up to 30 min) for *Vibrio fischeri* is about 18 mM. This value is very comparable with the 16 mM that was derived for both types of oil. The ratio between the EC50 and the EC10 appears to be in between 5 and 10 for acenaphthene, acenaphthylene (El-Alawi et al., 2002b), phenanthrene (El-Alawi et al., 2001), and toluene (Chang et al., 1981). The ratios for DMA (10) and HV46 (3) are thus comparable to this. It appeared that the inhibition of luminescence was well correlated with inhibition of growth of the bacteria. Further, UV-light strongly enhanced toxicity of the PAHs in long-term assays with *Vibrio*, while test conditions under darkness or UV-light led to the same results in the common short-term assays (El-Alawi et al., 2001; El-Alawi et al., 2002a; El-Alawi et al., 2002b). Thus, PAHs show some phototoxicity towards *Vibrio*.
Toxicity data for PAHs are also available for *Daphnia*, *Hyalella*, and *Chironomus*. The data for phenanthrene and fluoranthene tested under dark conditions in acute tests (48 h) with *Daphnia magna* (Verhiest et al., 2001) are similar to those for the gas oil DMA, when aqueous concentrations are recalculated to internal membrane concentrations (Equations 18 and 20). However, if white light or UV light is applied toxicity increases (Verhiest et al., 2001; Suedel and Rodgers Jr., 1996). It is concluded that these compounds are photoactivated (Verhiest et al., 2001). Benzo(*k*)fluoranthene is only slightly toxic in the sediment test (Verhiest et al., 2001), which is in accordance with the low solubility of this compound. The duration of exposure leads to increased toxicity after 48 h in comparison with 24 h (Verhiest

et al., 2001) but makes no difference between 10 d and 48 h (Suedel and Rodgers Jr., 1996). The internal membrane concentration derived from the sediment study leads for both studies to similar results as those derived from aqueous concentrations.
For *Hyalella azteca* three studies are available. The internal membrane concentrations calculated from the aqueous LC50s after 10 d of exposure for naphthalene, phenanthrene, fluorene, or pyrene (Lee et al., 2001) ranged between 18 and 29 mM. These concentrations are in between those estimated for the gas oil DMA and the lubricant HV46. The membrane concentration of 22 mM calculated from the 48-h LC50 for fluoranthene (Suedel and Rodgers Jr., 1996) is similar but the membrane concentration calculated from the 10-d LC50 decreased to 7.1-14 mM. Even lower values of 5.3 mM for phenanthrene and 1.1 mM for fluoranthene were estimated from the LC50s of a 14-d sediment toxicity study (Verhiest et al., 2001).
In the same study also a 10-d sediment toxicity test was performed with *Chironomus riparius* (Verhiest et al., 2001). The membrane concentrations calculated from the LC50s were 3.5-4.1 mM for phenanthrene and 3.1 mM for fluoranthene. These numbers are substantially smaller than those of both DMA and HV46 are. In an aqueous toxicity study with *Chironomus tentans* the 48-h LC50 of fluoranthene led to a calculated membrane concentration of 59 mM, but the 10-d LC50 (Suedel and Rodgers Jr., 1996) led to lower calculated membrane concentrations 5.6 to 8.9 mM. Because these values are considerably lower than those of the two petroleum products, phototoxicity of the PAHs may play an important role here. Phototoxicity is a well known mechanism of toxicity for PAHs but it was also observed for petroleum products tested with *Daphnia magna* (Wernersson, 2003). It should be stressed that most of these tests are performed in water and not in sediment.
The equilibrium partitioning method seems to work well in the sediment studies with *Chironomus riparius* and *Hyalella azteca* (Suedel and Rodgers Jr., 1996). The membrane concentrations calculated from NOECs based on sediment concentrations and aqueous concentrations are comparable. Similar to *Daphnia magna*, benzo(*k*)fluoranthene is not toxic in the sediment test to both *Chironomus riparius* and *Hyalella azteca* (Verhiest et al., 2001), which is in accordance with the low solubility of this compound.
In the pilot studies with the mud shrimp *Corophium volutator* also three pure compounds were tested (Huwer et al., 1997; Scholten et al., 1997). These were *n*-pentadecane (C15), *n*-heneicosane (C21), and *n*-octacosane (C28). These compounds are so hydrophobic that, based on equilibrium partitioning, toxicity is not expected. With the same method used for the petroleum mixtures the maximum internal membrane concentrations for these compounds are far less then 1 mM, while an LC50 of 28 mM is estimated for the gas oil DMA. For *n*-heneicosane and *n*-octacosane no toxicity is observed indeed. For *n*-pentadecane, however, toxicity is observed. The 10-d LC50 of *n*-pentadecane is 151 mg/kg_{dw}, the 28-d LC50 68 mg/kg_{dw} and the 28-d LC10 12 mg/kg_{dw}. These values are comparable to those for the gas oil and the hydrocarbon fluids. *n*-Pentadecane differs from *n*-heneicosane and *n*-octacosane in the fact that it is a fluid while the other two are solids. Therefore, it seems plausible that the effects of *n*-pentadecane are caused by physical soiling rather than narcosis. Another plausible explanation for the unexpected toxicity observed for n-pentadecane is oxygen depletion that is very likely associated with biodegradation of this linear structure during the sediment toxicity test.
Toxicity data for oil related compounds are also available for other species. Donkin et al. (1991) examined the effect of aliphatic as well as aromatic compounds on the feeding filtration rate of mussels (*Mytilus edulis*). The geometric mean of the calculated membrane concentrations from the EC50s was 17 mM for this sublethal effect. Further, the range of internal membrane concentration was rather narrow (15 out of 16 lying in between 8 and 26 mM, with one higher outlier). It was observed that for compounds for which the solubility was in the range of values too low to reach this internal membrane concentration, no toxicity

was observed. This was observed for the *n*-alkanes between *n*-decane and *n*-undecane, and for the *n*-alkylbenzenes between *n*-octyl- and *n*-decylbenzene. For the PAHs, no toxicity was observed for pyrene.
Also for terrestrial organisms some data are available. Sverdrup et al. examined the toxicity of PAHs and hetero-PAHs for *Folsomia fimetaria* (springtail) (2001; 2002b), *Eisenia veneta* (earthworm) (2002a) and *Enchytraeus crypticus* (olichaete) (2002). The geometric mean of the internal membrane concentrations that are estimated from the LC50s for *Folsomia* is about 23 mM. This value for the EC10 of reproduction is 6.7 mM. The internal membrane concentrations calculated from the EC10s for growth of the earthworm *Eisenia veneta* and reproduction of *Enchytraeus crypticus* are both 12 mM. For the LC50 of *Eisenia veneta* this value is 33 mM, leaving out 4 values that are estimated to be above water solubility (otherwise 54 mM). The LC50 for *Eisenia fetida*, estimated from the oil polluted soil of the PERF database (Gas Research Institute/PERF, 2000), is about a factor of 6 higher. A solubility cut-off is observed for the reduction in survival of earthworms for both aromatic and aliphatic compounds that can not be accumulated to concentrations in this range due to their low aqueous solubility. This point lies somewhere between decane and dodecane (Young et al., 14-18 April 1998).

4.2.3 Species sensitivity distributions

The derived endpoints for the 7 benthic species and water fleas tested with the gas oil DMA can be compared with other sets of data by looking at species sensitivity distribution as well. Here a comparison is made with the data presented by DiToro et al. (2000; 4th of April 2003). The lethal internal membrane concentrations (or EC50 in the case of algae) from this dataset range from 34.3 to 431 mM. When comparing this to the toxicity data for the petroleum products, it should be kept in mind that the majority of the lethal body burden is caused by the aromatic fractions in the case of the petroleum products. Therefore, for a good comparison the data for lethal body burden for the 38 species are multiplied by 0.546 to correct for PAHs (DiToro et al., 2000). It appears that the distribution for DMA is almost identical to the dataset of 38 species from DiToro et al. (DiToro et al., 2000; DiToro, 4th of April 2003) derived from toxicity data for single compounds (Figure 4.6), even more so if the EC50 for bioluminescence for *Vibrio* is omitted. However, for HV46 a different pattern is observed. The LC50s for HV46 are in a narrow range, which can probably be attributed to the low solubility of this product, which limits the accumulation of the compounds. In this case, another mechanism of toxicity might explain these results, for instance physical soiling. This process is not dependent on the accumulated amount of compounds.

4.3 Derivation of environmental risk limits

4.3.1 Selection of data

For the derivation of the risk environmental risk limits (ERLs) for mineral oil the chosen endpoints are based on the EC10 values for the tests with the gas oil DMA only, because of the following three reasons:
First, the data for HV46 show, especially for fresh water, an enhanced toxicity when expressed on internal membrane concentrations. This effect is most pronounced for the least sensitive effects and thus for the highest actual concentrations. Another toxic mechanism than narcosis is plausible in this cause. This enhanced toxicity might be attributed to phototoxicity, but the importance of this effect in sediment is not clear. Another explanation for the additional toxic effect for HV46 is physical soiling. Where the accumulation into organisms is

a very important factor for narcosis and photoinduced toxicity, it is not related to the effect of physical soiling.

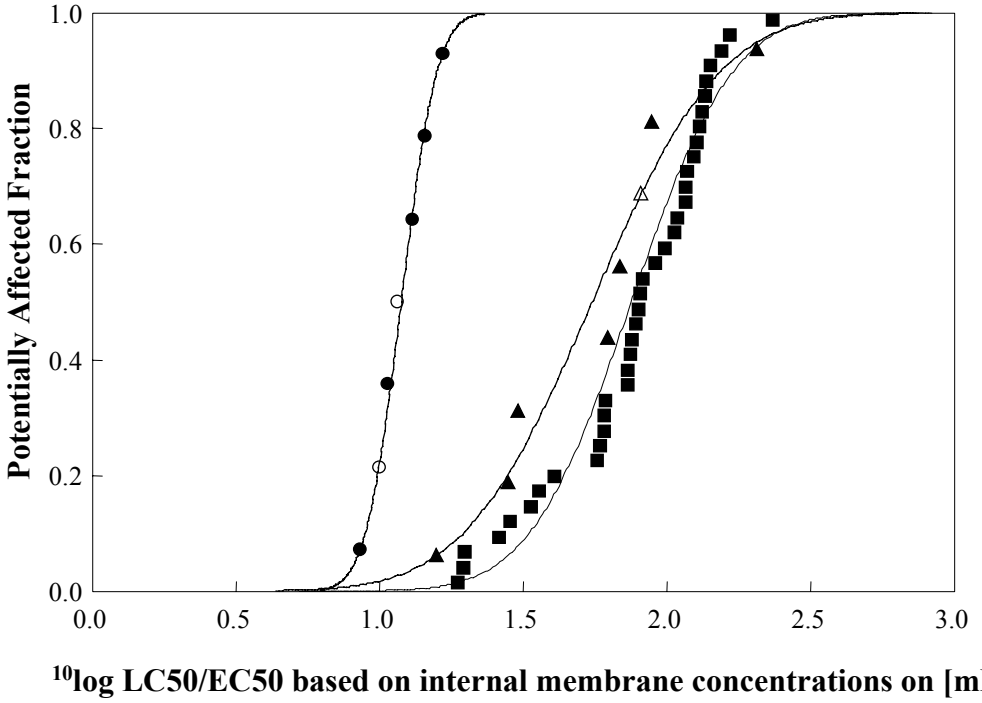

Figure 4.6: Species sensitivity distribution for baseline toxicity. ■: Data from DiToro; ▲: Estimated for the gas oil DMA; ●: Estimated for the lubricant HV46. Open symbols denote dose-response relationships with r^2 lower than 0.4.

Second, the fitting of the dose-effect relationships for HV46 is in several cases poor in comparison with DMA, especially for *Echinocardium* and to a lesser extent for *Vibrio*. There are two reasons for this. The estimated membrane concentrations are not continuously increasing with increasing nominal or actual oil concentrations. Further, also the toxicity data are not continuously increasing or decreasing with increasing concentration (Brils et al., 2002).
Third, the EC10 values are chosen in favour of the NOEC because the size of the groups is limited, which makes the statistical power of a post-test in the ANOVA limited.
Fourth, the control sediments in the series are actually no controls because both in terms of actual concentration and internal membrane concentration the concentration of the control sediments is not zero. Therefore, the use of the derivation of a NOEC by a post test in the ANOVA can be questioned.

4.3.2 Statistical extrapolation

No significant differences were found for fresh water sediment and marine sediment species, regardless whether the results were based on dry weight concentration or target lipid concentrations and regardless if DMA or HV46 or the geometric mean of both oil types was considered. Thus, the combined set of EC10s for the most sensitive parameters of the toxicity of DMA to the six benthic species (*Plectus* was excluded because of the poor dose-response relationship) was used in the statistical extrapolation. The fit was accepted by all goodness-of-fit tests included in ETX (Van Vlaardingen and Traas, 2002). The risk levels based on internal membrane concentrations are **0.27** mM (90% CI: 0.01-1.44 mM) for the HC5 and **7.7** mM (90% CI: 1.6-37.2 mM) for the HC50. For comparison, these levels are 0.70 mM (90% CI:

0.09-1.74 mM) for the HC5, and 4.3 mM (90% CI: 1.8-10.0 mM) if the internal membrane concentrations for HV46 were used. The difference in both HC5 and HC50 can almost solely be explained by the low EC10 for *Chironomus* of HV46 in comparison with DMA.

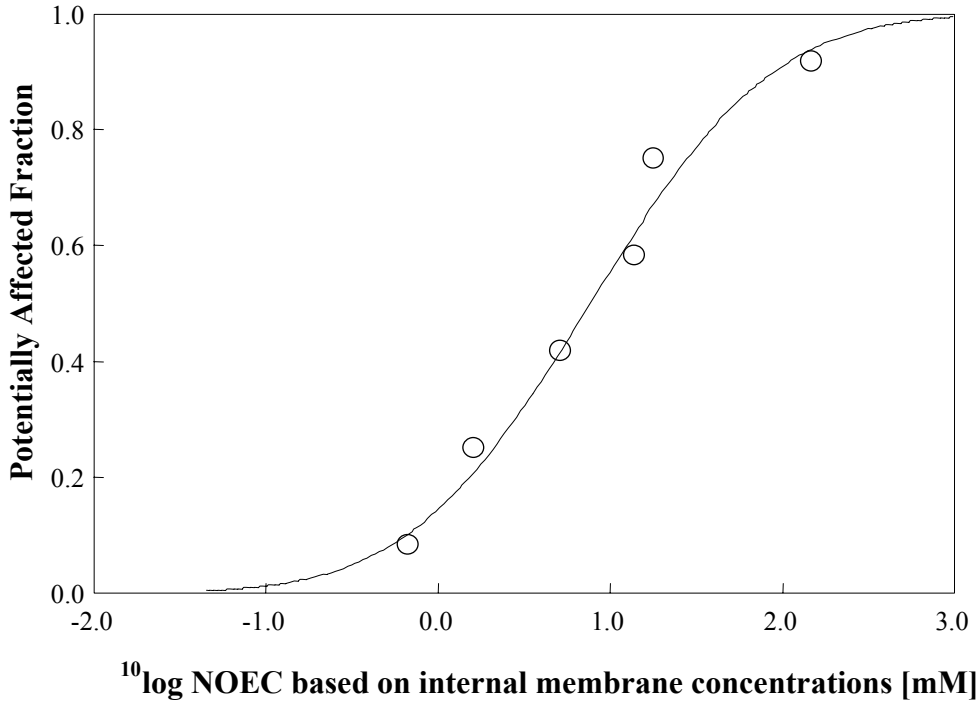

Figure 4.7: Statistical extrapolation of the internal effect concentration (mM in membranes) for the gas oil DMA.

4.3.3 ERLs for sediment

The risk limits based on internal molar membrane concentration can be recalculated to concentrations in the sediment by means of linear equilibrium partitioning with the organic matter of the sediment. From Figure 4.8 it can be concluded that for internal membrane concentrations below 10 mM these concentrations can be estimated well by a two phase equilibrium partitioning model, taking only organic matter as hydrophobic phase into account. Because both the HC5 and the HC50 are below this value of 10 mM, equilibrium partitioning between organic matter and water is used to calculate the ERLs on basis of sediment concentrations.

The derived risk limits for the 19 blocks that were used in the calculation are shown in Table 7. These blocks represent boiling point fraction in the analysis, each with a range of log K_{ow} values of 0.5 unit. Because of the additivity of narcotic effects a toxic unit approach has to be applied to the different fractions of mineral oil. Therefore, the ERLs for mineral oil are exceeded if the sum of all ratios between the actual concentration and the ERL for each fraction exceeds the value of 1 and not if the actual concentrations of each fraction do not exceed their corresponding ERL. Alternatively, the fractions can be categorised according to the TPHCWG (Gustafson et al., 1997). The derived ERLs based on this classification scheme are shown in Table 8. The division in these blocks was also used for the derivation of the human-toxicological serious risk concentration for soil (Franken et al., 1999).

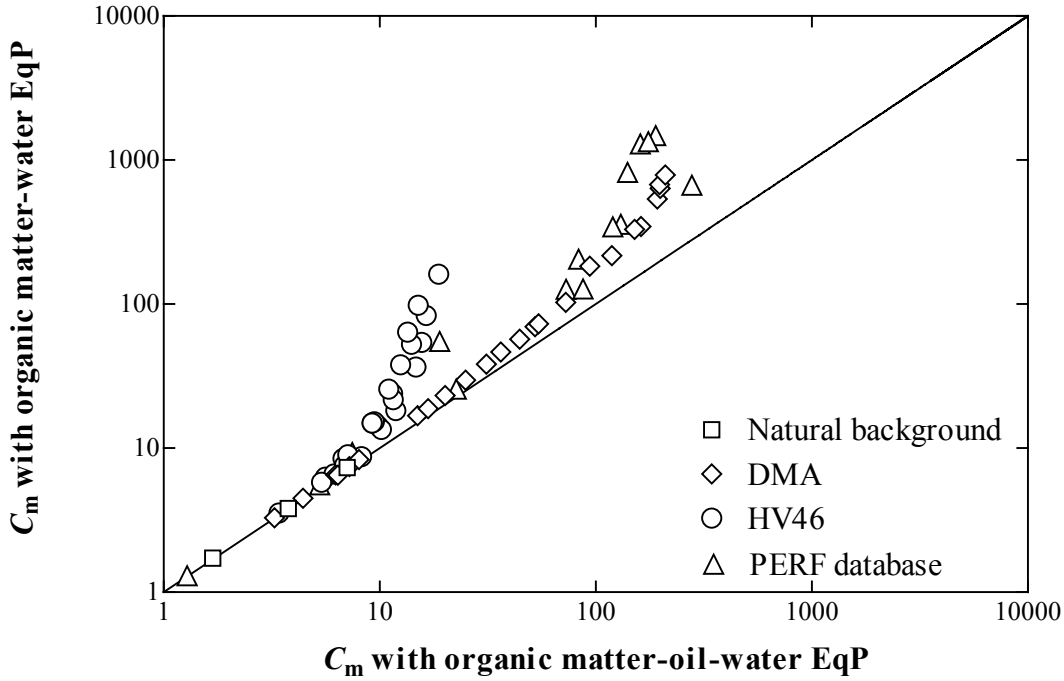

Figure 4.8: Two-phase versus three-phase equilibrium partitioning (see section 3.2). Substantial deviations occur at internal membrane concentrations higher than 10 mM. Drawn line represents the 1:1 relationship.

4.3.4 ERLs for soil

Because the used equilibrium partitioning method seems to work for soil as well as for sediment, the ERLs derived for sediment are also proposed as ERLs for soil. Implicitly it is assumed that the sensitivity of terrestrial and benthic organisms is equal. The sensitivity of aquatic organisms compared well with the sensitivity for benthic organisms (section 4.2.3). Internal effect concentrations for some terrestrial species lie in this range as well (section 4.2.2). In a recent evaluation of the equilibrium partitioning method it also appeared that there was no systematic difference in sensitivity between aquatic and terrestrial organisms (Van Beelen et al., 2003).

4.3.5 ERLs for water

ERLs for water can be derived from the internal levels based on membrane concentrations too by applying equilibrium partitioning. To calculate the ERLs for the dissolved fraction the internal HC and HC50 levels have to be divided by the membrane-water partition coefficient. ERLs can also be derived for total concentrations in water, by assuming that the content of suspended matter in surface water is 0.03 g/L and that the organic matter content of suspended matter is 20% (Traas, 2001). In this case the K_{oc} for each fraction is used to calculate the partition coefficient to suspended matter. The ERLs for dissolved concentrations apply to mineral oil in both surface and ground water. The ERLs for total concentrations are derived for surface water only.
Similar to the risk limits for sediment and soil, the ERLs for water can be based on windows of 0.5 log K_{ow} unit and on basis of the classification of the TPHCWG (Gustafson et al., 1997). These ERLs are shown in Table 9 and Table 10.

4.3.6 Comparison with other ERLs

The current MPC for mineral oil is 1000 mg/kg$_{dw}$ and the SRC$_{eco}$ 5000 mg/kg$_{dw}$. The proposed risk limits for the fractions are thus lower than the current risk limits, except from the risk limits for the higher aliphatic compounds. For mineral oil in soil a proposal for different fractions of mineral oil has already been made for the human toxicological part of the Intervention Values (Franken et al., 1999). The SRC$_{eco}$ and SRC$_{human}$ values for the different fractions are shown together for both soil and sediment in Table 11. The SRC$_{human}$ values are retrieved from Lijzen et al. (2001), These values are modified based on the adjustment of the exposure models CSOIL and SEDISOIL. The SRC$_{eco}$ values derived here are all lower than the human-toxicological values (except for benzene and toluene) by less than a factor of 2 for the lower aliphatic and aromatic fractions up to almost a factor 200 for the higher fractions.

For some aromatic compounds that also occur in mineral oil environmental risk limits have been derived previously (Verbruggen et al., 2001). It appears that the ERLs for the non-substituted monoaromatic compounds are very similar to those for the lower aromatic fractions of mineral oil (C5-C10), i.e. they lie almost all within the 90% confidence interval derived for the HC50 and HC5 of mineral oil (Figure 4.9). For the PAHs, it appears that the ERLs for the individual compounds are in general lower than the SRCs$_{eco}$ and MPCs derived for mineral oil. This can largely be attributed to the limited availability of toxicity data that were available for the derivation of these ERLs. The MPCs are therefore subject to the higher assessment factors that are applied. The HC50 is also influenced by the fact that the more hydrophobic PAHs are not toxic to many tested organisms. For this reason the HC50 is only based on the most sensitive organisms for which a NOEC of EC50 could be derived. It can therefore, be tentatively concluded that the derived risk limits are in line with what is expected from the risk limits for individual compounds that occur in mineral oil.

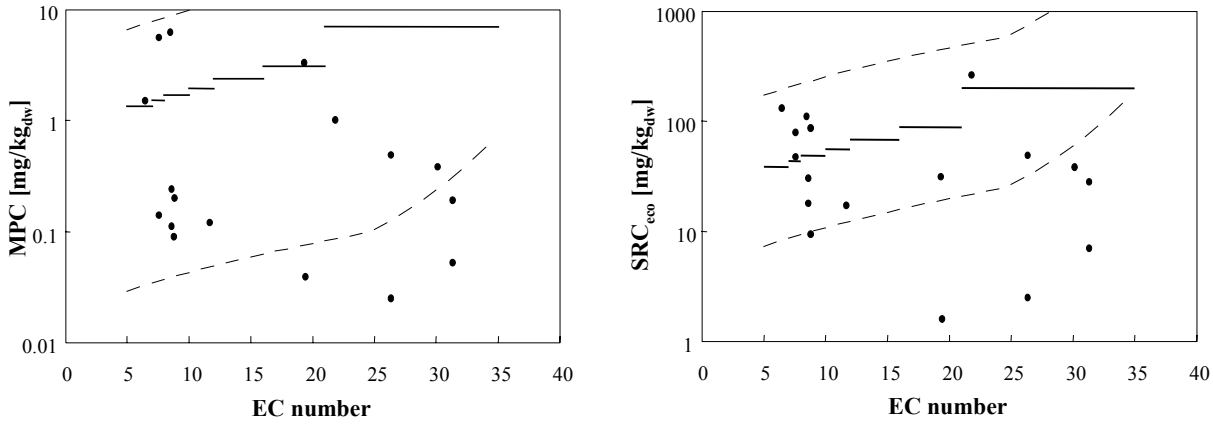

Figure 4.9: Comparison of ERLs for TPH fractions (drawn lines) and ERLs for non-substituted monoaromatic compounds and PAHs (symbols) (from Verbruggen et al., 2001). Dashed lines are based on the 90% confidence intervals of the statistical extrapolation.

Table 7: Derived risk limits for mineral oil in standard soil and sediment (10% organic matter) for blocks of equal hydrophobicity range. Because of additivity a toxic unit (TU) approach should be used.

Compounds	EC fraction	MPC [mg/kg$_{dw}$]	Max. TU	SRC$_{eco}$ [mg/kg$_{dw}$]	Max. TU
Aliphatic	7-10	0.48	> 1	14	> 1
	10-11	0.79	> 1	23	> 1
	11-12	1.3	> 1	38	> 1
	12-13	2.6	> 1	75	> 1
	13-14	6.3	> 1	180 [a]	0.54
	14-15	19	> 1	530 [a]	0.13
	15-16	70 [a]	0.68	2000 [a]	0.02
	16-17	340 [a]	0.10	- [a]	0.00
	17-40	3500 [a]	0.01	- [a]	0.00
Aromatic	7-12	1.8	> 1	51	> 1
	12-15	2.3	> 1	66	> 1
	15-18	2.8	> 1	79	> 1
	18-22	3.3	> 1	96	> 1
	22-25	4.0	> 1	110	> 1
	25-28	5.6	> 1	160	> 1
	28-32	10	> 1	290 [a]	0.15
	32-35	21 [a]	0.42	590 [a]	0.01
	35-38	50 [a]	0.03	- [a]	0.00
	38-40	- [a]	0.00	- [a]	0.00

[a] If no block of compounds with a lower ERL are present, these values may be neglected, because solubility is so low that the internal concentration corresponding to the ERL will not be reached. In the toxic unit approach, the contribution of this fraction can not exceed the maximum toxic unit value. If the maximum toxic unit value is zero, the contribution to toxicity is negligible.

Table 8: Derived risk limits for mineral oil in standard soil and sediment (10% organic matter) categorised according to the TPHCWG method (Gustafson et al., 1997). Because of additivity a toxic unit (TU) approach should be used.

Compounds	EC fraction	MPC [mg/kg$_{dw}$]	Max. TU	SRC$_{eco}$ [mg/kg$_{dw}$]	Max. TU
Aliphatic	>5-6	0.55	> 1	16	> 1
	>6-8	0.54	> 1	15	> 1
	>8-10	0.49	> 1	14	> 1
	>10-12	0.91	> 1	26	> 1
	>12-16	9.9	> 1	280 [a]	0.29
	>16-21	- [a]	0.00	- [a]	0.00
Aromatic	>5-7 (benzene)	1.3	> 1	39	> 1
	>7-8 (toluene)	1.5	> 1	44	> 1
	>8-10	1.7	> 1	49	> 1
	>10-12	2.0	> 1	56	> 1
	>12-16	2.4	> 1	68	> 1
	>16-21	3.1	> 1	88	> 1
	>21-35	7.0	> 1	200 [a]	0.49

[a] If no block of compounds with a lower ERL are present, these values may be neglected, because solubility is so low that the internal concentration corresponding to the ERL will not be reached. In the toxic unit approach, the contribution of this fraction can not exceed the maximum toxic unit value. If the maximum toxic unit value is zero, the contribution to toxicity is negligible.

Table 9: Derived risk limits for mineral oil in water for blocks of equal hydrophobicity range. Because of additivity a toxic unit (TU) approach should be used.

Compounds	EC fraction	MPC [µg/L]	MPC_{total} [µg/L] [b]	Max. TU	SRC_{eco} [µg/L]	$SRC_{eco,total}$ [µg/L] [b]	Max. TU
Aliphatic	7-10	0.36	3.2	> 1	10	93	> 1
	10-11	0.093	4.9	> 1	2.7	140	> 1
	11-12	0.061	8.0	> 1	1.7	230	> 1
	12-13	0.047	16	> 1	1.4	450	> 1
	13-14	0.045	38	> 1	1.3 [a]	1100 [a]	0.54
	14-15	0.052	110	> 1	1.5 [a]	3200 [a]	0.13
	15-16	0.077 [a]	420 [a]	0.68	2.2 [a]	12000 [a]	0.02
	16-17	0.15 [a]	2000 [a]	0.10	- [a]	- [a]	0.00
	17-40	0.60 [a]	21000 [a]	0.01	- [a]	- [a]	0.00
Aromatic	7-12	28	38	> 1	790	1100	> 1
	12-15	11	25	> 1	320	720	> 1
	15-18	4.3	21	> 1	120	600	> 1
	18-22	1.6	22	> 1	47	620	> 1
	22-25	0.62	24	> 1	18	700	> 1
	25-28	0.27	34	> 1	7.9	970	> 1
	28-32	0.15	60	> 1	4.4 [a]	1700 [a]	0.15
	32-35	0.10 [a]	120 [a]	0.42	2.9 [a]	3500 [a]	0.01
	35-38	0.078 [a]	300 [a]	0.03	- [a]	- [a]	0.00
	38-40	- [a]	- [a]	0.00	- [a]	- [a]	0.00

[a] If no block of compounds with a lower ERL are present, these values may be neglected, because solubility is so low that the internal concentration corresponding to the ERL will not be reached. In the toxic unit approach, the contribution of this fraction can not exceed the maximum toxic unit value. If the maximum toxic unit value is zero, the contribution to toxicity is negligible.
[b] The total concentration refers to the sum of the concentrations in the dissolved phase and in particulate matter.

Table 10: Derived risk limits for mineral oil in water categorised according to the TPHCWG method (Gustafson et al., 1997). Because of additivity a toxic unit (TU) approach should be used.

Compounds	EC fraction	MPC [µg/L]	MPC_{total} [µg/L] [b]	Max. TU	SRC_{eco} [µg/L]	$SRC_{eco,total}$ [µg/L] [b]	Max. TU
Aliphatic	>5-6	12	15	> 1	330	420	> 1
	>6-8	2.6	5.8	> 1	74	170	> 1
	>8-10	0.33	3.3	> 1	9.4	94	> 1
	>10-12	0.084	5.6	> 1	2.4	160	> 1
	>12-16	0.047	59	> 1	1.3 [a]	1700 [a]	0.29
	>16-21	- [a]	- [a]	0.00	- [a]	- [a]	0.00
Aromatic	>5-7 (benzene)	81	89	> 1	2300	2600	> 1
	>7-8 (toluene)	55	64	> 1	1600	1800	> 1
	>8-10	36	47	> 1	1000	1300	> 1
	>10-12	21	33	> 1	600	940	> 1
	>12-16	9.0	23	> 1	260	670	> 1
	>16-21	2.5	21	> 1	71	600	> 1
	>21-35	0.21	42	> 1	6.1 [a]	1200 [a]	0.49

[a] If no block of compounds with a lower ERL are present, these values may be neglected, because solubility is so low that the internal concentration corresponding to the ERL will not be reached. In the toxic unit approach, the contribution of this fraction can not exceed the maximum toxic unit value. If the maximum toxic unit value is zero, the contribution to toxicity is negligible.
[b] The total concentration refers to the sum of the concentrations in the dissolved phase and in particulate matter.

Table 11: Comparison of human toxicological and ecotoxicological SRC values.

Compounds	EC fraction	$SRC_{human,soil}$ [mg/kg_{dw}]	$SRC_{human,sed.}$ [mg/kg_{dw}]	$SRC_{eco,soil}$ [mg/kg_{dw}]	Max. TU
Aliphatic	>5-6	35	47000	16	> 1
	>6-8	109	>100000	15	> 1
	>8-10	28	10600	14	> 1
	>10-12	152	12100	26	> 1
	>12-16	55000 [b]	12200	280 [a]	0.29
	>16-21	>100000 [b]	>100000	- [a]	0.00
Aromatic	>5-7 (benzene)	29 (1.1) [c]	190 (5.5) [c]	39 (130) [c]	> 1
	>7-8 (toluene)	62 (32) [c]	280 (191) [c]	44 (47, 79) [c]	> 1
	>8-10	59	100	49	> 1
	>10-12	317	180	56	> 1
	>12-16	5900 [b]	420	68	> 1
	>16-21	17500 [b]	2600	88	> 1
	>21-35	19200 [b]	3600	200 [a]	0.49

[a] If no block of compounds with a lower ERL are present, these values may be neglected, because solubility is so low that the internal concentration corresponding to the ERL will not be reached. In the toxic unit approach, the contribution of this fraction can not exceed the maximum toxic unit value. If the maximum toxic unit value is zero, the contribution to toxicity is negligible.

[b] Stated to be above the pore water solubility

[c] For benzene and toluene separate risk limits are derived (Verbruggen et al., 2001; Lijzen et al., 2001). These values are shown between brackets. For the human-toxicological risk assessment these values should be used. For the ecotoxicological risk assessment it appears that the terrestrial SRC_{eco} for toluene is almost equal to the value derived here. The values derived for benzene and toluene by equilibrium partitioning are 2 to 3 times higher. It is advised to use the values derived here in the toxic unit approach when mineral oil is considered.

4.4 Effects due to physical soiling

For some petroleum mixtures as well as for pure petroleum related compounds the solubility of the products is so low that the observed toxic effects probably can not be attributed to the amount that is accumulated within the organism. A more likely explanation in this case is the physical soiling of organisms by the viscous oil products (see 4.2). It needs to be evaluated to what extent the derived ERL levels that are based on narcosis are protective against these effects of physical soiling. Effects that possibly could be attributed to physical soiling were observed in the studies with the fresh water Drontermeer sediment (0.94% organic carbon) and in the marine Mokbaai sediment (0.22% organic carbon) but not in the marine Oesterput sediment (1.7-1.9% organic carbon). The thresholds (EC10s) for these effects occur at in the order of 10-100 mg/kg_{dw} for the Mokbaai sediment and 100-1000 mg/kg_{dw} for the Drontermeer sediment (Huwer et al., 1997; Scholten et al., 1997; Aquasense, 2002; Rotteveel et al., 2002). These data suggest a correlation with organic carbon content. Further, it appeared that only liquid alkanes showed toxic effects and solid alkanes did not (Huwer et al., 1997; Scholten et al., 1997). Therefore, the highest SRC_{eco} for compounds that are liquids under normal environmental conditions (< 20 ºC) are considered. This is the SRC_{eco} of 280 mg/kg_{dw} for the aliphatic fraction C12-C16 in standard soil (containing 10% organic matter). For soil with respectively 0.94% and 0.22% organic carbon the SRC_{eco} for this fraction is 45 and 10 mg/kg_{dw}. Although the effect of physical soiling is not fully explored, the derived ERLs are probably protective against physical soiling.

4.5 SPME biomimetic extractions

Another analytical method to assess the exposure to complex mixtures such as mineral oil in the environment is the solid phase microextraction (SPME) biomimetic extraction procedure

(Parkerton et al., 2000; Verbruggen et al., 2000b). With this method the amount of accumulated compounds by organisms from the environment is mimicked. The SPME method was also used in two studies with the gas oil DMA, in which the accumulated amount on polyacrylate (PA) SPME fibers was compared with the acute toxicity to *Daphnia magna* (Verbruggen, 1999) and *Chironomus riparius* (Leslie, 2003). These biomimetic analyses are plotted in Figure 4.10 as a function of the estimated membrane concentration, based on three different partitioning models. Also included in this figure are blank Drontermeer sediment (Leslie, 2003) and clean demineralised laboratory water as well as some duplicates for DMA in water (Verbruggen, 1999, unpublished results).
Blank SPME fibers give rise to a concentration of 1.18 (s.d. 1.15, n=5) or 1.13 (s.d. 0.16, n=3) mM in polyacrylate of the fibers (Leslie, 2003). The biomimetic extracts of clean surface water and demineralised laboratory water had concentrations on the fiber of 1.57 (s.d. 0.39, n=3), 2.60 (s.d. 0.61, n=3) (Verbruggen, 1999, unpublished results). Blank sediment lead to fiber concentrations of 4.47 (s.d. 0.73, n=3) and 3.29 (s.d. 0.30, n=3). It can be concluded that blank sediment leads to slightly enhanced fiber concentrations.
The estimated membrane concentration of blank Drontermeer sediment is 1.7 mM. The concentration in polyacrylate of SPME fibers is estimated to be half of the concentration in membrane lipids (Verbruggen et al., 2000b). The fiber concentration of blank Drontermeer sediment corresponding to the estimated membrane concentration should be 0.8 mM. It can be concluded that this is in reasonable agreement with the difference between clean water and clean sediment presented above.
From Figure 4.10, it appears that for the lowest concentrations partitioning between organic carbon and water is in good agreement with the SPME analyses. At higher concentrations however, organic carbon-water partitioning overpredicts the concentrations from the biomimetic extraction method, as expected. In this case the three phase partitioning model seems to work well. Only at very high concentrations, at which oil-water partitioning according to Raoult's law takes over the partitioning process, the estimated concentrations are an underestimation of the concentrations from the biomimetic extraction method. This is also observed for the biomimetic extractions in water only.

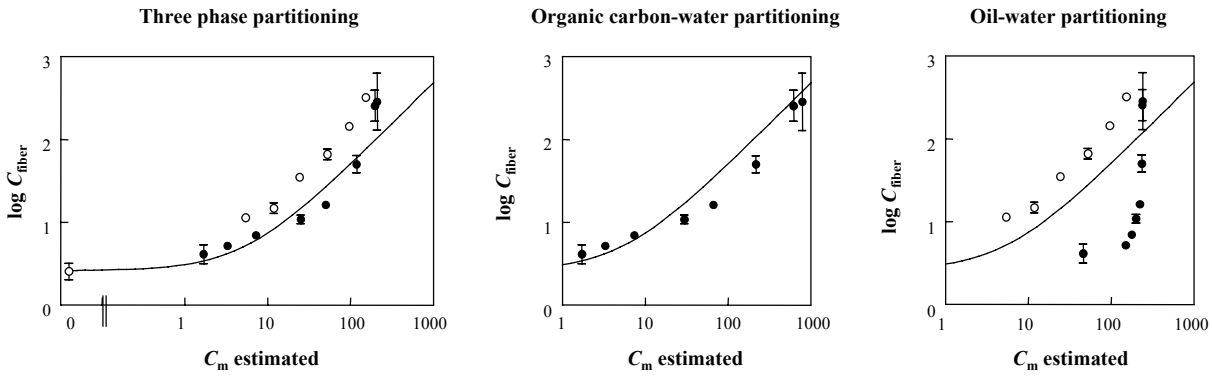

Figure 4.10: Amount accumulated by PA SPME fibers plotted versus the membrane concentration estimated from fraction analysis. Open symbols are the biomimetic extracts from water (Verbruggen, 1999), closed symbols are the biomimetic extracts from sediment (Leslie, 2003). The solid line is the theoretical relationship between membrane lipid and SPME fiber concentrations (Verbruggen et al., 2000b), with average background of 2.6 mM in pure water. The sediment concentrations are calculated with respectively partitioning between water and both oil and organic carbon, organic carbon only and oil only.

5. Preliminary risk analysis

5.1 Effects of field contaminated soils

Some polluted soils from the historically polluted Petroleum Harbour (Amsterdam, The Netherlands) have been tested with a set of acute and chronic bioassays (Van Gestel et al., 2001). Unfortunately, no detailed fraction analysis was performed in this study. It appears that the samples with very high oil concentrations (> 2000 mg/kg_{dw}) are toxic in all chronic tests (*Lactuca sativa* growth, *Eisenia fetida* cocoon production, *Folsomia candida* reproduction and bait lamina decomposition). The elutriate of these soils appears also to be toxic in some of the acute bioassays (Microtox, *Pseudokirchneriella subcapitata*, *Bacillus* sp.). Toxic effects were almost absent in the mobility tests with *Plectus acuminatus*, *Folsomia candida*, and *Daphnia magna* and for root length of *Lactuca sativa*. It can be concluded that for these samples the level of the SRC_{eco} is most likely exceeded. For samples containing oil at lower concentrations (1000 mg/kg_{dw} and less) toxic effects are less pronounced.
Two polluted sediments from the harbour of Rotterdam (Eemhaven and Waalhaven, Rotterdam, The Netherlands) have been tested with the same bioassays as used for the marine Oesterput sediment (Brils et al., 2000; Brils et al., 2002). In this study no detailed fraction analysis was performed. From the concentrations per boiling point range, it is concluded that at least the MPC for the sample with the highest oil concentration (1000 mg/kg_{dw}) is exceeded. No toxic effects were observed for mortality of *Corophium volutator* and *Echinocardium cordatum* and bioluminescence of *Vibrio fischeri* in both samples. It should be stressed however, that for all these three species the EC10 lies above the MPC and except for *Vibrio fischeri* also above the SRC_{eco}. On basis of this information, it can be concluded that the concentrations are most likely in the range between the MPC and the SRC_{eco}.
Most of the compounds of field-contaminated soils and sediments are in the higher aliphatic fractions (see 2.1.1 and 3.1.2.2). If it is assumed that 80% of the compounds belongs to the aliphatic fraction (see Figure 2.1), the lower levels of the polluted soils and sediments discussed above are in between the MPC and the SRC_{eco} while the higher levels are above the SRC_{eco} as well. The observed toxicity of these samples is in reasonable agreement with the derived risk limits and the endpoints for single species based on internal concentrations.

5.2 Implications of the background level of sediments

Background concentrations as high as 107 mg/kg_{dw} can be found in sediments with 1.9% organic carbon with the GC-FID analysis (Harmsen and Zweers, 2000). Because no detailed fraction analysis was available for the control sediments, some assumptions had to be made for the distribution over the different fractions. Based on the literature, the background level of compounds in the analysis of mineral oil was assumed to be equally distributed over the aromatic and aliphatic fraction (3.1.2.2). The estimated internal concentrations for the three control sediments in the benthic studies are ranging from 1.7 to 7.1 mM, largely due to the higher accumulation of the aromatic compounds. These levels are several times higher than the HC5 level and approach the HC50 level in the worst case. Even for individual fractions the derived MPC levels for some of the higher aromatic fractions (C16-C35) can sometimes be lower than estimated background values.
This raises some questions about the bioavailability of the compounds accounting for this background. On the other hand, when comparing the control sediments with literature data (Figure 2.1), the TPH concentrations of the control sediments are elevated in comparison with almost all background concentrations, which are probably more in line with the MPC. For the

marine control sediment with the highest estimated internal concentration, the aliphatic fraction is possibly underestimated (3.1.2.2). Therefore, the level of 7.1 mM is corresponding to a worst-case assumption.
Assuming different composition of the background concentrations or different bioavailability (i.e. omitting the background in the calculations) did not lead to very different results for the risk limits (data not shown). The MPC derived from the toxicity data for DMA does not differ more than a factor of three from the derived MPC, for the SRC_{eco} this difference is less than a factor of two. In conclusion, the uncertainty in the derived ERLs due to different assumptions about the composition and availability of the background concentrations is within a factor two to three, which is considered an acceptable precision in view of the uncertainty inherent to the statistical extrapolation (see 4.3.2).

6. Conclusions and recommendations

6.1 Summary of findings

- ERLs were derived for mineral oil based on internal concentrations from a set of six benthic species. The ERLs were derived for separate fractions of TPH to which the toxic unit approach was applied. The internal concentrations were calculated by means of equilibrium partitioning.
- For the lighter of the two tested oil types, the endpoints for single species as well as the species sensitivity distribution appeared to be consistent with data for single compounds and petroleum products.
- For the heavier oil type an additional toxic effect besides narcosis could probably be explained by physical soiling of the organisms. Nevertheless, the derived risk limits are considered to be protective against both types of effects.
- The derived environmental risk limits are comparable with those for single substances such as non-substituted monoaromatic compounds and PAHs. However, by applying statistical extrapolation instead of more conservative assessment factors, the ERLs for mineral oil are less stringent than ERLs for especially the PAHs.
- The new ERLs are based on a fraction analysis of TPH, for which no results were available in the Netherlands. The composition of reference sediments and of field polluted sediment and soils are relatively unknown. Therefore, at this moment the risk analysis of TPH in soils and sediments is hampered by this lack of information.
- For the risk assessment of oil polluted field samples, the pattern of the background concentration derived from the GC-FID measurement may have major implications. The assumption was made that 50% of the compounds of the background concentration of control sediments belongs to the aromatic fraction. With this assumption, the calculated risk quotients for the background were above the MPC level. TPH concentrations of these control sediments were also above reported background levels in literature. If the background concentration mainly exists of higher aliphatic hydrocarbons, the estimated risk for an analysed control sediment sample will be much less.
- It follows that more insight in the analytical response of control sediments with the current GC-FID method is necessary, together with information on the nature and bioavailability of these compounds and data on chronic toxicity of the control sediments compared with cleaner sediments.
- When assessing oil polluted field samples, the new ERLs are lower than the current ERLs for aromatic and lower aliphatic compounds. However, when the field sample consists entirely of higher aliphatic compounds, the ERLs are less stringent.

6.2 Implementation of the derived risk limits for analysis

6.2.1 Fraction analysis

To evaluate the potential risk from oil pollution of field samples with the proposed ERLs a fractionation step is needed before analysis. The method used for the analysis of the sediment toxicity studies with Drontermeer sediment was very similar to the TNRCC method and using silica as column material (source: conference call on analysis of mineral oil).
When comparing the ERLs with the limits of detection of the fraction analysis, it is evident that it is possible to measure $SRCs_{eco}$ by this method. However, the MPC is limited by the

limit of detection for the aliphatic fractions below C12 and the aromatic fraction below C16 or even C21.
For a routine determination of environmental samples it may be sufficient to perform a fraction analysis for only a few samples. These samples should be chosen on the basis of their chromatographic pattern in the TPH analysis (shape of the signal as a function of the retention time). For all samples with a similar pattern it can be assumed that the relative composition is more or less the same. This will reduce costs and time if large numbers of samples have to be analysed (source: conference call on analysis of mineral oil).

6.2.2 SPME biomimetic extraction

The level of the MPC converted to PA SPME fiber concentrations is 0.13 mM. It is evident that such an increase is below the detection limit due to the variance of the results of the method (see 4.5). From the data presented by Leslie (2003) for clean fiber and blank sediment it can be concluded that with a larger volume of fiber in the samples the variance can be reduced significantly. Still the MPC will be below the detection limit, also because of the variance in blank fiber response. It can be concluded that if there is an enhanced response in comparison with clean water, MPC level is probably exceeded.
For the SRC_{eco} the level for SPME fibers would be 3.7 mM. This level can be detected with the method above the background level of clean water. The fact that this method gives a direct measure of the bioavailability, in combination with the easy sample preparation and cost effectiveness of this method, can make it very useful in actual risk assessment of contaminated sediments (Sijm et al., 2001).

6.3 Recommendations for further research

Because of the lack of data on fraction analysis of mineral oil in the Dutch environment, it is recommended that some clean and some field-polluted sediments be analysed with the fraction analysis method. Preferably some ecotoxicological information of these samples should be available as well. However, it should be realised that the absence of toxic effects in a set of bioassays does not mean that the MPC is not exceeded. The MPC derived by statistical extrapolation lies below the level of the lowest endpoints in the evaluated toxicity studies.
Thus, only toxicity test organisms with endpoints for which estimated critical internal effect concentrations are available should be considered for further research. Moreover, it must be cautioned that interpretation of toxicity test results from field samples may be confounded by other causal factors that contribute to observed toxicity. Therefore, field samples cannot be really be used to validate the approach but rather only to invalidate predictions (i.e. toxic units >> 1, but no toxicity observed). Such results would suggest that the site-specific bioavailability has not been adequately taken into account or perhaps test organism behaviour is altered so that exposure (and hence effect) is less than predicted based on equilibrium partitioning theory.
Further, it is recommended that this fraction analysis be accompanied by a biomimetic extraction procedure with SPME fibers. The reason for this is twofold. First, the biomimetic extraction gives a more direct measure of the bioavailability of the constituents of mineral oil than the fraction analysis with a solvent extraction does. Second, at this moment it is not clear what method has the lowest detection limit for mineral oil. This can be clarified by simultaneously applying the two methods.

References

Aldenberg T, Jaworska J, Traas TP. 2002. Normal Species sensitivity distributions and probabilistic ecological risk assessment. In: Posthuma L, Suter II GW, Traas TP, eds. Species sensitivity distributions in ecotoxicology. Boca Raton, FL: CRC Press. pp. 49-102.

Aldenberg T, Slob W. 1993. Confidence limits for hazardous concentrations based on logistically distributed NOEC toxicity data. Ecotoxicol Environ Saf 25: 48-63.

Aldenberg T, Jaworska JS. 2000. Uncertainty of the hazardous concentration and fraction affected for normal species sensitivity distributions. Ecotoxicol Environ Saf 46: 1-18.

Aquasense. 2002. Bioassays op met olie belaste sedimenten. Testen met *Hyalella azteca* en *Ephoron virgo*. Amsterdam, The Netherlands: AquaSense. 1674-2.

Arfsten DP, Davenport R, Schaeffer DJ. 1994. UV-A coexposure enhances the toxicity of aromatic hydrocarbons, munitions, and metals to *Photobacterium phosphoreum*. Biomed Environ Sci 7: 101-108.

Banerjee S. 1984. Solubility of organic mixtures in water. Environ Sci Technol 18: 587-591.

Barron MG, Carls MG, Short JW, Rice SD. 2003. Photoenhanced toxicity of aqueous phase and chemically dispersed weathered Alaska North Slope crude oil to pacific herring eggs and larvae. Environ Toxicol Chem 22: 650-660.

Belfroid A, Seinen W, Van Gestel K, Hermens J. 1993. The acute toxicity of chlorobenzenes for earthworms (*Eisenia andrei*) in different exposure systems. Chemosphere 26: 2265-2277.

Bouloubassi I, Fillaux J, Saliot A. 2001. Hydrocarbons in surface sediments from the Changjiang (Yangtze River) Estuary, East China Sea. Mar Pollut Bull 42: 1335-1346.

Brils JM, Huwer SL, Scholten MCTh. 2000. Oil effect concentration in freshly spiked marine sediment for luminescent bacteria, mud shrimps and sea urchins. Apeldoorn, The Netherlands: TNO-MEP (Netherlands Organisation for Applied Scientific Research, Institute of Environment, Energy and Process Innovation. R 2000/150.

Brils JM, Huwer SL, Kater BJ, Schout PG, Harmsen J, Delvigne GAL, Scholten MCTh. 2002. Oil effect in freshly spiked marine sediment on *Vibrio fischeri*, *Corophium volutator*, and *Echinocardium cordatum*. Environ Toxicol Chem 21: 2242-2251.

Broderius S, Kahl M. 1985. Acute toxicity of organic chemical mixtures to the Fathead Minnow. Aquat Toxicol 6: 307-322.

CEFIC. 2002. The classification of petroleum solvent streams and related complex hydrocarbon solvents for aquatic effects under the dangerous substances directive. Brussels, Belgium: Hydrocarbon Solvent Producers Association.

Chang JC, Taylor PB, Leach FR. 1981. Use of the Microtox® assay for environmental

samples. Bull Environ Contam Toxicol 26: 150-156.

Cline PV, Delfino JJ, Rao PSC. 1991. Partitioning of aromatic constituents into water from gasoline and other complex solvent mixtures. Environ Sci Technol 25: 914-920.

CONCAWE. 1996. Environmental risk assessment of petroleum substances: the hydrocarbon block approach . Brussels, Belgium: CONCAWE Ecology group. 96/52.

CONCAWE. 2001. Environmental classification of petroleum products - Summary data and rationale. Brussels, Belgium: CONCAWE Petroleum Products Ecology Group. Report 01/54.

Daylight Chemical Information Systems, Inc. CLOGP. Available at http://www.daylight.com/release/index.html. Accessed 2002-2003.

De Maagd PG-J, Sinnige TL, Schrap SM, Opperhuizen A, Sijm DTHM. 1998. Sorption coefficients of polycyclic aromatic hydrocarbons for two lake sediments: influence of the bactericide sodium azide. Environ Toxicol Chem 17: 1899-1907.

De Maagd PG-J, Ten Hulscher DThEM, Van den Heuvel H, Opperhuizen A, Sijm DTHM. 1998. Physicochemical properties of polycyclic aromatic hydrocarbons: aqueous solubilities, *n*-octanol/water partition coefficients, and Henry's law constants. Environ Toxicol Chem 17: 251-257.

Deneer JW. 2000. Toxicity of mixtures of pesticides in aquatic systems. Pest Manag Sci 56: 516-520.

Denneman CAJ, Van Gestel CAM. 1990. Bodemverontreiniging en bodemecosystemen: Voorstel voor C-(toetsings)waarden op basis van ecotoxicologische risico's. Bilthoven, The Netherlands: National Institute of Public Health and the Environment. RIVM report 725201 001.

DiToro DM. Effect QSAR and the narcosis lipid model. Presented at the CONCAWE Workshop "Simplifying the Environmental Risk Assessment of Complex Substances", Bilthoven, The Netherlands, 4th of April 2003. CD-ROM. CONCAWE, Brussels, Belgium.

DiToro DM, McGrath JA, Hansen DJ. 2000. Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. I. Water and tissue. Environ Toxicol Chem 19: 1951-1970.

DiToro DM, Zarba CS, Hanssen DJ, Berry WJ, Swartz RC, Cowan CE, Pavlou SP, Allen HE, Thomas NA, Paquin PR. 1991. Technical basis for establishing sediment quality criteria for nonionic organic chemicals using equilibrium partitioning. Environ Toxicol Chem 10: 1541-1583.

Donkin P, Widdows J, Evans SV, Brinsley MD. 1991. QSARs for the sublethal responses of marine mussels (*Mytilus edilus*). Sci Total Environ 109/110: 461-476.

Doskey PV. 2001. Spatial variations and chronologies of aliphatic hydrocarbons in Lake Michigan sediments. Environ Sci Technol 35: 247-254.

Dulfer WJ, Govers HAJ. 1995. Membrane/water partitioning of polychlorinated biphenyls in small unilamellar vesicles of four saturated phosphatidylcholines. Environ Sci Technol 29: 2548-2554.

El-Alawi YS, Dixon DG, Greenberg BM. 2001. Effects of a pre-incubation period of polycyclic aromatic hydrocarbons to the luminescent bacterium *Vibrio fischeri*. Environ Toxicol 16: 277-286.

El-Alawi YS, Huang X-D, Dixon DG, Greenberg BM. 2002. Quantitative structure-activity relationship for the photoinduced toxicity of polycyclic aromatic hydrocarbons to the luminescent bacteria *Vibrio fischeri*. Environ Toxicol Chem 21: 2225-2232.

El-Alawi YS, McConkey BJ, Dixon DG, Greenberg BM. 2002. Measurement of short- and long-term toxicity of polycyclic aromatic hydrocarbons using luminescent bacteria. Ecotoxicol Environ Saf 51: 12-21.

Exxon Biomedical Sciences Inc. 1996. Physical chemical characterization of gasoline samples. East Millstone, NJ: Exxon Biomedical Sciences, Inc., Environmental Toxicology and Chemistry Laboratory. 104490C.

Franken ROG, Baars AJ, Crommentuijn GH, Otte P. 1999. A proposal for revised Intervention Values for petroleum hydrocarbons ('minerale olie') on base of fractions of petroleum hydrocarbons. Bilthoven, The Netherlands: National Institute of Public Health and the Environment. RIVM report 601501 012.

Gas Research Institute/PERF. 2000. Environmentally acceptable endpoints for hydrocarbon-contaminated soils: Petroleum Environmental Research Forum (PERF). GRI5094-250-2863/PERF 94-06 Research Area 1.

Gobas FAPC, Lahittete JM, Garofalo G, Shiu WY, Mackay D. 1988. A novel method for measuring membrane-water partition coefficients of hydrophobic organic chemicals: Comparison with 1–octanol–water partitioning. J Pharm Sci 77: 265-272.

Gogou A, Bouloubassi I, Stephanou EG. 2000. Marine organic geochemistry of the Eastern Mediterranean: 1. Aliphatic and polyaromatic hydrocarbons in Cretan Sea surficial sediments. Mar Chem 68: 265-282.

GraphPad Software Inc. 1996. GraphPad Prism [computer program]. version 2.01. San Diego, CA: GraphPad Software Inc.

Gustafson JB, Tell JG, Orem D. 1997. Selection of Representative TPH Fractions Based on Fate and Transport Considerations. Vol. 3. Amherst, MA: Amherst Scientific Publishers (Available at http://www.aehs.com/publications/catalog/contents/tph.htm).

Harmsen J, Zweers AJ. 2000. Effectconcentratie minerale olie in sediment. Chemische verschijningsvorm. Wageningen, The Netherlands: Alterra Research Instituut voor de Groene Ruimte. Alterra-rapport 011.

Hermens J, Broekhuyzen E, Canton H, Wegman R. 1985. Quantitative structure activity relationships and mixture toxicity studies of alcohols and chlorohydrocarbons: Effect on growth of *Daphnia magna*. Aquat Toxicol 6: 209-217.

Hermens J, Canton H, Janssen P, de Jong P. 1984. Quantitative structure-activity relationships and toxicity studies of mixtures of chemicals with anaesthetic potency: Acute lethal and sublethal toxicity to *Daphnia magna*. Aquat Toxicol 5: 143-154.

Huang X-D, Krylov SN, Ren L, McConkey BJ, Dixon DG, Greenberg BM. 1997. Mechanistic quantitative structure-activity relationship model for the photoinduced toxicity of polycyclic aromatic hydrocarbons: II. An empirical model for the toxicity of 16 polycyclic aromatic hydrocarbons to the duckweed *Lemna gibba* L. G-3. Environ Toxicol Chem 16: 2296-2303.

Huwer SL, Foekema EM, Scholten MCTh. 1997. Pilot study on the dose-effect responses of petroleum hydrocarbons in sediments (SLURP*olie). Report phase II: Test results. Apeldoorn, The Netherlands: TNO Institute of Environmental Sciences, Energy Research and Process Innovation. TNO-MEP - R 97/422.

Johnson BT, Long ER. 1998. Rapid toxicity assessment of sediment from estuarine ecosystems: A new tandem *in vitro* testing approach. Environ Toxicol Chem 17: 1099-1106.

Karickhoff SW. 1981. Semi-empirical estimation of sorption of hydrophobic pollutants on natural sediments and soils. Chemosphere 10: 833-846.

Karickhoff SW, Brown DS, Scott TA. 1979. Sorption of hydrophobic pollutants on natural sediments. Water Res 13: 241-248.

Kater BJ, Schout PG. 2000. Effecten van het verouderen van olie op respons van *Corophium volutator*. Kamperland, The Netherlands: RIKZ (National Institute for Coastal and Marine Management). RIKZ/OS-99.864x.

Könemann H. 1981. Fish toxicity tests with mixtures of more than two chemicals: A proposal for a quantitative approach and experimental results. Toxicology 19: 229-238.

Kooijman SALM. 1987. A safety factor for LC50 allowing differences in sensitivity among species. Water Res 21: 269-276.

Krylov SN, Huang X-D, Zeiler LF, Dixon DG, Greenberg BM. 1997. Mechanistic quantitative structure-activity relationship model for the photoinduced toxicity of polycyclic aromatic hydrocarbons: I. Physical model based on chemical kinetics in a two compartment system. Environ Toxicol Chem 16: 2283-2295.

LeDréau Y, Jacquot F, Doumenq P, Guiliano M, Bertrand JC, Mille G. 1997. Hydrocarbon balance of a site which had been highly and chronically contaminated by petroleum wastes of a refinery (from 1956 to 1992). Mar Pollut Bull 34: 456-468.

Lee J-H, Landrum PF, Field LJ, Koh C-H. 2001. Application of a ∑polycyclic aromatic hydrocarbon model and a logistic regression model to sediment toxicity data based on a species-specific, water-only LC50 toxic unit for *Hyalella azteca*. Environ Toxicol Chem 20: 2102-2113.

Leslie HA 2003. Biomimetic Solid-Phase Microextraction - Estimating Body Residues and Predicting Baseline Toxicity in Aquatic Organisms. Chapter 7: Biomimetic Solid-Phase Microextraction to estimate total body residue of petroleum products after exposure to

sediment: A feasibility study. University of Utrecht, Utrecht, The Netherlands.

Lide DR. 1998. CRC Handbook of Chemistry and Physics. 79th edition on CD-ROM. William Andrew Inc., Norwich, NY.

Lijzen JPA, Baars AJ, Otte PF, Rikken MGJ, Swartjes FA, Verbruggen EMJ, Van Wezel AP. 2001. Technical evaluation of the Intervention Values for Soil/sediment and Groundwater. Human and ecotoxicological risk assessment and derivation of risk limits for soil, aquatic sediment and groundwater. Bilthoven, The Netherlands: National Institute of Public Health and the Environment. RIVM report 711701 023.

Lourens JM, De Poorter LRM, Dulfer JW, Ferdinandy M. 2000. Effecten van minerale olie in zoute sedimenten. Onderzoek t.b.v. de afleiding van risicogrenzen voor ecosystemen. The Hague, The Netherlands: RIKZ (National Institute for Coastal and Marine Management), and RIZA (Institute for Inland Water Management and Waste Water Treatment). RIKZ-report 2000.035 / RIZA-report 2000.029.

MADEP. 1998. Method for the determination of extractable petroleum hydrocarbons (EPH). Boston, MA: Massachusetts Department of Environmental Protection and Commonwealth of Massachusetts (Available at http://www.state.ma.us/dep/bwsc/vph_eph.htm).

Maldonado C, Bayona JM, Bodineau L. 1999 . Sources, distribution, and water column processes of aliphatic and polycyclic aromatic hydrocarbons in the northwestern Black Sea water. Environ Sci Technol 33: 2693-2702.

Mazeas L, Budzinski H. 2002. Improved accuracy of GC-MS quantification of aliphatic and aromatic hydrocarbons in marine sediments and petroleums. Validation of reference matrices and application to the Erika oil spill. Int J Environ Anal Chem 82: 157-173.

McCarty LS, Mackay D. 1993. Enhancing ecotoxicological modeling and assessment. Environ Sci Technol 27: 1719-1728.

Mekenyan OG, Ankley GT, Veith GD, Call DJ. 1994. QSAR estimates of excited states and photoinduced acute toxicity of polycyclic aromatic hydrocarbons. SAR QSAR Environ Res 2: 237-247.

Mekenyan OG, Ankley GT, Veith GD, Call DJ. 1994. QSAR for photoinduced toxicity: I. Acute lethality of polycyclic aromatic hydrocarbons to *Daphnia magna*. Chemosphere 28: 567-582.

Mille G, El Jammal T, Doumenq P, Bertrand JC. 1992. Hydrocarbons and fatty acids in surficial sediments (French Mediterranean coastal zones). Sci Total Environ 113: 209-228.

Newsted JL, Giesy JP. 1987. Predictive models for photoinduced acute toxicity of polycyclic aromatic hydrocarbons to *Daphnia magna*, Strauss (Cladocera, crustacea). Environ Toxicol Chem 6: 445-461.

Parkerton TF, Stone MA, Letinski DJ. 2000. Assessing the aquatic toxicity of complex hydrocarbon mixtures using solid phase microextraction. Toxicol Lett 112: 273-282.

Patel H, Schultz TW, Cronin MTD. 2002. Physico-chemical interpretation and prediction of the dimyristoyl phosphatidyl choline-water partition coefficient. J Mol Struct 593: 9-18.

Pelletier MC, Burgess RM, Ho KT, Kuhn A, McKinney RA, Ryba SA. 1997. Phototoxicity of individual polycyclic aromatic hydrocarbons and petroleum to marine invertebrate larvae and juveniles. Environ Toxicol Chem 16: 2190-2199.

Peterson DR. 1994. Calculating the aquatic toxicity of hydrocarbon mixtures. Chemosphere 29: 2493-2506.

Poerschmann J, Kopinke F-D. 2001. Sorption of very hydrophobic organic compounds (VHOCs) on dissolved humic organic matter (DOM). 2. Measurement of sorption and application of a Flory-Huggins concept to interpret the data. Environ Sci Technol 35: 1142-1148.

Potter TL, Duval B. 2001. *Cerro negro* bitumen degradation by a consortium of marine benthic microorganisms. Environ Sci Technol 35: 76-83.

Potter TL, Simmons KE. 1998. Composition of Petroleum Mixtures. Vol. 2. Amherst, MA: Amherst Scientific Publishers (Available at http://www.aehs.com/publications/catalog/contents/tph.htm).

Readman JW, Fillmann G, Tolosa I, Bartocci J, Villeneuve J-P, Catinni C, Mee LD. 2002. Petroleum and PAH contamination in the Black Sea. Mar Pollut Bull 44: 48-62.

Ren S, Frymier PD. 2002. Modelling the toxicity of polar and nonpolar narcotic compounds to luminescent bacterium SHK1. Environ Toxicol Chem 21: 2649-2653.

Rotteveel S, Kamps MAAJ, Maas JL, Beek MA. 2002. De toxiciteit van minerale olie in zoete sedimenten. Lelystad, The Netherlands: RIZA (Institute for Inland Water Management and Waste Water Treatment). 2002.136X.

Rowland S, Donkin P, Smith E, Wraige E. 2001. Aromatic hydrocarbon "humps" in the marine environment: Unrecognized toxins? Environ Sci Technol 35: 2640-2644.

Sabljic A, Güsten H, Verhaar H, Hermens J. 1995. QSAR modelling of soil sorption. Improvements and systematics of log K_{oc} vs. log K_{ow} correlations. Chemosphere 31: 4489-4514.

Schluep M, Gälli R, Imboden DM, Zeyer J. 2002. Dynamic equilibrium dissolution of complex nonaqueous phase liquid mixtures into the aqueous phase. Environ Toxicol Chem 21: 1350-1358.

Schmidt TC, Kleinert P, Stengel C, Goss K-U, Haderlein.Stefan B. 2002. Polar fuel constituents: Compound identification and equilibrium partitioning between nonaqueous phase liquids and water. Environ Sci Technol 36: 4074-4080.

Scholten MCTh, Huwer SL, Foekema EM, Van Dokkum HP, Karman CC, Peters RJB. 1997. Pilot study on the dose-effect responses of petroleum hydrocarbons in sediments (SLURP*olie). Final report. Apeldoorn, The Netherlands: TNO Institute of Environmental Sciences, Energy Research and Process Innovation. TNO-MEP - R 97/420.

Schwarzenbach RP, Gschwend PM, Imboden DM. 1993. Environmental Organic Chemistry. New York, NY: John Wiley & Sons .

Sijm D, Lijzen J, Peijnenburg W, Sneller E, Traas T, Verbruggen E. 2001. Biobeschikbaarheid in beleid ... wat er aan vooraf ging en wat nog komt. Resultaten van een workshop en het beleidsvervolg. Bilthoven, The Netherlands: National Institute of Public Health and the Environment and Institute for Inland Water Management and Waste Water Treatment. RIVM report 607220 006 / RIZA-report 2002.003.

Slob W. 2002. Dose-response modeling of continuous endpoints. Toxicol Sci 66: 298-312.

Suedel BC, Rodgers Jr. JH. 1996. Toxicity of fluoranthene to *Daphnia magna*, *Hyalella azteca*, *Chironomus tentans*, and *Stylaria lacustris* in water-only and whole sediment exposures. Bull Environ Contam Toxicol 57: 132-138.

Sverdrup LE, Jensen J, Kelley AE, Krogh PH, Stenersen J. 2002. Effects of eight polycyclic aromatic compounds on the survival and reproduction of *Enchytraeus crypticus* (oligochaeta, clitellata). Environ Toxicol Chem 21: 109-114.

Sverdrup LE, Kelley AE, Krogh PH, Nielsen T, Jensen J, Scott-Fordsmand JJ, Stenersen J. 2001. Effects of eight polycyclic aromatic compounds on the survival and reproduction of the springtail *Folsomia fimetaria* l. (collembola, isotomidae). Environ Toxicol Chem 20: 1332-1338.

Sverdrup LE, Krogh PH, Nielsen T, Stenersen J. 2002. Relative sensitivity of three terrestrial invertebrate tests to polycyclic aromatic compounds. Environ Toxicol Chem 21: 1927-1933.

Sverdrup LE, Nielsen T, Krogh PH. 2002. Soil Ecotoxicity of Polycyclic Aromatic Hydrocarbons in Relation to Soil Sorption, Lipophilicity, and Water Solubility. Environ Sci Technol 36: 2429-2435.

TNRCC. 2000. Characterization of Nc_6 to Nc_{35} petroleum hydrocarbons in environmental samples. Austin, TX: Texas Natural Resource Conservation Commission. Draft TNRCC Method 1006 (Available at http://www.tnrcc.state.tx.us/permitting/analysis.htm).

Tolls J, Van Dijk J. 2002. Bioconcentration of *n*-dodecane and its highly branched isomer 2,2,4,6,6-pentamethylheptane in fathead minnows. Chemosphere 47: 1049-1057.

Tolls J, Van Dijk J, Verbruggen EMJ, Hermens JLM, Loeprecht B, Schüürmann G. 2002. Aqueous solubility-molecular size relationships: A mechanistic case study using C_{10}- to C_{19}-alkanes. J Phys Chem A 106: 2760-2765.

TPHCWG. 2000. Characterization of C_6 to C_{35} petroleum hydrocarbons in environmental samples. Revision: 5.1 Date: 3/2/98: Total Petroleum Hydrocarbon Criteria Working Group (Available at http://www.aehs.com/publications/catalog/tph.htm).

Traas TP. 2001. Guidance document in deriving environmental risk limits. Bilthoven, The Netherlands: National Institute of Public Health and the Environment. RIVM report 601501 012.

U.S. EPA. 1996a. Method 3611B Alumina column cleanup and separation of petroleum wastes *in* Test methods for evaluating solid waste, Physical/chemical methods: U.S. Environmental Protection Agency's Office of Solid Waste. SW-846 (Available at http://www.epa.gov/epaoswer/hazwaste/test/main.htm).

U.S. EPA. 1996b. Method 3630C Silica Gel Cleanup *in* Test methods for evaluating solid waste, Physical/chemical methods: U.S. Environmental Protection Agency's Office of Solid Waste. SW-846 (Available at http://www.epa.gov/epaoswer/hazwaste/test/main.htm).

U.S. EPA. 2000. EPI Suite. [computer program]. version 3.10. KOWWIN. Washington, DC: U.S. Environmental Protection Agency (EPA) Office of Pollution Prevention Toxics and Syracuse Research Company (SRC).

Vaes WHJ, Urrestarazu Ramos E, Hamwijk C, van Holsteijn I, Blaauboer BJ, Seinen W, Verhaar HJM, Hermens JLM. 1997. Solid Phase Microextraction as a tool to determine membrane/water partition coefficients and bioavailable concentrations in *in vitro* systems. Chem Res Toxicol 10: 1067-1072.

Vaes WHJ, Urrestarazu Ramos E, Verhaar HJM, Cramer CJ, Hermens JLM. 1998. Understanding and estimating membrane-water partition coefficients: Approaches to derive quantitative structure property relationships. Chem Res Toxicol 11: 847-854.

Van Beelen P, Verbruggen EMJ, Peijnenburg WJGM. 2003. The evaluation of the equilibrium partitioning method using sensitivity distributions of species in water and soil. Chemosphere 52: 1153-1162.

Van Gestel CAM, Van der Waarde JJ, Derksen JGM, Van der Hoek EE, Veul MFXW, Bouwens S, Rusch B, Kronenburg R, Stokman GNM. 2001. The use of acute and chronic bioassays to determine the ecological risk and bioremediation efficiency of oil-polluted soils. Environ Toxicol Chem 20: 1438-1449.

Van Hattum B, Hermens J, Verbruggen E. 2000. Toepassing van de hydrocarbon block methode bij de interpretatie van toxiciteitsexperimenten met minerale olie. Amsterdam, The Netherlands: Institute for Environmental Studies (IVM), Vrije Universiteit, Amsterdam. E-00/03.

Van Leeuwen CJ, Van der Zandt PTJ, Aldenberg T, Verhaar HJM, Hermens JLM. 1992. Application of QSARs, extrapolation and equilibrium partitioning in aquatic effects assessment. I. Narcotic industrial pollutants. Environ Toxicol Chem 11: 267-282.

Van Leeuwen CJ, Verhaar HJM, Hermens JLM. 1996. Quality criteria and risk assessment for mixtures of chemicals in the aquatic environment. Hum Ecol Risk Assess 2: 419-425.

Van Straalen NM, Denneman CAJ. 1989. Ecotoxicological evaluation of soil quality criteria. Ecotoxicol Environ Saf 18: 241-251.

Van Vlaardingen P, Traas TP. 2002. ETX-2000. Normal Distribution Based Hazardous Concentration and Potentially Affected Fraction [computer program]. version 1.407. Bilthoven, The Netherlands: National Institute of Public Health and the Environment.

Van Wezel AP, Opperhuizen A. 1995. Narcosis due to environmental pollutants in aquatic

organisms: Residue-based toxicity, mechanisms and membrane burdens. Crit Rev Toxicol 25: 255-279.

Verbruggen EMJ, Posthumus R, Van Wezel AP. 2001. Ecotoxicological Serious Risk Concentrations for soil, sediment and (ground)water: updated proposals for first series of compounds. Bilthoven, The Netherlands: National Institute of Public Health and the Environment. RIVM report 711701 020.

Verbruggen EMJ 1999. Predicting Hydrophobicity, Bioconcentration and Baseline Toxicity of Complex Organic Mixtures. Chapter 7: Measurement and calculation of the bioconcentration and toxicity of complex mixtures of organic chemicals using biomimetic extraction and hydrophobicity distribution profiles. Utrecht University, Utrecht, The Netherlands.

Verbruggen EMJ, Hermens JLM. 2001. Estimating accumulation and baseline toxicity of complex mixtures of organic chemicals to aquatic organisms: The use of hydrophobicity dependent analytical methods. In: Lipnick RL, Jansson B, Mackay D, Petreas M, eds. Persistent, bioaccumulative, and toxic chemicals II Assessment and new chemicals. ACS symposium series 773. Washington, DC: American Chemical Society. pp. 89-98.

Verbruggen EMJ, Hermens JLM, Tolls J. 2000. Physicochemical properties of higher nonaromatic hydrocarbons: A literature study. J Phys Chem Ref Data 29: 1435-1446.

Verbruggen EMJ, Vaes WHJ, Parkerton TF, Hermens JLM. 2000. Polyacrylate-coated SPME fibers as a tool to simulate body residues and target concentrations of complex organic mixtures for estimation of baseline toxicity. Environ Sci Technol 34: 324-331.

Verhiest G, Clément B, Blake G. 2001. Single and combined effects of sediment-associated PAHs on three species of freshwater macroinvertebrates. Ecotoxicology 10: 363-372.

Volkman JK, Holdsworth DG, Neill GP, Bavor Jr HJ. 1992. Identification of natural, anthropogenic and petroleum hydrocarbons in aquatic sediments. Sci Total Environ 112: 203-219.

VROM. 1989. Premises for risk management. Risk limits in the context of environmental policy. Tweede Kamer Der Staten-Generaal. Aanhangsel Van De Handelingen. Vergaderjaar 1988-1989: 21137, no. 5.

VROM. 1994. Environmental Quality Objectives in the Netherlands. The Hague, The Netherlands: Ministry of Housing, Spatial Planning and Environment.

Wagner C, Løkke H. 1991. Estimation of ecotoxicological protection levels from NOEC toxicity data. Water Res 35: 1237-1242.

Walraven SACM, Peters RJB. 2002. Het gehalte minerale olie, alifaten en aromaten in monsters zoetwatersediment. Apeldoorn, The Netherlands: TNO-MEP (Netherlands Organisation for Applied Scientific Research, Institute of Environment, Energy and Process Innovation. concept.

Webster L, McIntosh AD, Moffat CF, Dalgarno EJ, Brown NA, Fryer RJ. 2000. Analysis of sediments from Shetland Island voes for polycyclic hydrocarbons, steranes and

triterpanes. J Environ Monit 2: 29-38.

Weisman W. 1998. Analysis of Petroleum Hydrocarbons in Environmental Media. Vol. 1. Amherst, MA: Amherst Scientific Publishers (Available at http://www.aehs.com/publications/catalog/contents/tph.htm).

Wernersson A-S. 2003. Predicting petroleum phototoxicity. Ecotoxicol Environ Saf 54: 355-365.

Young LJ, Tattersfield LJ, Sherren AJ. Development of a matrix approach for ecotoxicological assessment of soils contaminated with hydrocarbons. Presented at 8th Annual Meeting of SETAC-Europe, Bordeaux, France, 14-18 April 1998.

Appendix: Details of calculation

In this appendix the results of the calculations are given together with the necessary information on composition, sediment or soil characteristics, and toxicity to calculate the endpoints based on internal concentrations.
Results of the toxicity experiments that are significantly different from the control (ANOVA, Dunnett's test) are shown italic. The highest level that is not significantly different from the control is shown bold. The corresponding sediment concentration or internal membrane concentration can be considered as NOEC.
The parameters from Equations 24 to 26 from the dose-response relationships are given at the end of each table.

Concentrations and toxicity of DMA in fresh water lake Drontermeer sediment, <0.5 mm																
Nominal concentration	[mg/kg$_{dw}$]	0	38	90			196	391			1260	4018			7995
Actual concentration	[mg/kg$_{dw}$]	34	25	55	52	49	130	250	210	260	710	2100	2700	2900	3700
Aliphatics	< C10	0.0%	0.0%	0.0%	0.0%	0.0%	0.2%	0.3%	0.3%	0.3%	0.3%	0.2%	0.3%	0.5%	0.3%
	>C10-C11	0.0%	0.0%	0.0%	0.0%	0.0%	0.4%	0.6%	0.6%	0.6%	0.7%	0.7%	0.7%	0.7%	0.7%
	>C11-C12	0.4%	0.1%	0.0%	0.0%	0.0%	1.5%	1.9%	1.8%	1.9%	2.2%	2.2%	2.4%	2.5%	2.4%
	>C12-C13	0.0%	0.6%	0.0%	0.7%	1.5%	1.5%	1.9%	1.8%	1.9%	2.1%	2.2%	2.2%	2.3%	2.2%
	>C13-C14	0.0%	0.9%	0.5%	1.0%	1.6%	1.8%	2.3%	2.2%	2.3%	2.6%	2.6%	2.7%	2.8%	2.7%
	>C14-C15	0.0%	1.1%	0.8%	1.2%	1.8%	1.7%	2.1%	2.1%	2.1%	2.4%	2.4%	2.5%	2.5%	2.5%
	>C15-C16	0.6%	1.2%	0.9%	1.3%	1.8%	2.3%	2.8%	2.7%	2.8%	3.1%	3.1%	3.2%	3.3%	3.2%
	>C16-C17	0.4%	1.6%	1.4%	1.8%	2.2%	2.4%	2.9%	2.8%	2.9%	3.2%	3.2%	3.3%	3.5%	3.3%
	>C17-C18	1.0%	2.4%	2.2%	2.6%	3.0%	3.4%	4.1%	4.0%	4.1%	4.5%	4.5%	4.6%	4.7%	4.6%
	>C18-C19	1.6%	3.5%	3.4%	3.9%	4.4%	4.0%	4.7%	4.6%	4.7%	5.0%	5.0%	5.1%	5.2%	5.1%
	>C19-C20	1.8%	2.7%	2.6%	2.9%	3.3%	3.8%	4.4%	4.4%	4.5%	4.8%	4.8%	4.9%	5.0%	4.9%
	>C20-C21	2.0%	2.3%	2.3%	2.5%	2.6%	3.7%	4.2%	4.1%	4.2%	4.5%	4.6%	4.7%	4.7%	4.7%
	>C21-C22	1.8%	2.1%	2.0%	2.2%	2.5%	3.4%	3.8%	3.8%	3.9%	4.2%	4.2%	4.3%	4.4%	4.3%
	>C22-C23	1.6%	1.6%	1.5%	1.6%	1.8%	2.7%	3.1%	3.0%	3.1%	3.4%	3.4%	3.5%	3.5%	3.5%
	>C23-C24	1.6%	1.5%	1.4%	1.5%	1.6%	2.3%	2.6%	2.5%	2.6%	2.7%	2.8%	2.8%	2.8%	2.8%
	>C24-C25	1.9%	1.2%	1.0%	1.1%	1.2%	1.9%	1.9%	1.9%	1.9%	2.0%	2.0%	2.0%	2.1%	2.0%
	>C25-C26	2.3%	1.1%	0.8%	0.9%	1.0%	1.6%	1.5%	1.5%	1.5%	1.5%	1.5%	1.6%	1.6%	1.6%
	>C26-C27	2.8%	0.9%	0.5%	0.6%	0.7%	1.4%	1.1%	1.1%	1.1%	1.1%	1.1%	1.1%	1.1%	1.1%
	>C27-C28	3.6%	1.1%	0.5%	0.6%	0.7%	1.4%	0.9%	1.0%	0.9%	0.8%	0.8%	0.8%	0.8%	0.8%
	>C28-C29	3.9%	1.1%	0.4%	0.5%	0.5%	1.4%	0.8%	0.8%	0.7%	0.6%	0.5%	0.5%	0.5%	0.5%
	>C29-C30	3.9%	1.1%	0.4%	0.4%	0.4%	1.3%	0.6%	0.6%	0.6%	0.4%	0.3%	0.3%	0.3%	0.3%
	>C30-C31	7.9%	2.1%	0.5%	0.6%	0.7%	2.3%	0.9%	0.9%	0.9%	0.4%	0.2%	0.2%	0.2%	0.2%
	>C31-C32	2.9%	0.8%	0.3%	0.4%	0.4%	0.9%	0.4%	0.4%	0.4%	0.2%	0.2%	0.2%	0.1%	0.1%
	>C32-C33	2.3%	0.7%	0.4%	0.4%	0.4%	0.7%	0.3%	0.3%	0.3%	0.1%	0.1%	0.1%	0.1%	0.1%
	>C33-C34	1.6%	0.4%	0.0%	0.0%	0.0%	0.5%	0.2%	0.2%	0.2%	0.1%	0.0%	0.0%	0.0%	0.0%
	>C34-C35	1.2%	0.3%	0.0%	0.0%	0.0%	0.3%	0.1%	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%
	>C35-C36	0.7%	0.2%	0.0%	0.0%	0.0%	0.2%	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C36-C37	0.7%	0.2%	0.0%	0.0%	0.0%	0.2%	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C37-C38	0.6%	0.2%	0.0%	0.0%	0.0%	0.2%	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C38-C39	0.5%	0.1%	0.0%	0.0%	0.0%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C39-C40	0.5%	0.1%	0.0%	0.0%	0.0%	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	Total	50.0%	33.1%	23.9%	28.7%	34.2%	49.6%	50.7%	49.8%	50.9%	52.9%	52.7%	54.1%	55.5%	54.1%
Aromatics	< C10	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C10-C11	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C11-C12	0.0%	0.0%	0.0%	0.0%	0.0%	0.6%	0.8%	0.8%	0.8%	1.0%	1.0%	1.0%	1.0%	1.0%
	>C12-C13	0.0%	0.0%	0.0%	0.0%	0.0%	1.1%	1.4%	1.4%	1.4%	1.7%	1.9%	1.8%	1.7%	1.8%
	>C13-C14	0.0%	0.0%	0.0%	0.0%	0.0%	1.6%	2.1%	2.0%	2.2%	2.5%	2.8%	2.7%	2.6%	2.7%
	>C14-C15	0.0%	0.6%	0.5%	0.7%	1.0%	1.7%	2.2%	2.1%	2.2%	2.5%	2.7%	2.6%	2.5%	2.6%
	>C15-C16	0.0%	1.0%	0.9%	1.2%	1.5%	2.4%	3.1%	3.0%	3.1%	3.5%	3.8%	3.7%	3.6%	3.7%
	>C16-C17	0.0%	1.1%	0.9%	1.3%	1.6%	2.2%	2.8%	2.8%	2.9%	3.2%	3.4%	3.4%	3.3%	3.4%
	>C17-C18	0.0%	1.5%	1.4%	1.8%	2.2%	2.5%	3.2%	3.1%	3.2%	3.5%	3.8%	3.7%	3.6%	3.7%
	>C18-C19	0.0%	1.8%	1.7%	2.1%	2.6%	3.0%	3.8%	3.7%	3.8%	4.2%	4.4%	4.4%	4.3%	4.4%
	>C19-C20	0.7%	2.2%	2.1%	2.5%	2.9%	3.2%	3.9%	3.8%	3.9%	4.3%	4.5%	4.4%	4.4%	4.4%
	>C20-C21	0.3%	2.5%	2.4%	2.9%	3.4%	2.9%	3.6%	3.6%	3.6%	3.9%	4.1%	4.0%	4.0%	4.1%
	>C21-C22	1.1%	2.3%	2.2%	2.5%	2.9%	2.8%	3.2%	3.2%	3.3%	3.5%	3.6%	3.6%	3.5%	3.6%
	>C22-C23	0.5%	1.6%	1.5%	1.8%	2.2%	1.9%	2.3%	2.3%	2.3%	2.5%	2.6%	2.5%	2.5%	2.5%
	>C23-C24	0.5%	1.2%	1.2%	1.4%	1.6%	1.5%	1.8%	1.8%	1.8%	1.9%	2.1%	2.0%	1.9%	2.0%
	>C24-C25	0.8%	1.1%	0.9%	1.1%	1.4%	1.2%	1.3%	1.3%	1.3%	1.4%	1.4%	1.4%	1.4%	1.4%
	>C25-C26	0.8%	1.3%	1.2%	1.4%	1.5%	1.0%	1.1%	1.1%	1.1%	1.1%	1.1%	1.1%	1.0%	1.1%
	>C26-C27	0.6%	1.0%	1.0%	1.1%	1.2%	0.8%	0.8%	0.8%	0.8%	0.7%	0.8%	0.7%	0.7%	0.7%
	>C27-C28	1.2%	1.7%	1.9%	1.8%	1.6%	0.9%	0.8%	0.8%	0.8%	0.6%	0.6%	0.6%	0.5%	0.6%
	>C28-C29	1.6%	2.2%	2.4%	2.4%	2.3%	1.0%	0.8%	0.8%	0.7%	0.5%	0.4%	0.4%	0.4%	0.4%
	>C29-C30	1.9%	2.4%	2.8%	2.5%	2.2%	1.0%	0.7%	0.8%	0.7%	0.4%	0.3%	0.3%	0.3%	0.3%
	>C30-C31	2.5%	2.8%	3.2%	3.0%	2.7%	1.2%	0.8%	0.9%	0.7%	0.4%	0.3%	0.3%	0.2%	0.3%
	>C31-C32	3.7%	4.0%	4.5%	4.1%	3.7%	1.6%	1.0%	1.1%	0.9%	0.5%	0.2%	0.2%	0.2%	0.2%
	>C32-C33	5.0%	4.8%	5.9%	4.9%	3.8%	2.1%	1.1%	1.3%	1.1%	0.5%	0.2%	0.2%	0.2%	0.2%
	>C33-C34	6.6%	6.7%	7.4%	6.9%	6.3%	2.7%	1.5%	1.8%	1.4%	0.6%	0.2%	0.2%	0.2%	0.2%
	>C34-C35	4.4%	4.3%	5.3%	4.4%	3.4%	1.8%	1.0%	1.2%	1.0%	0.4%	0.2%	0.2%	0.1%	0.1%
	>C35-C36	5.1%	5.2%	6.1%	5.4%	4.5%	2.1%	1.2%	1.4%	1.1%	0.5%	0.2%	0.2%	0.1%	0.1%
	>C36-C37	3.2%	3.3%	4.3%	3.4%	2.5%	1.4%	0.8%	0.9%	0.7%	0.3%	0.2%	0.1%	0.1%	0.1%
	>C37-C38	3.5%	3.8%	4.8%	4.0%	3.0%	1.5%	0.8%	1.0%	0.8%	0.3%	0.1%	0.1%	0.1%	0.1%
	>C38-C39	2.7%	3.0%	4.5%	3.2%	1.8%	1.2%	0.7%	0.8%	0.6%	0.3%	0.1%	0.1%	0.1%	0.1%
	>C39-C40	3.1%	3.3%	5.1%	3.5%	1.6%	1.3%	0.7%	0.9%	0.7%	0.3%	0.1%	0.1%	0.1%	0.1%
	Total	50.0%	66.9%	76.1%	71.3%	65.8%	50.4%	49.3%	50.2%	49.1%	47.1%	47.3%	45.9%	44.5%	45.9%
Fraction organic carbon		0.94%	0.94%	0.94%	0.94%	0.94%	0.94%	0.94%	0.94%	0.94%	0.94%	0.94%	0.94%	0.94%	0.94%
Fraction water	[L/kg$_{dw}$]	60%	60%	60%	60%	60%	60%	60%	60%	60%	60%	60%	60%	60%	60%
Internal concentration	[mM]	1.7	3.3	7.2			25.0	50.4			118.8	196.3			209.8
Chironomus riparius	Survival	100%	99%	98.8%			98.75%	100.0%			**98.75%**	*79%*			*31.2%*
	Development delay	0%	1.25%	1.25%			1.25%	0.0%			**5%**	*65%*			*96%*
	Dry weight [mg]	1.16	1.19	1.23			1.16	1.05			**1.23**	*0.25*			*0.17*
Plectus acuminatus	Survival	67.5%	**42.5%**	*23.75%*			*25%*	*25%*			*12.5%*	*13.75%*			*16.25%*
Hyalella azteca	Survival	93.75%	98.8%	93.8%			92.50%	**77.50%**			*32.50%*	*3.75%*			*1.25%*
	Dry weight [mg]	0.083	**0.068**	*0.051*			*0.041*	*0.033*			*0.022*				
Ephoron virgo	Survival	93.33%	90%	81.67%			**73.65%**	*60%*			*8.33%*	*3.33%*			*1.67%*
	Length [mm]	1.28	*1.10*	*1.08*			*1.07*	*0.92*			*0.70*	*0.80*			

Dose-response relationships									Actual sediment concentrations						
		Bottom	Top	log EC50	log EC10	A	C	B	Bottom	Top	log EC50	log EC10	A	C	B
Chironomus riparius	Survival	0 c	99.17	3.511	3.348				0 c	99.17	2.311	2.282			
	Development delay	1.454	100 c	3.372	3.235				1.458	100 c	2.286	2.262			
	Dry weight [mg]	0 c	1.173	3.282	3.035				0 c	1.171	2.25	2.172			
Plectus acuminatus	Survival					49.15	0 c	313.5					43.33	0 c	117.4
Hyalella azteca	Survival	0 c	96.41	2.702	2.224				0 c	94.75	1.948	1.635			
	Dry weight [mg]					0.08817	0.2684	96.72					0.0967	0.319	6.33
Ephoron virgo	Survival	0 c	88.61	2.488	2.068				0 c	86.77	1.794	1.465			
	Length [mm]					1.235	0.6052	233.9					1.206	0.6135	48.71

Concentrations and toxicity of HV46 in fresh water lake Drontermeer sediment, <0.5 mm															
Nominal concentration	[mg/kg$_{dw}$]	0	64	183			426	1255			3998	12611			39961
Actual concentration	[mg/kg$_{dw}$]	34	84	200	250	190	440	1200	1100	1100	3200	9600	8700	8000	33000
Aliphatics	< C10	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C10-C11	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C11-C12	0.4%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C12-C13	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C13-C14	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C14-C15	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C15-C16	0.6%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C16-C17	0.4%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C17-C18	1.0%	0.4%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C18-C19	1.6%	0.7%	0.5%	0.5%	0.6%	0.3%	0.1%	0.2%	0.2%	0.1%	0.1%	0.1%	0.1%	0.1%
	>C19-C20	1.8%	0.7%	0.5%	0.5%	0.5%	0.3%	0.3%	0.3%	0.3%	0.2%	0.2%	0.2%	0.2%	0.2%
	>C20-C21	2.0%	0.8%	0.5%	0.5%	0.5%	0.4%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%	0.5%
	>C21-C22	1.8%	1.0%	0.9%	0.8%	0.8%	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%	0.9%	1.0%	0.9%
	>C22-C23	1.6%	1.3%	1.3%	1.3%	1.2%	1.5%	1.6%	1.6%	1.6%	1.6%	1.6%	1.6%	1.7%	1.6%
	>C23-C24	1.6%	1.9%	2.1%	2.0%	2.0%	2.4%	2.6%	2.6%	2.6%	2.6%	2.6%	2.7%	2.7%	2.7%
	>C24-C25	1.9%	2.5%	2.6%	2.7%	2.7%	3.3%	3.7%	3.6%	3.6%	3.8%	3.8%	3.8%	3.9%	3.9%
	>C25-C26	2.3%	3.8%	4.2%	4.2%	4.1%	5.3%	5.8%	5.8%	5.8%	6.0%	6.0%	6.1%	6.3%	6.1%
	>C26-C27	2.8%	5.2%	5.8%	5.8%	5.7%	7.4%	8.2%	8.2%	8.2%	8.5%	8.5%	8.7%	8.8%	8.7%
	>C27-C28	3.6%	6.7%	7.4%	7.5%	7.6%	9.8%	10.9%	10.8%	10.8%	11.3%	11.4%	11.5%	11.7%	11.5%
	>C28-C29	3.9%	7.1%	7.9%	7.9%	7.9%	10.6%	11.9%	11.8%	11.8%	12.4%	12.6%	12.6%	12.7%	12.7%
	>C29-C30	3.9%	6.8%	7.4%	7.5%	7.6%	10.3%	11.7%	11.6%	11.6%	12.2%	12.5%	12.5%	12.4%	12.5%
	>C30-C31	7.9%	6.7%	6.3%	6.5%	6.7%	9.3%	10.8%	10.7%	10.7%	11.3%	12.8%	11.7%	10.5%	11.7%
	>C31-C32	2.9%	4.3%	4.5%	4.6%	4.8%	6.2%	6.9%	6.9%	6.9%	7.2%	7.4%	7.3%	7.3%	7.4%
	>C32-C33	2.3%	2.8%	2.9%	2.9%	2.9%	3.6%	3.9%	3.9%	3.9%	4.1%	4.1%	4.1%	4.1%	4.1%
	>C33-C34	1.6%	1.7%	1.7%	1.7%	1.8%	2.0%	2.1%	2.1%	2.1%	2.2%	2.2%	2.2%	2.2%	2.2%
	>C34-C35	1.2%	1.1%	1.1%	1.1%	1.0%	1.1%	1.2%	1.2%	1.2%	1.2%	1.2%	1.2%	1.2%	1.2%
	>C35-C36	0.7%	0.7%	0.7%	0.7%	0.7%	0.7%	0.7%	0.7%	0.7%	0.7%	0.7%	0.7%	0.7%	0.7%
	>C36-C37	0.7%	0.6%	0.5%	0.6%	0.6%	0.5%	0.5%	0.5%	0.5%	0.4%	0.4%	0.4%	0.4%	0.4%
	>C37-C38	0.6%	0.5%	0.4%	0.5%	0.6%	0.4%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%	0.3%
	>C38-C39	0.5%	0.3%	0.0%	0.3%	0.5%	0.3%	0.3%	0.3%	0.3%	0.3%	0.2%	0.2%	0.3%	0.2%
	>C39-C40	0.5%	0.4%	0.0%	0.3%	0.6%	0.3%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%	0.2%
	Total	50.0%	58.5%	59.2%	60.3%	61.4%	77.0%	85.2%	84.7%	84.7%	88.1%	90.3%	89.7%	89.2%	89.8%
Aromatics	< C10	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C10-C11	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C11-C12	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C12-C13	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C13-C14	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C14-C15	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C15-C16	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C16-C17	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C17-C18	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C18-C19	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C19-C20	0.7%	0.4%	0.4%	0.4%	0.3%	0.2%	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C20-C21	0.3%	0.3%	0.3%	0.3%	0.3%	0.2%	0.1%	0.1%	0.1%	0.0%	0.1%	0.0%	0.0%	0.0%
	>C21-C22	1.1%	0.6%	0.5%	0.4%	0.4%	0.2%	0.1%	0.1%	0.1%	0.1%	0.1%	0.1%	0.0%	0.0%
	>C22-C23	0.5%	0.5%	0.5%	0.5%	0.5%	0.3%	0.2%	0.2%	0.2%	0.1%	0.2%	0.1%	0.1%	0.1%
	>C23-C24	0.5%	0.5%	0.5%	0.4%	0.4%	0.3%	0.2%	0.2%	0.2%	0.2%	0.3%	0.2%	0.1%	0.2%
	>C24-C25	0.8%	0.6%	0.6%	0.6%	0.6%	0.5%	0.4%	0.4%	0.4%	0.4%	0.4%	0.3%	0.3%	0.3%
	>C25-C26	0.8%	1.0%	1.1%	1.1%	1.0%	0.8%	0.6%	0.7%	0.7%	0.6%	0.6%	0.6%	0.5%	0.6%
	>C26-C27	0.6%	1.2%	1.4%	1.3%	1.2%	1.1%	0.9%	0.9%	0.9%	0.9%	0.9%	0.8%	0.8%	0.8%
	>C27-C28	1.2%	1.9%	2.2%	2.1%	1.9%	1.6%	1.3%	1.3%	1.3%	1.2%	1.1%	1.2%	1.2%	1.2%
	>C28-C29	1.6%	2.3%	2.5%	2.4%	2.3%	1.8%	1.5%	1.5%	1.5%	1.4%	1.2%	1.3%	1.4%	1.3%
	>C29-C30	1.9%	2.6%	2.9%	2.7%	2.5%	1.9%	1.5%	1.6%	1.6%	1.4%	1.2%	1.3%	1.4%	1.3%
	>C30-C31	2.5%	2.7%	2.9%	2.8%	2.6%	1.9%	1.4%	1.4%	1.4%	1.2%	1.1%	1.2%	1.3%	1.2%
	>C31-C32	3.7%	3.3%	3.4%	3.3%	3.1%	1.9%	1.3%	1.3%	1.3%	1.0%	0.8%	0.9%	1.0%	0.9%
	>C32-C33	5.0%	4.1%	4.0%	3.9%	3.8%	2.0%	1.1%	1.2%	1.2%	0.8%	0.6%	0.6%	0.7%	0.6%
	>C33-C34	6.6%	4.8%	4.5%	4.5%	4.4%	2.1%	1.0%	1.1%	1.1%	0.6%	0.4%	0.4%	0.5%	0.4%
	>C34-C35	4.4%	3.4%	3.2%	3.1%	3.1%	1.5%	0.7%	0.8%	0.8%	0.4%	0.2%	0.3%	0.3%	0.3%
	>C35-C36	5.1%	3.6%	3.2%	3.3%	3.5%	1.5%	0.7%	0.7%	0.7%	0.4%	0.2%	0.2%	0.3%	0.2%
	>C36-C37	3.2%	2.1%	1.9%	1.9%	1.9%	0.9%	0.4%	0.5%	0.5%	0.3%	0.2%	0.2%	0.2%	0.2%
	>C37-C38	3.5%	2.3%	2.0%	2.0%	2.0%	0.9%	0.4%	0.5%	0.5%	0.3%	0.1%	0.2%	0.2%	0.2%
	>C38-C39	2.7%	1.6%	1.5%	1.4%	1.4%	0.7%	0.3%	0.4%	0.4%	0.2%	0.1%	0.2%	0.2%	0.2%
	>C39-C40	3.1%	1.7%	1.4%	1.4%	1.3%	0.7%	0.4%	0.4%	0.4%	0.2%	0.1%	0.2%	0.3%	0.2%
	Total	50.0%	41.5%	40.8%	39.7%	38.6%	23.0%	14.8%	15.3%	15.3%	11.9%	9.7%	10.3%	10.8%	10.2%
Fraction organic carbon		0.94%	0.94%	0.94%	0.94%	0.94%	0.94%	0.94%	0.94%	0.94%	0.94%	0.94%	0.94%	0.94%	0.94%
Fraction water	[L/kg$_{dw}$]	60%	60%	60%	60%	60%	60%	60%	60%	60%	60%	60%	60%	60%	60%
Internal concentration	[mM]	1.7	3.4	6.1			7.1	9.3			11.6	12.5			13.5
Chironomus riparius	Survival	100%	100%	97.5%			98.75%	**92.5%**			*51%*	*6%*			*0%*
	Development delay	0%	1.25%	2.5%			1.25%	**8.8%**			*66%*	*100%*			*100%*
	Dry weight [mg]	1.16	1.15	**1.04**			*0.89*	*0.58*			*0.09*				
Plectus acuminatus	Survival	62.5%	37.5%	37.5%			45%	40%			22.5%	30%			25%
Hyalella azteca	Survival	93.75%	97.5%	**92.5%**			*63.75%*	*61.25%*			*46.25%*	*18.75%*			*1.25%*
	Dry weight [mg]	0.083	**0.062**	*0.048*			*0.035*	*0.033*			*0.020*				
Ephoron virgo	Survival	93.33%	71.67%	85%			**80%**	*18.33%*			*5%*	*3.33%*			*0%*
	Length [mm]	1.28	**1.26**	*1.15*			*1.01*	*0.95*			*0.90*				

Dose-response relationships		Actual sediment concentrations							Internal membrane concentrations						
		Bottom	Top	log EC50	log EC10	A	C	B	Bottom	Top	log EC50	log EC10	A	C	B
Chironomus riparius	Survival	0 c	99.05	3.513	3.152				0 c	97.76	1.064	1.037			
	Development delay	1.285	100 c	3.414	3.11				2.76	100 c	1.061	1.053			
	Dry weight [mg]	0 c	1.142	3.016	2.447				0 c	1.133	0.9545	0.8142			
Plectus acuminatus	Survival					41.59	0 c	45170					61.45	0 c	15.43
Hyalella azteca	Survival	0 c	99.7	3.265	2.141				0 c	93.87	1.001	0.8136			
	Dry weight [mg]					0.09042	0.2984	184.9					0.1033	0.01196	7.181
Ephoron virgo	Survival	0 c	83.44	2.935	2.728				0 c	83.64	0.9344	0.8734			
	Length [mm]					1.332	0.6842	359.7					1.401	0 c	25.47

Concentrations and toxicity of DMA in salt water Oesterput sediment, <0.5 mm									<0.5 cm					
Nominal concentration	[mg/kg$_{dw}$]	0	40	86	186	400	1265	4000	0	40	186	400	1265	4000
Actual concentration	[mg/kg$_{dw}$]	31	40	99	104	178	680	1707	107	36	98	255	554	1601
Aliphatics	< C10	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C10-C11	0.3%	1.0%	1.1%	2.4%	2.4%	7.7%	1.6%	0.3%	1.1%	3.7%	3.0%	1.5%	3.5%
	>C11-C12	0.3%	3.1%	1.0%	3.5%	2.4%	2.6%	2.9%	0.3%	1.6%	2.7%	2.6%	1.6%	3.2%
	>C12-C13	0.7%	5.6%	3.5%	6.0%	3.8%	3.6%	3.7%	0.2%	2.3%	3.1%	3.1%	3.8%	4.0%
	>C13-C14	1.2%	5.3%	4.2%	2.9%	4.2%	3.7%	4.5%	0.3%	2.5%	3.1%	3.5%	4.7%	4.3%
	>C14-C15	1.8%	3.6%	3.8%	3.8%	4.5%	4.3%	4.8%	0.3%	2.2%	3.4%	3.8%	5.0%	4.9%
	>C15-C16	1.1%	2.2%	3.4%	3.4%	3.9%	3.7%	4.4%	0.4%	2.3%	3.1%	3.1%	4.5%	4.5%
	>C16-C17	3.5%	4.0%	7.3%	5.7%	5.9%	5.9%	6.4%	0.8%	4.2%	5.2%	5.9%	7.0%	6.8%
	>C17-C18	1.8%	2.5%	5.1%	4.4%	5.2%	5.2%	5.8%	0.1%	1.2%	4.2%	4.4%	5.7%	5.8%
	>C18-C19	2.8%	2.7%	5.2%	4.3%	4.6%	4.4%	5.1%	0.4%	1.5%	4.1%	4.9%	5.3%	5.5%
	>C19-C20	3.7%	3.2%	5.1%	3.8%	4.3%	4.5%	4.3%	0.9%	1.3%	3.3%	3.5%	3.8%	3.8%
	>C20-C21	3.9%	2.7%	3.7%	2.8%	3.2%	2.9%	2.8%	0.9%	1.6%	2.7%	2.6%	2.8%	2.6%
	>C21-C22	2.6%	1.8%	2.0%	1.7%	2.0%	1.9%	1.9%	1.6%	2.4%	2.5%	2.0%	2.2%	2.2%
	>C22-C23	3.7%	2.4%	2.4%	2.0%	2.1%	2.0%	1.9%	2.2%	2.4%	2.1%	1.5%	1.7%	1.5%
	>C23-C24	2.4%	1.8%	1.6%	1.2%	1.3%	1.3%	1.2%	3.0%	3.3%	1.8%	1.0%	1.1%	0.9%
	>C24-C25	2.1%	1.7%	1.2%	0.9%	0.9%	0.9%	0.8%	5.5%	5.0%	2.0%	1.2%	1.0%	0.7%
	>C25-C26	1.4%	1.1%	0.8%	0.7%	0.6%	0.6%	0.5%	4.1%	3.5%	1.2%	0.6%	0.4%	0.2%
	>C26-C27	1.8%	1.1%	0.7%	0.8%	0.5%	0.5%	0.4%	1.8%	1.2%	0.4%	0.2%	0.1%	0.0%
	>C27-C28	2.6%	1.5%	1.1%	0.8%	0.6%	0.4%	0.3%	2.5%	1.7%	0.6%	0.3%	0.2%	0.0%
	>C28-C29	2.2%	1.2%	0.7%	0.6%	0.3%	0.2%	0.2%	2.1%	1.0%	0.4%	0.2%	0.1%	0.0%
	>C29-C30	2.3%	1.3%	0.7%	0.6%	0.3%	0.2%	0.1%	2.2%	1.0%	0.5%	0.2%	0.1%	0.0%
	>C30-C31	1.4%	0.8%	0.3%	0.3%	0.1%	0.1%	0.1%	3.0%	1.9%	0.7%	0.3%	0.1%	0.0%
	>C31-C32	0.9%	0.5%	0.2%	0.2%	0.1%	0.0%	0.0%	1.9%	1.2%	0.4%	0.2%	0.1%	0.0%
	>C32-C33	1.1%	0.6%	0.1%	0.2%	0.1%	0.0%	0.0%	1.5%	0.9%	0.3%	0.2%	0.0%	0.0%
	>C33-C34	1.2%	0.7%	0.1%	0.3%	0.1%	0.0%	0.0%	1.7%	1.0%	0.3%	0.2%	0.0%	0.0%
	>C34-C35	0.8%	0.5%	0.1%	0.2%	0.0%	0.0%	0.0%	2.3%	1.4%	0.5%	0.2%	0.0%	0.0%
	>C35-C36	0.9%	0.5%	0.1%	0.2%	0.0%	0.0%	0.0%	2.4%	1.5%	0.6%	0.2%	0.0%	0.0%
	>C36-C37	0.6%	0.3%	0.0%	0.1%	0.0%	0.0%	0.0%	2.5%	0.0%	0.3%	0.5%	0.0%	0.0%
	>C37-C38	0.6%	0.3%	0.0%	0.1%	0.0%	0.0%	0.0%	2.6%	0.0%	0.3%	0.5%	0.0%	0.0%
	>C38-C39	0.2%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	1.0%	0.0%	0.0%	0.3%	0.0%	0.0%
	>C39-C40	0.2%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	1.1%	0.0%	0.0%	0.4%	0.0%	0.0%
	Total	50.0%	54.0%	55.4%	53.7%	53.5%	56.6%	53.6%	50.0%	51.4%	53.8%	50.5%	52.8%	54.4%
Aromatics	< C10	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C10-C11	0.3%	0.2%	0.3%	0.1%	0.1%	0.0%	0.0%	0.3%	0.3%	0.3%	0.2%	0.1%	0.0%
	>C11-C12	0.3%	0.1%	0.3%	1.5%	1.1%	1.1%	1.2%	0.3%	0.3%	1.3%	1.2%	0.8%	1.4%
	>C12-C13	0.7%	0.4%	0.9%	4.8%	3.0%	2.9%	3.0%	0.2%	0.4%	2.5%	2.6%	3.1%	3.2%
	>C13-C14	1.2%	0.7%	1.2%	2.8%	4.1%	3.6%	4.4%	0.3%	0.4%	3.1%	3.5%	4.6%	4.2%
	>C14-C15	1.8%	2.5%	3.0%	4.0%	4.7%	4.5%	5.1%	0.3%	1.5%	3.5%	3.9%	5.2%	5.2%
	>C15-C16	1.1%	2.1%	3.3%	3.9%	4.4%	4.3%	5.1%	0.4%	2.2%	3.5%	3.5%	5.2%	5.2%
	>C16-C17	3.5%	3.5%	6.3%	5.7%	6.0%	5.9%	6.4%	0.8%	3.4%	5.3%	6.0%	7.1%	6.8%
	>C17-C18	1.8%	2.1%	4.2%	3.6%	4.3%	4.2%	4.6%	0.1%	0.9%	3.4%	3.6%	4.6%	4.7%
	>C18-C19	2.8%	2.3%	4.1%	3.8%	4.0%	3.8%	4.4%	0.4%	1.1%	3.6%	4.3%	4.6%	4.8%
	>C19-C20	3.7%	3.1%	4.9%	3.5%	3.9%	4.1%	3.9%	0.9%	1.3%	3.0%	3.2%	3.4%	3.5%
	>C20-C21	3.9%	2.8%	4.0%	2.5%	2.9%	2.5%	2.4%	0.9%	1.9%	2.5%	2.3%	2.4%	2.3%
	>C21-C22	2.6%	1.9%	2.1%	1.5%	1.8%	1.6%	1.6%	1.6%	2.7%	2.2%	1.7%	1.9%	1.8%
	>C22-C23	3.7%	2.4%	2.5%	1.7%	1.7%	1.5%	1.4%	2.2%	2.7%	1.7%	1.2%	1.3%	1.1%
	>C23-C24	2.4%	1.8%	1.6%	1.0%	1.0%	1.0%	0.8%	3.0%	3.3%	1.6%	0.9%	0.9%	0.7%
	>C24-C25	2.1%	1.9%	1.2%	0.7%	0.8%	0.6%	0.5%	5.5%	5.4%	1.9%	1.2%	0.9%	0.5%
	>C25-C26	1.4%	1.4%	0.8%	0.6%	0.5%	0.4%	0.3%	4.1%	4.6%	1.2%	0.6%	0.4%	0.2%
	>C26-C27	1.8%	1.7%	0.7%	0.7%	0.4%	0.3%	0.3%	1.8%	1.7%	0.4%	0.2%	0.1%	0.0%
	>C27-C28	2.6%	2.5%	1.1%	0.8%	0.5%	0.3%	0.3%	2.5%	2.5%	0.6%	0.3%	0.2%	0.0%
	>C28-C29	2.2%	1.6%	0.7%	0.6%	0.3%	0.2%	0.1%	2.1%	1.0%	0.4%	0.2%	0.1%	0.0%
	>C29-C30	2.3%	1.7%	0.7%	0.6%	0.3%	0.2%	0.1%	2.2%	1.0%	0.5%	0.2%	0.1%	0.0%
	>C30-C31	1.4%	1.1%	0.3%	0.3%	0.1%	0.1%	0.1%	3.0%	2.4%	0.7%	0.3%	0.1%	0.0%
	>C31-C32	0.9%	1.0%	0.2%	0.2%	0.1%	0.1%	0.0%	1.9%	1.9%	0.4%	0.2%	0.1%	0.0%
	>C32-C33	1.1%	1.3%	0.1%	0.3%	0.1%	0.0%	0.0%	1.5%	1.1%	0.3%	0.2%	0.0%	0.0%
	>C33-C34	1.2%	1.6%	0.1%	0.3%	0.1%	0.0%	0.0%	1.7%	1.2%	0.3%	0.2%	0.0%	0.0%
	>C34-C35	0.8%	1.1%	0.1%	0.2%	0.0%	0.0%	0.0%	2.3%	1.6%	0.5%	0.2%	0.0%	0.0%
	>C35-C36	0.9%	1.3%	0.1%	0.2%	0.0%	0.0%	0.0%	2.4%	1.7%	0.6%	0.2%	0.0%	0.0%
	>C36-C37	0.6%	0.6%	0.0%	0.2%	0.0%	0.0%	0.0%	2.5%	0.0%	0.3%	0.5%	0.0%	0.0%
	>C37-C38	0.6%	0.7%	0.0%	0.2%	0.0%	0.0%	0.0%	2.6%	0.0%	0.3%	0.5%	0.0%	0.0%
	>C38-C39	0.2%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	1.0%	0.0%	0.0%	3.2%	0.0%	0.0%
	>C39-C40	0.2%	0.2%	0.0%	0.0%	0.0%	0.0%	0.0%	1.1%	0.0%	0.0%	3.1%	0.0%	0.0%
	Total	50.0%	46.0%	44.6%	46.3%	46.5%	43.4%	46.4%	50.0%	48.6%	46.2%	49.5%	47.2%	45.6%
Fraction organic carbon		1.7%	1.7%	1.7%	1.7%	1.7%	1.7%	1.7%	1.9%	1.9%	1.9%	1.9%	1.9%	1.9%
Fraction water	[L/kg$_{dw}$]	80.1%	80.1%	80.1%	80.1%	80.1%	80.1%	80.1%	79.6%	79.6%	79.6%	79.6%	79.6%	79.6%
Internal concentration	[mM]	3.8	6.3	15.0	20.1	31.2	93.8	161.9	7.1	4.4	16.8	36.3	72.7	151.1
Corophium volutator	Survival	92.9%	90.4%	87.9%	**76.7%**	*33.8%*	*1.25%*	*0.56%*						
Vibrio fischeri	Bioluminescence	100%	*62.5%*	*39%*	*43.5%*	*36%*	*21.5%*	*15%*						
Echinocardium cordatum	Survival								93.33%	100%	**93.33%**	*26.67%*	*3.33%*	*0%*
	Reburial								93.33%	100%	**73.33%**	*3.33%*	*3.33%*	*0%*

Dose-response relationships		Actual sediment concentrations							Internal membrane concentrations						
		Bottom	Top	log EC50	log EC10	A	C	B	Bottom	Top	log EC50	log EC10	A	C	B
Corophium volutator	Survival	0 c	92.06	2.2	1.998				0 c	91.79	1.447	1.252			
Vibrio fischeri	Bioluminescence	0 c	100 c	1.994	1.053	107.5	0 c	121.8	0 c	100 c	1.197	0.2114	96.43	0 c	25.08
Echinocardium cordatum	Survival	0 c	100 c	2.298	2.059				0 c	96.95	1.481	1.299			
	Reburial	0 c	96.57	2.176	1.994				0 c	96.83	1.312	1.143			

Concentrations and toxicity of HV46 in salt water Oesterput sediment, <0.5 mm										<0.5 cm					
Nominal concentration	[mg/kg$_{dw}$]	0	86	186	400	1265	4000	12650	40000	0	400	1265	4000	12650	40000
Actual concentration	[mg/kg$_{dw}$]	31	78	170	372	811	2708	7448	18957	107	328	734	1068	4163	20916
Aliphatics	< C10	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C10-C11	0.3%	0.5%	0.0%	0.1%	0.1%	0.9%	0.5%	0.2%	0.3%	1.6%	0.1%	0.4%	0.1%	0.1%
	>C11-C12	0.3%	1.4%	0.1%	0.1%	0.1%	0.1%	0.0%	0.2%	0.3%	1.3%	0.1%	0.6%	0.1%	0.1%
	>C12-C13	0.7%	2.1%	0.0%	0.1%	0.1%	0.1%	0.0%	0.1%	0.2%	1.0%	0.1%	0.4%	0.1%	0.1%
	>C13-C14	1.2%	1.7%	0.1%	0.1%	0.1%	0.0%	0.0%	0.3%	0.3%	0.9%	0.1%	0.4%	0.1%	0.1%
	>C14-C15	1.8%	1.6%	0.1%	0.1%	0.1%	0.0%	0.0%	0.2%	0.3%	0.6%	0.1%	0.3%	0.0%	0.1%
	>C15-C16	1.1%	1.1%	0.2%	0.2%	0.2%	0.0%	0.0%	0.1%	0.4%	0.7%	0.1%	0.4%	0.1%	0.1%
	>C16-C17	3.5%	2.5%	1.2%	0.9%	0.4%	0.1%	0.0%	0.0%	0.8%	0.8%	0.1%	0.4%	0.1%	0.1%
	>C17-C18	1.8%	1.0%	0.5%	0.4%	0.3%	0.1%	0.0%	0.1%	0.1%	0.1%	0.0%	0.1%	0.0%	0.1%
	>C18-C19	2.8%	0.8%	0.5%	0.4%	0.4%	0.1%	0.1%	0.4%	0.4%	0.4%	0.1%	0.2%	0.1%	0.1%
	>C19-C20	3.7%	1.2%	0.8%	1.0%	1.2%	0.5%	0.3%	1.7%	0.9%	0.3%	0.4%	0.3%	0.4%	0.3%
	>C20-C21	3.9%	1.4%	1.2%	1.7%	2.2%	1.3%	0.8%	2.5%	0.9%	1.2%	1.1%	0.8%	1.0%	0.6%
	>C21-C22	2.6%	1.7%	1.8%	2.4%	3.3%	2.1%	1.6%	2.8%	1.6%	2.3%	2.4%	1.6%	2.3%	1.6%
	>C22-C23	3.7%	3.0%	3.7%	5.3%	6.7%	4.9%	3.9%	3.9%	2.2%	3.1%	4.0%	2.8%	3.9%	2.8%
	>C23-C24	2.4%	4.9%	5.6%	6.9%	8.5%	6.9%	5.8%	5.9%	3.0%	4.3%	5.7%	4.0%	5.6%	4.2%
	>C24-C25	2.1%	5.6%	6.1%	7.4%	9.1%	8.2%	7.1%	7.5%	5.5%	8.2%	11.1%	9.9%	11.4%	8.7%
	>C25-C26	1.4%	4.8%	5.4%	7.0%	8.2%	8.0%	7.1%	7.5%	4.1%	6.8%	8.8%	6.9%	9.3%	7.7%
	>C26-C27	1.8%	5.3%	6.4%	7.9%	8.7%	9.7%	9.2%	9.2%	1.8%	3.1%	4.1%	3.6%	4.3%	3.8%
	>C27-C28	2.6%	6.9%	8.4%	10.6%	11.5%	12.9%	12.3%	12.2%	2.5%	4.2%	5.5%	4.8%	5.7%	5.1%
	>C28-C29	2.2%	4.4%	4.8%	6.5%	5.8%	8.4%	9.7%	9.9%	2.1%	3.6%	4.7%	4.7%	5.2%	4.9%
	>C29-C30	2.3%	4.2%	4.6%	6.4%	5.7%	8.3%	9.5%	9.8%	2.2%	3.6%	4.7%	4.7%	5.2%	4.9%
	>C30-C31	1.4%	2.3%	2.5%	4.2%	3.3%	5.2%	7.0%	6.4%	3.0%	4.7%	5.7%	6.0%	6.3%	6.7%
	>C31-C32	0.9%	1.8%	1.9%	2.6%	2.1%	3.3%	4.4%	4.0%	1.9%	3.0%	3.6%	3.8%	4.0%	4.2%
	>C32-C33	1.1%	1.3%	1.2%	2.8%	2.4%	3.0%	3.8%	2.6%	1.5%	3.0%	3.6%	4.3%	4.0%	5.1%
	>C33-C34	1.2%	1.0%	0.9%	1.5%	1.3%	1.6%	2.0%	1.4%	1.7%	1.9%	2.1%	2.4%	2.2%	2.7%
	>C34-C35	0.8%	0.7%	0.6%	1.7%	1.4%	1.6%	1.9%	1.2%	2.3%	3.3%	3.9%	4.4%	4.4%	6.2%
	>C35-C36	0.9%	0.6%	0.5%	1.0%	0.9%	0.9%	1.1%	0.7%	2.4%	2.3%	2.5%	2.7%	2.7%	3.7%
	>C36-C37	0.6%	0.5%	0.4%	0.8%	2.3%	0.6%	0.6%	0.0%	2.5%	2.2%	2.6%	3.3%	2.9%	3.9%
	>C37-C38	0.6%	0.4%	0.4%	0.6%	1.6%	0.4%	0.4%	0.0%	2.6%	1.9%	2.0%	2.5%	2.1%	2.8%
	>C38-C39	0.2%	0.2%	0.9%	2.1%	0.1%	0.3%	0.2%	0.0%	1.0%	2.0%	1.2%	2.6%	1.5%	2.3%
	>C39-C40	0.2%	0.2%	1.0%	1.9%	0.1%	0.3%	0.2%	0.0%	1.1%	1.9%	1.1%	2.4%	1.3%	2.0%
	Total	50.0%	65.2%	61.5%	84.9%	88.3%	89.8%	89.8%	91.0%	50.0%	74.1%	81.5%	81.6%	86.1%	85.0%
Aromatics	< C10	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C10-C11	0.3%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	0.1%	0.0%	0.0%	0.0%	0.0%
	>C11-C12	0.3%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%	0.3%	0.1%	0.0%	0.0%	0.0%	0.0%
	>C12-C13	0.7%	0.2%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.2%	0.1%	0.0%	0.0%	0.0%	0.0%
	>C13-C14	1.2%	0.4%	0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	0.3%	0.1%	0.1%	0.0%	0.0%	0.0%
	>C14-C15	1.8%	0.6%	0.1%	0.1%	0.1%	0.0%	0.0%	0.0%	0.3%	0.1%	0.1%	0.0%	0.0%	0.0%
	>C15-C16	1.1%	0.3%	0.2%	0.1%	0.0%	0.0%	0.0%	0.0%	0.4%	0.1%	0.1%	0.0%	0.0%	0.0%
	>C16-C17	3.5%	1.1%	1.1%	0.3%	0.1%	0.0%	0.0%	0.0%	0.8%	0.3%	0.1%	0.1%	0.0%	0.0%
	>C17-C18	1.8%	0.6%	0.5%	0.2%	0.1%	0.0%	0.0%	0.0%	0.1%	0.0%	0.0%	0.0%	0.0%	0.0%
	>C18-C19	2.8%	0.8%	0.5%	0.2%	0.1%	0.0%	0.0%	0.0%	0.4%	0.1%	0.1%	0.0%	0.0%	0.0%
	>C19-C20	3.7%	1.2%	0.8%	0.4%	0.2%	0.1%	0.0%	0.1%	0.9%	0.3%	0.2%	0.1%	0.0%	0.0%
	>C20-C21	3.9%	1.6%	1.2%	0.4%	0.3%	0.1%	0.1%	0.2%	0.9%	0.4%	0.2%	0.1%	0.1%	0.0%
	>C21-C22	2.6%	1.2%	1.3%	0.3%	0.3%	0.1%	0.1%	0.1%	1.6%	0.7%	0.4%	0.3%	0.2%	0.1%
	>C22-C23	3.7%	2.0%	2.3%	0.7%	0.6%	0.4%	0.3%	0.3%	2.2%	0.9%	0.6%	0.4%	0.3%	0.2%
	>C23-C24	2.4%	1.6%	1.9%	0.7%	0.7%	0.6%	0.5%	0.5%	3.0%	1.3%	0.9%	0.6%	0.5%	0.3%
	>C24-C25	2.1%	1.6%	1.9%	0.8%	0.9%	0.7%	0.6%	0.7%	5.5%	2.5%	1.8%	1.4%	1.1%	0.8%
	>C25-C26	1.4%	1.5%	1.8%	0.8%	0.8%	0.8%	0.7%	0.7%	4.1%	2.0%	1.4%	1.1%	1.0%	0.7%
	>C26-C27	1.8%	1.6%	2.1%	0.9%	0.9%	1.0%	0.9%	0.9%	1.8%	0.9%	0.7%	0.5%	0.5%	0.4%
	>C27-C28	2.6%	2.5%	3.1%	1.3%	1.3%	1.3%	1.3%	1.2%	2.5%	1.2%	0.9%	0.7%	0.6%	0.5%
	>C28-C29	2.2%	1.8%	2.0%	0.8%	0.7%	0.9%	1.0%	1.0%	2.1%	1.0%	0.8%	0.7%	0.6%	0.5%
	>C29-C30	2.3%	1.9%	2.2%	0.9%	0.7%	0.9%	1.0%	1.0%	2.2%	1.1%	0.8%	0.7%	0.6%	0.5%
	>C30-C31	1.4%	1.3%	1.4%	0.5%	0.4%	0.5%	0.7%	0.6%	3.0%	1.4%	1.0%	0.9%	0.7%	0.7%
	>C31-C32	0.9%	1.2%	1.3%	0.4%	0.3%	0.4%	0.5%	0.5%	1.9%	1.0%	0.7%	0.6%	0.5%	0.5%
	>C32-C33	1.1%	1.5%	1.3%	0.5%	0.4%	0.4%	0.6%	0.4%	1.5%	0.9%	0.7%	0.8%	0.6%	0.7%
	>C33-C34	1.2%	1.7%	1.5%	0.4%	0.3%	0.3%	0.4%	0.2%	1.7%	0.8%	0.6%	0.6%	0.4%	0.5%
	>C34-C35	0.8%	1.5%	1.2%	0.4%	0.3%	0.4%	0.4%	0.3%	2.3%	1.4%	1.2%	1.2%	1.0%	1.4%
	>C35-C36	0.9%	1.6%	1.2%	0.3%	0.3%	0.3%	0.3%	0.2%	2.4%	1.3%	1.0%	1.0%	0.8%	1.1%
	>C36-C37	0.6%	1.1%	0.8%	0.4%	0.9%	0.2%	0.2%	0.0%	2.5%	1.4%	1.3%	1.5%	1.2%	1.6%
	>C37-C38	0.6%	1.1%	0.8%	0.3%	0.8%	0.2%	0.2%	0.0%	2.6%	1.4%	1.2%	1.3%	1.0%	1.4%
	>C38-C39	0.2%	0.6%	3.2%	1.3%	0.1%	0.2%	0.2%	0.0%	1.0%	1.4%	0.8%	1.7%	0.9%	1.4%
	>C39-C40	0.2%	0.5%	2.7%	1.4%	0.1%	0.2%	0.2%	0.0%	1.1%	1.5%	0.9%	1.8%	1.0%	1.6%
	Total	50.0%	34.8%	38.5%	15.1%	11.7%	10.2%	10.2%	9.0%	50.0%	25.9%	18.5%	18.4%	13.9%	15.0%
Fraction organic carbon		1.7%	1.7%	1.7%	1.7%	1.7%	1.7%	1.7%	1.9%	1.9%	1.9%	1.9%	1.9%	1.9%	1.9%
Fraction water	[L/kg$_{dw}$]	80.1%	80.1%	80.1%	80.1%	80.1%	80.1%	80.1%	79.6%	79.6%	79.6%	79.6%	79.6%	79.6%	79.6%
Internal concentration	[mM]	3.8	5.6	8.2	6.8	9.8	15.7	16.4	18.7	7.1	11.9	10.2	11.5	14.7	15.1
Corophium volutator	Survival	93.7%	92.9%	95%	91.25%	90.8%	**86.25%**	*55.00%*	*10.6%*						
Vibrio fischeri	Bioluminescence	100%	**100%**	*74%*	*57.5%*	*66.5%*	*55.5%*	*39%*	*38%*						
Echinocardium cordatum	Survival									93.33%	90%	**76.67%**	*20%*	80%	*0%*
	Reburial									93.33%	90%	**66.67%**	*16.67%*	*63.33%*	*0%*

Dose-response relationships		Actual sediment concentrations							Internal membrane concentrations						
		Bottom	Top	log EC50	log EC10	A	C	B	Bottom	Top	log EC50	log EC10	A	C	B
Corophium volutator	Survival	0 c	92.51	3.935	3.548				0 c	92.98	1.221	1.195			
Vibrio fischeri	Bioluminescence	0 c	100 c	3.553	1.466	79.05	0 c	15480	0 c	100 c	1.159	0.6328	122.9	0 c	15.88
Echinocardium cordatum	Survival	0 c	100 c	3.393	1.958				0 c	100 c	1.117	0.8517			
	Reburial	0 c	100 c	3.175	1.873				0 c	100 c	1.088	0.8393			

PERF soils	designation	AT1	AC1	BT1	BT2	CT1	CC1	DT1	DC1	ET1	EC1	FT1	FC1	IT1	IC1
Actual TPH concentration	[mg/kg$_{dw}$]	11800	25	47000	42000	10900	330	10100	370	4200	46	1210	154	6700	48
Concentration in fractions	[mg/kg$_{dw}$]	11711	0	46101	42073	10901	327.2	10104.3	370	4180.8	34.8	1205	154.7	6687.1	26.9
Aliphatics	< C10	1.2%		0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	2.1%	0.0%	0.0%	0.0%	1.5%	0.0%
	>C10-C11	6.8%		0.2%	0.1%	0.0%	0.0%	0.3%	0.0%	5.7%	0.0%	1.0%	0.0%	5.5%	0.0%
	>C11-C12	6.8%		0.2%	0.1%	0.0%	0.0%	0.3%	0.0%	5.7%	0.0%	1.0%	0.0%	5.5%	0.0%
	>C12-C13	11.3%		0.7%	0.7%	0.3%	0.7%	5.4%	3.1%	10.2%	0.0%	4.4%	0.0%	9.7%	0.0%
	>C13-C14	11.3%		0.7%	0.7%	0.3%	0.7%	5.4%	3.1%	10.2%	0.0%	4.4%	0.0%	9.7%	0.0%
	>C14-C15	11.3%		0.7%	0.7%	0.3%	0.7%	5.4%	3.1%	10.2%	0.0%	4.4%	0.0%	9.7%	0.0%
	>C15-C16	11.3%		0.7%	0.7%	0.3%	0.7%	5.4%	3.1%	10.2%	0.0%	4.4%	0.0%	9.7%	0.0%
	>C16-C17	2.4%		2.2%	2.2%	2.0%	1.3%	0.8%	3.1%	2.8%	4.5%	0.3%	0.7%	3.9%	6.6%
	>C17-C18	2.4%		2.2%	2.2%	2.0%	1.3%	0.8%	3.1%	2.8%	4.5%	0.3%	0.7%	3.9%	6.6%
	>C18-C19	2.4%		2.2%	2.2%	2.0%	1.3%	0.8%	3.1%	2.8%	4.5%	0.3%	0.7%	3.9%	6.6%
	>C19-C20	2.4%		2.2%	2.2%	2.0%	1.3%	0.8%	3.1%	2.8%	4.5%	0.3%	0.7%	3.9%	6.6%
	>C20-C21	2.4%		2.2%	2.2%	2.0%	1.3%	0.8%	3.1%	2.8%	4.5%	0.3%	0.7%	3.9%	6.6%
	>C21-C22	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	>C22-C23	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	>C23-C24	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	>C24-C25	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	>C25-C26	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	>C26-C27	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	>C27-C28	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	>C28-C29	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	>C29-C30	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	>C30-C31	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	>C31-C32	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	>C32-C33	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	>C33-C34	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	>C34-C35	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	>C35-C36	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	>C36-C37	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	>C37-C38	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	>C38-C39	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	>C39-C40	0.1%		1.9%	2.1%	2.2%	1.8%	0.1%	1.7%	0.2%	4.1%	1.7%	2.7%	0.1%	3.5%
	Total fractions	74.5%		51.0%	54.6%	52.6%	42.8%	28.2%	60.5%	72.2%	100.0%	54.4%	55.5%	73.1%	100.0%
	Total TPH	74.6%	48.0%	51.1%	54.8%	52.3%	42.4%	27.7%	59.5%	71.4%	76.1%	54.5%	55.8%	73.1%	56.3%
Aromatics	< C10	0.0%		0.2%	0.2%	0.0%	0.0%	0.0%	0.0%	0.3%	0.0%	0.0%	0.0%	0.1%	0.0%
	>C10-C11	0.9%		0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	2.0%	0.0%	0.0%	0.0%	1.3%	0.0%
	>C11-C12	0.9%		0.1%	0.1%	0.0%	0.0%	0.0%	0.0%	2.0%	0.0%	0.0%	0.0%	1.3%	0.0%
	>C12-C13	3.6%		0.4%	0.3%	0.0%	0.2%	7.2%	0.0%	3.6%	0.0%	0.3%	0.0%	3.6%	0.0%
	>C13-C14	3.6%		0.4%	0.3%	0.0%	0.2%	7.2%	0.0%	3.6%	0.0%	0.3%	0.0%	3.6%	0.0%
	>C14-C15	3.6%		0.4%	0.3%	0.0%	0.2%	7.2%	0.0%	3.6%	0.0%	0.3%	0.0%	3.6%	0.0%
	>C15-C16	3.6%		0.4%	0.3%	0.0%	0.2%	7.2%	0.0%	3.6%	0.0%	0.3%	0.0%	3.6%	0.0%
	>C16-C17	1.4%		1.6%	1.6%	1.0%	1.3%	2.2%	1.2%	1.4%	0.0%	0.2%	0.2%	1.7%	0.0%
	>C17-C18	1.4%		1.6%	1.6%	1.0%	1.3%	2.2%	1.2%	1.4%	0.0%	0.2%	0.2%	1.7%	0.0%
	>C18-C19	1.4%		1.6%	1.6%	1.0%	1.3%	2.2%	1.2%	1.4%	0.0%	0.2%	0.2%	1.7%	0.0%
	>C19-C20	1.4%		1.6%	1.6%	1.0%	1.3%	2.2%	1.2%	1.4%	0.0%	0.2%	0.2%	1.7%	0.0%
	>C20-C21	1.4%		1.6%	1.6%	1.0%	1.3%	2.2%	1.2%	1.4%	0.0%	0.2%	0.2%	1.7%	0.0%
	>C21-C22	0.1%		2.2%	2.0%	2.1%	2.1%	1.8%	1.4%	0.1%	0.0%	0.5%	1.1%	0.1%	0.0%
	>C22-C23	0.1%		2.2%	2.0%	2.1%	2.1%	1.8%	1.4%	0.1%	0.0%	0.5%	1.1%	0.1%	0.0%
	>C23-C24	0.1%		2.2%	2.0%	2.1%	2.1%	1.8%	1.4%	0.1%	0.0%	0.5%	1.1%	0.1%	0.0%
	>C24-C25	0.1%		2.2%	2.0%	2.1%	2.1%	1.8%	1.4%	0.1%	0.0%	0.5%	1.1%	0.1%	0.0%
	>C25-C26	0.1%		2.2%	2.0%	2.1%	2.1%	1.8%	1.4%	0.1%	0.0%	0.5%	1.1%	0.1%	0.0%
	>C26-C27	0.1%		2.2%	2.0%	2.1%	2.1%	1.8%	1.4%	0.1%	0.0%	0.5%	1.1%	0.1%	0.0%
	>C27-C28	0.1%		2.2%	2.0%	2.1%	2.1%	1.8%	1.4%	0.1%	0.0%	0.5%	1.1%	0.1%	0.0%
	>C28-C29	0.1%		2.2%	2.0%	2.1%	2.1%	1.8%	1.4%	0.1%	0.0%	0.5%	1.1%	0.1%	0.0%
	>C29-C30	0.1%		2.2%	2.0%	2.1%	2.1%	1.8%	1.4%	0.1%	0.0%	0.5%	1.1%	0.1%	0.0%
	>C30-C31	0.1%		2.2%	2.0%	2.1%	2.1%	1.8%	1.4%	0.1%	0.0%	0.5%	1.1%	0.1%	0.0%
	>C31-C32	0.1%		2.2%	2.0%	2.1%	2.1%	1.8%	1.4%	0.1%	0.0%	0.5%	1.1%	0.1%	0.0%
	>C32-C33	0.1%		2.2%	2.0%	2.1%	2.1%	1.8%	1.4%	0.1%	0.0%	0.5%	1.1%	0.1%	0.0%
	>C33-C34	0.1%		2.2%	2.0%	2.1%	2.1%	1.8%	1.4%	0.1%	0.0%	0.5%	1.1%	0.1%	0.0%
	>C34-C35	0.1%		2.2%	2.0%	2.1%	2.1%	1.8%	1.4%	0.1%	0.0%	0.5%	1.1%	0.1%	0.0%
	>C35-C36	0.1%		1.7%	1.5%	2.6%	4.3%	1.3%	2.9%	0.0%	0.0%	7.3%	5.7%	0.1%	0.0%
	>C36-C37	0.1%		1.7%	1.5%	2.6%	4.3%	1.3%	2.9%	0.0%	0.0%	7.3%	5.7%	0.1%	0.0%
	>C37-C38	0.1%		1.7%	1.5%	2.6%	4.3%	1.3%	2.9%	0.0%	0.0%	7.3%	5.7%	0.1%	0.0%
	>C38-C39	0.1%		1.7%	1.5%	2.6%	4.3%	1.3%	2.9%	0.0%	0.0%	7.3%	5.7%	0.1%	0.0%
	>C39-C40	0.1%		1.7%	1.5%	2.6%	4.3%	1.3%	2.9%	0.0%	0.0%	7.3%	5.7%	0.1%	0.0%
	Total fractions	25.5%		49.0%	45.4%	47.4%	57.2%	71.8%	39.5%	27.8%	0.0%	45.6%	44.5%	26.9%	0.0%
	Total TPH	25.4%	52.0%	48.9%	45.2%	47.7%	57.6%	72.3%	40.5%	28.6%	23.9%	45.5%	44.2%	26.9%	43.8%
Fraction organic carbon		1.90%	1.37%	11.23%	10.34%	5.20%	1.27%	4.45%	4.12%	0.68%	1.13%	2.10%	1.12%	1.15%	1.22%
Fraction water	[L/kg$_{dw}$]	15.0%	15.0%	17.3%	17.3%	13.5%	13.5%	15.7%	15.7%	8.2%	8.2%	6.9%	6.9%	16.0%	16.0%
Internal concentration	[mM]	160.9	ND	130.5	120.2	72.9	22.7	278.9	7.5	189.8	0.0	19.0	5.3	175.6	0.0
Eisenia fetida	Survival	15%	100%	100%	ND	95%	95%	0%	95%	100%	100%	100%	100%	55%	100%

Dose-response relationships			Internal membrane concentrations					
			Bottom	Top	log EC50	log EC10	A	C	B
Eisenia fetida	Survival		0 c	98.4	2.292	2.078			

PERF soils	designation	JT1	JC1	KT1	KC1	*Daphnia magna*						
Actual TPH concentration	[mg/kg_{dw}]	33000	4500	7800	146	Nomina TPH concentration	[mg/L]	0.089	0.267	0.89	2.67	8.9
Concentration in fractions	[mg/kg_{dw}]	33198	4461.6	7726.3	146.5							
Aliphatics	< C10	0.0%	0.3%	0.0%	0.0%	Aliphatics	< C10	0.7%	0.7%	0.7%	0.7%	0.7%
	>C10-C11	0.1%	1.1%	0.0%	0.0%		>C10-C11	1.0%	1.0%	1.0%	1.0%	1.0%
	>C11-C12	0.1%	1.1%	0.0%	0.0%		>C11-C12	2.3%	2.3%	2.3%	2.3%	2.3%
	>C12-C13	1.1%	3.5%	0.5%	0.0%		>C12-C13	2.3%	2.3%	2.3%	2.3%	2.3%
	>C13-C14	1.1%	3.5%	0.5%	0.0%		>C13-C14	3.0%	3.0%	3.0%	3.0%	3.0%
	>C14-C15	1.1%	3.5%	0.5%	0.0%		>C14-C15	2.7%	2.7%	2.7%	2.7%	2.7%
	>C15-C16	1.1%	3.5%	0.5%	0.0%		>C15-C16	3.1%	3.1%	3.1%	3.1%	3.1%
	>C16-C17	2.5%	3.1%	1.2%	0.3%		>C16-C17	3.3%	3.3%	3.3%	3.3%	3.3%
	>C17-C18	2.5%	3.1%	1.2%	0.3%		>C17-C18	4.5%	4.5%	4.5%	4.5%	4.5%
	>C18-C19	2.5%	3.1%	1.2%	0.3%		>C18-C19	4.9%	4.9%	4.9%	4.9%	4.9%
	>C19-C20	2.5%	3.1%	1.2%	0.3%		>C19-C20	4.7%	4.7%	4.7%	4.7%	4.7%
	>C20-C21	2.5%	3.1%	1.2%	0.3%		>C20-C21	4.4%	4.4%	4.4%	4.4%	4.4%
	>C21-C22	2.1%	1.5%	1.5%	2.3%		>C21-C22	4.0%	4.0%	4.0%	4.0%	4.0%
	>C22-C23	2.1%	1.5%	1.5%	2.3%		>C22-C23	3.2%	3.2%	3.2%	3.2%	3.2%
	>C23-C24	2.1%	1.5%	1.5%	2.3%		>C23-C24	2.6%	2.6%	2.6%	2.6%	2.6%
	>C24-C25	2.1%	1.5%	1.5%	2.3%		>C24-C25	1.8%	1.8%	1.8%	1.8%	1.8%
	>C25-C26	2.1%	1.5%	1.5%	2.3%		>C25-C26	1.3%	1.3%	1.3%	1.3%	1.3%
	>C26-C27	2.1%	1.5%	1.5%	2.3%		>C26-C27	0.9%	0.9%	0.9%	0.9%	0.9%
	>C27-C28	2.1%	1.5%	1.5%	2.3%		>C27-C28	0.7%	0.7%	0.7%	0.7%	0.7%
	>C28-C29	2.1%	1.5%	1.5%	2.3%		>C28-C29	0.4%	0.4%	0.4%	0.4%	0.4%
	>C29-C30	2.1%	1.5%	1.5%	2.3%		>C29-C30	0.3%	0.3%	0.3%	0.3%	0.3%
	>C30-C31	2.1%	1.5%	1.5%	2.3%		>C30-C31	0.2%	0.2%	0.2%	0.2%	0.2%
	>C31-C32	2.1%	1.5%	1.5%	2.3%		>C31-C32	0.1%	0.1%	0.1%	0.1%	0.1%
	>C32-C33	2.1%	1.5%	1.5%	2.3%		>C32-C33	0.0%	0.0%	0.0%	0.0%	0.0%
	>C33-C34	2.1%	1.5%	1.5%	2.3%		>C33-C34	0.0%	0.0%	0.0%	0.0%	0.0%
	>C34-C35	2.1%	1.5%	1.5%	2.3%		>C34-C35	0.0%	0.0%	0.0%	0.0%	0.0%
	>C35-C36	2.1%	1.5%	1.5%	2.3%		>C35-C36	0.0%	0.0%	0.0%	0.0%	0.0%
	>C36-C37	2.1%	1.5%	1.5%	2.3%		>C36-C37	0.0%	0.0%	0.0%	0.0%	0.0%
	>C37-C38	2.1%	1.5%	1.5%	2.3%		>C37-C38	0.0%	0.0%	0.0%	0.0%	0.0%
	>C38-C39	2.1%	1.5%	1.5%	2.3%		>C38-C39	0.0%	0.0%	0.0%	0.0%	0.0%
	>C39-C40	2.1%	1.5%	1.5%	2.3%		>C39-C40	0.0%	0.0%	0.0%	0.0%	0.0%
	Total fractions	56.5%	61.2%	36.4%	46.1%		Total	52.6%	52.6%	52.6%	52.6%	52.6%
	Total TPH	57.6%	62.2%	37.2%	45.9%							
Aromatics	< C10	0.0%	0.0%	0.0%	0.0%	Aromatics	< C10	0.0%	0.0%	0.0%	0.0%	0.0%
	>C10-C11	0.0%	0.0%	0.0%	0.0%		>C10-C11	0.0%	0.0%	0.0%	0.0%	0.0%
	>C11-C12	0.0%	0.0%	0.0%	0.0%		>C11-C12	2.2%	2.2%	2.2%	2.2%	2.2%
	>C12-C13	0.3%	0.8%	0.0%	0.0%		>C12-C13	2.8%	2.8%	2.8%	2.8%	2.8%
	>C13-C14	0.3%	0.8%	0.0%	0.0%		>C13-C14	3.2%	3.2%	3.2%	3.2%	3.2%
	>C14-C15	0.3%	0.8%	0.0%	0.0%		>C14-C15	2.9%	2.9%	2.9%	2.9%	2.9%
	>C15-C16	0.3%	0.8%	0.0%	0.0%		>C15-C16	4.0%	4.0%	4.0%	4.0%	4.0%
	>C16-C17	1.8%	2.1%	0.8%	0.0%		>C16-C17	3.6%	3.6%	3.6%	3.6%	3.6%
	>C17-C18	1.8%	2.1%	0.8%	0.0%		>C17-C18	3.9%	3.9%	3.9%	3.9%	3.9%
	>C18-C19	1.8%	2.1%	0.8%	0.0%		>C18-C19	4.6%	4.6%	4.6%	4.6%	4.6%
	>C19-C20	1.8%	2.1%	0.8%	0.0%		>C19-C20	4.4%	4.4%	4.4%	4.4%	4.4%
	>C20-C21	1.8%	2.1%	0.8%	0.0%		>C20-C21	4.1%	4.1%	4.1%	4.1%	4.1%
	>C21-C22	1.9%	1.4%	2.7%	0.8%		>C21-C22	3.3%	3.3%	3.3%	3.3%	3.3%
	>C22-C23	1.9%	1.4%	2.7%	0.8%		>C22-C23	2.5%	2.5%	2.5%	2.5%	2.5%
	>C23-C24	1.9%	1.4%	2.7%	0.8%		>C23-C24	1.9%	1.9%	1.9%	1.9%	1.9%
	>C24-C25	1.9%	1.4%	2.7%	0.8%		>C24-C25	1.2%	1.2%	1.2%	1.2%	1.2%
	>C25-C26	1.9%	1.4%	2.7%	0.8%		>C25-C26	0.9%	0.9%	0.9%	0.9%	0.9%
	>C26-C27	1.9%	1.4%	2.7%	0.8%		>C26-C27	0.6%	0.6%	0.6%	0.6%	0.6%
	>C27-C28	1.9%	1.4%	2.7%	0.8%		>C27-C28	0.5%	0.5%	0.5%	0.5%	0.5%
	>C28-C29	1.9%	1.4%	2.7%	0.8%		>C28-C29	0.3%	0.3%	0.3%	0.3%	0.3%
	>C29-C30	1.9%	1.4%	2.7%	0.8%		>C29-C30	0.2%	0.2%	0.2%	0.2%	0.2%
	>C30-C31	1.9%	1.4%	2.7%	0.8%		>C30-C31	0.1%	0.1%	0.1%	0.1%	0.1%
	>C31-C32	1.9%	1.4%	2.7%	0.8%		>C31-C32	0.1%	0.1%	0.1%	0.1%	0.1%
	>C32-C33	1.9%	1.4%	2.7%	0.8%		>C32-C33	0.0%	0.0%	0.0%	0.0%	0.0%
	>C33-C34	1.9%	1.4%	2.7%	0.8%		>C33-C34	0.0%	0.0%	0.0%	0.0%	0.0%
	>C34-C35	1.9%	1.4%	2.7%	0.8%		>C34-C35	0.0%	0.0%	0.0%	0.0%	0.0%
	>C35-C36	1.4%	1.2%	4.4%	8.6%		>C35-C36	0.0%	0.0%	0.0%	0.0%	0.0%
	>C36-C37	1.4%	1.2%	4.4%	8.6%		>C36-C37	0.0%	0.0%	0.0%	0.0%	0.0%
	>C37-C38	1.4%	1.2%	4.4%	8.6%		>C37-C38	0.0%	0.0%	0.0%	0.0%	0.0%
	>C38-C39	1.4%	1.2%	4.4%	8.6%		>C38-C39	0.0%	0.0%	0.0%	0.0%	0.0%
	>C39-C40	1.4%	1.2%	4.4%	8.6%		>C39-C40	0.0%	0.0%	0.0%	0.0%	0.0%
	Total fractions	43.5%	38.8%	63.6%	53.9%		Total	47.4%	47.4%	47.4%	47.4%	47.4%
	Total TPH	42.4%	37.8%	62.8%	54.1%							
Fraction organic carbon		0.98%	3.56%	3.67%	2.96%							
Fraction water	[L/kg_{dw}]	10.8%	10.8%	18.8%	18.8%							
Internal concentration	[mM]	83.3	140.3	1.3	87.3			11.0	23.5	51.9	95.9	152.5
Eisenia fetida	Survival	90%	90%	10%	10%							
Daphnia magna	Survival							100%	100%	68%	30%	0%

Dose-response relationships								
			Bottom	Top	log EC50	log EC10	A	C	B
Eisenia fetida	Survival								
Daphnia magna	Survival		0 c	100 c	1.835	1.541			