Framework Contract No ECHA/2008/2
Reference No ECHA/2008/02/SR30

Service Request on a critical review of the environmental and physicochemical methodologies commonly employed in the environmental risk assessment of petroleum substances in the context of REACH registrations

Emiel Rorije, Eric M.J. Verbruggen & Joop A. de Knecht

National Institute for Public Health and the Environment
Ministry of Health, Welfare and Sport

DHI

Contact:
E.Rorije
Expertise Centre for Substances (SEC)
National Institute for Public Health and the Environment (RIVM)
P.O.Box 1, 3720 BA Bilthoven
The Netherlands
Emiel.Rorije@rivm.nl

Date: 05 August 2012

Version: 4

Internal Review by:
Theo Vermeire
Dick Sijm
Theo Traas
ZhiChao Dang

This review is performed in order of the European Chemical Agency (ECHA)

Summary

This project aimed to provide information on the scientific validity and the general applicability of the Petrotox and Hydrocarbon Block Method tools as developed by CONCAWE for predicting the environmental hazards, exposure and subsequently the environmental risks associated with petroleum hydrocarbons in the context of REACH registrations. The tools are intended to be applicable to any (petroleum) hydrocarbon substance. RIVM was asked by the European Chemicals Agency to review the validity and implementation of the underlying theories and the validity and quality of the QSAR models used to estimate effect and exposure properties of all representative hydrocarbon structures (the so-called CONCAWE library) used to establish reference hydrocarbon blocks.

CONCAWE Library of model substances and QSARs

In general it can be stated that almost all the obvious classes of hydrocarbon structures, present in hydrocarbon products, are also present in the library of substances used by CONCAWE for the read across of toxicological and physico-chemical properties to the hydrocarbon blocks. One concern is the absence of O(xygen) and N(itrogen) containing petroleum substances in the library of model substances. A substantial part of the substance library (5.9%) are S(ulfur) containing substances, but nitrogen and oxygen containing petroleum components are absent. This implies that for petroleum product containing O/N containing petroleum substances the CONCAWE models are not suitable.

To generate the physico-chemical properties of the library substances for which no experimental data is available, both the PetroTox and HBM tool use several QSAR models. Unfortunately, these two tools apply different QSAR models in generating these properties. Although the performance of the QSAR models applied both in PetroTox and the HBM tool seems to be sufficient, when these QSAR predictions are compared to available experimental data, the different models do show large differences for the estimations of the same physico-chemical properties for hydrocarbon substances. The use of the same QSAR model for estimating a property in the hazard as well as the environmental fate assessment would be logical, desirable and advisable. As octanol-water partitioning (K_{ow}) is the basis for the toxicity QSARs (Target Lipid Model), the use of the SPARC QSAR model for predicting K_{ow} seems to be required and defensible in the PetroTox model. There is no such requirement for the use of the KOWWIN model for estimating K_{ow} in the HBM tool. A comparison between log K_{ow} predictions from SPARC and KOWWIN reveals large differences between these models, adding to the uncertainty in the risk characterization. The same can be said for other physico-chemical properties. In conclusion, we realize that there is no absolute best model, nevertheless we propose to use one model for all relevant physico-chemical properties to avoid inconsistencies.

In estimating the fate of hydrocarbon products in the environment, the HBM tool applies the BIOHCWIN model, developed to estimate the half lives in surface water. This model seems to underestimate the degradation half lives of short chain alkanes and branched alkanes, although, for for other compounds the model seems to be sufficiently conservative. The factors used to extrapolate half lives to soil and sediment are not well founded and based on a proposed ratio from a study of Boethling et al. (1995) in which only a few petroleum compounds are present. Using a conservative approach, the ratios proposed in the REACH guidance to extrapolate half lives from water to soil and sediment, based on the ready biodegradability test, are recommended. Most critical is the equation used to estimate the half lives in an STP based on the estimated half lives in surface water with BIOHCWIN. More experimental data are needed for several classes of petroleum components to have a broader coverage of the petroleum

compounds and to gain more confidence in the validity of the calculation method. At present there is a possibility that the fraction degraded in an STP will be overestimated and consequently the environmental concentrations predicted may be too low.

Based on our analysis of the BCF model values used in the HBM tool it can be concluded that by using (the outdated) BCFWin v2.16 estimates for the BCF in the HBM tool bioconcentration will be underestimated. We recommend using a more up to date estimate (e.g BCFBAF v3.00 model estimates, or following the REACH guidance recommendations for calculation of BCF based on K_{ow}).

Target Lipid Model

Based on the available data the assumptions and formulas used by PETROTOX to predict toxicity with LL50 and toxic units (TU) are considered scientifically valid for use in the classification of petroleum mixtures.

A number of weaknesses were identified in the target lipid model with respect to

a) the assumption of the normal distribution of the log CTLBB and log ACR,
b) the assumption of independence of parameters, which was not met for the combination of CTLBB and the universal slope for narcosis, and
c) the numerical values used for the ACR, including the use of chronic values instead of NOECs. Consequently, the PNEC estimation is considered too optimistic by a factor of 3 to 7 in general, which warrants the adjustment of the model and/or the application of an additional assessment factor.

Overall conclusion

In view of the shortcomings identified with respect to the assumptions made in the HBM and Petrotox we recommend improving both models. In the current stage we believe there is a serious possibility that the tool PETRORISK, which integrates both models, will lead to an underestimation of the (environmental) risk related to the production and use of petroleum products.

Abbreviations

ACR	Acute-to-Chronic Ratio
AF	Assessment Factor
BABs	Branched Alkyl Benzenes
BAF	BioAccumulation Factor
BATs	Branched Alkyl Tetralins
BINs	Branched Indanes and Indenes
BCF	BioConcentration Factor
BMF	BioMagnification Factor
BP	Boiling Point
BTEX	Benzene, Toluene, Ethylbenzene, and Xylene
ChV	Chronic Value, geometric mean value of the NOEC and LOEC.
CONCAWE	CONservation of Clean Air and Water in Europe, the oil companies' European association for environment, health and safety in refining and distribution.
CTLBB	Critical Target Lipid Body Burden
CTPHT	Coal Tar Pitch High Temperature
C&L	Classification and Labelling
DBT	DiBenzoThiophene
ECHA	European CHemicals Agency
EC10	Effect Concentration causing 10% effect
EC50	Effect Concentration causing 50% effect
ELS	Early Life Stage
EP	Equilibrium Partitioning
EUSES	European Union System for the Evaluation of Substances
EU RAR	European Union Risk Assessment Report
FAV	Final Acute Value
FCV	Final Chronic Value
GC/MS	Gas Chromatography / Mass Spectrometry
GCxGC	two-dimensional Gas Chromatography
GIT	GastroIntestinal Tract
GMAV	Genus Mean Acute Value
HBM	Hydrocarbon Block Method
HC5	Hazardous Concentration affecting 5% of the species
HLC	Henry's Law Constant (Air-Water partition coefficient)
HOM	Humic Organic Matter
HPC	Hydrocarbon Petroleum Compound
IR	Infra Red
(log) Kbc	(10-base logarithm of the) Black Carbon / water partition coefficient
(log) Kmw	(10-base logarithm of the) Membrane (or Micelle) / Water partition coeff.
(log) Koa	(10-base logarithm of the) Octanol / Air partition coefficient
(log) Koc	(10-base logarithm of the) Organic Carbon / water partition coefficient
(log) Kow	(10-base logarithm of the) Octanol / Water partition coefficient
LABs	Long-chain Alkyl Benzenes
LC50	Lethal Concetration causing 50% lethality
LL50	Lethal Loading 50%
LOEC	Lowest Observed Effect Concentration
MATC	Maximum Acceptable Toxicant Concentration
MP	Melting Point

NMR	Nuclear Magnetic Resonance
NOEC	No-Observed Effect Concentration
NO3	Nitrate
OC	Organic Carbon
OH	Hydroxyl
PAH	PolyAromatic Hydrocarbons
PBT	Persistent, Bioaccumulative and Toxic
PEC	Predicted Environmental Concentration
PNEC	Predicted No-Effect Concentration
QSAR	Quantitative Structure-Activity Relationship
QSPR	Quantitative Structure-Property Relationship
RCR	Risk Coefficient Ratio (PEC/PNEC ratio)
REACH	Registration, Evaluation, Authorization and restriction of CHemicals
RIVM	Dutch National Institute for Public Health and the Environment
s.e.	standard error
SSD	Species-Sensitivity Distribution
STP	Sewage Treatment Plant
TGD	Technical Guidance Document
TLM	Target Lipid Model
TU	Toxic Units
USEPA	United States Environmental Protection Agency
UV	Ultra Violet
VP	Vapour Pressure
WAF	Water Accomodated Fraction
WS	Water Solubility

PetroTOX / PetroRISK classes of hydrocarbons

AlS	sulphur bearing aliphatics compounds
ArS	sulfur bearing aromatic structures
DiAr	diaromatic structures
di-N	di-naphthenic structures
i-N	other naphtenics
i-olefins	branched olefinic structures
i-P	linear alkanes: iso-parrafins
MoAr	parent and substituted Mono Aromatics
NDiAr	naphthenic diaromatic structures
NmAr	naphthenic mono Aromatic structures
n-CC5	parent and substituted cyclopentanes
n-CC6	parent and substituted cyclohexanes
n-olefins	linear olefinic structures
n-P	linear alkanes: n-paraffins
PolyAr	polyaromatic structures
PolyN	poly naphthenics

QSAR Models and Software

AOP	or AOPWIN™: QSAR model to estimates the gas-phase reaction rate for the reaction between the most prevalent atmospheric oxidant, hydroxyl radicals, and a chemical. Gas-phase ozone radical reaction rates are also estimated for olefins and acetylenes. Part of USEPA EPISuite
BCFBAF	The BCFBAF Program is an update and expansion of the previous BCFWIN Program. It has been expanded to include estimation of the Biotransformation Rate (kM) in fish and estimation of Bioaccumulation Factor (BAF) by the Arnot-Gobas method.
BCFWin	QSAR model to calculate the bioconcentration factor from log Kow. Part of USEPA EPISuite
BIOHCWIN	QSAR models to estimate biodegradation half-life for compounds containing only carbon and hydrogen (i.e. hydrocarbons). Part of USEPA EPISuite.
ClogP	QSAR model to calculate log P, which is equal to log Kow
EPISuite	The EPI (Estimation Programs Interface) Suite™ is a Windows® based suite of physical/chemical property and environmental fate estimation models developed by the EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC).
ETX	A Program to Calculate Hazardous Concentrations and Fraction Affected, Based on Normally Distributed Toxicity Data. RIVM Report no. 601501028.
HenryWIN	QSAR model to Calculate the Henry's Law constant (air/water partition coefficient) using two different methods (group and/or bond contribution). Part of USEPA EPISuite.
KOWWIN	QSAR model for log Kow calculation. Part of USEPA EPISuite
MPBPVP	or MPBPWIN™: QSAR models for melting point, boiling point, and vapor pressure of organic chemicals. Part of USEPA EPISuite.
SPARC	Sparc Performs Automated Reasoning in Chemistry - a predictive modeling system, which calculates a large number of physical/ chemical parameters from molecular structure and basic environmental information (media, temperature, pressure, pH, etc.).
SimpleTreat	Fate model to predict the distribution and elimination of chemicals by sewage treatment plants. RIVM report no. 719101025/1996
STPWin	Fate model which predicts the percent of a compound that will be removed from wastewater. Part of USEPA EPISuite.
WSKOW	or WSKOWWIN™: QSAR model to estimate an octanol-water partition coefficient using the algorithms in the KOWWIN™ program and estimates a chemical's water solubility from this value. Part of USEPA EPISuite.
WATERNT	QSAR model to estimate water solubility directly using a "fragment constant" method similar to that used in the KOWWIN™ model. Part of USEPA EPISuite.

Contents

RIVM PetroRisk data analysis.xls

1 Introduction

1.1 Objective

Commissioned by the European Chemicals Agency a critical review has been performed on the environmental and physicochemical methodologies employed in the PetroTox and HBM tools developed by CONCAWE to perform hazard and environmental risk assessment for petroleum substances in the context of REACH registrations.

The main objective of the project is to provide information on the scientific validity and the general applicability of the models currently available for predicting the environmental hazards, environmental exposure and subsequently the environmental risks (including risk to humans indirectly exposed via the environment) associated with petroleum hydrocarbons in the context of REACH registrations. Towards this end the hydrocarbon block method approach as implemented by CONCAWE in their PetroTOX and HBM [Van de Meent, 2008] tool and any QSPR/QSAR models used therein are to be investigated.

1.2 Method

This project was subdivided into three work packages. Based on the available data and internal knowledge on the fate and behaviour of petroleum substances we have assessed the scientific validity of the calculated physicochemical and environmental fate parameters within the models PETROTOX and HBM, in work package 1.

In the second work package, the available data were used to determine the scientific validity of the assumptions and formulas used in PETROTOX to predict toxicity, with LL50, toxic units (TU) and HC5 being of particular interest in this respect. The available ecotoxicity data and SAR evidence were used to determine the possible mode(s) of action of petroleum hydrocarbons in aquatic and terrestrial species.

Based on the scientific validation, the PETROTOX and HBM tools were evaluated for their efficacy in the prediction of hazards, exposures and risks for petroleum hydrocarbons and related substances. The final aim of this evaluation was to determine whether the quality of the data underlying the models and the accuracy (in terms of uncertainty of the predictions compared to uncertainty in the experimental values) in the predictions of these models is sufficient to predict environmental hazard, environmental exposure and environmental risk for a given hydrocarbon petroleum substance. This was dealt with in the third work package. Based on the short-comings detected, recommendations are given on how these models can be adapted to enhance their acceptance.

2 Materials and Methods

2.1 Work Package 1: CONCAWE library and Fate QSARs

2.1.1 Objectives

To obtain information on the scientific validity of the CONCAWE library of models for assessing the environmental risks of petroleum hydrocarbon components and any related QSPR/QSAR calculated properties used by the PETROTOX and HBM models. The overall aim of this work package is to determine the amount of the high quality data present in the CONCAWE library and hence provide detail on the accuracy and quality consistency within the hydrocarbon block as used by the PETROTOX and HBM models to predict hazard, exposure and risk for a given petroleum substance.

2.1.2 Approach

The CONCAWE library consists of a large number of unique hydrocarbon structures and their associated physicochemical properties. We have evaluated whether this library sufficiently covers the range and variety of true petroleum hydrocarbons components so that accurate fate and hazard profiles are obtained. For this purpose, we have investigated which sources were used to build the library and how well these sources reflect the composition of products that are available on the market. This information has been obtained by evaluating compositional information from different products, crude oil and/or field samples from a variety of sources.

For each library component a number of physicochemical properties are provided as calculated by SPARC (in PetroTOX) or experimental data are used supplied with EpiSuite QSAR model estimates (in the HBM tool). SPARC is a suite of thermodynamically based QSAR models for physico-chemical properties of organic substances, developed by the University of Georgia through grants from USEPA. EpiSuite is a suite of more classical QSAR models for physico-chemical properties of organic substances. EpiSuite models mostly use substructure fragments as descriptors, and were developed by SRC Inc. in close cooperation with and through grants from the USEPA.

In order to evaluate the applicability of SPARC and EpiSuite QSAR models to calculate the melting point, boiling point, vapour pressure and water solubility of petroleum hydrocarbon substances properties, experimental data have been compiled by RIVM and summarized in the report. The experimental data have been obtained from earlier project on environmental risk limits for total petroleum hydrocarbons [Verbruggen et al., 2008] and the European risk assessment on coal tar pitch high temperature [EU, 2008]. In the tender vapour pressure was explicitly mentioned as one of the (four) physico-chemical parameters to be evaluated. However, both in PetroTox as well as in the HBM tool vapour pressure is not used, and no data (experimental or estimated) for vapour pressure is present in the substance libraries provided with these two tools. Instead the Henry's Law Constant (water-air partitioning coefficient) has been evaluated, as this parameter is used both in PetroTOX and the HBM tool to estimate the partitioning over the aqueous and atmospheric/head-space compartments.

The accuracy of the calculated properties within the CONCAWE library has been determined by comparison of the available experimental data with those calculated by SPARC. In addition, a comparison is made with other QSAR programs, to assess the uncertainty and any structural deviations of the data obtained with SPARC.

In order to assess the validity of calculated environmental fate parameters within PETROTOX and HBM such as log K_{ow}, log K_{aw}, log K_{oa}, log K_{mw}, BCF values in fish and biodegradation half lives, the methods used have been evaluated based on the underlying data and by comparison with experimental data. In addition, other available QSPR/QSAR models were compiled and compared to those used in PETROTOX and HBM. Special attention was paid to the assumption made for unbranched and branched aliphatic and alkylated aromatic hydrocarbons with respect to their degradability and metabolism in fish. For substances with a log $K_{ow} > 5$, which are not metabolized, uptake from environmental sources other than water (e.g food and sediment) might contribute to the bioaccumulation in higher organism. This assumption has been critically evaluated. It has also been assessed whether the K_{oa} should be included in the estimation biomagnification potential in air-breathing species.

The outcome of the analyses is presented in section 3.1. The details of all the data used in the analyses are presented in Annex I.

2.2 Work Package 2: Target Lipid Model

2.2.1 Objectives

To obtain information on the validity of the Target Lipid Model and its applicability to petroleum hydrocarbon components.

2.2.2 Approach

In order to determine the scientific validity of the terms, assumption and formulas in PETROTOX to predict ecotoxicity, LL50, toxic units (TU) and HC5, in section 3.2 the underlying data are analysed and compared with the available data obtained from in-house projects and the literature review.

The core of the target lipid model, on which PETROTOX is based, are QSARs for acute toxicity. One of the crucial elements in PETROTOX is the application of an estimated acute-to-chronic ratio (ACR) for a broad range of petroleum hydrocarbons. This method was critically reviewed in respect to the underlying data used to derive the ACR, taking into consideration structural differences and differences in mode of action as well as differences between species. The justification in terms of uncertainty towards all the different petroleum hydrocarbons was further assessed by comparison with ACRs found in additional data sources for hydrocarbon petroleum compounds and related substances.

It can be assumed that ecotoxicity of a large number of hydrocarbon petroleum components is mainly caused by narcosis or baseline toxicity. On the other hand phototoxicity is also observed for different petroleum mixtures, which might be due to the presence of PAHs which are known to exert an enhanced toxicity due to this mode of action. It was also investigated whether the toxic unit concept, as the basis for the derivation of the ecotoxicity of a mixture of Hydrocarbon Petroleum Compounds (HPCs), it is also applicable to components which act via another mode of action, like PAHs, for which the highest toxicity is based on phototoxicity

It will further be determined to which extent possible acute and chronic toxic effects of hydrocarbons on sediment and terrestrial organisms are accounted for in the PETROTOX model.

2.3 Work Package 3: Evaluation of Uncertainties

2.3.1 Objectives

To investigate some specific uncertainties related to the methodologies applied using the hydrocarbon Block Method approach to environmental hazard, environmental exposure and environmental risk assessment of petroleum hydrocarbon substances.

2.3.2 Approach

Based on the results obtained in work package 1 and 2 on the representativeness of the CONCAWE library of model compounds for different petroleum streams, the accuracy of the calculated fate and behaviour properties of the HPCs and the derivation of PNECs, in section 3.3 it will be investigated to which extent the use of HBM and PETROTOX could under- or overestimate the environmental risk of release of HPCs in the environment. Based on the shortcomings detected, recommendations are given on how these models can be adapted to minimize uncertainties in the risk assessment outcome.

3 Results

3.1 WP1. CONCAWE library and Fate QSARs

3.1.1 Composition of petroleum products

All petroleum products are derived from crude oil whose major constituents are hydrocarbons. These hydrocarbons can be saturated, unsaturated, linear, branched, cyclic, polycyclic, and can be heterogeneous with major hetero-atoms: oxygen, nitrogen and sulphur. Petroleum contains many thousands of different compounds that vary in molecular weight from 16 (methane) to more than 2000. This broad range in molecular weights results in boiling points that range from –160 ºC to temperatures in excess of 1000°C (see Table 1). Crude oil is rarely used in its raw form but must instead be processed into its various products, generally as a means of forming products with a hydrogen content different from that of the original feedstock.

As shown in Figure 2, each of these refineries has its own range of preferred petroleum feedstock from which a desired distribution of products is obtained. In general, refinery processes can be divided into three major types:

1. Separation: division of petroleum into various streams (or fractions) depending on the nature of the crude material.
2. Conversion: production of salable materials from petroleum, usually by skeletal alteration, or even by alteration of the chemical type, of the petroleum constituents.
3. Finishing: purification of various product streams by a variety of processes that essentially remove impurities from the product; for convenience, processes that accomplish molecular alteration, such as reforming, are also included in this category.

Table 1. General summary of the carbon number and boiling point of PHC product types (from Speight, 2002)

Product	Lower carbon limit	Upper carbon limit	Lower boiling point (°C)	Upper boiling point (°C)
Refinery gas	C1	C4	-161	-1
Liquefied petroleum gas	C3	C4	-42	-1
Naphtha	C5	C17	36	302
Gasoline	C4	C12	-1	216
Kerosine/diesel fuel	C8	C18	126	258
Aviation turbine fuel	C8	C16	126	287
Fuel oil	C12	>C20	216	421
Lubricating oil	>C20		>343	
Wax	C17	>C20	302	>343
Asphalt	>C20		>343	
Coke	>C50		>1000	

The separation and finishing processes may involve distillation or even treatment with a wash solution, either to remove impurities or, in the case of distillation, to produce a material boiling over a more narrow range, and the chemistry of these processes is quite simple. Conversion processes are, in essence, processes that change the number of carbon atoms per molecule, alter the molecular hydrogen-to-carbon ratio, or change the molecular structure of the material without affecting the number of carbon atoms per molecule ( ).These latter processes (isomerization processes) essentially change the shape of the molecule(s) and are used to improve the quality of the product (Speight, 2002 and references cited therein). Table 1 gives an overview of the products types and boiling points ranges.

Petroleum components can be separated into four fractions, the saturated, aromatic, resin and asphaltene fractions, by absorption chromatography. Each of these fractions contains a large number of compounds, examples are given in Figure 1 [Speight, 1999]. Saturates are hydrocarbons containing no double bonds. They are further classified according to their chemical structures into alkanes (paraffins) and cycloalkanes (naphthenes). Alkanes have either a branched or unbranched (normal) carbon chain(s), and have the general formula C_nH_{2n+2}.

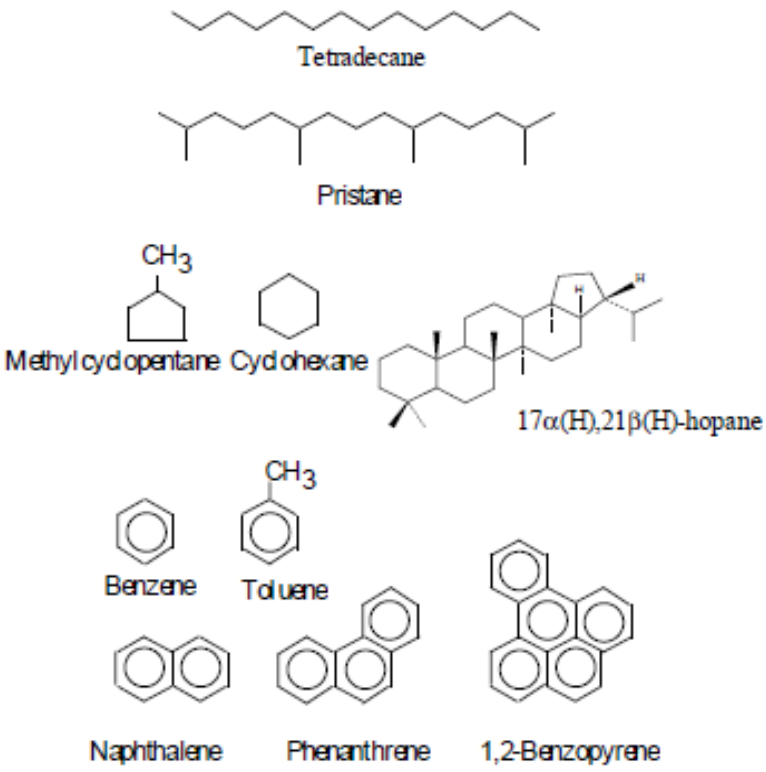

Figure 1. Representative Hydrocarbons
Tetradecane (an *n*-alkane), pristane (a branched alkane), and methylcyclopentane, cyclohexane and 17α[H],21ß[H]-hopane (cycloalkane compounds) are present in the saturated fraction of crude oil. The other compounds shown in this figure are present in the aromatic fraction.

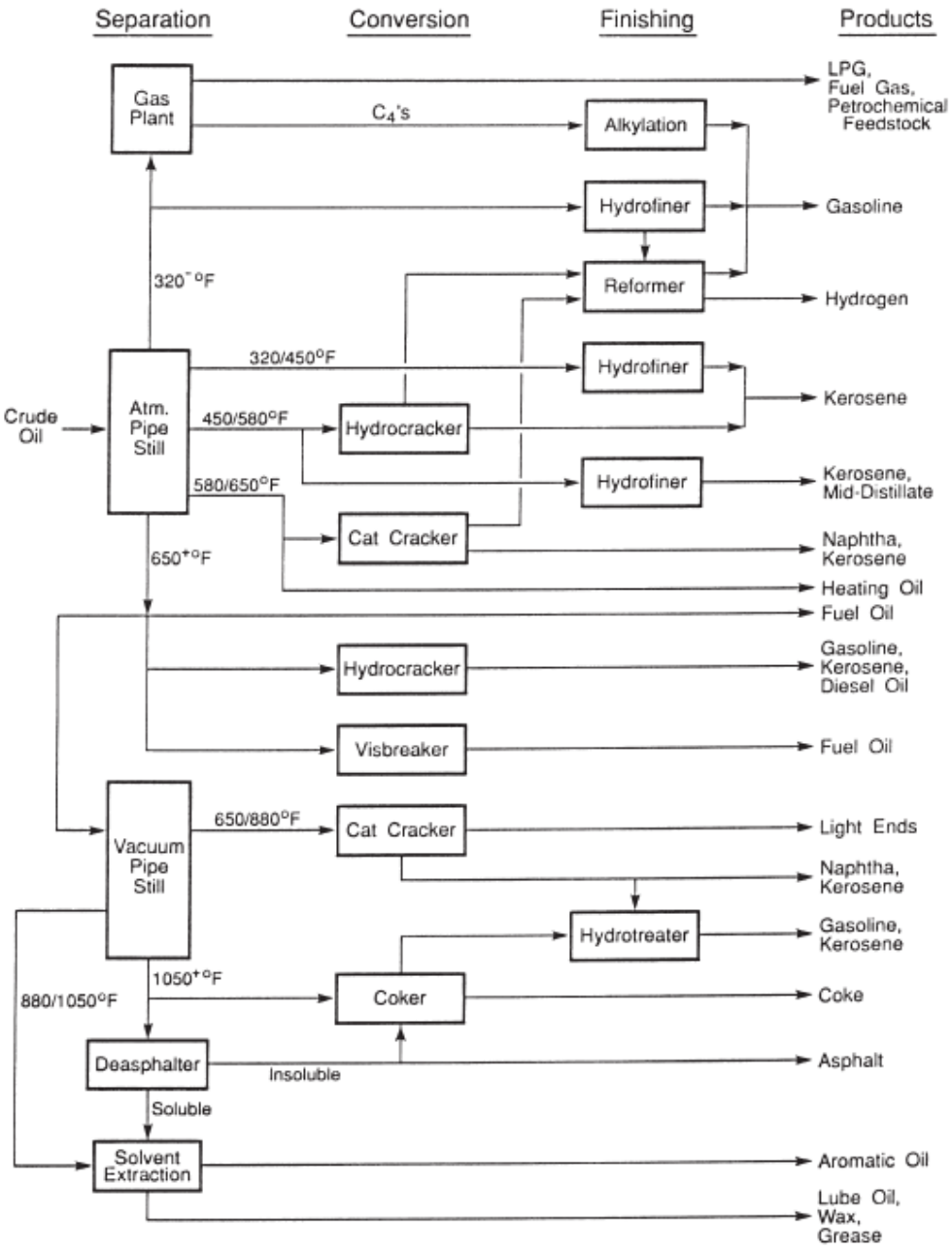

Figure 2. Schematic of a petroleum refinery (from Speight, 1999)

Alkanes

In crude oil and several refined petroleum products the n-alkanes and branched alkanes form the major components (see Table 2). Crude oil can contain n-alkanes from C1 to C40 in size, with the most abundant n-alkanes typically being n-pentane to n-dodecane. Numerous branched-chain

alkanes up to C10 have been identified in crude oil. Branched C6- to C8-alkanes commonly are found in the greatest abundance in this structural group [Jensen, 1994]. This group also includes the isoprenoids, which are isoalkanes with a single methyl branch on every fourth carbon atom. The isoprenoids found in the greatest amounts in crude oil are pristane (C19) and phytane (C20).

Alkenes

Although alkenes are not commonly found in crude oil, they are more common in refined petroleum products.

Cycloalkanes

Cycloalkanes have one or more rings of carbon atoms (mainly cyclopentanes and cyclohexanes), and have the general formula C_nH_{2n}. The majority of cycloalkanes in crude oil and refined petroleum products have an alkyl substituent(s). The cycloalkanes are present in high concentrations in crude oil and several refined petroleum products (see Table 2), but they also are the least well characterized because of analytical difficulties. They can be either single, fused, or linked by a single carbon–carbon bond; contain aromatic rings; and/or have alkyl substituents, either branched or linear. Cyclopentane and cyclohexane derivatives tend to be the main cycloalkanes in petroleum products. Mono- and dicycloalkanes compose 50 to 55% of the cycloalkanes with more than 10 carbon atoms; heavier members of this group typically are found with one long alkyl chain (linear or slightly branched) and several methyl or ethyl groups [Potter & Simmons, 1998; Howard et al., 2005 and references therein].

Aromatics

Aromatics have one or more aromatic rings with or without an alkyl substituent(s). Benzene is the simplest one, but alkyl-substituted aromatics generally exceed the non-substituted types in crude oil [Potter & Simmons, 1998]. Alkylated benzenes found at the highest concentration in petroleum are those with one or two methyl or ethyl groups, although other mono- and dialkylbenzenes with chains from 12 to 40 carbon atoms in length have been identified [Potter & Simmons, 1998; Howard et al., 2005 and references therein].

Resins and asphaltenes

In contrast to the saturated and aromatic fractions, both the resin and asphaltene fractions contain non-hydrocarbon polar compounds. Their elements contain, in addition to carbon and hydrogen, trace amounts of nitrogen, sulfur and/or oxygen. Typically, of the S-containing heterocyclics, dibenzothiophene and its alkylated derivatives are found in the greatest abundance in crude oil [American Petroleum Institute, 1994].

Asphaltenes consist of high-molecular weight compounds which are not soluble in a solvent such as n-heptane, while resins are n-heptane-soluble polar molecules. Resins contain heterocyclic compounds, acids and sulfoxides.

Analysis of an unresolved complex mixture from crude oil and petroleum products in the environment (biota and abiotic media) can identify several branched alkylbenzenes (BABs), tetralins (BATs), and indanes and indenes (BINs), cyclic and aromatic sulfoxide compounds and benzothiophenes. In addition, residues of acyclic isoprenoids such as farnesane, norpristane, pristane, and phytane are identified. The presence of these compounds is mostly likely due to their persistence against biodegradation [Rowland et al., 2001; Booth et al., 2007; Frenzel et al, 2009, 2010; Melbye et al., 2009].

Table 2. Overview of the carbon number, composition and end use of several petroleum fuel mixtures [from Potter & Simmons, 1998]

Petroleum fuel mixtures	Alkane carbon nr. range	Compound classes	End use
gasoline	n-C4 – C12	High conc. BTEX, monoaromatics and branched alkanes Lower conc. n-alkanes, cycloalkanes and naphthalenes Very low conc. PAHs	Automotive spark-ignition engine
kerosene	n-C7 – C21	High conc. cycloalkanes and n-alkanes Lower conc. monoaromatics and branched alkanes Very low conc. BTEX and PAHs	Critical kerosene burners
JP-4, fuel	n-C5 – C18	High conc. cycloalkanes, n-alkanes, branched alkanes, Low conc. n-alkanes, BTEX and monoaromatics Very low conc. PAHs	Aviation turbine engines
Diesel	n-C8 – C21	High conc. n-alkanes and cycloalkanes Lower conc. branched alkanes, monoaromatics , naphthalenes and PAHs Very low conc. of BTEXs	High speed engines Domestic burners Medium capacity commercial industrial burners
No 6 fuel oil	n-C12 – C34	High conc. n-alkanes and cycloalkanes Lower conc. naphthalenes and PAHs, Very low conc. BTEXs	Commercial burners Industrial burners
Lubricating and motor oils	n-C18 – C34	High conc. branched alkanes and cycloalkanes Very low conc. BTEXs and PAHs	Internal combustion engines
Crude oil	n-C1 – C34	High conc. n-alkanes , branched alkanes and cycloalkanes Lower conc. BTEXs, PAHs and naphthalenes Variable conc. of sulfur heterocyclics	

Analysis of heavy fraction in crude oil

The components of petroleum in crude oil are analyzed mainly by using gas chromatography in combination with mass spectrometry (GC/MS). Consequently, the chemical structures of the higher molecular-weight components (the heavy fractions) that cannot be identified by GC are mostly unknown. Furthermore, the compositions of many branched alkanes and alkyl cyclo-alkanes have not been determined because their isomers are numerous and cannot be resolved by GC [Killops and Al-Juboori, 1990; Gough and Rowland, 1990]. Therefore, a multitude of analytical techniques such as flame ionization detection, IR- and UV-absorption spectrometry, NMR and elemental analysis in combination with appropriate separation techniques such as

various chromatographic methods and/or chemical conversion is necessary to characterize petroleum, and especially its heavy fractions.
In the CONCAWE library these fraction are not included and consequently these fraction are not taken into account in the risk assessment. We however assumed that this fraction is of less relevance for the overall risk of petroleum compounds.

3.1.2 Contents of the CONCAWE library

The CONCAWE library which is used to select representative substances for each hydrocarbon block both in the PETROTOX model as well as the Hydrocarbon Block Method by van de Meent [2008] consists of 1512 single hydrocarbon structures in the case of PETROTOX v3.05, and 1518 single hydrocarbon structures in the case of the HBM model (version 15-07-2008). From personal communication with CONCAWE it was understood that the library has been built using hypothetical structures in an attempt to fill the foreseen maximum number of Hydrocarbon Blocks as well as possible within a practical approach. The matrix reflects the characterization which is currently possible using GCxGC analysis. This task was outsourced to Syracuse Inc. (Ph.Howard) and subsequently the library does not necessarily reflect a specific hydrocarbon product such as crude oil.

In Table 3 the matrix of Hydrocarbon Blocks which were the basis of the library composition is given, with the final number of representative structures in each hydrocarbon block indicated. The abbreviations used in Table 3 are explained in Table 4.

Table 3. Matrix of Hydrocarbon Blocks in the High Resolution mode of PetroRISK, with the associated number of substances in the CONCAWE library that would fall into a specific hydrocarbon block. Abbreviations of classes explained in Table 4.

C#	n-P	i-P	n-CC5	n-CC6	i-N	Di-N	n-Olefins	i-Olefins	Poly-N	AlS	MoAr	NMAr	DiAr	NDiAr	PolyAr	ArS	Total
C3-5	2	2	2	NA	NA	NA	2	2	NA	NA	NA	NA	NA	NA	NA	NA	10
C6-8	3	6	11	6	1	3	3	6	NA	1	6	NA	NA	NA	NA	2	48
C9-11	3	26	11	23	7	8	6	14	NA	4	25	7	3	NA	NA	4	141
C12-14	3	45	10	28	6	38	6	19	1	6	36	30	37	7	5	22	299
C15-17	3	33	5	19	6	19	2	8	4	4	32	28	34	22	33	20	272
C18-20	3	11	3	3	6	6	0	0	10	2	6	31	24	31	75	13	224
C21-23	3	6	3	3	6	4	0	0	11	2	6	27	8	20	93	9	201
C24-26	3	5	3	3	6	6	0	0	12	1	6	21	6	14	39	0	125
C27-29	3	7	3	3	6	5	0	0	15	0	6	17	6	12	28	0	111
C30-32	3	4	3	1	5	5	0	0	10	0	5	13	2	4	11	0	66
C33+	2	0	4	0	0	5	0	0	3	0	0	0	1	0	0	0	15
Total	31	145	58	89	49	99	19	49	66	20	128	174	121	110	284	70	1512

Although there are several blocks in the matrix which end up with no representative structure, this was seen (by CONCAWE) as not problematic as these were blocks that are in general not determining the toxicity of hydrocarbon petroleum compounds. Structures denoted with NA in Table 3 do not exist.

The HBM model has an additional 6 substances present in the library when compared to the library used in the PetroTOX model as reviewed in this study. This difference is discussed further under the next heading. Apart from these additional six compounds, the substances in the CONCAWE library used by both methods are identical.

Furthermore, it is observed that the ID numbers used by Petrotox and the HBM model are identical up to nr 685, but from then on the library constituents are numbered differently. This might be confusing when comparing the choice of representative structures for specific hydrocarbon blocks

between Petrotox v3.05 and HBM model (v.15-07-2008). In the library used in the newly developed PetroRISK tool (which combines the PetroTOX and HBM excel worksheet tools into one, an altogether new identification number is given to the library of substances, but reference to the ID-numbers used in both Petrotox and the HBM model is given.

When discussing the CONCAWE library in the present report, we refer to the larger 1518 compound containing version, as used in the HBM tool [van de Meent, 2008], dated 15-07-2008, unless it is explicitly otherwise indicated.

The library is divided in functional chemical classes for the purpose of defining Hydrocarbon Blocks. Two divisions are applied, a coarse separation (called LowRes) and a fine separation into classes (called HighRes).

The first division used in both PETROTOX and HBM, used to define hydrocarbon blocks in the Low Resolution mode, distinguishes between aliphatic and aromatic substances, and subsequently defines the hydrocarbon blocks based on boiling point fractions. The library contains 544 saturated aliphatic substances and 158 unsaturated aliphatic substances (olefins), together 702 aliphatic substances, roughly half of the library. The aromatic substances in the library make up a slightly larger part, with 816 of the 1518 substances having at least one aromatic ring in their structure.

It should be noted that when a substance is defined as "aromatic" this does not imply that the chemical structure is mainly aromatic. A substance like 1-ethyl 4-2,6,10,14 tetramethylhexadecyl-indane, Figure 3, is characterized as "aromatic", but the number of aromatic carbon atoms is only 6, on a total of 31 carbon atoms. Its chemical behaviour is more likely to reflect aliphatic substances than aromatic substances. There is a large fraction of aromatics in the CONCAWE library with large aliphatic substituents.

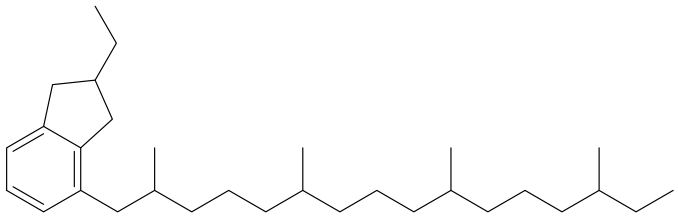

Figure 3. Structure of 1-ethyl 4-2,6,10,14-tetramethylhexadecyl-indane

The division used for the High Resolution mode uses sixteen different chemical classes, and subsequently uses the number of carbon atoms (instead of the boiling point fractions used in the LowRes mode) to define the hydrocarbon blocks. These sixteen classes and the number of substances present in the CONCAWE library in each class are given in Table 4.

From this table it follows that polyaromatic structures, and the naphthenic mono-aromatic structures are highly represented (in numbers) whereas relatively few of the olefinic (both linear and branched) structures are present in the CONCAWE library.

The division in subclasses as used in the HBM is less straight forward, as no numbered classes are indicated in the CONCAWE library incorporated in the HBM, and the class names are not equal to the names used in the PETROTOX model. For instance, the HBM library has a class named "alkane, mono-cyclo", with 197 representatives in the library. This HBM class is a merger of PETROTOX classes n-CC5 (58 substances), n-CC6 (89), i-N (59) and one substance from the class naphthenics, mono aromatic.

Table 4. Composition of the CONCAWE library

Class:	Petrotox description:	#	%
n-P	linear alkanes: n-paraffins	31	2.1
i-P	linear alkanes: iso-parrafins	145	9.6
n-CC5	parent and substituted cyclopentanes	58	3.8
n-CC6	parent and substituted cyclohexanes	89	5.9
i-N	other naphtenics	49	3.2
di-N	di-naphthenic structures	99	6.5
n-olefins	linear olefinic structures	19	1.3
i-olefins	branched olefinic structures	49	3.2
PolyN	poly naphthenics	66	4.4
AIS	sulphur bearing aliphatics compounds	20	1.3
MoAr	parent and substituted Mono Aromatics	128	8.5
NmAr	naphthenic mono Aromatic structures	174	11.5
DiAr	diaromatic structures	121	8.0
NDiAr	naphthenic diaromatic structures	110	7.3
PolyAr	polyaromatic structures	284	18.8
ArS	sulfur bearing aromatic structures	70	4.6
		1512	100%

Differences between the libraries used in PetroTOX and HBM [Van de Meent, 2008]

The 6 substances which are added in the HBM model (15-07-2008) which are not present in the CONCAWE library used in PETROTOX v3.05 [PetroTox, 2006] are:

adamantane	2-methyl-adamantane	2-ethyl-adamantane
diadamantane	1-methyldiadamantane	1,3,5-Trimethyladamantane.

These are all adamantane based structures. Adamantane is shown in Figure 4.

In the 1512 structure PETROTOX library no other tricyclo-aliphatic compounds are present. The structurally most similar reference substances in the library used by CONCAWE are the mono-cyclohexane- and bicyclohexane-like structures (decalines). Decaline is shown in Figure 5.

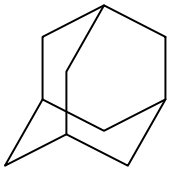

Figure 4. Structure of Adamantane

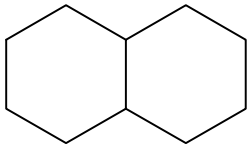

Figure 5. Structure of Decaline

Petroleum is the only natural source of adamantane; its content varies between 0.0001 and 0.03% depending on the oil field and is too low for commercial production [Bagriy, 1989]. In terms of representativity one could argue that 6 adamantane structures on a total of 1518 substances in the library (i.e. 0.4%) is a factor of 13 (0.4/0.03) too much, when natural oils should be represented.

However, for a product in which adamantanes have been enriched, the representativity could also turn out to be too low. This example shows in general the problem with defining a representative library of structures, when it will be applied to an unforeseen diversity of products, which are not even all based on or derived from the same crude (oil) product.

Analysis of the validity of the CONCAWE library

In general, comparing the composition of petroleum products and the composition of the CONCAWE library it can be stated that all obvious classes of hydrocarbon structures, which might be present in hydrocarbon petroleum products, are also present in the library, with the exception of Nitrogen and Oxygen petroleum components. Several hydrocarbon blocks that are present in the High Resolution mode in the PetroTox and HBM tools are empty, but this does not necessarily indicate a lack of representative substances in the library. For example olefins with a carbon number above 18 are not encountered in oil products.

It is however impossible to state in general whether the number of structures in the library is over- or under-representing fractions in oil or more in general in hydrocarbon products, because fractions can vary indefinitely for (commercial) products, and the (size of) the hydrocarbon blocks is up to the user to define. Given the fact that any representative structure present in the CONCAWE library is given an equal weight (within each hydrocarbon block that is defined by the user) this can give rise to a large deviation in representativity from the real composition of the mixture which is being assessed.

From the sensitivity analysis of the HBM performed by Van de Meent [2008] for CONCAWE, it appears that a Hydrocarbon Block definition using the Low Resolution (distinction between aliphatic and aromatic only) and using 7 different boiling point fractions (total: 14 different hydrocarbon blocks) gave the lowest uncertainty in a MonteCarlo analysis based on the uncertainty of the different input parameters.

The reason that the LowRes mode gave a lower uncertainty than the HighRes mode is probably due to the fact that increasing the resolution, by introducing the 16 different chemical classes, gave rise to very sparsely populated or even empty hydrocarbon blocks. This indicates that the CONCAWE library of 1518 substances is apparently not large enough to allow for such a subdivision into 16 separate chemical classes. When a hydrocarbon block, as defined by the user, is found to have no representative structures in the CONCAWE library, the PetroTOX and HBM methods “borrow” the properties of the next neighbouring hydrocarbon block that does have representative structures in the library. It is concluded that, when this situation is encountered in the risk assessment of a specific product, it should be seen as an indication that PetroTOX and HBM tools might not be appropriate tools to estimate the risk for that specific product.

Since the whole exercise is based on QSAR generated values for the physicochemical properties used in deriving the PEC and PNEC, theoretically the library can be filled with hypothetical chemical hydrocarbon structures until the required number is reached to have a good representation in each of the 16 structural classes. However, in doing so one has to wonder how “representative” such theoretical structures are of the real properties of a hydrocarbon product, given the fact that each structure is given equal weight (within its hydrocarbon block) in determining the PEC and PNEC of that specific hydrocarbon block.

An alternative solution which might improve representativity, is not using average properties for a hydrocarbon block, where the average is determined (in part) by the (assumed equal) weight (representativity) assigned to *each* structure in the CONCAWE library. Instead it could be considered to assign/determine Quantitative Structure-Activity Relationships following the trend in each specific chemical class, where the properties which are now taken as *averages* of the

representative structures in each hydrocarbon block, are calculated as dependent on either the Boiling Point (LowRes mode) or Carbon Number (HighRes mode). This approach has been followed by Verbruggen et al. [2004, 2008]. It brings the added benefit that the estimate for a hydrocarbon block is not dependent on whether there are actual components present in the library for representing a certain hydrocarbon block. Therefore no "empty" hydrocarbon blocks can occur and "borrowing" properties from a nearest neighbour block is not necessary. However, in such an approach it is even more important to limit the size of the blocks, because average properties are used, and extremes within a block are disregarded.

In general both the PetroTox and the HBM tools reviewed here allow the user to freely choose the way he wants to divide his product/mixture into hydrocarbon blocks, i.e. the boiling point intervals in the case of the LowRes method, and the intervals based on the number of carbon atoms can be chosen very small or very broad, as the user seems fit. This could lead to the incorrect assumption that the HBM will always give the same quality of results. However, a higher resolution, also in the boiling point ranges or the number of carbon atoms ranges, will lead to a better characterization of the model, which will in turn lead to a more accurate risk assessment, until the method starts to generate "empty" hydrocarbon blocks, indicating the resolution becomes too high in comparison to the size of the library.

In the very recent PetroRISK implementation [available through personal communication with CONCAWE] of PetroTox and the HBM tool, the user can only choose between two different resolutions; LowRes using the distinction between Aliphatics and Aromatics, and with prescribed Boiling Point ranges of 50°C, and HiRes, distinguishing the 16 chemical classes and with a prescribed carbon number range of 3 (each block only spanning 3 carbon numbers, i.e. C6-C8, C9-C11 etc.). This seems a reasonable limitation, forcing the user to work with sensible hydrocarbon blocks. However, for the aliphatics a shift in three carbon atoms comprises a log Kow range of ~1.5 unit, which is too large to consider this as a homogeneous block for the lower aliphatic substances.

3.1.3 Physicochemical properties

Physico-chemical properties for the various hydrocarbon blocks (high or low resolution) used within PETROTOX and HBM are derived from the CONCAWE library of chemical properties for individual representative structures which are derived from basic structure types typically found in petroleum. The chemical properties include sub-cooled liquid solubility, Henry's Law Constant (HLC), log Kow, molecular volume, boiling point, chemical class and molecular weight. The parameters were estimated from SPARC v4.2 (May 2008), an on-line program that computes chemical properties from chemical structure [Carreira et al. 1994].

SPARC and EPISuite QSAR models

Most models that predict a given physicochemical property (e.g., solubility, boiling point, etc.) are based, in a very direct way, on experimental data for that property for a limited training set of chemicals. Model development involves finding the best correlations between various descriptors of chemical structure and the observed property values. These descriptors are subsequently used to construct a model that adequately "recalculates" the training (or calibration) data set. Then, to validate, one must demonstrate that the empirical model also accurately predicts property values for chemicals not included in the training set, but whose experimental values are known. These data are often called the validation set. In order to predict a new physicochemical property (e.g., octanol/water partition coefficient), the entire process must be repeated, requiring new training and validation data sets for each new property. This applies to all the models of the US EPA EpiSuite as applied in the HBM tool [Van de Meent, 2008).

On the other hand, with SPARC, experimental data for physicochemical properties (such as boiling point) are not used to develop (or directly impact) the model that calculates that particular property. Instead, physicochemical properties are predicted using a few models that quantify the underlying phenomena that drive all types of chemical behavior (e.g., resonance, electrostatic, induction, dispersion, H-bonding interactions, etc.). These mechanistic models were parameterized using a very limited set of experimental data, but not data for the end-use properties that will subsequently be predicted. After verification, the mechanistic models were implemented in various software modules that calculate properties (such as boiling point). It is critical to recognize that the same mechanistic model (e.g., H-bonding model) will appear in all the software modules that predict the various end-use properties (e.g., boiling point) for which that phenomenon is important. Thus, any comparison of SPARC-calculated physicochemical properties to an adequate experimental data set is a true model validation test as there is no training (or calibration) data set in the traditional sense for that particular property.

Validation of the models

For an indication of the performance of the SPARC models on different physicochemical endpoints reference is being made to Hilal et al. [2003]. In general the validation data presented here are extremely good. There is no reason to think that performance of the SPARC models specifically for the CONCAWE library of hydrocarbon structures would be worse (or better) than the averages presented in this validation report. For model performance and validation results of the EPISuite models for physico-chemical properties, fate and degradation reference is made to the manuals and help-files that are provided with the EPISuite set of models (available online from EPA).

The comparison of the SPARC models to the data generated using the EpiSuite models shows, for the different physicochemical endpoints, that both are capable of reproducing the experimental

data very well, but despite similar performance on substances for which experimental data is available, large differences within the CONCAWE library are observed between the two estimation methods. Without data it is difficult to say that one method is correct and the other is incorrect. However, if the SPARC models have not been trained to reproduce the experimental data in the PhysProp database (as the EpiSuite models definitely have been optimized to do) than one would have more confidence in the SPARC predictions over the EpiSuite models for those substances that do not have experimental data.

However, the publication on solubility and log Kow estimation modules within SPARC [Hilal et al., 2004] clearly shows that some optimization of statistical parameters for SPARC-calculated Solubility, Activity and Distribution coefficients has been performed, using training and testing datasets. Therefore it is difficult to give a clear recommendation on which physico-chemical QSAR models (from SPARC or EpiSuite) would be preferable.

In the following, the validity of the calculated environmental fate parameters as used within PETROTOX and HBM is assessed. The formulas used and any inherent assumptions made in their derivation were critically evaluated based on the underlying data and by comparison with experimental data. Also other available QSPR/QSAR models are compiled and compared to those used in PETROTOX and HBM.

Melting Point, MP

Melting Point is used in the CONCAWE Hydrocarbon Block Model but it is not used in PetroTOX. Its use in the HBM is to determine the state of physical state of a substance (i.e. solid, liquid). As such, it does not have a large influence on the calculation of the Fate Factors, and the subsequent PECs.

The values used in the HBM are the "Experimental database" values from the EPISuite, i.e. the experimental values as gathered in the PhysProp database [PhysProp], when available. For the substances which have no experimental value in the PhysProp database the estimate from the MPBPVP model is used v.1.43 (2008), from US EPA/Syracuse, as provided in the EPISuite.

A comparison of the values used in the HBM CONCAWE library versus experimental values is therefore not useful, as all experimental values have been used in the library. In general the quality of the estimate of the melting point is described in the EPISuite user manuals, and its recommendation (at the end of the quotation) is that the estimates can be used for screening purposes *at best*. However, its use to determine the physical state seems to be acceptable.

The following qualification and Figure 6, indicating the performance of the MPBPWin model in predicting Melting Point, are taken from the EPISuite user manual, available online. It should be noted that this applies to organic substances in general, not only hydrocarbon substances:

"The ability to Predict Liquid versus Solid: The PHYSPROP Database contains 3246 compounds designated as liquids or having melting point less than 25°C. In addition, it contains 8225 compounds with melting points greater than 25°C. For these datasets, MPBPWIN predicts the following (assuming compounds with MP of 25°C or less are liquids & compounds with 25°C or greater MP are solids):

> *For 8225 solid compounds - MPBPWIN predicts 93% of the compounds will be solids*
> *For 3246 liquid compounds - MPBPWIN predicts 70% of the compounds will be liquids*
> *For all 11471 compounds - MPBPWIN correctly predicts the physical state of 86% of all compounds.*

Estimated melting points from MPBPWIN can only be recommended for screening purposes (at best)."

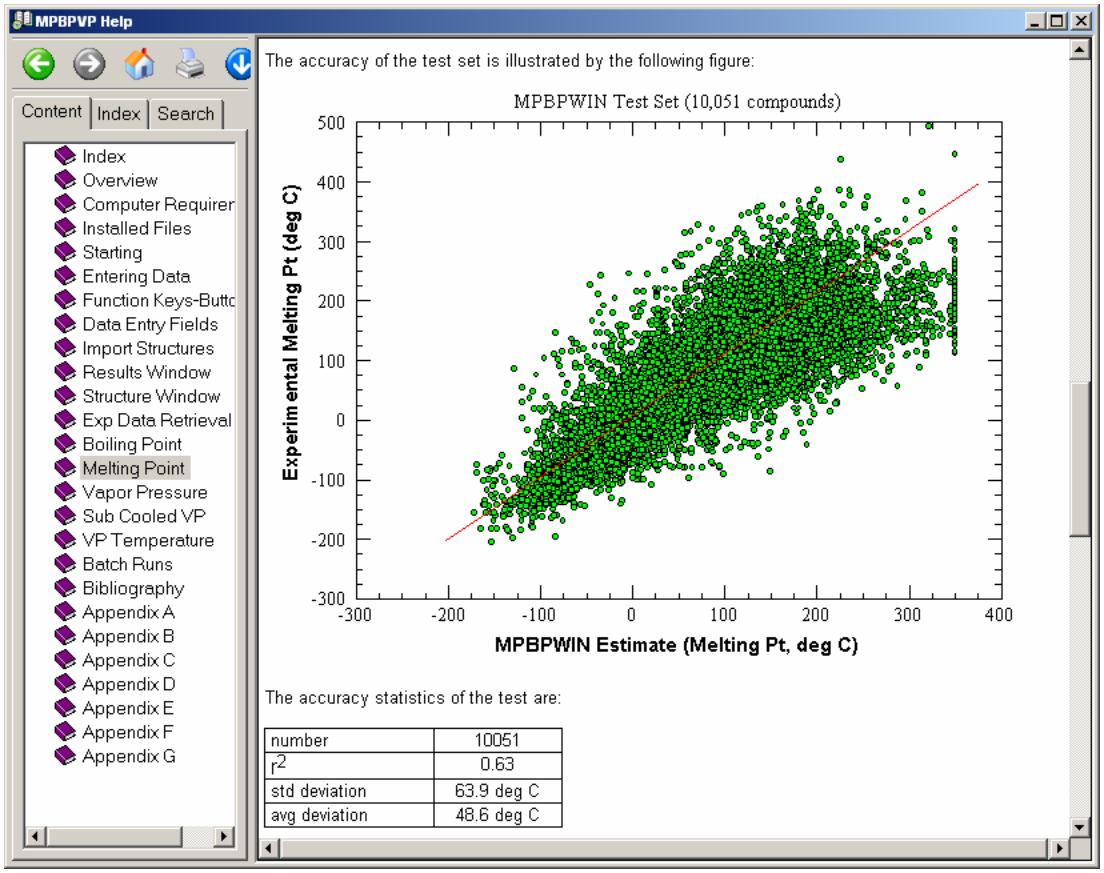

Figure 6. **Accuracy of the Melting Point model from EPISuite for a 10051 substance test set (taken from the MPBPVP Help file).**

The evaluation of the quality of the Melting Point data underlying the QSAR models is performed by comparing the PhysProp database reported melting point values to the melting point data assembled by Verbruggen et al. [2008] for 262 single hydrocarbon substances. These values have been plotted against each other in Figure 7. It should be concluded that the sources for most substances are the same.

It is observed that for allmost all single hydrocarbon structures for which evaluated experimental values are given by Verbruggen [2008], the data comply with the referenced values in the PhysProp database. For only one substance, 1-methylnaphthalene, the reported values for Melting Point are so different that it would also affect the aggregation state of a substance at room temperature (-30.4°C, Verbruggen, 2008 and 34°C, PhysProp database)

The differences observed for the other substance will have very limited or no impact on the evaluation of the subsatances/hydrocarbon blocks in both PetroTOX and the HBM tool.

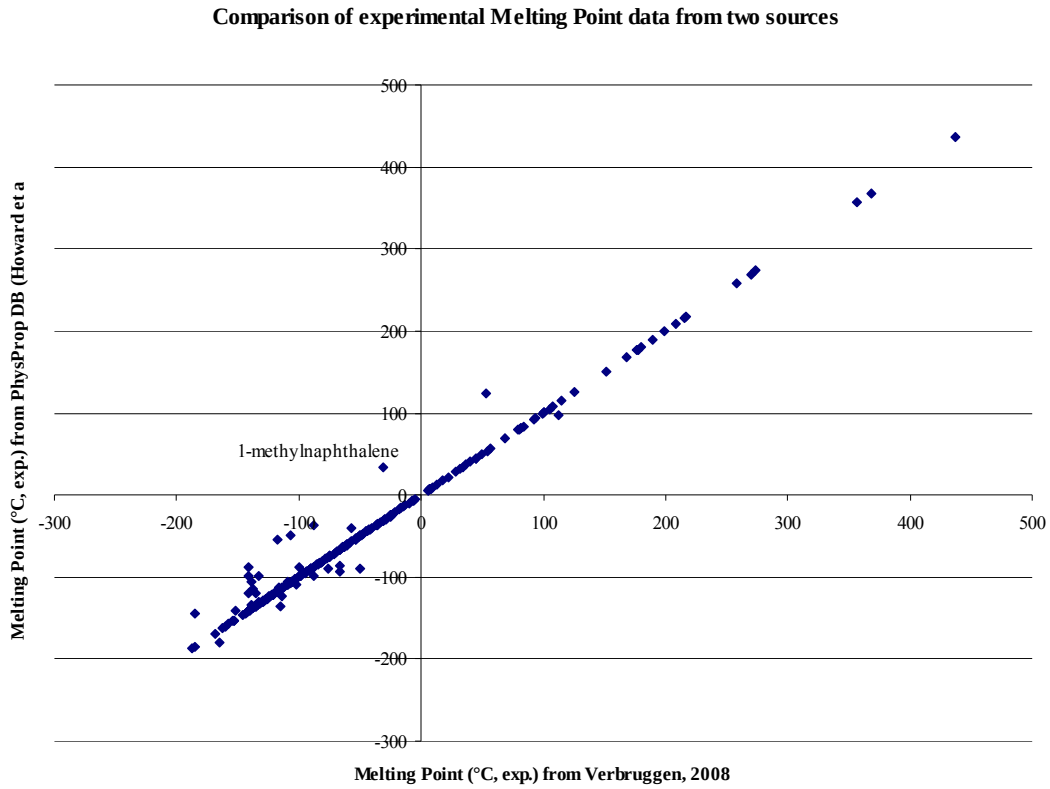

Figure 7. **Comparison of experimental Melting Point data compiled by two different sources, for 262 Hydrocarbon structures. Individual data is presented in Annex I**

Boiling Point, BP

Boiling point is used both in PetroTOX and CONCAWE HBM. However, the values used in PetroTOX are the SPARC v4.2 calculated values, and the source of the values used in the HBM tool are not identified in the different accompanying documents. It is assumed that the boiling points used in the HBM have been estimated with a newer version of SPARC, but not the most recent version. This is deduced from a small attempt to reproduce the SPARC estimates as used by the PETROTOX model:

Example calculations for the substance BENZENE:

CONCAWE library in HBM excel sheets	BP = 77.96 °C
SPARC 4.2 (CONCAWE library in Petrotox)	BP = 79.05 °C
SPARC 4.5 (current version, online)	BP = 77.72 °C
MPBPVP v1.43 calc'd	BP = 102.24 °C
experimental Database (PhysProp)	BP = 80.00 °C

The assumption that the boiling points used in the HBM tool have been generated with a newer version of SPARC is also in line with the updated CONCAWE library (six extra compounds) applied in the HBM tool. It is not in line with the calculations for other physico-chemical

properties, which are only used in the HBM model and not in Petrotox. Most physico-chemical estimates come from the EPISuite models / USEPA, not from the also available SPARC models.

The accuracy of the different estimates when compared to the experimental values is not very different, as graphically visible in Figure 8. The correlation coefficient R^2 of the regression line plotted in the graph for the SPARC v4.2 model is slightly better than for the data used in the HBM tool (SPARC v4.x?), but this is statistically not a relevant difference. The concordance of the predictions from the USEPA model for boiling point MPBPVP v1.43 with the experimental data is slightly less than for the SPARC models (both versions), but still very well reproducing the experimental data available for the hydrocarbons present in the CONCAWE library. It should be noted that the experimental values to which the boiling points are compared come from the PhysProp database from Syracuse inc. [PhysProp DB], i.e. these are the data on which the MPBPVP model, and very likely also the SPARC models, were fitted (data given in Annex I).

However, when comparing the QSAR estimated boiling point values for the whole CONCAWE library, there is much more deviance between the models than would be expected based on the comparison of the different SPARC versions versus the experimental data. It turns out that the values used in the HBM model sometimes markedly differ from the values used to establish the Hydrocarbon blocks in PetroTOX.

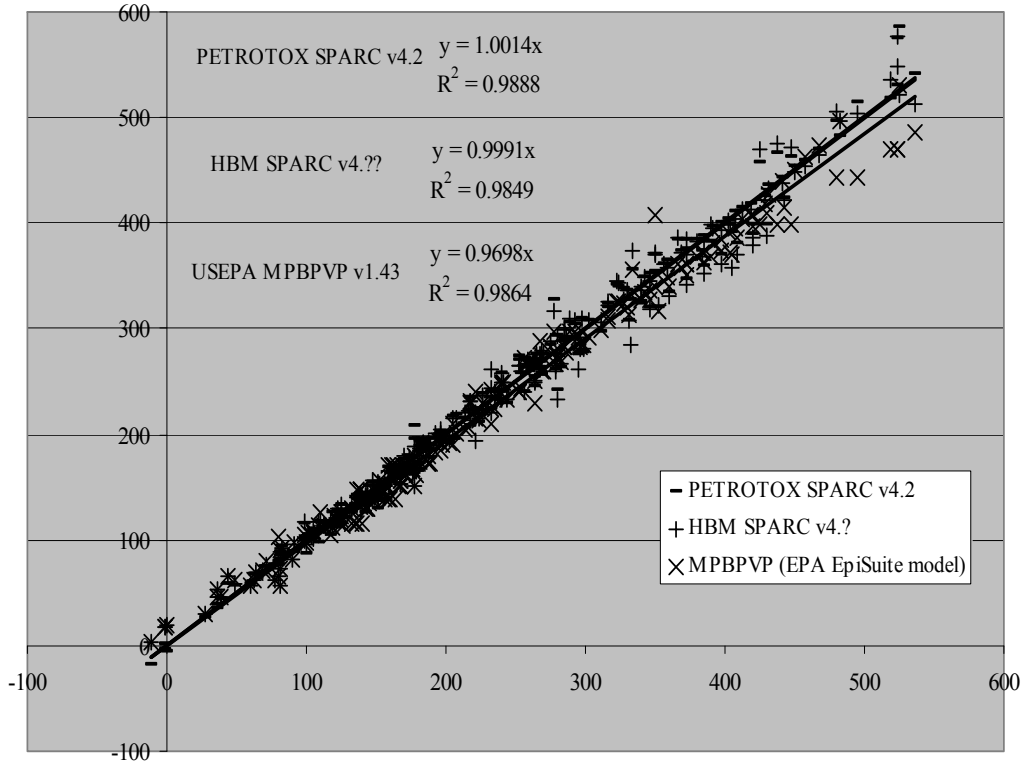

Figure 8. **Comparison of three Boiling Point QSAR estimates on the subset of the CONCAWE library for which experimental boiling point data is available. Individual data is presented in Annex I.**

Several (groups of) substances can be identified in Figure 9 where the difference between the calculated boiling points from different methods is > 100 degrees. It depends very much on the definition and resolution of the hydrocarbon blocks whether this will have significant influence on the outcome of the hydrocarbon block method. The HBM LowRes3 example in the vdMeent analysis uses BP intervals of 50 degrees Celsius to define 7 different blocks (both aliphatic and aromatic). In the graph a 50°C interval around the 1:1 line is also plotted. At least the substances that are out of this boiling point interval will probably be assigned to a different hydrocarbon block. The fraction of the substances outside the interval compared to the total number of substances should give some insight of the number of substances that would end up in a different hydrocarbon block, when using the LowRes3 scheme as used in the HBM analysis from Van de Meent [2008]. There are in total 197 substances outside this 50°C range. That amounts to ~13% which certainly will end up in a different block, but even substances with a much smaller difference can end up in a different block, if they are close to the extreme values of the blocks. It seems very well possible that this change of 13% of the substances will have a significant influence on the average PEC and PNEC calculated for the different blocks. This also very much depends on the number of representative structures in a specific hydrocarbon block. In situations where a single hydrocarbon block is represented by only few substances (as is the case for some of

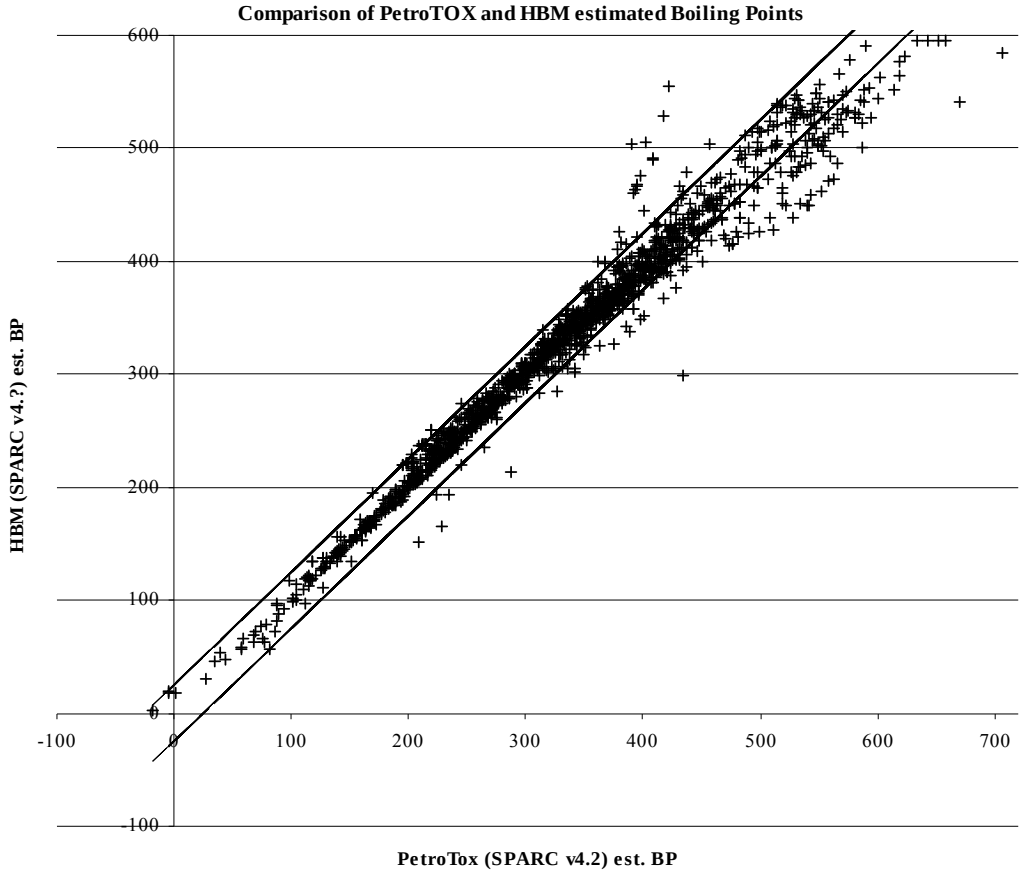

Figure 9. **Comparison of the Boiling Point predictions from two different QSAR models, for the whole CONCAWE library (1512 compounds). Individual data is given in Annex I.**

the blocks in the LowRes3 scheme as used by Van de Meent [2008]), the change of only one substance from or to such a hydrocarbon block can have a very significant influence on the average value of the PEC or PNEC derived for that hydrocarbon block.

Overall it is not consistent or logical that two methods, which should be combined to calculate a risk, use two different methods (boiling point estimates) to define their hydrocarbon blocks. It would make much more sense if both methods use the same physicochemical properties for the whole library of representative structures. This issue with the different boiling points might have been resolved by CONCAWE in their combined PetroRISK tool which integrates the PetroTOX and HBM tools. This was not checked for this evaluation

An additional evaluation of the quality of the Boiling Point data underlying the QSAR models is performed by comparing the PhysProp database reported Boiling Point values to the boiling point data assembled by Verbruggen et al. [2008] for 309 single hydrocarbon compounds. This comparison is visualized in Figure 10. It is seen that for all 309 single hydrocarbon structures for which evaluated experimental values are given by Verbruggen et al. [2008], the data comply with the referenced values in the PhysProp database. Although differences of several tenths of degrees Celsius are frequently noted, the overall picture is that there are no substances for which the experimental data disagrees between the two independent sources.

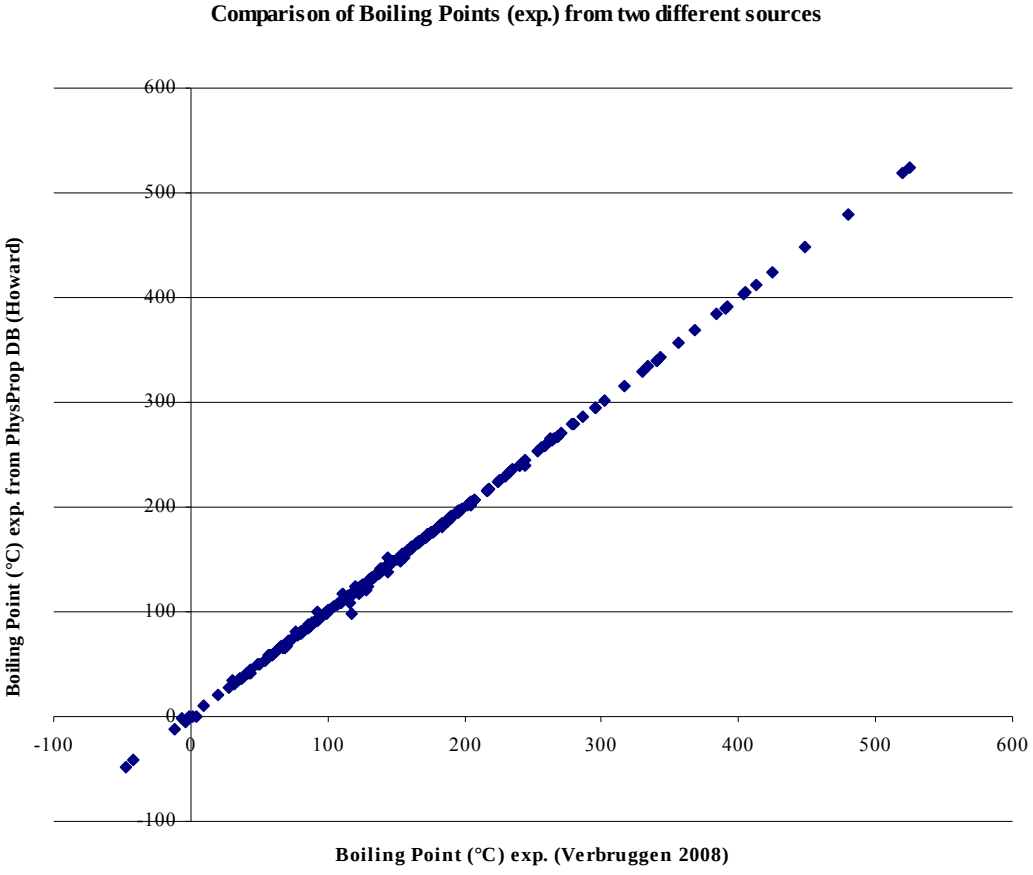

Figure 10. **Comparison of experimental boiling point data from two different (compiled) data sources, for 309 hydrocarbon compounds. Individual data is presented in Annex I.**

Water solubility

The values for water solubility used in the PETROTOX model are calculated by SPARC v4.2. The values used in the HBM model are not properly accounted for, but seem to come predominantly from the experimental values [PhysProp database] or from the WSKOW model as supplied in the EPISuite from USEPA. For some substances, the values as calculated by the WATERNT model from the same EPISUITE have been preferred. We could not (within the limited timeframe) figure out in which case preference was given to a specific model, or why. For one substance an error in the HBM library of three orders of magnitude was made (mg/l vs. g/l), n-nonane, experimental water solubility 0.22 mg/l and the value used in the HBM library is 220 mg/l.

The performance of all three models (SPARC, WSKOW and WATERNT) versus the experimental values for water solubility in the PhysProp database is very comparable, as shown Figure 11, and as indicated by the R^2 values for the linear regression lines plotted in this graph.

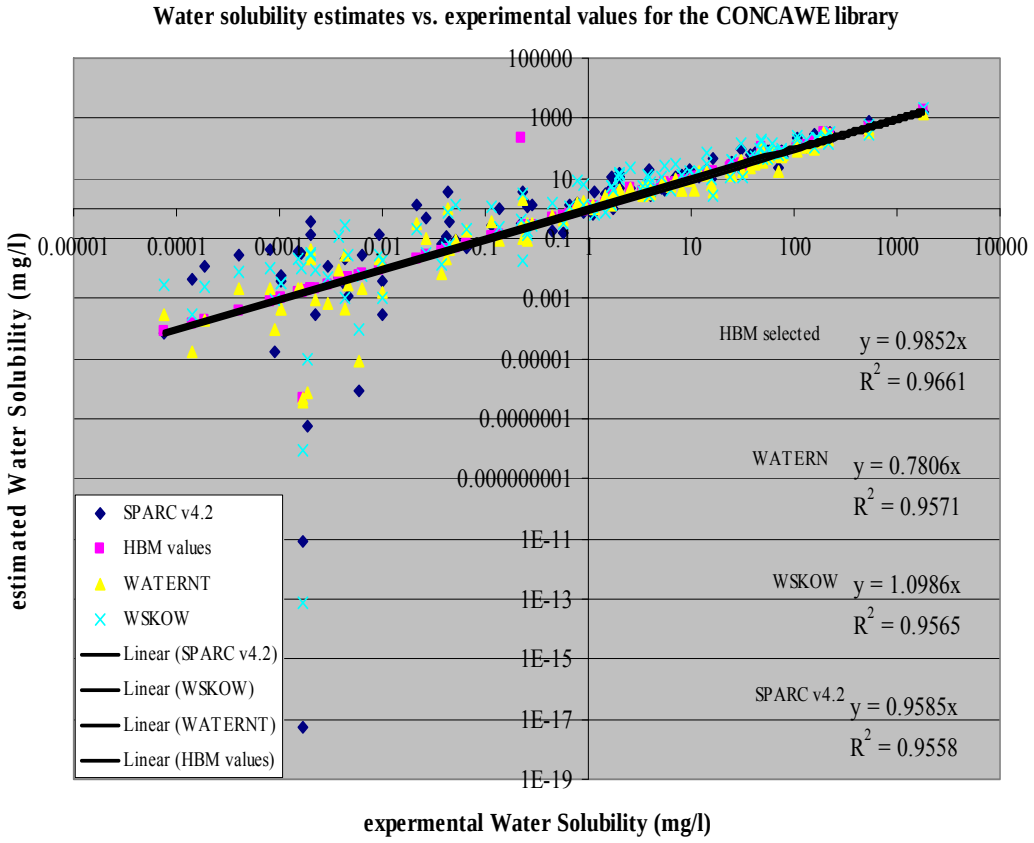

Figure 11. **Comparison of water solubility estimates from three different QSARs, and the dataset used in the HBM tool [Van de Meent, 2008] with experimental data from the PhysProp database [PhysProp db] for the subset of substances in the CONCAWE library for which experimental data was available.**

The HBM selected values perform best, in terms of regression coefficient (r2) and also with the slope of the regression line being closest to 1. This is to be expected as the majority of the values are taken from the PhysProp database, only for a minority the calculated values are preferred in the HBM model over the experimental values. The SPARC model performs better than the WSKOW and WATERNT models on this hydrocarbon substance data set.

An important observation is that there are significant differences in solubility between the models used in PetroTOX (SPARC v4.2) and the HBM tool (using WSKOW or WATERNT), although their statistical performance for the subset of substances in the CONCAWE library for which we have experimental values is comparable. This is similar to what was seen for the boiling point estimates used in the two tools.

The difference in predictions for the whole CONCAWE library between the values from PETROTOX (SPARC 4.2) and the HBM (WATERNT or WSKOW) is visualized in Figure 12.

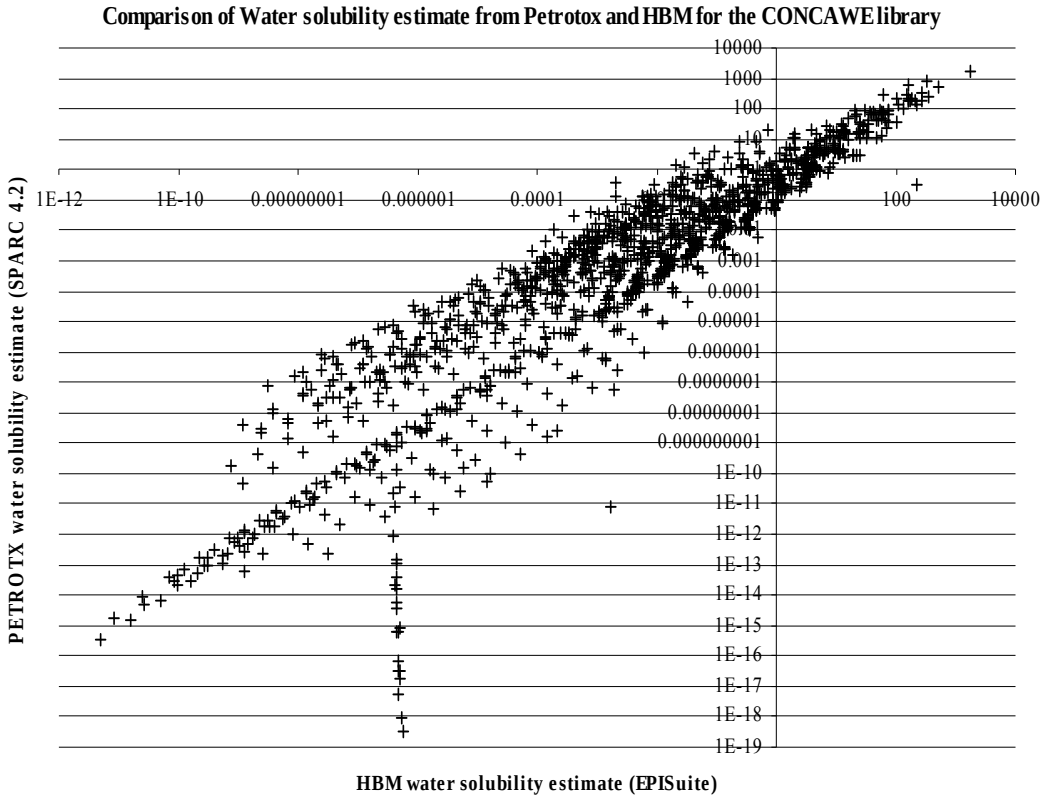

Figure 12. Comparison of water solubility estimates from two different QSAR models for the whole CONCAWE library (1512 compounds). Individual data is presented in Annex 1.

Assessing the validity of the water solubility data underlying the QSAR models applied in PetroTOX and HBM is performed by comparing the experimental data as compiled in the PhysProp database to experimental data assembled and evaluated independently by Verbruggen et al. [2000a, 2008]. The data from Verbruggen can be considered to be thoroughly evaluated and more recently evaluated. The experimental data in the PhysProp databases also formed the training

data set for both the EpiSuite QSAR models (WATERNT and WSKOW in this case) as well as the data on which the SPARC models have been calibrated. Visual inspection of the two datasets is possible by plotting them against each other, as given in **Error! Reference source not found.**. For almost all 88 substances, the experimental data from both sources match extremely well. The only 6 exceptions (difference between experimental data a factor of 10 or higher) are given in Table 5.

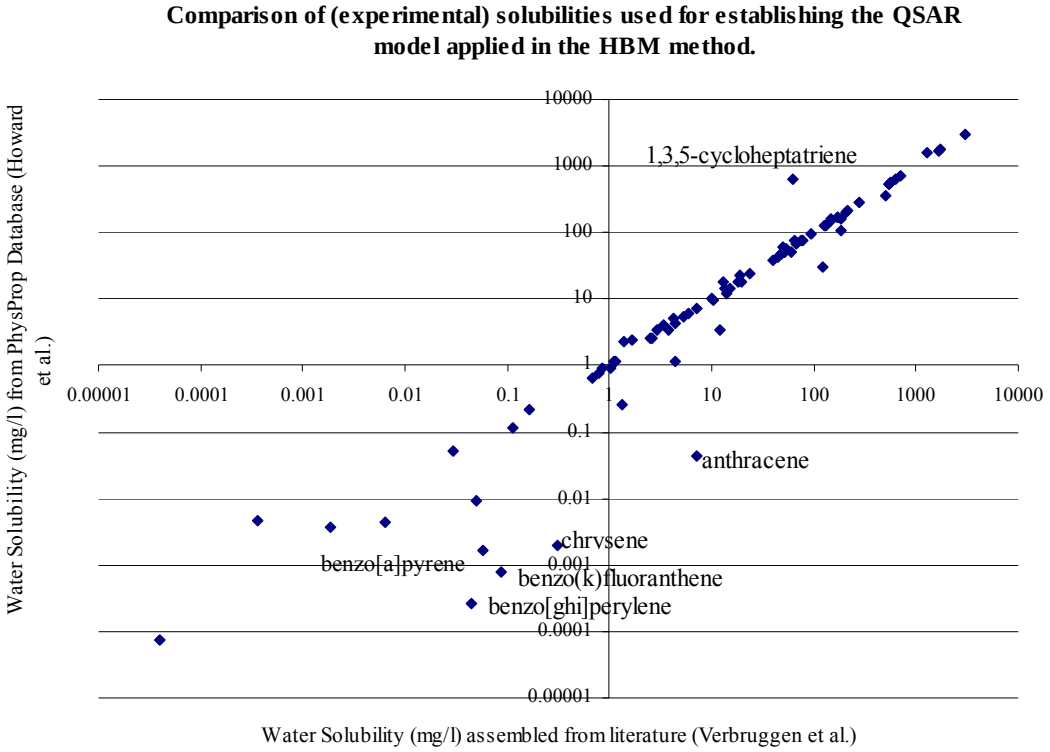

Figure 13. **Comparison of experimental boiling point data from two different (compiled) data sources, for 309 hydrocarbon compounds. Data from Verbruggen et al. [2000a] represent subcooled liquid solubility. Individual data is presented in Annex I.**

Table 5. Experimental water solubilities from two different (compiled) data sources where the reported water solubilities differ more than a factor of 10.

	WS* [Verbruggen 2000a]		WS [PhysProp DB]		factor	WS (WSKOW estimate)	
Anthracene	7.07	mg/l	0.043	mg/l	163	0.69	mg/l
Chrysene	0.31	mg/l	0.002	mg/l	153	0.026	mg/l
Benzo[k]fluoranthene	0.087	mg/l	0.0008	mg/l	108	0.011	mg/l
Benzo[ghi]perylene	0.044	mg/l	0.00026	mg/l	168	0.0028	mg/l
Benzo[a]pyrene	0.058	mg/l	0.00162	mg/l	36	0.010	mg/l
1,3,5-cycloheptatriene	62	mg/	620	mg/l	10	202	mg/l

* Subcooled liquid solubility

The difference in solubility found for 1,3,5-cycloheptatriene might be due to a typing error in one of the sources (difference is exactly a factor of 10), but the solubility of PAH structures seems to be consistently reported much higher by Verbruggen et al. [2000a] than in the PhysProp database. It is observed that the QSAR estimates for these 5 PAHs are consistently around a factor of 10 higher than the experimental value from the PhysProp database (which served as the training dataset), but still lower than the experimental values for subcooled water solubility reported by Verbruggen et al. [2000a, 2008].

The quality of the water solubility estimates is found to be disputable for a number of PAH structures. The QSAR estimates for these PAHs are closer to the experimental data on subcooled liquid solubility assembled by Verbruggen et al. [2000a] than to the experimental data from the PhysProp database on which the QSAR model was trained.

For environmental risk assessment, solubility only plays a role in the determination of environmental fate factors, but not in the determination of the HC5 coming from PetroTOX. In PetroTOX the (subcooled liquid) solubility estimates are used to calculate the loadings in order to derive an LL50 value, which is directly used to determine the actual concentration at different loadings. These loadings are used for the hazard characterization, i.e. C&L.

Although the different estimates for solubility are not mixed up in one assessment (risk or hazard) it seems incorrect to use different QSAR models in the PetroTOX and HBM tools, generating different solubility estimates, and subsequently base the hazard characterization on one solubility estimate, and the risk characterization on another. However, it should be noted that for the determination of the lethal loadings in PetroTOX the subcooled liquid solubility should be used for solids, instead of the normal solubility. Raoult's law is applied in the calculation of the lethal loading and to apply Raoult's law subcooled liquid solubility is needed instead of normal water solubility.

Further it should be noted that for the highest n-alkanes for which experimental data are available, the experimental data used in the HBM model do not represent true aqueous solubility, but are more likely to reflect colloidal accommodation of hydrocarbons [Verbruggen et al., 2000a]. This colloidal accommodation is not representative of environmental distribution and should thus not be used instead of the true aqueous solubility. Unfortunately, the QSAR estimates by EPIWIN, used if such experimental values were not available, are partly based on these colloidal influenced experimental values in the PhysProp database and deviate from true aqueous solubility as well. This has strong influences on the properties of the higher aliphatic substances. For example, the estimates for true aqueous solubility based on molar volume at boiling point [Verbruggen et al., 2000a], would be in the order of 1 femtogram per liter (1 fg $=10^{-15}$ g) for n-hexacosane (n-C26). The value used in the HBM model is 1.7 µg/L. The estimate from WSKOWWIN (EPIWIN) is 9 pg/L, while the SPARC estimate results in 8 fg/L.

3.1.4 Fate properties; partitioning and degradation

Octanol-Water Partitioning coefficient (Kow)

As noted for boiling point and water solubility, PETROTOX uses SPARC 4.2 to estimate the log Kow, whereas the HBM uses experimental values from the PhysProp database when available, and the KOWWIN v.1.67a estimate from the USEPA EpiSuite when no experimental value is available.

Again, when comparing the estimates with the experimental values for those substances in the CONCAWE library where an experimental value is available, the two methodologies do not seem to qualitatively differ very much, and both are capable of reproducing the log Kow values with a high degree of reliability. This is visualized in Figure 14, and the regression equations given in this figure confirm the close fit of the estimates to the experimental data. It can be concluded from these regression equations that in general the KOWWIN model is slightly underestimating the experimental values (slope of 0.95 in the regression equation) whereas the SPARC model is slightly overestimating the experimental values (slope of 1.05 in the regression equation). These differences only become significant at log Kow values above 6, as can be seen by the separation of the two regression lines in Figure 14 .

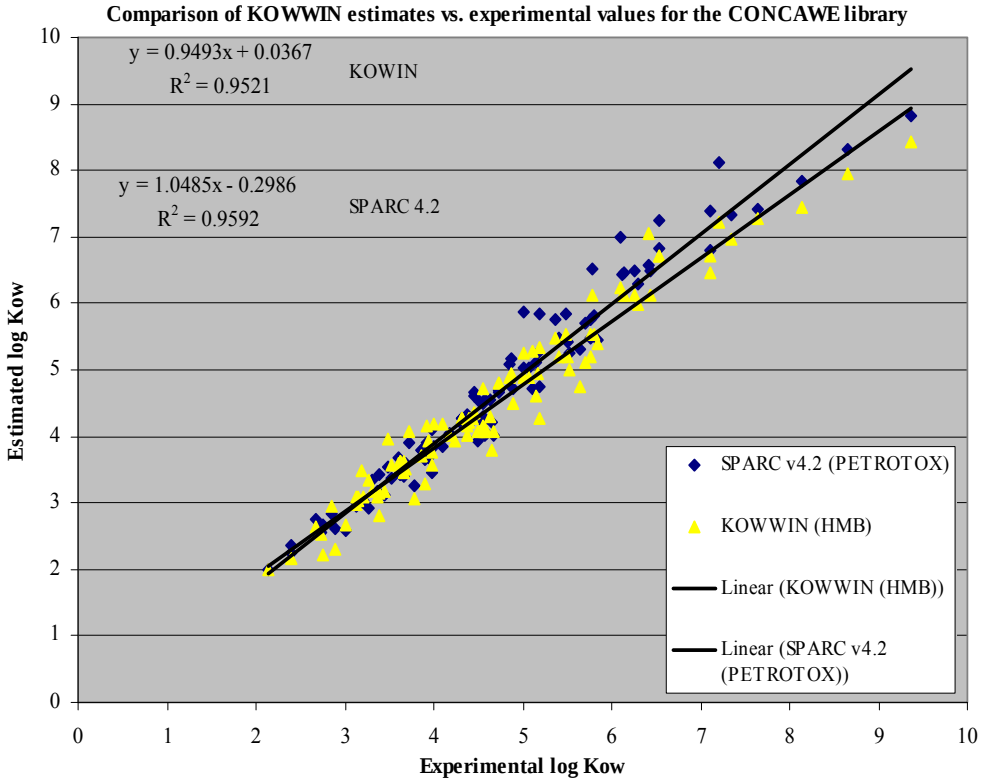

Figure 14. **Comparison of QSAR estimated octanol-water partition coefficients from two different QSAR models against experimental partition coefficients, for the subset of substances from the CONCAWE library for which experimental data was available. Individual data is presented in Annex I.**

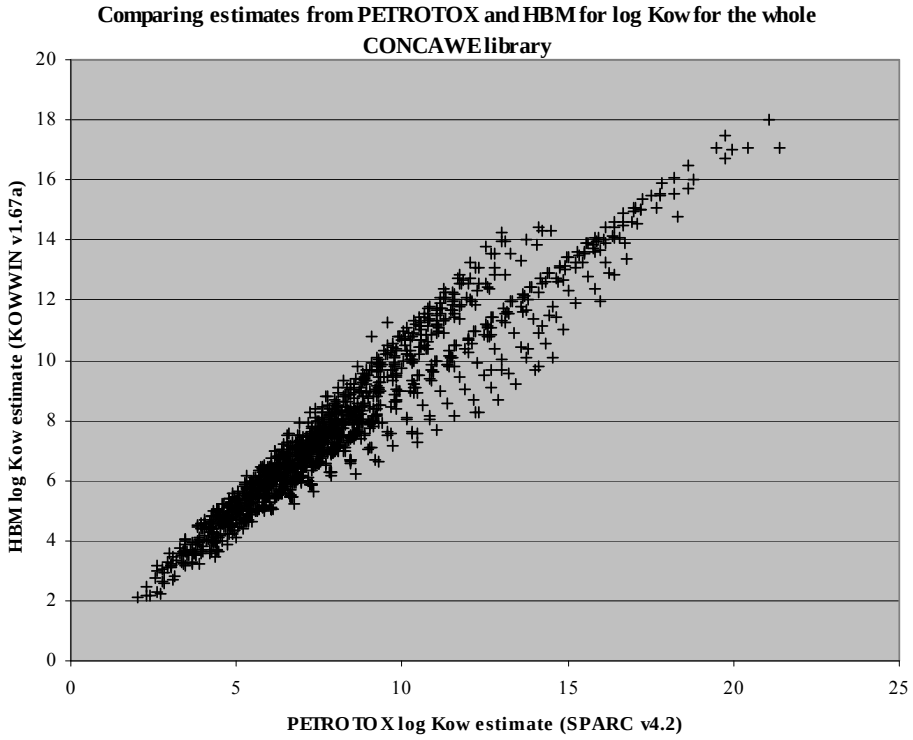

Figure 15. **Comparison of QSAR estimates from SPARC v4.2 and KOWWIN v1.67a QSAR models to each other for the whole CONCAWE library (1512 compounds). Individual data are presented in Annex I.**

However, when comparing the estimates from the two methods for the whole CONCAWE library, see Figure 15, large individual differences in the estimates between the two methodologies are visible. Figure 15, where the whole dataset is used for comparison, also seems to indicate that in general the SPARC estimates are higher than the KowWin estimates (which was also observed in the comparison of the model estimates to the experimental data), as practically all of the values in this figure are at or below the 1:1 line. However, when the more relevant fraction of the CONCAWE library with a log Kow<8 is looked at in more detail (Figure 16) these structural higher estimates from the SPARC model are not observed anymore, although there still is considerable scatter in the data. When the data is split into aliphatics and aromatics (according to the LowRes mode indication provided in PetroTOX) it is observed that for the aromatics there is no systematic over- or underprediction of one model against the other, but for the aliphatics all SPARC estimates are higher than the KOWWIN estimates (figures not given, but individual data which allows to reproduce this observation, including the graphic representation, is given in Annex I). The variability between the two methodologies below a log Kow (estimated) of 8 seems to be more limited than for the very high (theoretical) log Kow domain. However, we still see a very large spread of the values up to two log units. A difference of two log units between the log Kow estimation used to calculate the PEC and PNEC introduces large uncertainties in the risk characterization in terms of RCR.

In our view it is not logical that the two tools which supply each one part of the risk assessment, use different estimates of the physicochemical properties. The log Kow in PetroTox (the SPARC 4.2 estimate) is directly used to determine the log Kmw (micelle-water or membrane-water partition coefficient) which in turn is used to derive directly the HC5 (the chronic PNEC value).

The log Kow entered in the HBM tool (KOWWIN estimates and experimental values from the PhysProp database) is used to determine the fate factors of the same substances in the environment. This implies that for the same substance the PEC and PNEC values are derived from different Kow values, which introduces additional uncertainties in the risk characterization.

In defense of this approach CONCAWE argued that the Target Lipid Model was completely based on the SPARC log Kow estimates, and therefore it is necessary to use SPARC estimates to calculate the HC5, with the alternative being to re-calibrate the whole TLM on a different log Kow estimate (e.g. KOWWIN). Their argument to use KOWWIN estimates for the estimation of the fate factors was less convincing, stating that this methodology is the most accurate. There is no reason why the fate factors could not have been calculated using the SPARC log Kow estimate. At least the extrapolation from PNEC water to PNEC soil (or sediment) using the partitioning equilibrium method, used the same log Kow (KOWWIN estimate) as applied for the calculation of the fate factors, which makes sense.

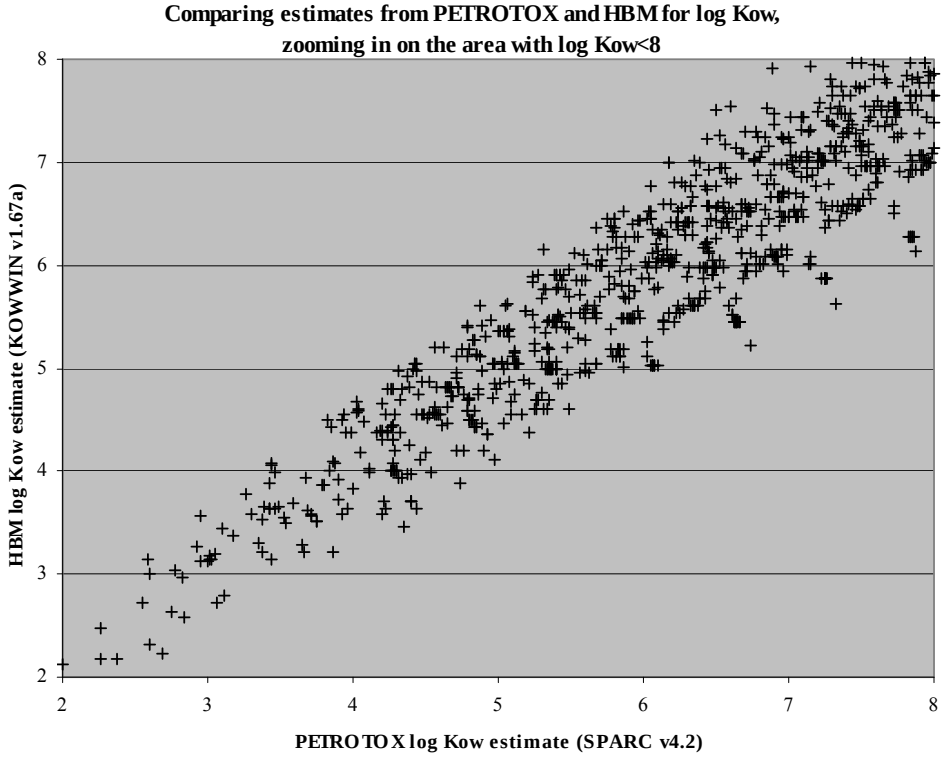

Figure 16. **Comparison of QSAR estimates from SPARC v4.2 and KOWWIN v1.67a QSAR models for the subset of substances of the CONCAWE library where the (predicted) log Kow is below 8. Individual data are presented in Annex I.**

For 321 substances in the CONCAWE library the difference between log Kow used in PetroTOX and in the HBM tool is more than 1 log units. This is more than 21% of the substances in the CONCAWE library. The fraction of the library for which the QSAR estimates differ substantially are given in
Table 6.

Table 6. Indication of the number of substances that show a substantial difference between the two QSAR models used for log Kow estimation.

Difference in log units between PetroTOX and HBM	nr. of substances in CONCAWE Library	
2	103	(6.8%)
1.5	175	(11.6%)
1	321	(21.2%)
0.7	510	(33.7%)
0.5	718	(47.5%)
0.3	938	(62.0%)

Again, the quality of the data underlying the QSARs used in PetroTOX and HBM methods (SPARCv4.2 and the KOWWIN model,) is assessed by comparing the data from the PhysProp database (which served as the training dataset for KOWWIN and possibly also to calibrate the SPARC models) to an independent assembly and thorough evaluation of Kow data by Verbruggen et al. [2008] for a set of 125 individual hydrocarbon substances. The concordance between log Kow from the two different data sources is very high, as can be visually seen in Figure 17. Therefore no discussion on the (quality of the) experimental values used for deriving the SPARC or KOWWIN QSAR models is required.

The only substance for which a difference in log Kow is noted between the two sources is 9,10-dimethyl-anthracene, where Verbruggen et al. [2000a] reports a value of 5.44 and the PhysProp database gives a reference value of 5.69. This difference, in the light of the experimental difficulties of actually measuring such higher log Kow values, and the observed variability between the QSAR estimates from the two models used in PetroTOX and the HBM tool seems negligible.

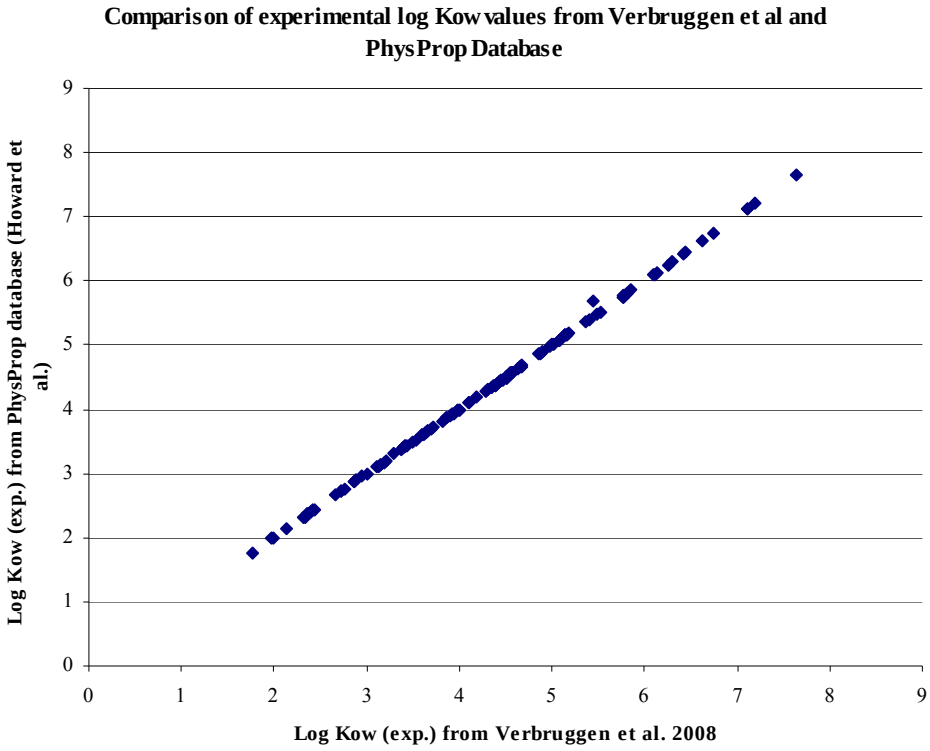

Figure 17. **Comparison of experimental octanol-water partitioning data from two different (compiled) data sources, for 125 hydrocarbon compounds. Individual data is presented in Annex I.**

Water-air partitioning coefficient, Henry's Law constant

Henry's law constant (the water-air partitioning coefficient) is used in PetroTOX to determine the headspace concentration and subsequent water concentration in the calculation of the Lethal Loading of a hydrocarbon mixture. This parameter is therefore relevant to determine the actual concentrations used to classify a mixture, at different loadings. In the calculation of the HC5 (the chronic PNEC) which is compared to the PECs resulting from the HBM tool, Henry's Law Constant (HLC) is not used. In the calculation of the Fate Factors in the HBM tool, HLC is determining very strongly which environmental compartments a substance will partition to.

The HLC used in PetroTox is calculated with SPARC v4.2, whereas the HLC used in the HBM model to determine the fate factors for all library components is taken from HenryWin v3.20 (Oct. 2008) provided as part of the EPISuite models from US EPA. Experimental values reported in the PhysProp database are used wherever available, and QSAR estimates are used when no experimental value is present in the database.

The specific HenryWin v3.20 QSAR model used by the HBM tool is the Bond model, not the Group model. The bond contribution methodology splits a compound into smaller units (two atom fragments only), and includes individual hydrogen bond values; the group method does not. Both the Group Method and Bond Method are susceptible to estimates resulting in "Missing

Fragments". When a compound is split into groups or bonds, one or more of the resulting groups or bonds may not have a value in the library of available values. The Group Method is much more likely to have a "Missing Fragment" occurrence (meaning an HLC estimate is not possible). Although the performance statistics from the training dataset show a smaller standard error for the Group method estimates (HenryWin Help file) an independent evaluation [Altschuh et al., 1999] for a diverse set of organic chemicals found the bond method more accurate than the group method. The group method generates inaccurate estimates for certain types of structures, such as hexachlorocyclohexanes [Altschuh et al., 1999]. It seems that these consideration formed the basis for the choice for the bond method in favour of the group method. From the HenryWin calculations for the 97 substances in the CONCAWE library for which an experimental HLC was available regression lines have been calculated.

For the Group method this yields:

HLC (exp.) = 0.88 x HLC (group method) – 0.25 $R^{2=}$0.96, s.e.= 0.41, n=97

And for the Bond method this yields

HLC (exp.) = 0.99 x HLC (bond method) – 0.14 $R^{2=}$0.97, s.e.= 0.40, n=97

Although the bond model does not have a significant lower uncertainty in the prediction of the log HLC (standard error of prediction 0.40 vs. 0.41 log units), it shows much less systematic deviation, with a slope in the regression very close to 1, and the intercept of -0.14 much closer to 0 than for the group method (intercept -0.25).

For the SPARC HLC estimate the regression analysis yields

HLC (exp.) = 0.97 x HLC (SPARC) – 0.07 $R^{2=}$0.96, s.e.= 0.45, n=98

The concordance of both the SPARC v4.2 estimates and the HenryWin Bond method estimates with the experimental HLC data as taken from the PhysProp database is high, and one model does not seem particularly better than the other, although the HenryWin Bond method seems to give a significantly lower standard error in the estimate of 0.40 log units HLC versus 0.45 log units for the SPARC model. Figure 18 is a visualization of this concordance.

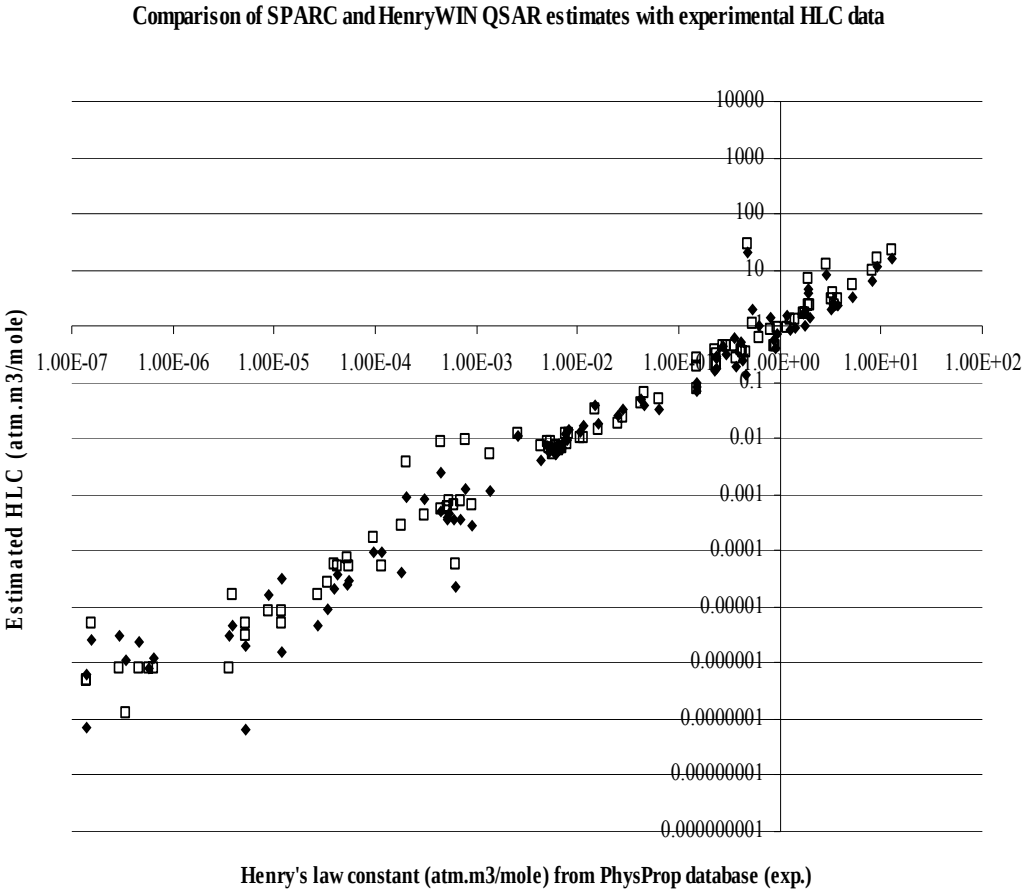

Figure 18. **Comparison of QSAR estimated Henry's Law Constants (HLC) from two different QSAR models against experimental HLCs, for the subset of substances from the CONCAWE library for which experimental data was available. Outlined squares representing the HenryWin v3.20 data and filled diamonds representing the SPARC v4.2 estimates. Individual data is presented in Annex I.**

Despite the similar behaviour of the two models for the subset of substances in the CONCAWE library for which experimental data is available, the concordance between the SPARC v4.2 estimated and the HenryWin Bond method estimated HLC's for the whole CONCAWE library is not very high, as can be seen in Figure 19. However, as the HLC is not used in PetroTox to determine the HC5, but only the LL50, this difference between the two QSAR models is not relevant for environmental risk assessment; only the HenryWin v3.20 estimates are used to determine the fate factors of the CONCAWE library components. The HenryWin Bond model, at least when comparing with the available experimental data, seems to predict the HLC with a sufficient accuracy for use in risk assessment.

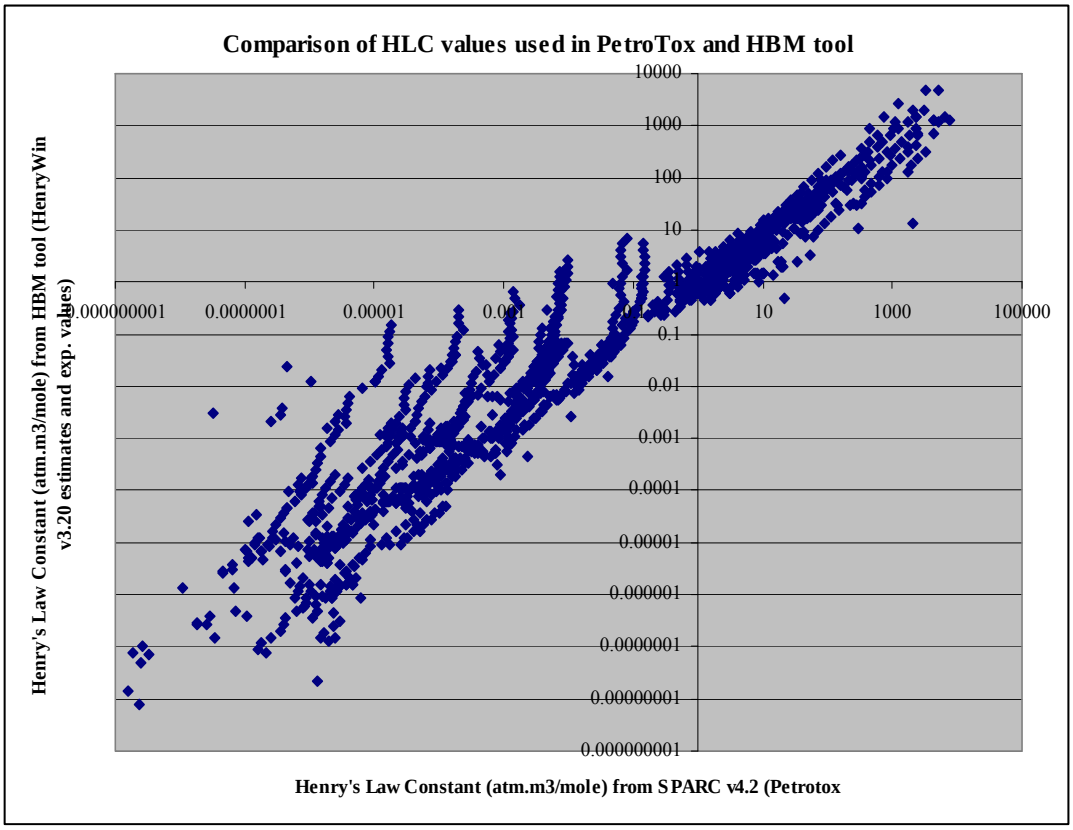

Figure 19. **Comparison of QSAR estimates from SPARC v4.2 and HENRYWIN v3.20 Bond Method QSAR models to each other for the whole CONCAWE library (1512 compounds). Individual data are presented in Annex I.**

Organic carbon-water partitioning coefficient, Koc

Many studies have been performed to determine the organic carbon-water partition coefficient (Kow) of aromatic hydrocarbons, both monoaromatic and polycylic compounds. A well known relationship between Koc and Kow 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:

$$\text{Log Koc} = \log \text{Kow} - 0.21$$

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., 1996]. For non-substituted aliphatic compounds no data for log Koc 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 of 1.7), the resulting log Koc values for PAHs are in accordance with the other data for PAHs (Figure 20). The data for n-

alkanes, however, are not in line with these data. The well-known relationship between log Koc and log Kow for predominantly hydrophobic compounds from Sabljić et al. [1995] seems to describe these data more accurate (Figure 20). Therefore, the Sabljić equation is used for the aliphatic compounds:

$$\log Koc = 0.81 \log Kow + 0.1$$

The Koc also plays a role in PetroTOX in determining correction factors for bioavailability in the calculation of the Lethal Loadings. The bioavailability correction is however not used in the determination of the HC5 (chronic PNEC) used for the risk assessment. In the calculation of the individual Fate Factors for the CONCAWE library in the HBM [Van de Meent, 2008] tool the Koc plays an important role. In the HBM the Koc is calculated directly from the log Kow, and the predominantly hydrophobics QSAR from the TGD(2003) is used to do this. This is in line with the REACH guidance. It could be argued that the Karickhoff equation would be better suited for the (poly)aromatic hydrocarbons (see above), but the actual dataset used to derive the Sabljić equation for predominantly non-hydrophobics also contains a considerable fraction of (poly)aromatic hydrocarbons, whereas it does not contain any aliphatic substances, apart from halogenated aliphatic hydrocarbons.

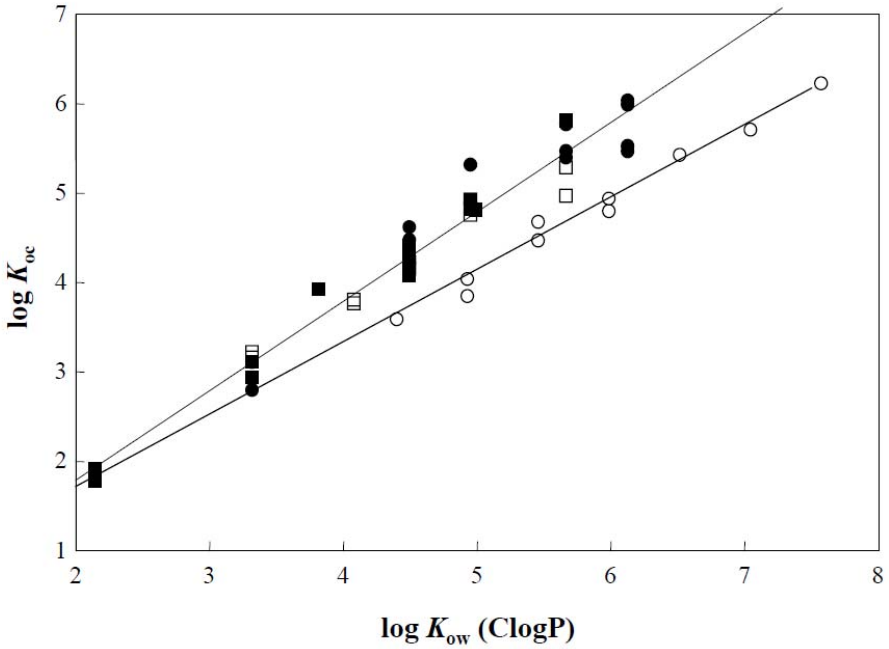

Figure 20: **Organic carbon-water partition coefficients as a function of log Kow. Upper drawn line is the selected QSAR for the aromatic compounds [Karickhoff et al., 1979], lower drawn line is the selected QSAR for the aliphatic compounds [Sabljic et al., 1995]. Data: ■: PAHs and benzene from [Karickhoff et al., 1979; Karickhoff, 1981] ●: PAHs from [De Maagd et al., 1996]; □: PAHs and ○: n-alkanes from [Poerschmann and Kopinke, 2001].**

BCF in fish (biota-water partitioning coefficient)

The BCF used in the HBM model are only used to estimate the concentration of fish as input for human intake. The PetroTox model uses a relationship with log Kow to calculate the lethal body burdens. The assumptions and the uncertainties of this approach are discussed under 3.2 Target Lipid Model.

The values used in the HBM tool for the BCF in fish are estimates from the EPA BCFWin v2.16 program. This estimation software has been updated recently to v3.00 and there are a number of marked differences. The estimates for BCF derived with USEPA BCFWin v2.16 are considered to be under-estimation of the bioconcentration potential of substances, possibly due to a too aggressive correction for metabolism. This is reflected by the graph of 35 CONCAWE components for which evaluated experimental BCF values were present in the PhysProp database.The concordance between the experimental values and the estimates used in HBM is shown in Figure 21.

In the area where the experimental BCF is above 1000, there is one substance that is most correctly predicted, 2,2,4,4,6,8,8-heptamethylnonane, with an experimental BCF of 6600 and an estimated BCF of 5300. There are however 9 substances where BCFs are underestimated, with one extreme outlier; n-Hexadecane with experimental BCF of 5011 but an estimated BCF of 15.

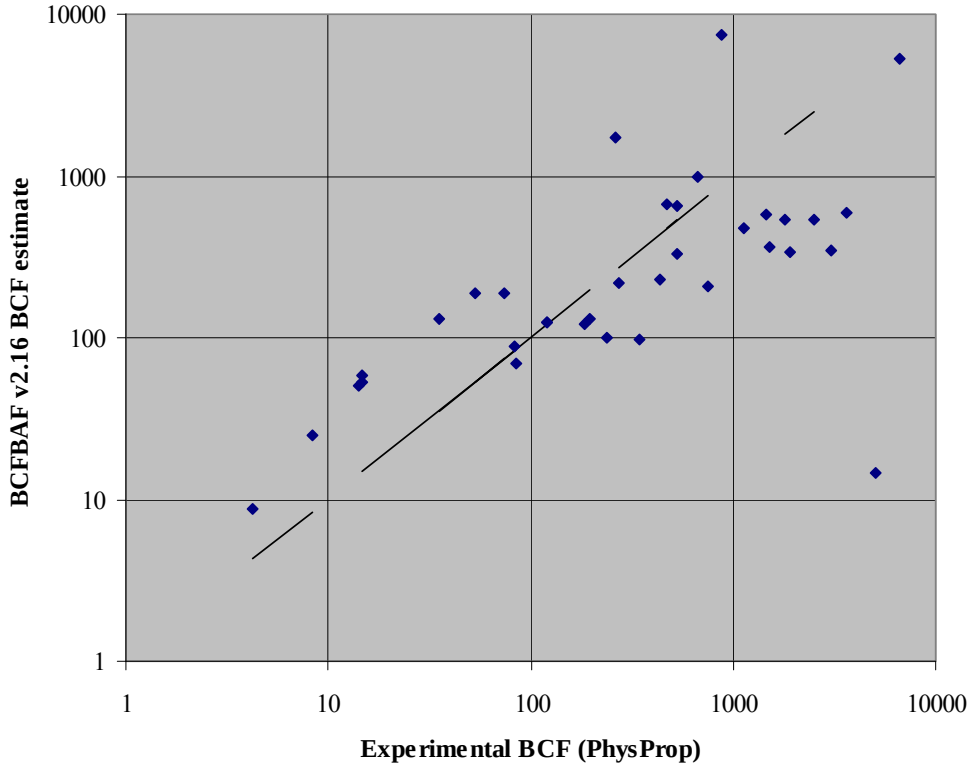

Figure 21. **Comparison of QSAR estimated BioConcentration Factors against experimental partition coefficients, for the subset of substances from the CONCAWE library for which experimental data was available. Individual data is presented in Annex I.**

However, the experimental BCF for this substance can not be considered a reliable value, because it was determined at concentrations far above the water solubility, and were subsequently recalculated with the water solubility as exposure concentration. It should be noted that reliable BCF estimates for linear alkanes generally results in low BCF values, possibly as a consequence of extensive metabolism [e.g. Tolls & van Dijk, 2002]. Especially for a substance like decalin, the estimated BCF seems to be far below the experimental value. Also the BCFs of several of the PAHs are rather strongly underestimated. In the range where the experimental BCF is between 100 en 1000 there are two overestimated substances; 2,2,4,6,6-pentamethylheptane, exp.BCF=880 vs. est. BCF=7464, and benz[a]anthracene, exp. BCF=260 vs. est. BCF=1719. Five substances are clearly underestimated.

The trend of underestimation was also recognized by the USEPA, which becomes clear when comparing the v2.16 estimates for the CONCAWE library with the BCFBAF v3.0 estimates for BCF. In Figure 22 BCF estimates for the whole CONCAWE library are plotted against each other from BCFBAF v3.0 and BCFWin v2.16. It is observed that the estimates in the newer version of the USEPA model are overall much more conservative (points above the 1:1 line in figure 22, i.e. the BCF estimated by BCFBAF v3.0 is higher than estimated with v2.16) than the version used in the HBM tool (v2.16) to calculate the fate factors. Those substance for which v2.16 is slightly more conservative (points below the 1:1 line in figure 22) do not belong to specific PetroRisk classess of hydrocarbons, all 16 classes of hydrocarbons are represented in the subgroup of substances for which v2.16 is (slighly) more conservative in its estimate of the BCF.

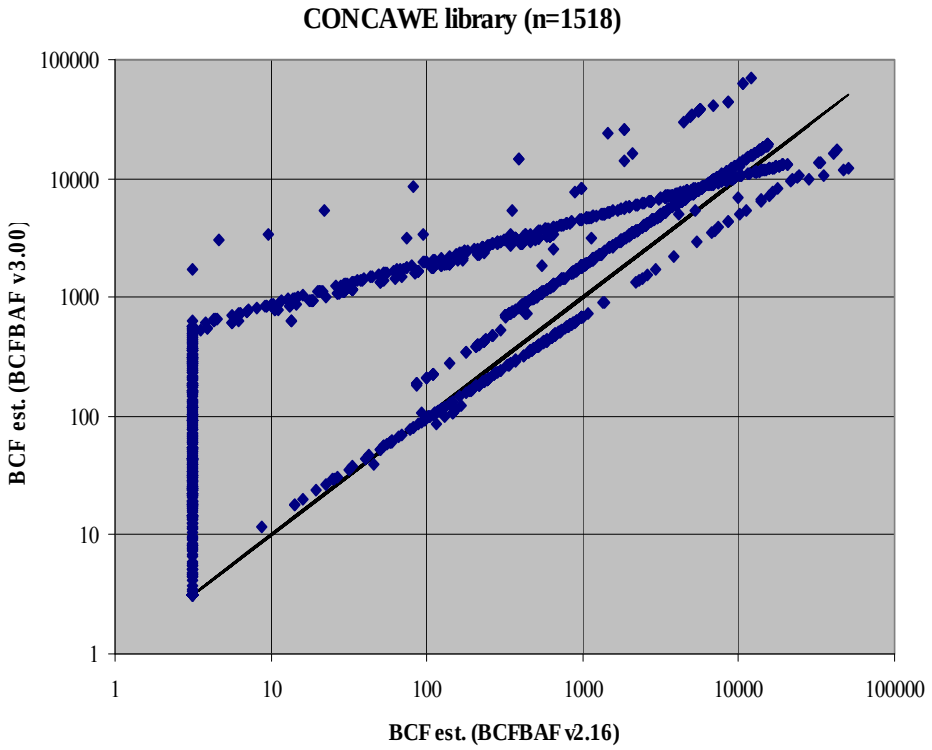

Figure 22. **QSAR estimated BioConcentration Factors from two different model versions (BCFBAF v3.00 vs BCFBAF v2.16) for the whole CONCAWE library of 1518 compounds. Individual data is presented in Annex I.**

When the v2.16 estimates are compared to the EU REACH Guidance and TGD(2003) recommended equations for BCF (as implemented in the latest EUSES versions) the underestimation of BCF values by BCFBAF v2.16 is even more pronounced. This is visible in figure 23. In the EU estimates for BCF no correction is present for possible metabolism, and these values can therefore be considered as maximum (passive) BCF values.

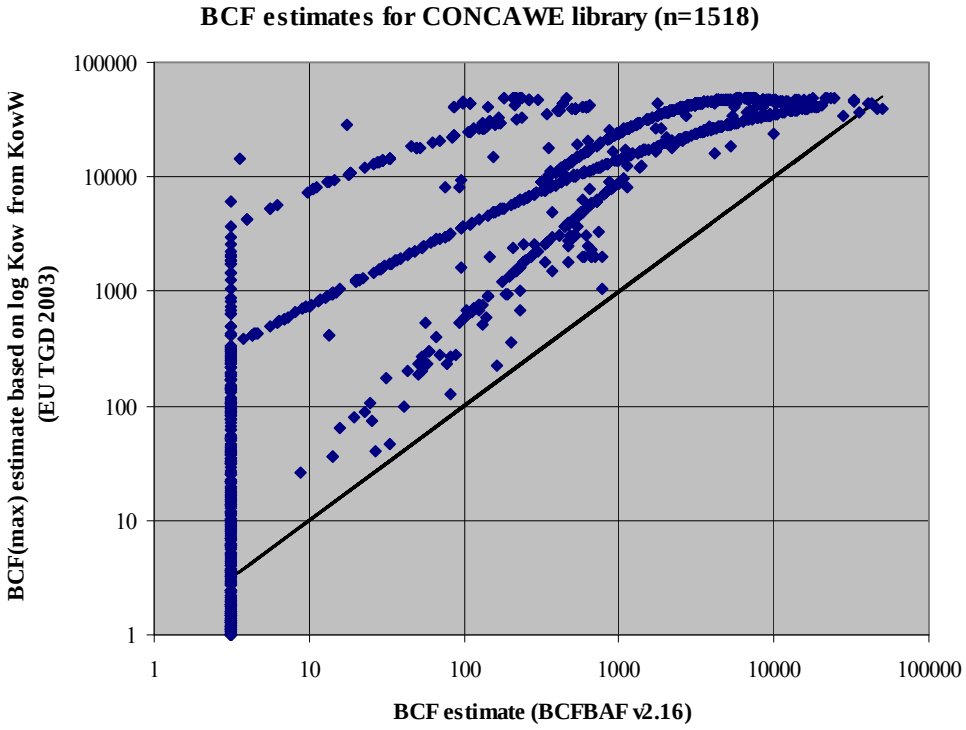

Figure 23. **QSAR estimated BioConcentration Factors from two different models (BCFBAF v2.16 vs EU TGD2003 BCFmax) for the whole CONCAWE library of 1518 compounds. Individual data is presented in Annex I.**

The Gaussian function for the maximum BCF-value on the basis of the hydrophobicity (log Kow in the following equation) for apolar organic chemicals in fish was retrieved from REACH Guidance R.11:

$$\log BCF_{\max} = \frac{34.43}{2.93 \cdot (2 \cdot \pi)^{0.5}} \cdot e^{-0.5 \cdot \left(\frac{\log K_{ow} - 6.52}{2.93} \right)^2}$$

The parameters in this equation are slightly different from those of the curve shown in Figure R11-4 of the REACH Guidance R.11, because in the calculations in the CONCAWE HBM the log Kow was estimated by USEPA KowWin instead of ClogP.

A regression analysis of the three estimation procedures using the very limited, 34 substance (excluding n-hexadecane), dataset of hydrocarbon substances for which experimental values are available, yields the following regression equations:

Log BCF(exp.) = 1.08 x log BCF(v3.00) - 0.20	R2= 0.70, s.e.=0.45, n=34
Log BCF(exp.) = 1.08 x log BCF(v2.16) - 0.15	R2= 0.63, s.e.=0.50, n=34
Log BCF(exp.) = 0.87 x log BCFmax(EU guidance) - 0.40	R2= 0.66, s.e.=0.48, n=34

From this limited dataset it cannot be concluded that BCFBAF v2.16 is under-estimating, as both the slope (1.08) and intercept (-0.20 vs -0.15) are very similar slope for both versions (v3.00 and v2.16). It can however be clearly observed in Figure 22 that the BCFs from v.2.16 are always equal to or below the estimates from v3.00. This shows the limitations of the analysis using this very small experimental dataset.

The estimates from the EU REACH Guidance equations, giving a BCFmax, and lacking any correction for metabolism, are clearly more conservative, also in predicting the limited experimental dataset. The slope of the regression line is 0.87 (vs. 1.08 for the BCBAF models), and the regression analysis shows a significant systematic error (over-estimating) as indicated by the intercept of -0.40.

If a conclusion on the quality of the three models should be based on this limited dataset, it would be that BCFBAF v3.00 would be the best choice for predicting BCF for (petroleum) hydrocarbons. Based on the comparison of the two BCFBAF versions an update of the BCF estimates to v.3.00 used in the HBM tool would be required to avoid underestimation of the bioconcentration potential.

(Bio)Degradation

Introduction

Biodegradation of petroleum hydrocarbons is a complex process that depends on the nature and on the amount of the hydrocarbons present. There are different factors influencing the hydrocarbon degradation such as oxygen levels, pH, available nutrients, humidity, salinity and temperature [Cooney et al. 1985]. Another important factor is the availability to microorganisms. In addition to bacteria, fungi, cyanobacteria, yeasts, and protozoa are all able to degrade mineral oil. Hydrocarbons differ in their susceptibility to microbial attack. The biodegradation rate of different constituents groups generally decreases in the order of: linear alkanes, branched alkanes, low-molecular weight aromatics, PAHs and cyclic alkanes [Das&Chandra, 2010 and refs. therein]. Alkanes are usually terminally oxidized to the corresponding alcohols, aldehydes and fatty acids. The degradation of long-chain alkanes can be slowed down due to the poor water solubility and sorption to organic matter. Branched alkanes are more difficult to degrade than linear alkanes, which is due to the limited substrate range of the monooxygenases and the more difficult metabolism in the β-oxidation route. Degradation of mineral oil can also occur under anaerobic conditions. However, the rate is much slower than under aerobic conditions.

Degradation half lives estimates for surface water, soil and sediment

The estimated aerobic primary half-lives in soil, water and sediment in the HBM/Petrorisk model are derived from the model BIOHCWIN. This model was developed based on a training set of

environmentally-relevant experimental data for 121 hydrocarbons (see Table 7) and was validated using 54 compounds.

Table 7. Overview of the petroleum compounds used for the training set to develop BIOHCWIN

Compound class	No. of compounds	Carbon nr range
n-Alkanes	20	C5–C34
Alkenes	1	C14
Branched alkanes	28	C6–C20
Monocycloalkanes	7	C5–C8
Fused-ring cycloalkanes	3	C10–C14
Hopanes	3	C25–C30
Monoaromatics	17	C6–C10
Unsubstituted PAHs	16	C10–C24
Alkylated PAHs	12	C11–C19
Hetero-PAHs	5	C8–C16
Biphenyls	1	C12
Indans and Indenes	5	C9–C10
Naphthenoaromatics	3	C16–C21

The "experimental" data are not actually measured (primary degradation) half lives, but "recommended half lives" based on an evaluation of all available biodegradation test and monitoring data available in the Environmental Fate DataBase [EFDB, SRC Inc.]. The original fragments from the MITI BIOWIN model were initially used as structural descriptors and additional fragments were then added to better describe the ring systems found in petroleum hydrocarbons and to adjust for non-linearity within the experimental data.

It was highlighted that not for all groups of petroleum compounds sufficient information was available. The majority of available data were for benzene, toluene, ethylbenzene, and xylene (BTEX); the isoprenoids phytane and pristane; PAHs; and n-alkanes. Many of the other compounds had only one or two experimental half-lives, and the level of uncertainty in the recommended half-life was greater. Howard et al [2005] did not consider the lack of data to be particularly problematic for structures expected to biodegrade either very quickly (i.e., 1–15 d) or very slowly (i.e., half-lives greater than one year). However, for those compounds with moderate rates of biodegradation, the uncertainty in the recommended biodegradation half-lives has a more pronounced effect on the ultimate results of the modeling effort. Although a distribution of half-lives would be more appropriate for describing the biodegradation half-life of a compound, in most cases insufficient data were available. Figure 24 shows the recommended experimental half lives versus the predicted half lives.

Preference was given to field data when these were available. However, when field half-life data were considerably longer than those reported in grab-sample studies, a half-life from the grab sample studies was chosen. It was noted that this might not result in the best value, but according to the authors most compounds had only grab-sample data, and the preferred use of the grab-

sample data in these instances resulted in a consistent relative ranking of compounds based on their half-life data.

In many cases, insufficient data were available to support the use of fragments that, at least theoretically, are expected to affect the biodegradation rate. These fragments include alkanes in which secondary or tertiary structures at the ends of the molecules should prevent beta-oxidation from occurring or, in the case of cyclic or aromatic structures, in which any applicable alkyl groups had secondary or tertiary structure inhibiting the biodegradation of the alkyl group. Programming of such fragments for inclusion into the model was considered to be too difficult because of problems in interpreting the structure. Too few data were available for branched alkanes in general, and no data were obtained that would clarify whether a compound blocked at both ends but containing a methyl or ethyl group in the middle of the molecule would have a lower half-life than one without a methyl or ethyl group. Fragments in which the number of adjacent hydrogen atoms on aromatic or cyclic ring structures was specified also were unsuccessful because of a lack of data. In this case, it would be expected that different isomeric substitution patterns would affect the potential ability of microbes to attack the underlying ring structure. Below the performance of the BIOHCWIN model in comparison to measured data will be discussed. More recently the degradation half lives of petroleum compounds in unacclimated fresh surface water and seawater were investigated [Prince et al, 2007 and 2008, CONCAWE, 2009], using purge-and-trap and extraction methodologies, both coupled to GC/MS, and hexachloroethane and hexachlorobenzene as conserved internal markers. Also these half lives will be compared with those calculated with BIOHCWIN.

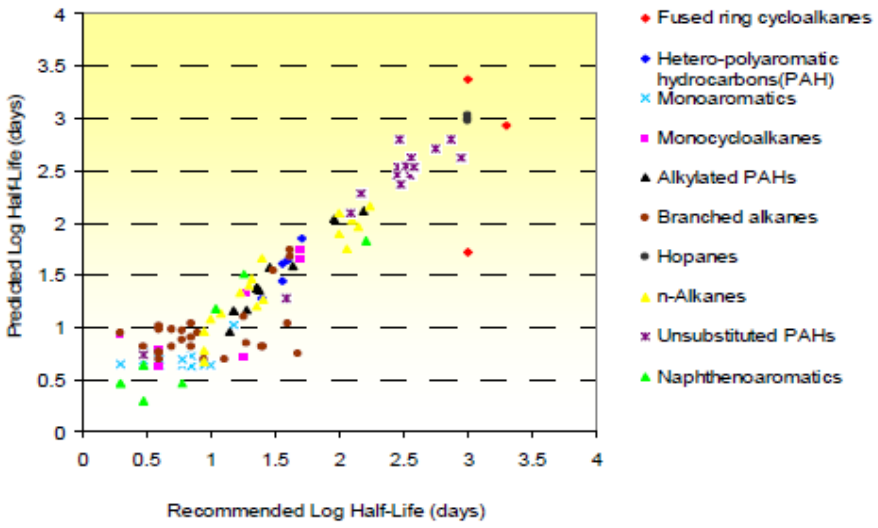

Figure 24. **Comparison of the (recommended) experimental and estimated log half-life for the 121 training set compounds by BIOHCWIN**

n-alkanes

It appears that the recommended primary biodegradation half-lives for the n-alkanes were not linear. For this reason two fragments were added to the BIOHCWIN model to correct for the nonlinearity of the n-alkane experimental biodegradation data. Despite the addition of these corrective fragments, it becomes apparent, as illustrated in Figure 25, that the model in general underestimates the environmental half lives of the longer n-alkanes, starting from n=12; docosane

and up to n=30; triacontane. This limitation of the model was also acknowledged by Howard et al.[2005]. In comparison to the data obtained by Prince et al [2007, 2008], the predicted half lives by BIOHCWIN are lower for the short-chain alkanes (i.e. butane, factor 4; pentane, factor 2,5 -7; hexane, factor 1.5), but increasingly higher for the long-chain (i.e. octane, factor 1.5; undecane, factor 3.5; heptadecane, factor 11)

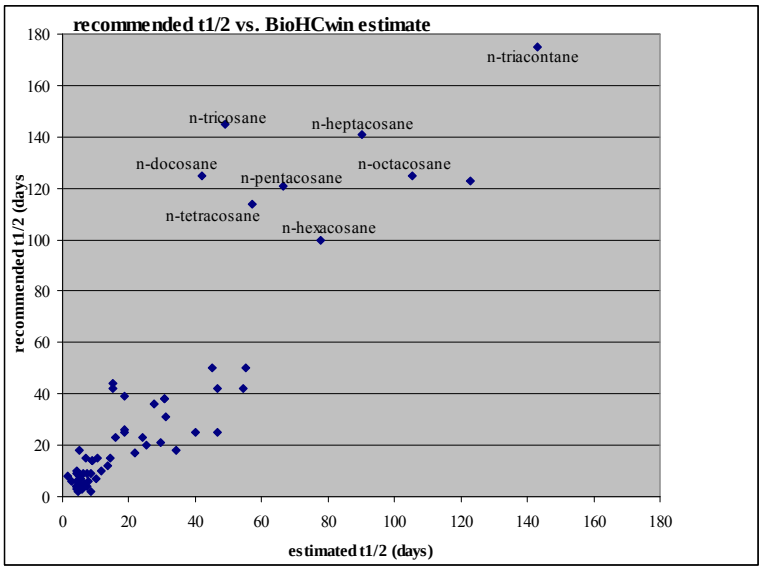

Figure 25. **Comparison of the HCBIOWIN estimated environmental half-life (in days) against the "recommended" half-life taken from the PhysProp database [PhysProp DB]. Individual data are presented in Annex I.**

n-alkenes

Due to the limited information on the degradability of alkenes the performance of the BIOHCWIN model on this group of compounds could be not assessed. Only one alkene (C14) was part of the training data set.

Branched alkanes

Based on a comparison with experimental and predicted half lives it be concluded that the BIOHCWIN does not perform well for branched alkanes. For several branched alkanes the half lives were significantly under-predicted as shown in the Table 8. It was noticed that the data for the branched-alkanes class was particularly variable and appeared to be correlated to increased branching, particularly when it was present at both ends of the carbon chain.

No corrections were made in the BIOHCWIN model to predict the degradability of compounds with quaternary carbons, particularly at an end site, or branched compounds with methyl groups successively located along the carbon chain, as it was considered not trivial to program the model specifically for compounds with these types of structures. Data for branched alkanes with more than 10 carbon atoms (i.e., norpristane, pristane, phytane, and squalane) showed that degradation may be non-linear, however the data were too limited to apply correction fragments to the longer-chain branched alkanes within this structural class. Overall, care must be taken with branched compounds, as it seems their environmental half-lives are generally underestimated.

Based on the data obtained by CONCAWE, the half lives of the short chain branched alkanes are under-predicted with BIOHCWIN ((i.e. branched butanes (factor 4) up to pentanes (factor 1.5), whereas BIOHCWIN over-predicts the half-lives of the longer chain branched alkanes (generally a factor 2 to 4).

Table 8. Comparison of the measured and estimated half lives (BIOHCWIN) for an number of branched alkanes [Howard et al., 2005]

compound	measured half life reported by Howard (days)	Measured half life reported by CONCAWE (days)	Estimated half life (days)
2,4-dimethylpentane	**48**	**9.1**	**5.6**
2,3,3-trimethylpentane	**53**	**13**	**10.3**
2,2,4-trimethylpentane	**40**	**8.4**	**10.9**
2,3-dimethylhexane	**26**	**7.5**	**6.6**
2,5-dimethylhexane	**25**	**6.5**	**6.6**
1,2-dimethylcyclohexane	**18**	**8.1**	**5.1**

Cycloalkanes

Only measured data where available to assess the performance of the model for the monocycloalkanes. Although the predicted half lives by BIOHCWIN were lower than the measured half lives reported by Howard, overall they are comparable with the measured values reported by CONCAWE. For a limited number of compounds, like for 1,2-dimethylcyclohexane the predicted DT50 values are lower than measured (see Table 8).

The half-lives of the tricyclohexane compounds adamantane and diadamantane are very strongly underestimated (residual ~1000 days). As these are the only two tricycle compounds in the dataset (for which a recommended half life is available) it is likely that for this class of compounds the half-life in the environment will in general be grossly underestimated. Also the persistence of Perylene is underestimated significantly (residual 475 days, see Figure 26). However, based on the data obtained by CONCAWE, the BIOHCWIN model seems to predict the half lives in seawater within a factor of 2.

Monoaromatics

In general, the estimated half lives of the simple substituted benzenes (included mono-, di-, tri-, and tetramethyl as well as dimethylethyl, methylpropyl, n-propyl, isopropyl, and isobutyl groups) is rapid (<15 days) which corresponds well with the their experimental half-lives. The measured data in fresh water obtained by Prince et al. [2007] were in most case lower than those predicted by BIOHCWIN.

For the linear long-chain alkylbenzenes (LABs) BIOHCWIN estimates longer half lives than the short alkyl chain benzenes as expected. However like for the n-alkanes there may not be a direct linear increase in DT50 with an increasing number of carbon atoms in the alkyl substituent and estimates will therefore be more uncertain and likewise underpredicted. This might also apply to branched LABs. BIOHCWIN model makes also no distinction between internal and external isomers of LABs, whereas several investigations showed that external LAB isomers are more rapidly biodegraded than internal LAB isomers [Takada, 1990].

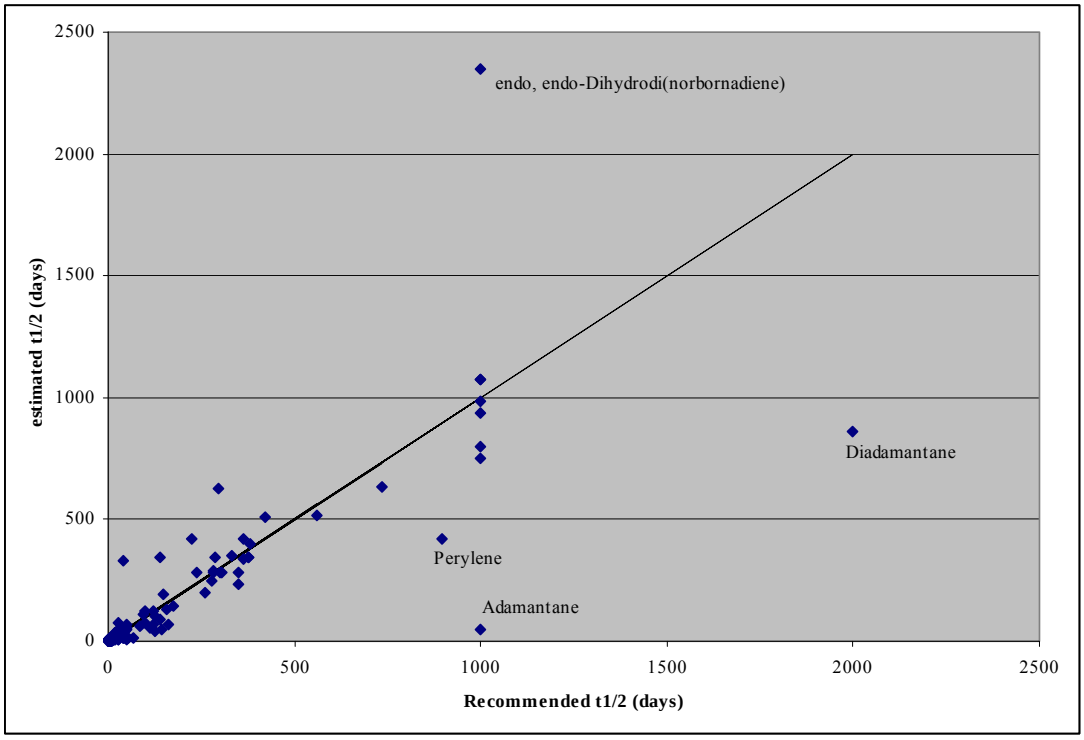

Figure 26. **Comparison of the HCBIOWIN estimated environmental half-life (in days) against the "recommended" half-life taken from the PhysProp database, for all substances in the CONCAWE library which have recommended half-lives. Individual data are presented in Annex I.**

PAHs

The biodegradation of PAHs has been reviewed in the EU RAR on CTPHT. In general the biodegradation rate decreases with increasing number of aromatic rings. According to Volkering and Breure [2003], two factors are considered responsible for the difference in degradation rate. First, the bacterial uptake rates of the compounds with higher molecular weight have been shown to be lower than the uptake rates of the low molecular weight PAHs. The second and most important factor is the bioavailability of PAHs, due to sorption on suspended organic matter and sediment. Since the Kow and the Koc are strongly correlated, high molecular weight PAHs will degrade slower than low molecular weight PAHs.

Based on their analysis of the experimental data Howard et al. [2005] concluded that in general BIOHCWIN under-predicts the half lives of three-ring PAHs, i.e., the experimental versus estimated half lives was for fluorene 44 versus 15 d; phenanthrene 42 versus 15 d; acenaphthylene 38 versus 7 d; acenaphthene 39 versus 19 d. For the higher ring PAHs the predicted half lives are closer to the experimental data and might even be over-predicted. When comparing the half lives suggested by Mackay *et al*. (1992), which were also used in the risk assessment of CTPHT, the half lives in water of the three ring PAHs are in the same order of magnitude whereas the half lives of the higher ring PAHs are over-predicted.

However, the half lives in soil and sediment are underpredicted for all PAHs (see

Table 9) .

Based on the available data methylation results in an increase in PAH half life C2-, C3-, or C4-PAHs and therefore in BIOHCWIN a correction factor was added for methylated PAHs. For polycyclic aromatic hydrocarbons with alkyl substitutions other than methyls no additional corrections were made. The half-lives for benzothiophene, dibenzothiophene and its alkylated derivatives and C1-DBT, C2-DBT and C3-DBT naphthobenzothiophene were reasonably well predicted. The half lives of the alkyl-substituted naphthobenzothiophenes were, however, considerably underpredicted, i.e. the predicted half-lives for methyl-, dimethyl-, and trimethyl-naphthobenzothiophenes are 16, 28, and 51 d versus 66, 87, and 125 d, respectively. This discrepancy occurs because the methyl substituent is being assessed as an aromatic mono-CH3 (four or more fused rings).

Biphenyls

Only limited data are available, though the data for biphenyl and 4-methylbiphenyl were considered sufficient to add a separate fragment to the model. Whether this is appropriate to estimate the half-lives for multiple methylated biphenyls is difficult to determine, though based on the data obtained by Prince et al. [2007 and 2008] BIOHCWIN generally provides higher half lives in surface water than measured.

Naphtheno-aromatic structures

The few data available for this group were mainly for a small group of tetralins and several mono-aromatic and tri-aromatic steranes. For the tetralins a corrective fragment was incorporated, though based on the half lives determined by Prince et al. [2007 and 2008] in surface water, BIOHCWIN under-predicted the half lives of tetralins (approx 1.5 versus 3.5 days).

Evaluation

For the simple structural molecules, like the linear alkanes up to C12, the mono-aromatics and PAHs, the BIOHCWIN seems to give a reasonable prediction of half lives in surface water. However, for the more lipophilic and complex compounds, especially the branched petroleum compounds, the predictions are less certain and most often the half lives are *under*-predicted. Howard et al. [2005] also acknowledged that the available data were for relatively simple compounds, and that the BIOHCWIN should be used with caution in predicting half-lives for compounds that are considerably more complex. However, according to the authors the BIOHCWIN model should function well for those compounds expected to be present in the greatest abundance in petroleum and related products, because these typically are compounds without great structural complexity. The validity of this statement will be discussed in work package 3.

Table 9. Comparison of the degradation half lives of PAHs between those used in the EU RAR for CTPHT [EU, 2008] and estimates by BioHCWIN [Howard et al. 2005].

Compound	Water	Soil	Sediment	BioHCWIN water/soil Days	BioHCWIN sediment
Naphthalene	4-13 (7)	42- 125 (71)	125 – 420 (229)	5.6	22.4
Acenaphthene	4-13 (7)	42- 125 (71)	125 – 420 (229)	19	76
Acenaphthylene	4-13 (7)	42- 125 (71)	125 – 420 (229)	31	124
Fluorene	13 -42 (23)	125 – 420 (229)	420 – 1250 (708)	15	60
Anthracene	13 -42 (23)	125 – 420 (229)	420 – 1250 (708)	12	49
Phenanthrene	13 -42 (23)	125 – 420 (229)	420 – 1250 (708)	15	60
Fluoranthene	13 -42 (23)	420 – 1250 (708)	> 1250	191	764
Pyrene	42- 125 (71)	420 – 1250 (708)	> 1250	284	1136
Benzo(a)anthracene	42- 125 (71)	420 – 1250 (708)	> 1250	344	1376
Chrysene	42- 125 (71)	420 – 1250 (708)	> 1250	344	1376
Benzo(a)pyrene	42- 125 (71)	420 – 1250 (708)	> 1250	422	1688
Benzo(b)fluoranthene	42- 125 (71)	420 – 1250 (708)	> 1250	285	1140
Benzo(k)fluoranthene	42- 125 (71)	420 – 1250 (708)	> 1250	285	1140
Benzo(ghi)perylene	42- 125 (71)	420 – 1250 (708)	> 1250	517	2068
Dibenzo(a,h)anthracene	42- 125 (71)	420 – 1250 (708)	> 1250	-	-
Indeno(1,2,3-cd)pyrene	42- 125 (71)	420 – 1250 (708)	> 1250	349	1396

Intermedia extrapolation factors for (bio)degradation

Howard et al. [2005] did not make clear from which compartment the data were obtained and whether any differences in degradation between compartments were observed. Based on a comparative analysis of biodegradation data from grab samples by Boethling et al. [1995] the HBM tool applies an intermedia factor of 1:1:4 to the biodegradation half-lives in water:soil:sediment. It should however be noted that Boethling et al. did recommend to set the degradation rate in surface water equal to that in soil only for screening purposes. Based on their analysis the ratio between surface water and soil is 1.6. It should also be noticed that the data set used by Boethling et al. was very limited (n=14) in which only two petroleum compounds were included. Therefore we see no reason to deviate from the standard ratio 1 : 2 taken in the REACH guidance in the case that half lives in surface water are sufficiently reliable. This ratio should then be further adjusted based on the Kp value (see table R16-8 of the REACH guidance). We also believe the data set for the extrapolation from surface water to sediment is too limited and not specifically related to petroleum compounds. Assuming that degradation of petroleum compounds is mainly due to aerobic processes, it seems more appropriate at this stage to apply a ratio of 1 : 20 as applied in EUSES and recommended in the REACH Guidance, assuming that the fraction of the sediment compartment that is aerobic is only 0.1.

Degradation half live estimates for activated Sludge waste water Treatment Plants

In order to estimate the half lives in an STP, experimental total removal values from STPs were obtained from the open literature and used to back-calculate biodegradation half-lives using the SimpleTreat model [Aronson et al., 2005; Howard et al., 2005]. Based on a regression of these SimpleTreat-derived biodegradation half-lives and the BIOHCWIN biodegradation half-life the following equation was determined which was considered suitable for PetroRISK for the correction of environmental half-lives to an appropriate half-life in an STP:

$$\text{Log SimpleTreat biodeg T1/2(hrs)} = 1.15\,[\log \text{ENVbiodeg T1/2(hrs)}] - 2.56 \qquad \text{Equation 1}$$

The selection of representative total removal values was based on a statistically-determined percent total removal value from the range of collected values. Selection based on characteristics of the operating plant was thought to be less representative in view of the limited information on the design and operation of the treatment plants and the influent pollutant loadings. The majority of the studies also reported very low influent concentrations.

Percent total removal values reported as >0 to >89% were not used in the determination of a representative total removal value; only values reported as >90% or higher were included. These data show that for these compounds, the median percentage total removal value is generally higher than the average value. Therefore, when differences were large between these values a biodegradation half-life range was determined and the average was ultimately used in the regression analysis.

For the back calculation two models were used: the STPWIN Model and SimpleTreat. The first model was not considered suitable because of the limited number of compounds on which this was modeled, which were also expected to exhibit rapid biodegradation in an activated sludge treatment plant. The SimpleTreat v3.11 model was run as a 9-box model (with primary sedimentation), surface aeration, and the assumption that biodegradation occurs in both water and solid phases (Method II).

It was noted that for several compounds, notably xylene, benzene, toluene, and ethylbenzene, it was difficult to derive an appropriate average half life in an STP. Even with the input of a first-order rate constant of 0 $hour^{-1}$, signifying no biodegradation, the lower total removal value for benzene, xylene, and toluene could not be reached by the SimpleTreat model. For example, benzene had a median total removal value of 92.15% and a geometric mean value of 82.34%. The input of 0 $hour^{-1}$ as the rate constant into the model resulted in a total removal of 88.6% based solely on transport processes. While the model was able to reach the lower total removal value for ethylbenzene, the model output showed that nearly 83% of the removal was due to release to air and this was not felt to be a reasonable estimation given the relative ease with which this compound is biodegraded under aerobic conditions.

Data were most commonly found for compounds such as benzene, toluene, ethylbenzene, xylene, and several PAHs. A major weakness of the collected data is the presence of only a single data point for 58 of 88 compounds. Of the 58 compounds with only a single total removal value in an STP, 51 of 58 compounds had total removal values >99% and only 2 of 58 compounds had removal values <90%. In comparison, there were 103 values for the total removal of toluene in an activated sludge plant. These values ranged from 0 to 100% removal and median and average values of 95 and 85% total removal, respectively, were calculated from these data.

In order to determine whether SimpleTreat was adequately estimating the biodegradation of a compound in an activated sewage treatment plant, a comparison was made of the SimpleTreat estimated data with experimental data where researchers had measured removal in terms of individual processes. If the model is working well, these data would be expected to be similar. However, only 6 of 88 compounds in the database had information on removal separated by process. Based on this analysis (see appendix) the authors concluded that using SimpleTreat as a means to obtain biodegradation rate constants from experimental total removal values is a relatively crude process. However, very few compounds have experimental data separating individual loss processes and the available data suggest that using total removal values to back-calculate biodegradation half-lives is reasonable in most cases.

Only for a limited number of compounds both SimpleTreat and environmental biodegradation half-lives were available. The equation shown above was finally based on 22 compounds (see Figure 27). The majority of these compounds were monoaromatics and PAHs.

Evaluation

The method to derive half lives in an STP based on half lives in the environment has a number of weaknesses which creates a large uncertainty in the determining the fate of petroleum compound in an STP and consequently in the estimation of the local surface water concentration when petroleum compounds are indirectly discharged via an STP.

First, the final data set on which the equation is based is very limited: the majority of the substances were monoaromatics and PAHs. Unfortunately, we do not have excess to the raw data, but the data point look very scattered, which means that the confidence limits should be high. Furthermore, no branched compounds were included which are known to degrade slower than the unbranched compounds. As discussed above, BIOHCWIN might underestimate the degradation half lives of the short chain alkanes and branched alkanes and consequently also the half lives in the STP are likely to be under-predicted.

Second, the validation of the predicted half lives in the STP was limited to only a few compounds, all having a relative low log Kow (i.e. benzene (log Kow 2), toluene (log Kow 2.5), ethylbenzene (log Kow 3.1), xylene (log Kow 3.1), Naphthalene (log Kow 3.3), anthracene (log Kow 4.7)).

These compounds are not expected to adsorb to sludge as strong as compounds with a log Kow > 5. Therefore there is little evidence that the back calculation would also fit for compounds where the total removal will be much more determined by adsorption to sludge.

Third, the variability of the removal of the substances that were used to validate the predicted half lives in an STP was in most cases very high (i.e benzene, toluene, ethylbenzene, xylene). It is therefore somewhat surprising that precisely based on these compounds it was concluded that this approach can provide a reasonable estimate of biodegradation in a sewage treatment plant.

Overall, based on the data available it is difficult to estimate to which extent the half lives in the STP could be underpredicted by the Petrorisk model. At present the approach is considered too premature and too crude to provide reliable half lives in an STP.

Alternatively, for those substances where reliable half lives in surface water can be predicted a ratio of 1 : 21 would be preferred, as indicated in the REACH guidance (see section R16.4.4.4 and R16.4.4.5). This is based a.o. on differences in microbial activity, corresponding to the dotted line in Figure 27 (the solid line is related to the proposed equation of Aronson et al. [2005]).

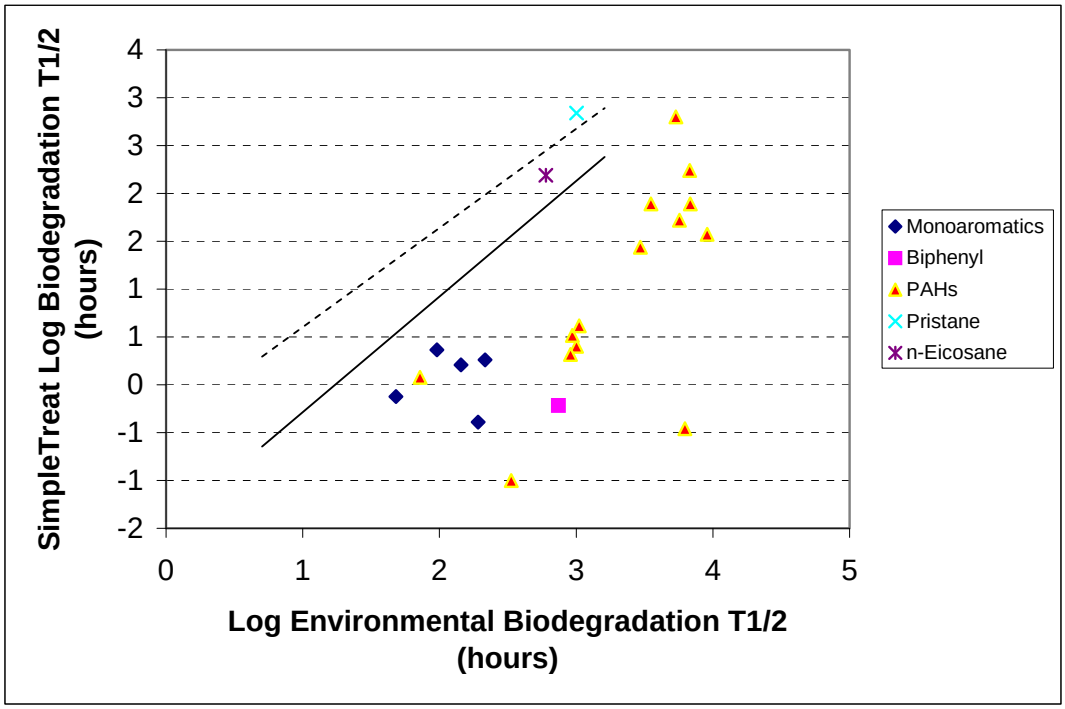

Figure 27. **Comparison of SimpleTreat estimation STP half-lives against environmental half-lives in the aqueous compartment. The solid line represents equation 1, used in the HBM tool. The dotted line represents the ratio between STP half-life and aqueous compartment half-life as suggested by the TGD2003 and REACH Guidance (see Annex 1).**

The difference between the two lines is approximately an order of magnitude (~1:220 as assumed in HBM vs. 1:21 as assumed in the REACH guidance), leading to a tenfold underestimation in the HBM

tool of the half-life in an STP. Although this does not directly translate to a 10-fold lower concentration in the fresh water compartment, it does have a very significant influence on the calculated PECs.

Atmospheric half life

Van der Meent [2008] uses the reaction rate constant for reaction with OH-radicals to calculate a half life in hours, assuming a (24 hour average) concentration of 5E5 OH-radicals/cm3 (as has been determined through back-calculation from the reported half-lives in the HBM tool). The value of the rate constant used is the experimental rate constant if this is present in the PhysProp database, otherwise the AOP1.92a (sep 2008) QSAR model is used to generate a prediction.

The AOP program allows the user to select 12 or 24 hour time frames and any average hydroxyl radical concentrations, but the default is originally set at 1.5 x 10^6 molecules (radicals)/cm3 per 12-h of daylight. Twelve hour daylight OH radical concentrations are reasonable for fast reacting chemicals but for chemicals that react more slowly (> a few days) 24 hour averages might be more appropriate. Atkinson [1985] suggested seasonally and diurnally 24 hour averaged hydroxyl radical concentrations at 298 K of:

5 x 10^5 molecules/cm3 in the northern hemisphere, and
6 x 10^5 molecules/cm3 in the southern hemisphere

In the HBM the more conservative value (leading to longer half-lives in the atmosphere) for the OH-radical concentration in the atmosphere of 5x10^5 molecules/cm3 has been chosen.

Atmospheric degradation due to ozone degradation (of relevance specifically for olefins and acetylenes) is also taken into account, using a 24-hour average concentration of ozone molecules in the atmosphere of 7 x 10^{11} O3/cm3. An experimental value for the rate constant for reaction with ozone is used if present in the PhysProp Database, otherwise the AOP v1.93a (sep 2008) QSAR estimate (for olefins and acetylenes only) is used.

Degradation due to reaction with NO3-radicals (nighttime atmospheric degradation) is not taken into account.

No comparison of the half-lives in the atmosphere with actual observed half-lives has been made, as such observed data is not available.

3.1.5 Uptake routes

The uptake of nonpolar organic chemicals with Log Kow < 5 by fish is primarily from the water (via the gills), while it can be assumed that for substances with a log K_{ow} > 5, which are not metabolised, uptake from environmental sources other than water (e.g food and sediment) might contribute to the bioaccumulation in higher organism. The same is likely true for benthic invertebrates [Belfroid et al., 1996; Loonen et al., 1997; Meador et al., 1995; Neff, 2002]. In order to take the uptake route via ingestion of sediment and soil particles into account, an additional safety factor of 10 for very hydrophobic compounds is often used when assessing the risk for soil and sediment dwelling organisms based on equilibrium partitioning (EP) [ECHA, 2008].

Due to their hydrophobic nature, petroleum compounds (including PAHs) have a low aqueous solubility and a high affinity for adsorption to soil and sediment organic matter and are therefore preferentially associated with carbon phases of particles. The extent to which petroleum compounds accumulate in a sediment- or soil dwelling organisms depends primarily on the ratio of the petroleum compounds uptake rate to the depuration rate, the capacity of the organism to metabolize petroleum compounds, the mobility and habitat of the organism, and various physicochemical properties of the individual compounds.

Hydrocarbons in solution in sediment porewater are more bioavailable and toxic to sediment-dwelling organisms than hydrocarbons adsorbed to sediment particles. For low molecular weight hydrocarbons (log Kow < 5.5) with high solubility, sufficient levels of hydrocarbons are present in the porewater to allow rapid bioaccumulation across external permeable membranes or across the gut epithelium when particles and pore water are ingested. In the gut, "solubilization" of particulate hydrocarbons is aided by surfactants and enzymes secreted by the animal [Mayer et al., 1996]. Voparil et al. [2004] showed that the gut fluids of *A. marina* solubilize much greater concentrations of PAHs from some anthropogenic particles than are available to water. This enhanced exposure likely is due to surfactant micelles in the digestive fluids of this animal. For high molecular weight hydrocarbons (log Kow> 5.5) with low solubility and high affinity for particles, few hydrocarbons are able to partition from the particles into bulk pore water. However, if hydrocarbon-contaminated sediment particles come into direct contact with permeable epithelia (e.g., gill, gut epithelium), some high molecular weight hydrocarbons may dissolve in the thin film of water between the particle and membrane surfaces and partition into the membrane.

In theory gastrointestinal uptake can lead to body residues greater than those predicted by Equilibrium Partitioning (EP) due to an increase in the fugacity of the chemical in the GIT, caused by a decrease of the sorption capacity of the food upon passage through the GIT (digestion of sorption sites: lipids and organic matter) and compaction (a decrease in the volume of the gut contents due to food absorption). Whether this process actually leads to greater body residues depends on the magnitude of the fugacity increase and the magnitude of the elimination rate in the feces compared to the other elimination routes [Gobas et al., 1993]. It is often assumed that EP concerns only uptake from pore water through the skin. However, Jager [2003] argued that the fact that steady-state body residues are not expected to deviate too much from the EP estimate does not mean that the route across the gut wall is not important. In fact, his data with *Eisenia andrei* showed that the gut route was the dominant route of exposure. The same conclusion was drawn for PAHs in sediment oligochaetes [Leppänen and Kukkonen, 1998; Lu, 2004].

Jager [2004] explained that the highest deviation from EP in any species and for any chemical can be predicted when Organic Carbon (OC) digestion is high, elimination across the outer skin is slow (to keep the fugacity in the organism above that of the soil), and retention time is not too long (to ensure sufficient refreshment of the GIT). However, digestion and retention time are likely to be inversely related [Willows, 1992]. Thus, even though digestion may be high in a species like

the litter-feeding earthworms of deposit feeding sediment organisms, the long retention time may prevent the organisms from exceeding the equilibrium estimate. Geophageous species like *A. rosea* seem adapted to a lifestyle of eating their way through soil with a high throughput and a low digestion efficiency. This implies only a limited increase of the fugacity in the GIT and therefore a limited additional uptake via this route. Given the reported data for digestion and retention times, and using extreme values for the chemical-specific parameters, it is highly unlikely that body residues will exceed the EP estimate by a factor of 1.5 for any chemical or any species of earthworm.

As mentioned in the EU RAR, the origin of the organic carbon to which the PAHs are associated may have its influence on the partition coefficients and the kinetic rate of desorption. In particular combustion soot or nonaqueous phase liquid, such as petroleum, creosote, or tar are known to reduce the bioavailability of the organic compounds such as PAHs [DiToro et al., 1991; Hansen et al., 2003; Thorsen et al., 2004, Neff et al., 2005]. In this way, strong sorbing carbonaceous materials may limit the bioavailability of PAHs to soil and sediment species more than amorphic organic carbon does, on average. Especially the role of carbonaceous materials such as black carbon, coals and kerogen is subject of discussion. This has been reviewed extensively by Cornelissen et al. [2005] and Koelmans et al. [2006]. The higher partition coefficients to black carbon indicate that soot-like materials may have a major influence on the bioavailability to soil and sediment species. The effect of the sorption to carbonaceous materials on uptake of PAHs by biota is still unclear. Where some studies show that uptake of PAHs is significantly decreased in the presence of carbonaceous materials, others show that this effect is not present or negligible (see for more information the EU RAR on CTPHT).

The last years, more evidence becomes available that sorption of organic chemicals into soils and sediments can be better described by a two-phase model. This model assumes that two main types of organic carbon exist: amorphous organic carbon, with a linear sorption, and black carbon (or carbonaceous geosorbents) with non-linear (Freundlich) sorption [Cornelissen et al., 2005]. A model to describe a two-phase system is that of Bucheli and Gustafsson [2000] and Accardi-Dey and Gschwend [2003]:

$$K_{PM} = K_{POC} f_{POC} + K_{BC} f_{BC} [PAH]^{(n-1)}_{Dissolved}$$

From several studies [Burgess *et al*., 2004; Lohmann *et al*., 2004; Vinturella *et al*., 2004; Jonker and Koelmans, 2001] it appears that the partition coefficients to soot-like particles (black carbon) (K_{BC}) are much higher than the partition coefficients normalised to the total of organic carbon in the sediment or soil (K_{oc}). These values for K_{BC} are a factor of 10 to 59 higher than the values used in the risk assessment, except from the data by Jonker and Koelmans [2001], which are 59 times higher than the values used in the risk assessment, but only 3.5 to 22 times as high as the K_{oc} values for amorphous organic carbon determined in the same way. Overall, the partitioning to carbonaceous materials can be up to 60 times higher than the partitioning to the commonly used organic carbon.

Overall, we believe that when toxicity data for sediment and soil organisms are not available these can be estimated from the aquatic toxicity data using equilibrium partitioning based on the Koc values derived with the one-phase model proposed by Karickhoff et al. [1979], which incorporates field-derived sediments with mixtures of all types of organic carbon (including both black carbon and amorphous organic carbon). Based on the conservative nature of these Koc values (lower Koc values, resulting in a higher bioavailability) and the arguments given by Jager, the use of an additional assessment factor for substances with a log Kow > 5 could be reconsidered. As this will not affect the RA of petroleum compounds only, this needs to be discussed in a broader context of REACH guidance before a decision can be made to deviate from the current guidance.

Biomagnification

The risk for secondary poisoning of petroleum compounds in the HBM model is based on the same approach as recommended in the REACH guidance. The risk to (marine) predators is calculated as the ratio between the concentration in their food (marine) fish and the no-effect concentration for oral intake (PNECoralpredator). The concentration in the fish (Cfish) is obtained from the BCF and for very hydrophobic substances an additional biomagnification factor (BMF) as a result of bioaccumulation from the food the fish consumes (which consists of different types of aquatic organisms). To assess the risk to marine top-predators an additional biomagnification factor (BMF2) is applied.

By establishing these factors it is assumed that a relationship exists between the BMF, the BCF and the log Kow. Although this might still be valid for water-respiring organisms, more and more information becomes available that in air-breathing animals biomagnification also occurs with hydrophobic chemicals (e.g., chlorobenzenes, lindane and perfluorinated sulfonic acids), with log Kow < 5 and with BCFs in fish-based experiments below the regulatory criterion of 5000. These findings indicate that very hydrophobic chemicals with a log Kow ≥ 5 are not the only chemicals with biomagnification potential and that lipid-water partitioning does not serve as a universal model for identifying bioaccumulative substances in wildlife and humans. As indicated by Kelly et al. [2007], in water-respiring organisms, elimination becomes sufficiently slow to cause biomagnification if the log Kow of the chemical exceeds ~5. In the air-breathing organisms, this occurs for chemicals with a high log Koa (≥6), which causes slow respiratory elimination, and a log Kow > 2, causing slow elimination in urine or nitrogenous wastes. Although these findings have not yet been implemented in the REACH guidance, it was considered worthwile to investigate whether the CONCAWE library contain petroleum compounds which might not biomagnify based on their log Kow but do have a log Koa > 6 which give them the potential to biomagnify in air-breathing animals.

A biomagnification factor (BMF) is used to account for accumulation through dietary uptake for aqueous species in the CONCAWE HBM (variable named BMF1 in the HBM). Biomagnification for airbreathing mammals is taken into account using the same biomagnification factor (variable named BMF2 in the HBM).

The assumptions made for accounting for biomagnification in the HBM model are as follows:

if	BCF< 2000	then	BMF = 1
if	2000> BCF >5000 and log Kow < 8	then	BMF = 2
if	2000> BCF >5000 and log Kow > 8	then	BMF = 3
if	BCF > 5000	then	BMF = 10

This is according to the REACH Guidance recommendations, and the same assumptions are implemented in EUSES.

The number of substances in the CONCAWE library of 1518 substances that fulfill these different criteria are:

BMF=1	1055 substances
BMF=2	193 substances
BMF=3	0 substances
BMF=10	270 substances

Should K_{oa} be accounted for in biomagnification for air-breathing species?

The BMF as a property which could serve to indicate potential bioaccumulation in air-breathing mammals on higher trophic levels (BMF2 in the HBM), is often regarded to be a function of the octanol-air partitioning coefficient [Kelly et al., 2007]. The quantitative relationship derived by Kelly et al. [2009] is:

$$BMF = -0.753 \cdot (\log K_{oa})^2 + 13.0 \cdot \log K_{oa} - 46.2$$

This Koa dependent BMF is visually compared to the BMF value used in the CONCAWE HBM (the BMF2) in Figure 28

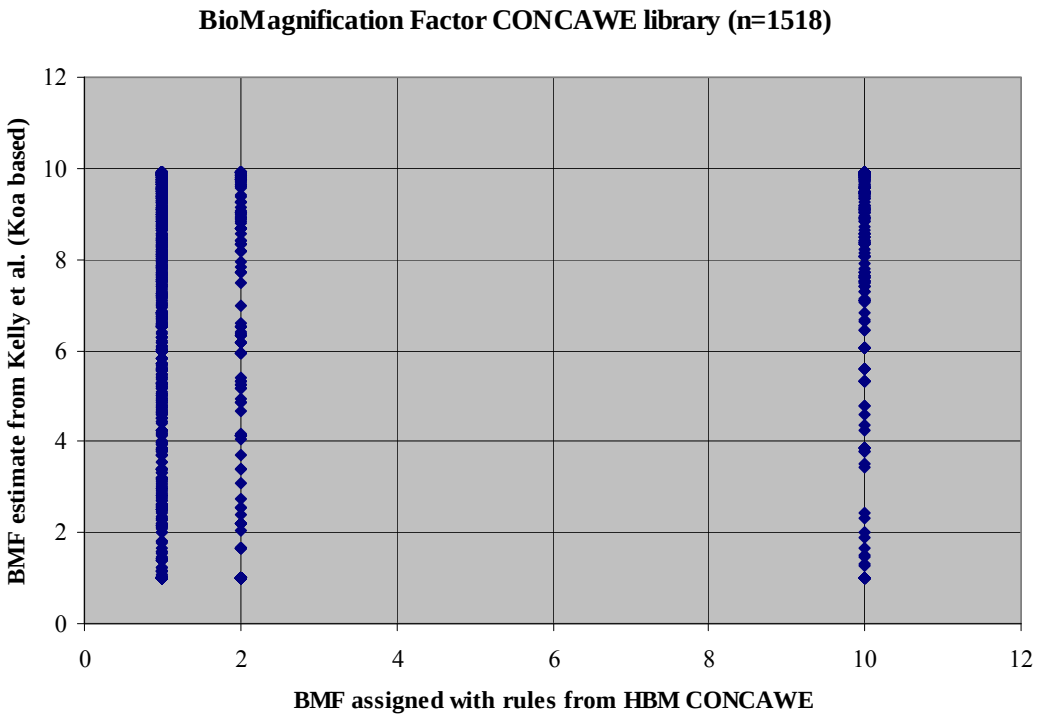

Figure 28. **BioMagnification Factor (BMF) as estimated based on Kelly et al. [2009] versus BMF as applied in the HBM tool, and as recommended in TGD2003 and REACH Guidance. Individual data is presented in Annex I.**

It is observed that substances in the CONCAWE library with an assigned BMF of 1 are calculated to have BMFs calculated from their (estimated) log Koa anywhere between 1 and 10. The same is true for substances with an assigned BMF of 2 and 10.

Kelly et al. [2007] state that there should be an extra concern for those substances with high log Koa (i.e. > 6), but log Kow below 5 (but still above 2). By examining both the log Koa and log Kow values of all substances in the CONCAWE library it is determined how many substances in the library would give rise to this extra concern for biomagnification in air breathing animals. As can be seen in figure 29, there are few substances with these specific properties.

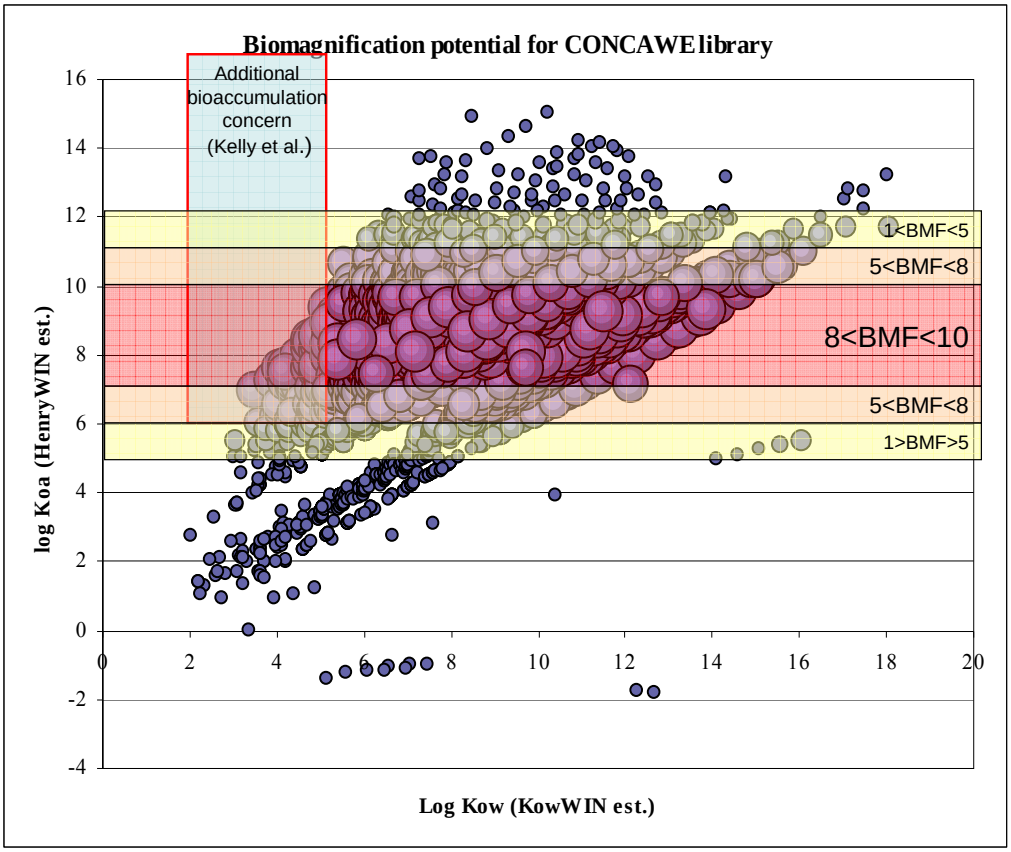

Figure 29. **Octanol-air partitioning coefficient against the octanol-water partitioning coefficient for the complete CONCAWE library of 1512 substances. In the graph the area of concern [Kelly et al. 2007[is indicated, as well as the areas of high biomagnification potential.**

In effect there are 16 substances (~1%) in the 1518 substance library which have log Koa>6 and 2<log Kow<5, the borders specifically mentioned by Kelly et al [2007]. These 16 substances, with their calculated BMF based on the equation from Kelly et al. [2009] are given in Table 10.

The substances mainly concern PAHs (anthracene, phenantrene, fluorene acenaphthene, substituted naphthalenes, pyrene), one biphenyl and one highly substituted benzene. The estimate of the BMF based on log Koa from Kelly et al. yields an estimated average BMF for these substances of 6.85. It seems that for the polyaromatic hydrocarbons in general the HBM tool (but also risk assessments performed with EUSES) might give rise to an underestimation of their bioaccumulating behaviour in air breathing mammals and/or at higher trophic levels.

Table 10. **16 substances from the CONCAWE library with log Koa>6 and 2<log Kow<5, which could give rise to added concern about their biomagnification behaviour in air breathing mammals.**

Nr.	BMF HBM	BMF Kelly	Substance Name
1	1	4.88	Acenaphthene
2	1	6.75	Fluorene
3	1	4.84	2-Ethylnaphthalene
4	1	8.45	Dibenzothiophene
5	1	4.70	2,4-Dimethylnaphthalene
6	1	4.78	2,8-Dimethylnaphthalene
7	1	8.13	Anthracene
8	1	8.41	Phenanthrene
9	1	8.93	Naphtho[21b]thiophene
10	1	5.84	1,2,3,4,5-Pentamethylbenzene
11	1	6.02	4-Methylbiphenyl
12	1	5.70	2,3,6-Trimethylnaphthalene
13	1	5.70	2,3,7-Trimethylnaphthalene
14	1	9.89	4-Thiacyclopenta[def]phenanthrene
15	1	9.76	Pyrene
16	1	6.82	1,2,3,4,5,6-Hexamethylbenzene

3.2 WP2. Target Lipid Model

3.2.1 History and overview of the target lipid model

The target lipid model (TLM) was developed by Di Toro and co-workers [Di Toro et al 2000a, b]. The model is a QSAR based model for narcosis type of chemicals that is based on the general accepted assumption that partitioning into lipophilic tissues such as cell membranes (the target lipids) is the determining factor in the toxicity of this type of chemicals. It was assumed that partitioning in target lipids is the same for different species. The difference in sensitivity for different species is related to the concentrations in these target lipids species at which critical effects occur, i.e. the critical target lipid body burden (CTLBB).

The approach that is followed is shortly described here. Log-transformed acute toxicity data for several species and compounds are expressed as a function of log K_{ow} with an equal slope but with (possibly) different intercepts for each species. On top of that, some correction factors were fitted for a few chemical classes that were slightly more toxic than standard baseline toxicants (narcotic chemicals). The intercept is regarded as the logarithm of the CTLBB.

In the original version of the target lipid model, a final acute value (FAV) is derived from the genus mean acute values (GMAV) from the set of CTLBBs for 33 species. Only for one genus (*Daphnia*) more than one species was available. Therefore, the final acute value of 35.3 $\mu mol/g_{octanol}$ is the 5th percentile of 31 genera. The final chronic value (FCV) is derived from the final acute value by dividing it by the acute to chronic ratio (ACR). For this purpose, the geometric mean of the presented ACRs. It should be noted that this value could not be exactly reproduced from the data presented (geometric mean of 4.97 instead of 5.09).

In later publications, the derivation of an acute and a chronic HC_5s (hazardous concentrations to 5% of the species) was introduced [McGrath et al 2004; Redman et al 2007; McGrath and Di Toro 2009]. The acute HC5 for a substance with a certain log K_{ow} was calculated from the species sensitivity distribution (SSD) based on the CTLBBs and product of the log K_{ow} and the universal slope for narcosis in combination with the variances herein. For the chronic HC_5 this calculation is extended with the ACR and its variance. The equation used to calculate HC_5s is:

Equation 2

$$\log(HC_5) = \log(K_{ow}) \cdot E[slope] + E[\log(CTLBB)] - E[\log(ACR)] - k_Z \cdot \sqrt{[\log(K_{ow})]^2 \cdot V[slope] + V[\log(CTLBB)] + V[\log(ACR)]}$$

In this equation E represents the mean and V the variance of the bracketed parameters. In the cited studies C^*_L is used as alternative for CTLBB as well, but this is the same parameter. The parameter k_Z is the extrapolation constant that is used in statistical extrapolation to arrive at the HC_5 value. By applying this equation, it is assumed that the universal slope for narcosis, CTLBBs, and the ACR are independent of each other. This assumption will be further evaluated below for the combination of the universal slope and the CTLBBs. In this equation the chemical class correction (Δc_i) is not included. In McGrath et al. [2004] it is introduced as a fixed parameter to correct the HC_5. It could be argued if this is correct and if the variance in the chemical class correction should be included in the calculation as well.

The model was subject to development over the years. Addition of new data changed the values of the parameters used in the model. It is clear that such an update of the model will result in different HC_5s as well. As shown in Table 11, the height of the acute to chronic ratio and the correction factor for the chemical classes are the parameters that were subject to the biggest changes.

The acute to chronic ratio was initially based on data for all baseline toxicants, except algae [Di Toro et al 2000a] which resulted in a geometric mean value of 5.09. Then, some data for algae were added, reducing the geometric mean to 4.47. By taking only aliphatic hydrocarbons, monoaromatic hydrocarbons and polycyclic aromatic hydrocarbons into account, the geometric mean of the value was reduced to 3.83. Because of the lower number of ACRs (29), the extrapolation constant k_Z was raised to 2.30. Remarkably, this higher value for k_Z was used by Redman et al. [2007] as well, although the old value of 4.47 for the geometric mean of the ACR was still used.

Table 11. Development of the parameters of the target lipid model. Parameters are presented as mean ± standard deviation

Parameter	Di Toro et al. 2000a	McGrath et al. 2004	PetroTox User's guide	Redman et al. 2007	McGrath and Di Toro 2009
Universal slope	-0.945±0.014	-0.945±0.014	-0.936±0.015	-0.936±0.15	-0.936±0.015
Final acute value	35.3	36.2			
Final chronic value	6.94				
mean CTLBB [a]		137		119.0	119
log CTLBB		2.14±0.28	2.13±0.12	2.076±0.335	2.076±0.335
ACR [a]	5.09±0.95	4.47	4.47	4.47	3.83
log ACR		0.650±0.392	0.650±0.355	0.650±0.283	0.583±0.323
Extrapolation constant	fifth percentile	2.21	2.21	2.30	2.3
Corrections (log basis)					
Aliphatic	0	0		0	0
Alcohol	0	0		0	0
Ketone	-0.245±0.059	-0.245		0	0
Ether	0	0		0	0
Halogenated	-0.244±0.033	-0.244		-0.339	-0.339±0.032
PAH	-0.263±0.057	-0.263	-0.352	-0.352	-0.352±0.053
Monoaromatic	0	0	-0.109	-0.109	-0.109±0.034

[a] These parameters represent geometric means.

In Di Toro *et al.* [2007] the same values for the parameters of the target lipid model are used as in Di Toro *et al.* [2000a]. In the PetroTox program file the data from the updated target lipid model [McGrath and Di Toro 2009] have been used. However, the variances of the ACR and the CTLBB are switched erroneously in the PetroTox model. This is probably caused by the order in which they occur in the equation presented in the original study [McGrath and Di Toro 2009]. The variance 0.105 (s.d. 0.323) refers to the 29 presented ACRs and the variance 0.112 (s.d. 0.335) refers to the set of 47 CTLBBs.

The values in the PetroTox model are partly deviating from the values reported in the PetroTox user's guide. The value for the universal slope from the user's guide is the value from the updated version from McGrath et al [2009]. However, the values for CTLBB and ACR are from the former model described by McGrath et al [2004]. The variance of the CTLBB in the user's guide is probably a typing error (0.015 instead of 0.105, i.e. standard deviation of 0.12 instead of 0.28).

Because the mean and variance of the universal slope, the CTLBB, the ACR and the extrapolation constant are parameters that are fixed in the model, it is evident from Equation 2 that the HC_5 is in fact solely dependent on the log K_{ow}. Thus, for any substance or hydrocarbon block, the log K_{ow} alone is sufficient to determine the HC_5.

3.2.2 Mixture toxicity and additivity

Considering that a range of petroleum compounds are emitted simultaneously, there is reason to assess the risk for the mixture and not for the compounds individually. In principle the use of concentration additivity is the correct method if substances of the mixture have the same mode of toxic action and any other interactions are absent. It is assumed that this is the case for petroleum mixtures and other mixtures consisting of baseline toxicants. Therefore, this method has been applied in the target lipid model. It has the additional advantage that it can be applied in a simple way by applying the toxic unit approach. A toxic unit (TU) is defined as the ratio of the concentration in a medium to the effect concentration in that medium. The toxicity of the mixture is the sum of the individuals TUs. Use of the toxic unit concept requires that the dose-response relationships of the individual compounds have similar shapes, which in general holds for compounds with the same mode of action. The additivity of the toxicity of narcotic chemicals has been demonstrated by a number of investigators before and has been applied in the target lipid model [Di Toro *et al*., 2000a,b; McGrath *et al.*, 2005; Di Toro *et al.*, 2007 ; DiToro & McGrath, 2009].

If substances have a dissimilar mode of toxic action, it would be theoretically more correct to apply response addition instead of concentration addition to the data. However, the differences with concentration addition are in general small, and generally concentration addition yields slightly more conservative estimates [Kortenkamp *et al.*, 2009]. Therefore, from a policy point of view, concentration addition could be supported. Apart from that, concentration addition seems indeed to be the right model to apply to petroleum substances, given their apolar, non-specific toxicity in most cases.

3.2.3 Phototoxicity, enhanced toxicity

It can be assumed that toxicity of a large number of petroleum components is mainly caused by narcosis or baseline toxicity. On the other hand phototoxicity is also observed for different petroleum mixtures, which is most likely due to the presence of PAHs which are known to exert an enhanced toxicity due to this mode of action. For example, the toxicity of four petroleum products to larvae of the bivalve *Mulinea lateralis* and juveniles of the mysid shrimp *Mysidopsis bahia* was enhanced under ultraviolet light. This effect was limited in the lightest of the four products, probably due to the absence of PAHs in this product [Pelletier *et al* 1997]. In another study most of 22 petroleum products appeared to be phototoxic to Daphnia magna. Again the presence of phototoxic PAHs explained the occurrence of phototoxic effects [Wernersson, 2003]. For the newt (*Pleurodeles waltl*) genotoxicity of an oil refinery effluent was enhanced due to the presence UV light [Fernandez & L'Haridon, 1994].

However, it appeared that the chronic toxicity of PAHs under less extreme phototoxic conditions, closer to real environmental conditions, is at the same level as the most sensitive phototoxic effects [Verbruggen 2012], see also Figure 33 in section 3.2.4 on the Validation of the Target Lipid Model. Therefore, provided that the chronic toxicity is correctly determined (in the case of the target lipid model, the data underlying the ACRs should be sufficiently protective), then this would

also sufficiently cover the phototoxic effects of petroleum products.Validity of the model for the risk assessment of aquatic organisms

Adaptation of the target lipid model in PetroTox

In order to account for the reduced uptake of very hydrophobic chemicals (log K_{ow}>6) the PetroTox model applies a correction factor. It is stated in the user's guide that the membrane-water partition coefficient (K_{mw}) is equal to the K_{ow} up to a log K_{ow} of 6, but in principle this is not correct. In the target lipid model (McGrath & Di Toro, 2009) the partition coefficient to target lipids (K_{LW}), which can be considered to correspond with K_{mw}, is 0.936 · log K_{ow}. In the target lipid model the slope of 0.936 accounts for the fact that partitioning to target lipids is not equal to partitioning to octanol. In the PetroTox model, a correction to this slope between log K_{mw} and log K_{ow} is made for highly hydrophobic substances. No correction is made until a log K_{ow} cut-off of 6, i.e. K_{mw} = 0.936 · log K_{ow} until log K_{ow} 6. Thereafter, the log K_{mw} stays virtually constant with a slope of 0.037 · 0.936 instead of 0.936. This is indeed in accordance with the statement from the PetroTox user's guide that log K_{mw} is in between 5.5 and 6.0 even at very high log K_{ow} values. No information is given on the origin of the value 0.037.

In Figure 30 this relationship between log K_{mw} and log K_{ow} is depicted. It appears that the estimated log K_{mw} by PetroTox is indeed closely following the experimental data for membrane-water partition coefficients that were summarized before [Verbruggen 2004; Verbruggen et al 2008]. In contrast to these publications, PetroTox does not use a continuous function for log K_{mw}, but instead uses a cut-off at log K_{ow} 6. As a consequence, the relative toxicity of substances with a log K_{ow} of 6.5 to 9 could be slightly underestimated (up to a factor 2.5). According to personal communication with CONCAWE, this cut-off level has been raised in the PetroRisk model, which would results in higher K_{mw} values for the highly hydrophobic substances.

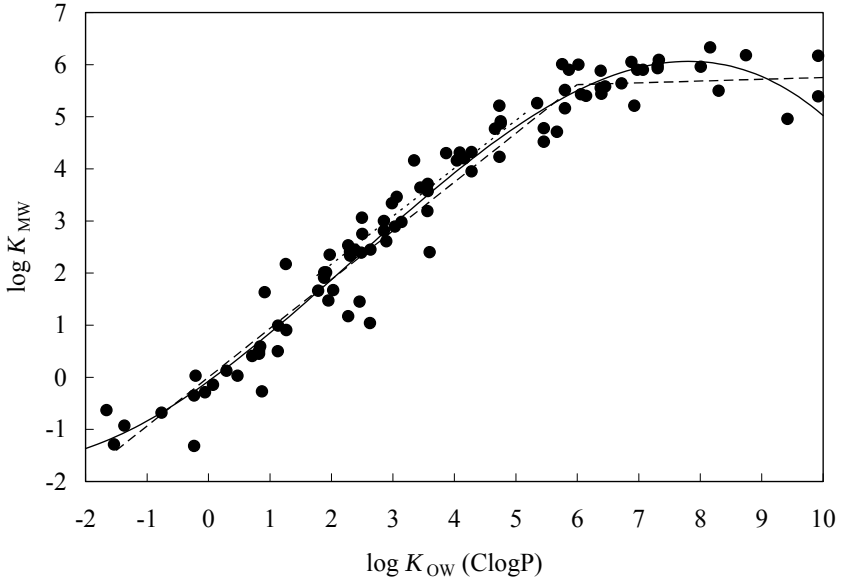

Figure 30. **Partition coefficients to artificial dimyristoylphosphatidylcholine membrane vesicles (K_{MW}) as a function of n-octanol-water partition coefficients (K_{ow}) from Verbruggen et al 2008 with —— best fit polynomial function; – – – PetroTox; ------- Verbruggen et al 2000b.**

The justification of such deviation from linearity is difficult. Several explanations can be given, including experimental artifacts. However, it should be noted that for the assessment of bioaccumulation within the context of the PBT assessment within REACH a similar plot was constructed based on data from bioaccumulation tests [see REACH guidance R11 and section on bioaccumulation of this report]. These data show a similar pattern, but the maximum value is already obtained at log K_{ow} 6.6, while the membrane-water partitioning peaks at log K_{ow} 7.7 (see Figure 30). In general, it seems justified to state that accumulation of extremely hydrophobic substances is limited and that the linear relationship with log K_{ow} breaks down at some point.

To calculate a chronic HC5 in the target lipid model the uncertainty (variance) in the universal slope for narcosis is needed. The modification in the PetroTox model results in a slope different from the universal slope for narcosis in the range up to log K_{ow} 6. It is not mentioned how the uncertainty in the slope is calculated for substances with a log K_{ow} in excess of 6.

Calculation of critical internal concentrations

In the TLM the internal concentrations are used as a metric for toxicity. This assumption is one of the key aspects for narcosis type of toxic action. To calculate the critical target lipid body burden, the logarithm of the LC50s is plotted as function of log K_{ow}. The assumption is that the intercept in this plot is equal to the critical target lipid body burden (CTLBB), which is only true if the intercept of the relationship between log K_{LW} and log K_{ow} is zero.

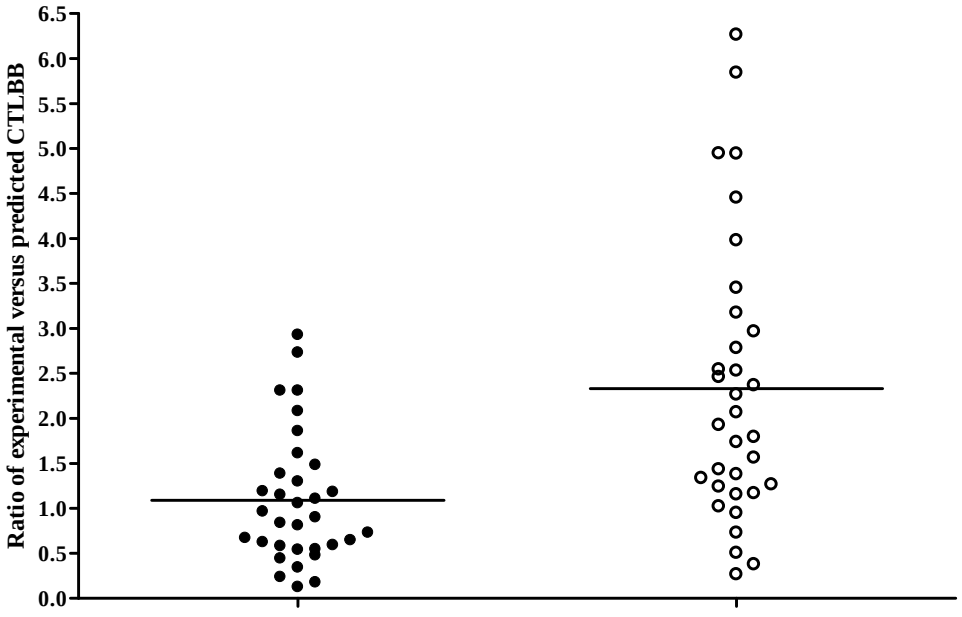

Figure 31. **Ratio between experimental critical target lipid body burden and values predicted values by the target lipid model. Chemical class corrections were applied to all values (0.458 for halogenated compounds, 0.445 for polycyclic aromatic hydrocarbons and 0.778 for monoaromatic compounds [McGrath et al. 2009]. Open symbols represent the values as**

presented by McGrath et al. [2009]. Closed symbols are corrected $10^{0.33}$ to account for the intercept between log K_{ow} and log K_{mw} .

For artificial membrane-water partition coefficients a higher intercept of 0.33 ± 0.05 was found [Verbruggen et al. 2000b]. It appears that the experimental values for critical body burdens as quoted by DiToro et al [2000a] after application of the relevant chemical class correction correspond better with the derived CTLBBs if these values are multiplied by $10^{0.33}$. This is shown in Figure 31.

In principle this is not particularly important for the concentrations recalculated to environmental media such as water. However, the fixation of the intercept between log K_{ow} and log K_{LW} has implications for the calculation of the HC5 if only the slope is assumed to vary, while variance in the intercept is neglected (see below on the evaluation on the equation to calculate the HC5).

Validation of lethal loadings

Lethal loadings (LL50s) are a measure of effect, i.e. 50% lethality, based on the loading of a product to water. Only a part of the loading will actually dissolve into the water. LL50s are used as a measure of toxicity in the framework of Classification and Labeling.

Raoult's Law is used to estimate oil-water partitioning. As such it is only used in the calculation of dissolved concentrations for systems where oil-water partitioning is important, e.g. laboratory toxicity tests with water accommodated fractions (WAF) and is mainly used in the hazard classification. When petroleum products are released in the environment, their compositions change. In the risk assessment of these products this is covered by fate modeling in the hydrocarbon block approach. PetroTox uses this calculation of aqueous concentrations by means of Raoult's Law in combination with the TLM to calculate LL50s for petroleum products to different species.

McGrath et al [2005] tested the accuracy of Raoult's law for the six gasolines tested, by measuring BTEX and naphthalene in the water accommodated fraction. The analyses were compared to the estimated concentrations of C6 to C10 aromatic compounds (BTEX and naphthalene constitute at least two third of this fraction). The results showed a strong correlation, except in the lower concentrations, for which analytical detection was limited. The toxicity of water accommodated fractions of the six gasolines was tested with algae (*Pseudokirchneriella subcapitata*), water flea (*Daphnia magna*) and juvenile rainbow trout (*Oncorhynchus mykiss*). The water concentrations for the different hydrocarbon blocks estimated by Raoult's Law in combination with the TLM yielded a very good correlation with the experimentally observed toxicity, especially after correction for volatilization to the headspace. PetroTox has an option to calculate volatilisation to the headspace as well. Inclusion of this option yields slightly less conservative results, but the data from McGrath *et al.* [2005] show that this is indeed an improvement in the prediction of toxicity.

The use of Raoult's law to calculate aqueous concentrations appeared to be useful in other experiments as well. It was for example applied to explain differences between toxicity of kerosene and gas oil to *Daphnia magna* [Verbruggen *et al.*, 2001] and was also applied to calculate pore water concentrations in benthic toxicity experiments [Verbruggen *et al.*, 2008].

The data from Foster *et al.* [2005] have been used in the validation of the TLM for gasolines in the study by McGrath *et al.* [2005]. It is stated that only if log K_{ow} differed significantly from the values estimated by SPARC, the latter values were used, because SPARC was used in the development of the TLM. It is not clear why the SPARC estimates were not used on forehand.

For oil-water partitioning, a relationship between subcooled liquid solubility and log K_{ow} has been used by McGrath and Di Toro [2009] that is strongly deviating from earlier derived values for total petroleum hydrocarbons, both aromatics and aliphatics [Verbruggen *et al.*, 2008]. The consequence is that the estimated liquid solubility is up to orders of magnitude too high. However, also the tabulated values for solubility are erroneous by a factor of 1000. Therefore, there is most likely an error in the data presented.

The correlation that is presented by Di Toro *et al.* [2007] between subcooled liquid solubility and log K_{ow} is similar to the equation presented by Verbruggen et al [2008], except from the difference in unit for solubility (a factor 3 in intercept). Nevertheless, the correlation of the evaluated data in Verbruggen *et al.* [2008] is more accurate, while it covers a wider range at the same time. It is not clear from the data, whether this is caused by the use of tabulated and partly estimated data for subcooled liquid solubility by Di Toro *et al*. [2007], or the fact that Di Toro *et al*. [2007] have used SPARC to calculate log K_{ow}, while Verbruggen *et al.* [2008] have used ClogP. However, the SPARC data from the CONCAWE library in PetroTox for log K_{ow} and log subcooled liquid solubility show a straight line with an equal relationship between the two parameters as included in Verbruggen *et al.* [2008].

PetroTox provides also the possibility to apply a bioavailability correction to the estimated water concentrations, because reduced bioavailability might lower the toxicity. However, the TLM is based on similar experimental data including similar organic carbon and biota loading as the lethal loading experiments to be simulated by the model. It will be evaluated below whether it is justified to apply such correction to the model outcome.

It could be argued that this bioavailability correction applies only the highly hydrophobic compounds, while the model is constructed of compounds containing compounds of lower hydrophobicity. However, for a substance like *n*-nonane, which is within the domain of the model with a calculated log K_{ow} of 5.301, the default bioavailability correction with 2 mg/L particulate organic carbon already results in a decrease in bioavailability of 24%. McGrath *et al.* [2004, 2005] indicate that the TLM has been validated for substances with a log K_{ow} up to 5. In that case, *n*-undecane falls within the domain of the model. For this substance with a calculated log K_{ow} of 6.419, the default bioavailability correction results in 80% decrease.

Further, it should be noted that it are mainly the components in this range of hydrophobicity that contribute to the largest part to the toxic unit in a lethal loading experiment, because of their relatively high solubility and partitioning to the water phase [e.g. Di Toro *et al.*, 2007; Verbruggen *et al.*, 2008].

In the bioavailability correction, the presence of a possible third phase (small oil droplets) in the loading experiments might become relevant. Small oil droplets might serve as a replenishment of the highly hydrophobic substances, for which binding to organic carbon is most important.

Overall, it can be concluded that the methodology of PetroTox gives accurate estimates of the LL50s for petroleum products and is considered adequate together with the TU units approach (see section 3.2.2) to determine the classification and labeling of petroleum mixtures.

The bioavailability correction included in PetroTox should not be applied to calculate the lethal loading of a petroleum product. Further, the PetroTox user's guide mentions the formation of dimers and trimers for highly hydrophobic substances. Formation of such di- and trimers results in an aqueous activity that is only a small part of the subcooled liquid solubility. No reference is given for this statement. In the user's guide it is stated that this activity is input from the CONCAWE library. However, in the worksheet with the CONCAWE library in the PetroTox

model, there is no column with aqueous activity. It is therefore not clear if and how the PetroTox model deals with this reduced activity.

SSD on acute data

An evaluation of the most recent version of the TLM [McGrath & Di Toro, 2009] shows that the CTLBBs do not follow a log-normal distribution. Normality is rejected by all three goodness-of-fit tests included in the program ETX (Anderson-Darling, Kolmogorov-Smirnov, and Cramer von Mises tests) at the 0.1 and 0.05 significance level. The Anderson-Darling and Cramer von Mises tests reject the normal distribution at the 0.025 level as well. However, in our view this will probably have a minor influence on the overall risk characterization.

Further, it should be noted that although the SSD contains 47 species, there is no higher aquatic plant (macrophyte) or blue-green algae (cyanophyte) included. Because higher plants belong to the eight taxonomic groups defined as minimum requirement to perform an SSD, this minimum number of taxonomic groups is thus not met according to the REACH guidance, although CTLBBs are present for almost fifty species. Here as well, this omission will probably have a minor impact on the overall risk.

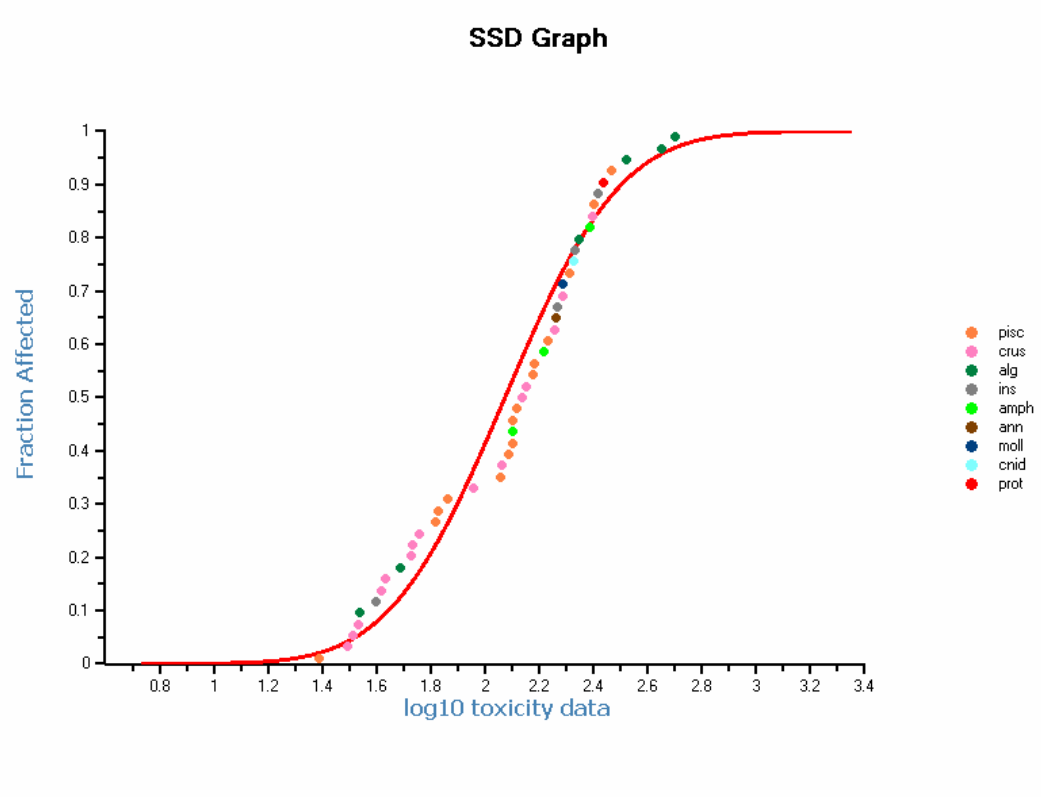

Figure 32. **Species sensitivity distribution (SSD) based on the critical target lipid body burdens (CTLBBs) as presented by McGrath & Di Toro [2009]. Taxonomic groups are sbown: pisc: fish, crus: crustaceans, alg: algae, ins: insects, amph: amphibians, ann: annelids, moll: molluscs, cnid: cnidarians, prot: protozoans.**

Acute to chronic ratio

The input for the acute to chronic ratios (ACRs) used in the TLM was critically evaluated. With respect to the acute toxicity it is noted that in case real acute effects were not observed in the studies, behavioral effects were used instead (e.g. for fluoranthene tested with fathead minnows (*Pimephales promelas*) the EC50 for behavioral effects was 69 µg/L, while the LC50 was > 212 µg/L. Behavioral effects occur at lower concentrations than the standard acute endpoints such as mortality, immobility or population growth and consequently will lead to an underestimation of the ACRs. Because these behavioral effects are not considered in the construction of the target lipid model, it is not appropriate to use ACR which are derived based on these effects.

Concerning the chronic toxicity it appears that chronic values (ChV) in stead of NOECs have been selected. These ChV are equal to the geometric mean of the NOEC and the LOEC (sometimes also denoted as maximum acceptable toxicant concentration (MATC). In general the difference between the NOEC and the ChV is the logarithm of the spacing factor (i.e. the factor between two consecutive concentrations, which is often a factor of 2 in the chronic studies considered for the TLM. Consequently, the used chronic endpoints are mostly slightly too high (√2) leading to an underestimation of the ACRs.

More important is that for several substances considered in the EU RAR on Coal Tar Pitch, high temperature (CTPHT) data were available that in several cases lead to much higher ACRs than the values reported by McGrath and Di Toro [2009]. In Table 12 only values are considered that were evaluated to be reliable and for which acute and chronic data were from the same source and performed under the same conditions.

Table 12: Acute to chronic ratios (ACRs) for polycyclic aromatic compounds. Acute and chronic tests are performed under similar conditions and are reported in the same study.

Species	Compound	Acute EC50	Chronic EC10	Chronic NOEC	ACR (EC50/EC10)	ACR (EC50/NOEC)	Source
Champia parvula	Naphthalene	1400	810	(<)950	1.70	(>)1.45	Thursby *et al.*, 1985
Pseudokirchnriella subcapitata	Phenanthrene	233	15.5		**15.0**		Halling-Sørensen *et al.*,., 1996
Pseudokirchneriella subcapitata	Anthracene	3.9	1.5	1.42	2.6	2.75	Gala & Giesy, 1992
(growth rate)		6.6	2.5	2.35	2.64	2.81	
		5.3	2.3	<5.03	2.30	>1.05	
		12.1	8.7	5.93	1.39	2.04	
		37.4	7.8	6.2	4.79	6.03	
(primary production)		3.3	1.7	1.36	1.94	2.43	
		5.9	2.7	2.26	2.19	2.61	
		4.9	2.2	<4.87	2.23	>1.01	
		8.1	2.5	5.75	3.24	1.41	
		24.0	3.9	2.81	6.15	8.54	
(number of viable cells)		4.5	1.7		2.65		Gala & Giesy, 1994
		10.2	4.1		2.49		
		15.8	6.8		2.32		
Scenedesmus vacuolatus	Naphthalene	3800	1700	1200	2.24	3.17	Walter *et al.*, 2002
Scenedesmus	Phenanthrene	590	150		3.93		Altenburger *et al.*, 2004

Species	Compound	Acute EC50	Chronic EC10	Chronic NOEC	ACR (EC50/EC10)	ACR (EC50/NOEC)	Source
vacuolatus							
Scenedesmus vacuolatus	Pyrene	49	21		2.33		Altenburger *et al.*, 2004
Scenedesmus vacuolatus	Fluoranthene	34	14		2.33		Altenburger *et al.*, 2004
		36		13		2.77	Walter *et al.*, 2002
Scenedesmus vacuolatus	Benz[a]anthracene	14	8.0		1.75		Altenburger *et al.*, 2004
Acartia tonsa	Phenanthrene	422	69	107	6.15	3.94	Bellas & Thor, 2007
Acartia tonsa	Pyrene	>129	22	32	>5.96	>3.99	Bellas & Thor, 2007
		28	1.7	5.1	**16.8**	5.52	
Acartia tonsa	Fluoranthene	120	41	51	2.94	2.38	Bellas & Thor, 2007
Americamysis bahia	Fluoranthene	31		11.1		2.79	Spehar *et al.*, 1999
		1.4		0.6		2.33	Spehar *et al.*, 1999
Cancer magister	Naphthalene	>2000		21		**>95.2**	Caldwell *et al.*, 1977
Ceriodaphnia dubia	Fluoranthene	45		43		1.05	Oris *et al.*, 1991
Daphnia magna	Fluorene	282		15		**18.8**	Finger *et al.*, 1985
Daphnia magna	Fluoranthene	117		17		6.88	Spehar *et al.*, 1999
		105.7		90		1.17	Suedel & Rodgers, 1996
Eurytemora affinis	Naphthalene	3800		<14		**>271**	Ott *et al.*, 1978
Hyalella azteca	Fluoranthene	183	28		6.54		Schuler *et al.*, 2004
		92.2		18		5.12	Suedel & Rodgers, 1996
Paracartia grani	Naphthalene	2535	530	≥130	4.78	≤19.5	Calbet *et al.*, 2007
Chironomus riparius	Fluorene	1539		142		10.8	Finger *et al.*, 1985
Chironomus tentans	Fluoranthene	208	14.0		**14.9**		Schuler *et al.*, 2004
		>250		30		>8.33	Suedel & Rodgers, 1996
Cyprinodon variegates	Acenaphthene	3100	610	520	5.08	5.96	Ward *et al.*,, 1981
Lepomis macrochrius	Fluorene	525		42		12.5	Finger *et al.*, 1985
Oncorhynchus mykiss	Naphthalene	2100	460	370	4.57	5.68	Moles *et al.*, 1981
Oncorhynchus gorbuscha	Naphthalene	1200	260	120	4.62	10	Moles & Rice, 1983
Pimephales promelas	Naphthalene	7900		450		**17.6**	DeGraeve *et al.*, 1982
Pimephales promelas	Acenaphthene	608	289	338	2.10	1.80	Cairns & Nebeker, 1982
Pimephales promelas	Fluoranthene	9.46		<6.2		>1.53	Diamond *et al.*, 1995
		>212		10.4		**>20.4**	Spehar *et al.*, 1999
Rana pipiens	Fluoranthene	366		125		2.93	Hatch & Burton Jr, 1998
Xenopus laevis	Fluoranthene	193	30	25	6.43	7.72	Hatch & Burton Jr ,1998

Values in bold exceed the 95th percentile of 13 used in TLM

The target lipid model assumes a geometric mean ACR of 3.83 and a variance in the log ACR of 0.112 [McGrath & Di Toro, 2009]. These values refer to the distribution of all ACR values available, not to species means. However, if geometric species means are calculated first, the geometric mean ACR would be virtually the same (3.90), while the variance would be substantially lower (0.240, s.d. 0.058). Therefore, the fact that species means were not used is not leading to an underestimation of the ACR. In Table 12 it can be seen that for most species the geometric mean value is in excess of 5.

The assumption that the ACR is log-normally distributed is also subject to discussion. This is because the ACR cannot theoretically be lower than one, due the fact that (1) the NOEC is supposed to occur at lower concentrations than the EC50, while the EC10 from a dose-response relationship is by definition lower than the EC50 and (2) more sensitive endpoints such as reproduction that only become manifest after a prolonged period of time, are not affecting the acute toxicity endpoint such as lethality. As the ACR can not be below one (apart from some scatter in toxicity data), the distribution of the ACR will be skewed at the lower side. For the derivation of an HC5 not the lower but the higher end of the distribution of ACRs is of interest. At the high end the distribution of the ACR could still be close to a log-normal distribution. For this reason, the fact that the ACR is not normally distributed at the lower end of the distribution does not necessarily hamper the use of a log-normal distribution, because the upper 5% of the distribution is of interest for calculating the chronic HC5.

In the evaluation of the ACR in the TLM, high ACR values as found for some crustaceans are not taken into account. This has resulted in a relatively narrow distribution of the ACR, for which the 95^{th} percentile is estimated to be around 13. If species geometric means would have been used instead of all ACRs as single entries, this 95^{th} percentile would be even lower.

It is obvious from Table 12 that a relatively high number of ACR values for PAHs show a value in excess of 13 (shown in bold). Also for two oil types, large ratios between the EC50 for mortality and the EC10 for sublethal endpoints were recorded for toxicity to some benthic species [Verbruggen et al 2008]. The geometric mean ACR was 5.73 for the gas oil, which is very similar to the data for PAHs listed in Table 12. The standard deviation of the log ACR was 0.65 in this case, which is twice as high as that of the relatively narrow distribution used in the target lipid model. Similar median ACR values were recorded in other studies [i.e. Ahlers *et al.*, 2006; Raimondo *et al.*, 2007].

Overall, the distribution of ACR values used for petroleum substances in the target lipid model do not cover the full range of ACR values which we believe is higher than assumed in the TLM. Consequently the chronic toxicity is most likely underestimated.

Equation used to arrive at an HC5

Theoretically, the equation to calculate the chronic HC5 is correct, provided that the individual parameters for which the uncertainty is accounted for are all independent of each other and are log normally distributed. It should be noted that the extrapolation constant (see below) from Aldenberg and Slob [1993] used the log logistic distribution instead of the log normal distribution, although the differences between these two distributions are small.

However, a remark could be made to how the uncertainty in the regression analysis of the CTLBB and the universal slope is taken into account. The CTLBB is the species specific intercept of the regression between the logarithms of EC50s and K_{ow}. The intercept and the slope of a linear regression equation are however not independent parameters, which is one of the most important assumptions to apply this equation. For the intercept, the variance is only based on the variance in estimated CTLBBs. However, the variance in the intercept is correlated to the variance of the slope as well (i.e. the higher the slope, the lower the intercept). On top of that, the general intercept of the relationship between the logarithms of K_{mw} and K_{ow} is now assumed to be zero but should be added to these CTLBBs. This intercept has its own variance and is also not independent of the universal slope as well.

Further, two of the three parameters used in the equation (slope, CTLBB, and ACR) did not follow a normal distribution. It appeared that the SSD on the CTLBB did not follow a normal distribution as expected, and the ACR should not be normally distributed based on theoretical considerations, although the choice of data for this parameter is probably more influential on the final result. Therefore, the requirement that the parameters are normally distributed is not met.

Choice of the extrapolation constant for the calculation of the HC5

In the statistical extrapolation method, an extrapolation constant (k_Z) is used to determine the hazardous concentration to a certain percentage of the species (e.g. HC5). This constant is not a single value in all cases, but depends on the number of species for which toxicity data are available and the percentage affected species (for each set of number of species and protection level there is a separate extrapolation constant). The smaller the number of data, the larger the extrapolation constant becomes, resulting in a lower HC5 value.

For these extrapolation constants (k_Z), median values are presented, as well as 5^{th} and 95^{th} percentiles for both the log-logistic distribution [Aldenberg & Slob, 1993] and the log-normal distribution [Aldenberg & Jaworska, 2000]. In the target lipid model the extrapolation constant for the one-sided left (lower) confidence limit of the HC5 has been used. This can be considered as a conservative value, because normally not the lower confidence limit is chosen for the HC5 but the median estimate.

The k_Z used in the target lipid model is stated to be conservative, because the number that determines the k_Z for the derivation of the HC5 is the smallest of the number of species in the acute species sensitivity distribution and the number of acute-to-chronic ratios [McGrath & Di Toro, 2009]. However, the latter number is the number of individual tests, which contains several values for one species tested with different compounds. It can be argued that in a species sensitivity distribution the number of species, for which acute-to-chronic ratios are available, should determine the value of k_Z and not the number of individual acute-to-chronic ratios. The number of individual species is rather low, only eleven species are listed.

Moreover, the eleven species for which an acute-to-chronic ratio is available do certainly not meet the conditions for performing a species sensitivity distribution according to the REACH guidance. The set contains two algal, three crustacean, four fish, one insect, and one rotifer species. The set misses thus a family in any order of insect or any phylum not already represented and higher plants, i.e. two of the eight taxonomic groups that are considered as the minimum requirement to perform an SSD according to the REACH guidance.

At the same time, it should be noted that k_Z for the lower confidence limit of 29 species is still higher, and thus more conservative, than the k_Z for the median estimate of 11 species. For the log-logistic distribution the k_Z for the median estimate in the case of 11 species is 1.72 [Aldenberg & Slob, 1993]. For the log-normal distribution, the k_Z for the lower confidence limit of 29 species is 2.232 (interpolated), while the k_Z for the median estimate in the case of 11 species is 1.696 [Aldenberg & Jaworska, 2000].

We believe that the dependency of the slope and the CTLBB will have an influence on the outcome of the risk assessment. By assuming that these parameters are not correlated, underestimation of the chronic toxicity is most likely.

3.2.4 Validation of the Target Lipid Model

Validation of the target lipid model for acute toxicity of petroleum hydrocarbons

McGrath and Di Toro [2009] have performed a cross-validation of the target lipid model with acute toxicity data for mono-aromatic compounds and polycyclic aromatic hydrocarbons. Although the model predicts the EC50s fairly well, it should be noted that accuracy is within a factor of three to five.

The validity of the target lipid model was also tested with some oil and PAH mixtures. For crude oil, at 0.6 toxic units for fathead minnow (*Pimephales promelas*) already 80% mortality is observed, where less than 50% is expected (50% at 1 toxic unit). This 80% mortality is even below the lower 5% confidence limit for 1 toxic unit. Therefore, there is a small discrepancy between the model and the observed toxicity, although the prediction is still within a factor of 2. Besides that, the number of compounds that have been measured is limited and is restricted to mono-aromatic and poly-aromatic compounds. The higher toxicity observed could be caused by the presence of lower aliphatic hydrocarbons. It can therefore be concluded that the model gives a rather good prediction the acute toxicity for weathered and unweathered crude oil.

In the analysis of Di Toro *et al.* [2007], the target lipid model is used to predict the toxicity for unweathered and weathered oil in water and sediment. Although only parent and alkylated mono-aromatic and polycyclic aromatic compounds were measured, the toxicity to fathead minnows exposed to Alaska North Slope crude oil is accurately predicted. The LC50 is at about 0.7-0.8 toxic units. Also the observed toxicity to the amphipod *Ampelisca abdita* exposed to fuel oil no.2 in sediment corresponds well with toxic unit above or below one.

Validation of the target lipid model for chronic toxicity of petroleum hydrocarbons

For mono-aromatic and polycyclic aromatic hydrocarbons, the TLM was tested with chronic data as well [McGrath & Di Toro, 2009]. An investigation of typically chronic effects on fish was made. Three studies with rainbow trout (*Oncorhynchus mykiss*) were evaluated by McGrath & Di Toro [2009]. The data for eggs and fry (until 4-d post-hatching) of rainbow trout from the study by Black *et al.* [1983] were compared with the predicted chronic endpoints from the TLM for rainbow trout. The chronic endpoints from the TLM were 880 µg/L for naphthalene and 70 µg/L for phenanthrene, while the experimental 27-d LC50s were 110 and 40 µg/L. In the EU RAR for CTPHT the LC10s from this study were also derived. These LC10s were 20 and 28 µg/L for naphthalene and phenanthrene, respectively. It can therefore be concluded that for this case the TLM does not predict the chronic toxicity well.

Another study with rainbow trout (*Oncorhynhus mykiss*) tested with phenanthrene showed effects at 500 µg/L [Hawkins *et al.* 2002], but the use of this study is limited as the concentration was not verified, only one concentration was tested and the percentage abnormal larvae was 100%. Therefore, this study can not be regarded as being in support of the chronic TLM. It only confirms that toxicity indeed occurs once the chronic endpoint is exceeded.

For benzo[*a*]pyrene, the chronic endpoint from the TLM was compared with the early life-stage (ELS) study with rainbow trout from Hannah *et al.* [1982]. The chronic endpoint from the TLM was 1.9 µg/L for this species and compound, while the LOEC from the experimental study was quoted to be 0.21-2.4 µg/L. Actually, from the original study it appears that this should be 0.08 to 2.4 µg/L (LOEC for mortality >2.99 µg/L), but the lowest LOEC for length is not accompanied by

a clear dose response-relationship. The NOEC for abnormalities is 1.48 µg/L. In the EU RAR for CTPHT this study was also evaluated where the EC10 for abnormalities of 2.9 µg/L derived. Thus, if the data for the endpoint length are considered inconclusive, in this case the TLM seems to be sufficiently protective.

For benzo[*k*]fluoranthene, the chronic endpoint for the TLM was compared with the early life-stage (ELS) study with zebrafish (*Danio rerio*) from Hooftman and Evers-De Ruiter [1992]. The chronic endpoint from the TLM was 3.8 µg/L for this species and compound, while the LOEC from the experimental study was quoted to be 0.72 µg/L, which is based on nominal concentrations. Based on actual concentrations, the lowest NOEC for length was <0.19 µg/L, while the NOEC for weight and mortality were 0.35 µg/L. In the EU RAR for CTPHT the EC10 values for these endpoints were calculated to be 0.17, 0.31, and 0.62 µg/L. Based on these results the TLM appears again to underestimate the chronic toxicity.

For phenanthrene, the chronic endpoint from the TLM was compared with the ELS study with Japanese medaka (*Oryzias latipes*) from Rhodes *et al.* [2005]. The chronic endpoint from the TLM was 135 µg/L for this species and compound, while it was stated that no effects were observed in the experimental study up to a concentration of 200 µg/L. The study was considered to be of low quality by McGrath & Di Toro [2009], because concentrations were not measured. However, the study was performed in bottles with Teflon-lined caps and test solutions were renewed daily. Indeed a NOEC could not be derived, but blue-sac disease and percentage normal medaka correlated significantly with exposure concentrations. The EC10 for malformations that can be derived from the data presented in the study is 93 µg/L. The TLM prediction is therefore in the same range.

Other data are difficult to interpret. For example, the ELS study for retene resulted in an EC50 of only two times the chronic endpoint for Japanese medaka (*Oryzias latipes*) and 85% blue sac disease at 3.5 times the chronic endpoint for rainbow trout (*Oncorhynchus mykiss*) The other data for the PAHs that are used in the comparison by McGrath and DiToro [2009] should be considered as unreliable, because exposure concentrations were not verified. Nevertheless, the data for the inland silverside (*Menidia beryllina*) tested with naphthalene and fathead minnow (*Pimephales promelas*) tested with benzo[*a*]pyrene show that the TLM also underestimates the observed toxicity.

In the study by Rhodes *et al.* [2005] the ELS test for Japanese medaka (*Oyzias latipes*) was performed with a mixture of parent PAHs, a mixture of methylated PAHs and an extract of oil sands. The recorded endpoints were blue sac disease score, percentage abnormalities, percentage hatching and hatch length. In general the TLM predicted the observed toxicity well, except for percentage hatching and hatch length for the oil sand extract, for which the observed toxicity was higher than predicted [McGrath and DiToro, 2009]. It has to be added that for this species the TLM did predict the toxicity for single PAHs correctly, while the deviations occurred for the other species tested (see above: *Danio rerio*, *Oncorhynchus mykiss*).

Comparison of risk limits derived with data for TPH and PAHs

The effect of the uncertainties and assumptions described above is difficult to describe quantitatively. To give an impression in the differences of several approaches the final HC5 for different log K_{ow} fractions is given in Table 13. It appears that there are relatively small differences between the 2004 and 2009 version of the target lipid model. As expected the final chronic value as presented by Di Toro *et al.* [2000a] is higher than the HC5 values from the later versions of the target lipid model. The difference with the values derived by the methodology presented by Verbruggen *et al.* [2008] is interesting, because toxicity studies with petroleum products as whole mixtures were used as a starting point to derive these values. It appears that the difference between the values derived by the target lipid model and the method of Verbruggen *et al*. is a factor 6 to 7.

Table 13. Overview of final chronic values (FCV) and chronic hazardous concentrations to 5% of the species (HC5)

log K_{ow}	Di Toro 2000a FCV	McGrath *et al.*, 2004 chronic HC5	McGrath & Di Toro 2009 chronic HC5	Verbruggen *et al.*, 2008 chronic HC5
0	6,940	2,620	2650	407
0.5	2,340	881	900	137
1	788	297	306	46.2
1.5	265	99.8	104	15.6
2	89.4	33.6	35.2	5.24
2.5	30.1	11.3	11.9	1.77
3	10.1	3.79	4.05	0.595
3.5	3.42	1.27	1.37	0.200
4	1.15	0.427	0.464	0.0675
4.5	0.388	0.143	0.157	0.0228
5	0.131	0.048	0.053	0.0077
5.5	0.044	0.016	0.018	0.0026

These HC5s for petroleum substances were derived based on a limited set of benthic species. However, the results were validated with data for the same or similar species but other narcotic chemicals and data for aquatic and terrestrial species tested with petroleum substances. The model resulted in consistent results with these data it was compared with. Moreover, more recently the same methodology was applied to PAHs instead of petroleum products [Verbruggen, 2012] using all reliable data from the risk assessment report on coal tar pitch extended with some new data. This resulted in a data set for toxicity of PAHs to 54 different species, containing freshwater, marine, benthic, and terrestrial species. The data resulted in almost the same HC5 value as for total petroleum hydrocarbons (about 25% lower).

The difference between the data that are presented for PAHs and mono-aromatic compounds by McGrath & Di Toro [2009] and the estimates for the same substances [Verbruggen, 2012] determined by the methodology by Verbruggen *et al.* [2008] is in general lower than a factor of 6-7 and on average less than a factor of 4 (see Figure 33). This is due to the application of the chemical class corrections for polycyclic aromatic compounds and mono-aromatic compounds in the target lipid model. However, individual differences might become higher due to the use of different log K_{ow} in both studies. The log K_{ow} for benzene and toluene calculated by SPARC are 0.2 to 0.3 units lower than experimental values and ClogP. The HC5 estimated by the target lipid model for these substances is therefore a factor of eight higher than the estimates by Verbruggen *et al.* [2008], even though the chemical class correction of 0.778 has been applied in the target lipid

model. Larger differences in both directions are also observed for the PAHs with log K_{ow} higher than 6 considered in the study by McGrath & Di Toro [2009], with the largest difference for indeno[1,2,3-*c*,*d*]pyrene, for which log K_{ow} estimated by SPARC is 0.43 unit lower than the estimate by ClogP.

It should be noted that the discrepancy in HC5 values is not due to the methodology applied by Verbruggen *et al.*, [2008]. If for data rich substances, such as naphthalene and fluoranthene, the HC5 is calculated on basis of chronic toxicity data for these substances, the HC5 is 25 µg/L for naphthalene and 0.60 µg/L for fluoranthene. The HC5 for these substances presented by McGrath & Di Toro [2009], after application of the chemical class correction, is 132 µg/L for naphthalene and 3.17 µg/L for fluoranthene. In both cases the difference between the HC5 directly determined from the selected toxicity data and the HC5 estimated by the target lipid model is a factor of 5.3.

Overall, the HC5 estimated by the TLM as implemented in PetroTox is significantly higher than HC5 values that were directly obtained from chronic toxicity data for PAHs and petroleum products. This will lead to a significant and systemic underestimation of the risk of petroleum products.

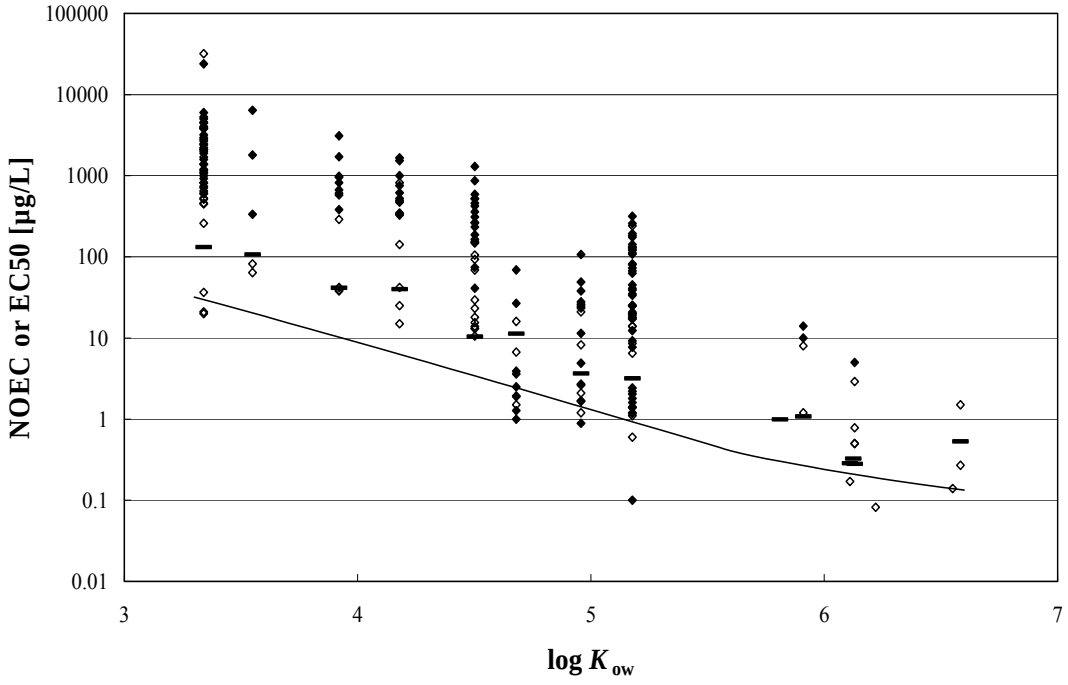

Figure 33. **Acute and chronic toxicity data for PAHs. Only reliable data are shown. Open symbols refer to chronic toxicity data, closed symbols are acute toxicity data. Drawn line is the level of the HC5 based on internal concentrations calculated from the shown chronic toxicity data. Small horizontal bars are HC5 for PAHs by McGrath & Di Toro [2009], including chemical class correction.**

3.2.5 Validity of the model for benthic and terrestrial organisms

In the target lipid model, it is assumed that toxicity to benthic organisms can be calculated by means of equilibrium partitioning [Di Toro *et al.*, 2000b]. This assumption seems to be confirmed by other data for petroleum products and PAHs. HC5 values for petroleum compounds [Verbruggen *et al.*, 2008] were derived from tests with benthic organisms. To validate this approach a comparison with aquatic as well as terrestrial toxicity data was made. This resulted in the observation that the equilibrium partitioning method was valid to calculate toxicity in the different compartments. In a recent evaluation of PAHs [Verbruggen, 2012], it was shown that the sensitivity of freshwater, marine, benthic (freshwater and marine) and terrestrial species was completely comparable. It can be concluded that equilibrium partitioning can be used to calculate the toxicity for benthic and terrestrial species.

4 Conclusions. WP3. Uncertainties in the HBM tools

4.1 Uncertainty in the exposure assessment

Melting Point

Although the Melting Point estimation applied in the HBM tool has a relatively large uncertainty (R^2=0.63, n=10051, standard deviation in the estimates of ~64°C), this uncertainty has very little influence on the result of the environmental risk assessment performed with PetroTOX and HBM tool.

Assessment of the quality of the data underlying the Melting Point QSAR model did not show additional uncertainty. The experimental Melting Point data used is considered correct.

Boiling Point

The boiling point estimates used in the PetroTOX model and the HBM tool are different, the PetroTOX tool uses SPARC v4.2 whereas the source of the boiling points used in the HBM tool is not documented, but is hypothesized to come from a newer version of the SPARC models. All other phys-chem parameters in the HBM tool are estimated using EPA EpiSuite models, and it is unclear why for Boiling Point an exception has been created.

SPARC v4.2, the newer version applied in the HBM tool and the EPISuite model (MPBPVPWin) all perform similarly well in reproducing the part of the CONCAWE library for which experimental data is available. The difference in estimated boiling points as used in the PetroTox model and the HBM tool could lead, in a worst-case scenario, to the allocation of CONCAWE library components to different Hydrocarbon Blocks for 13% of the substances, in the Low Resolution mode. In the High Resolution mode carbon numbers are used to define the Hydrocarbon Blocks, so differences in boiling points will not have any effect. The effect of this different allocation in the Low Resolution mode on the RCRs cannot be quantified.

Water solubility

PetroTox and the HBM tools apply different QSAR models to estimate the water solubility of the CONCAWE library compounds. Both models (SPARC and WSKOW) perform similarly well in reproducing the experimental data available for the CONCAWE library compounds. When comparing predictions from the two models to each other large variation in the estimates is observed. Based on the comparison between the models there is no trend that one model will on average give higher or lower water solubility estimates. Similarly, when comparing the models with experimental data it cannot be concluded that one model is (statistically significantly) better than the other.

The fact that one water solubility is used to establish a LL50 which will form the basis for Classification and Labelling conclusions, and another water solubility is used to perform the quantitative Risk Assessment is not consistent.

Octanol-Water Partition coefficient (Kow)

PetroTox and the HBM tools apply different QSAR models to estimate log Kow. In this case a valid reason for applying the SPARC estimated log Kow values is given by CONCAWE since the

whole Target Lipid Model has been fitted using SPARC estimated log Kow values. However, there is no reason to use a specific Kow estimate in the HBM tool, and the comparison of the two models against the available experimental data for the CONCAWE library does not indicate that one model is better than the other.

The large differences between the SPARC and KOWWIN estimates observed for the CONCAWE library (>21% of the library with a difference of > 1 log unit in log Kow) will impact the risk assessment directly, as log Kow is influencing the PEC as well as the PNEC. Therefore the uncertainty observed in the log Kow estimates seems to be of higher relevance than the uncertainties found for *e.g.* water solubility. It could be argued that since the TLM has been calibrated with SPARC estimated log Kow values, these estimates should be used to calculate the HC5. However, using a different model to calculate the fate factors does not seem appropriate.

When comparing the model predictions in the most relevant log Kow range of 2-8 the SPARC estimates for aliphatic compound are higher than the estimates from KOWWin. The average factor for all aliphatics in the CONCAWE library is KOWWIN = 0.86 x SPARC, i.e. 14%. For the aromatics the difference between the models is much less (<5% difference).

As the Koc value is derived directly from the Kow value the KOWWIN estimate would result in lower sorption. When emitted via an STP a larger fraction of the HPC will remain in water, when compared to the SPARC estimate. Consequently the PEC in surface water might become higher. This higher PEC in surface water could be counteracted a possible overestimation of the (bio)degradation rate in an STP. As discussed in section 3.1 on biodegradation, the biodegradation rates for STP could be overestimated (by a factor of 10 when compared to the default setting of the REACH guidance). This would possibly result in even lower environmental concentrations.

Since petroleum products will have a very diverse composition, with mixtures of aliphatics and aromatics, it is within the scope of this evaluation not possible to quantify what the exact effects (under- or over-estimation of the RCRs) will be within the scope of this evaluation.

Water-air partitioning coefficient, Henry's Law Constant

PetroTox and the HBM tools apply different QSAR models to estimate the Henry's Law Constant of the CONCAWE library compounds. Both models (SPARC and HenryWin) perform similarly well in reproducing the experimental data available for the CONCAWE library compounds. When comparing predictions from the two models to each other large variation in the estimates is observed.

In general the estimations from HenryWin seem to be higher than the estimates from SPARC. A higher HLC would imply higher partitioning to air, which in an STP would lead to lower effluent concentrations, and lower soil concentrations (via the application of sludge to agricultural soil). In addition, for some substances this will also increase the biomagnification potential in air breathing organisms. In that respect the HBM tool, in applying the HenryWin model is less conservative compared to using the SPARC estimate for the HLC.

Biodegradation and adsorption

In section 3.1, several uncertainties in the estimation of the degradation rate in different environmental compartments and sewage treatment plant have been identified. The BIOHCWIN model developed to estimate the half lives in surface water seems to under-predict the degradation half lives of short chain alkanes and branched alkanes, though, for the other group of compounds the model seems to be sufficiently conservative when compared to recently measured data

provided by CONCAWE [Princen, 2008, 2009; CONCAWE 2010]. The extrapolation to soil and sediment is however not well founded and simply based on the proposed ratio made in a study of Boethling et al. [1995] in which only a few petroleum compounds were present. In a conservative approach, the ratios used in the REACH guidance to predict the half lives in water, soil and sediment based on the ready biodegradability test is at present recommended.

As explained in section 3.1, the method used to estimated the half lives in an STP is not well founded. Most critical, is that the estimation of the half lives in a STP based on the half lives estimated in water is based on only a limited number of components. For several important classes which are expected to be present in a large number of petroleum products, like the linear and branched alkanes, no information was available. We believe that more experimental data are needed for several classes of petroleum components to have a broader coverage of the petroleum compounds and to build more confidence in the validity of the calculation method. At present there is a possibility that the fraction degraded in an STP will be over-predicted and therefore the environmental concentrations might be too low.

Based on the default residence time of 4 h in EUSES at which biodegradation in a STP will occur, only a half life of < 55 h will significantly impact on the PECs. With that half-life taken as a cut-off, the calculated ratio between the degradation rate in surface water and an STP is 180 or more. This is much more than the default ratio of 21 used in REACH guidance for cases where the degradation rate is estimated based on the results of a ready biodegradability study.

In Table 14 it is illustrated to which extent the distribution in an STP and PEC sludge, surface water and soil will differ when the half lives are estimated based on the ratio 1 : 21 between surface water and an STP instead of the half lives based on the equation used in the HBM model. In addition we have calculated the PECs based on half live ratio in soil versus surface water of 2, as proposed by the REACH guidance, instead of 1 as used in the HBM. We also made PEC calculations for two aromatics compounds using a Koc value based on the equation proposed by Karichoff [1979] instead of a Koc value based on the equation of Sabljic [1995] as used in the HBM.

Based on this analysis, it is obvious that the degradation half life in an STP is most critical for estimation the local PEC surface water. Depending on the adsorption capacity of the substance the PEC surface water can be up to four times higher using the default setting of the REACH guidance. The PEC soil will be around 2 times higher using a half life in soil twice of that in surface water. If a Koc value is used based on Karickoff [1979] for aromatics the PEC soil can be up to a factor of 4 higher.

BCF in fish (biota-water partitioning coefficient)

Based on our analysis of the BCF model values used in the HBM tool it can be concluded that by using BCFWin v2.16 estimates for the BCF in the HBM tool bioconcentration will be underestimated. A more conservative estimate (e.g BCFBAF v3.00 model estimates) would be recommendable. This optimistic estimate of the bioconcentration factor will give significant underestimation of the risk for secondary poisoning and man indirectly exposed via the environment.

Biomagnification

On the basis of the analysis the HBM method is found to correctly take into account the extra concern for biomagnification, in accordance with REACH Guidance. For a small subset of the CONCAWE library, those substances which have a high Koa but relatively low Kow, there might be an underestimation of their potential to bioaccumulate in higher, air breathing organisms. This subset consists of 16 substances (on a total of 1512) and concerns mainly PAHs.

Table 14 EUSES v2.3 distribution and PEC calculations for a number of illustrative hydrocarbon substances

	Distribution in STP				PECsludge mg/kg	PEC sw µg/kg	PEC soil µg/kg
	% to air	% to water	% to sludge	% deg			
n-octane							
HBM: Kow: 5.18, Koc: 19800 ½T STP: 0.84 h ½T soil/water: 6.4d	0.16	8.37	53.4	38.1	10.1	0.06	4.4
½T STP: 7.3 h	0.36	24.2	62.4	13	11.8	0.176	5.1
½T soil: 12.8d	0.36	24.2	62.4	13	11.8	0.176	8.6
2-methylundecane							
HBM: Kow: 6.16, Koc: 123000 ½T STP: 1.5 h ½T soil/water: 11 d	0.32	8.9	79.3	11.5	15.1	0.056	9.9
½T STP: 12.7 h	0.44	12.2	85.4	1.95	16.2	0.078	10.7
½T soil: 22 d	0.44	12.2	85.4	1.95	16.2	0.078	15.4
2,3-dimethylhepatane							
HBM: Kow: 4.61, Koc: 6830 ½T STP: 1 h ½T soil/water: 7.6d	0.5	11.9	34.7	52.9	6.59	0.088	3.3
½T STP: 8.9 h	1.25	38.6	40.7	19.5	7.72	0.29	3.9
½T soil: 15.2 d	1.25	38.6	40.7	19.5	7.72	0.29	6.1
1-methyl-3 ethylbenzene							
HBM: Kow: 3.98, Koc: 2110 ½T STP: 0.63 h ½T soil: 4.9 d	0.001	10.1	15.5	74.4	3.0	0.073	1.0
½T STP: 5.6 h	0.001	44.7	18	37.3	3.4	0.33	1.17
Koc: 5888	0.001	35.3	36.4	28.3	6.9	0.26	2.36
½T soil/water: 22 d	0.001	35.3	36.4	28.3	6.9	0.26	4.2
1-Phenyl-5-iso-propylnaphthalene							
HBM: Kow: 6.39, Koc: 189000 ½T STP: 2.3 h ½T soil/water: 16d	0	9.44	84.4	6.1	16	0.055	13.2
½T STP: 18.3 h	0	11.1	88	0.9	17	0.064	13.7
½T soil: 32 d	0	11.1	88	0.9	17	0.064	18.1
Koc: 1513561	0	8.4	91.5	0.1	17	0.019	18.8

4.2 Uncertainty in the effect assessment

Validation of the target lipid model:

The evaluation presented in Chapter 3 reveals some weaknesses of the target lipid model. The subjects identified, in arbitrary order, are the assumption of a normal distribution, which was not met for log CTLBB and log ACR, the assumption of independent parameters, which was not met for the combination of CTLBB and the universal slope for narcosis, and the numerical values used for the ACR, including the use of chronic values instead of NOECs.

It is difficult to assess what the influence is of each of these parameters individually. However, the final outcome for the HC5 of baseline narcotic chemicals is a factor of 7 lower than HC5 values based on chronic toxicity for petroleum products. Even after chemical class correction for the PAHs has been applied, the HC5 is on average a factor of 3-5 higher than would be derived from chronic toxicity data for PAHs.

Application of an additional assessment factor

In addition to the final value for the HC5 the REACH guidance requires an assessment factor varying from one to five applied to the median estimate of the HC5. In the following evaluation, only the data underlying the target lipid model are taken into account. If the HC5 would be based on other data, this would result in a different evaluation of the points influencing the assessment factor on the HC5.

- The endpoints from most of the studies used should be considered as true chronic endpoints, but are not necessarily the most sensitive endpoints. Some studies are not readily available and therefore, the relevance of the study remains unknown. However, the endpoints considered in the ACRs are not NOECs but chronic values (ChVs) and can thus not be considered as true chronic NOECs. Therefore, the set of ACRs from the target lipid model should not be used as it is done now, because it does not represent the ratio of chronic NOECs (or EC10s) to acute EC50s. The magnitude difference between chronic values and NOECs is at least 1.4. In our view, the most logic step would be to recalculate the ACRs with NOECs in stead of chronic values. Alternatively, an additional AF could be considered.
- The minimum requirement for the number of species (10) is amply exceeded by the number of CTLBBs (47), but marginally by the number of ACRs (11). The required eight taxonomic groups are not met by the set of CTLBBs as well as the set of ACRs. In both sets higher plants are lacking. Apart from that, the set of ACRs misses also a family in any order of insect or any phylum not already represented. This rather limited data set, especially for the ACR, would imply the use of an assessment factor higher than one.
- The assumed mode of toxic action is narcosis. Although the mechanism of chronic toxicity could be different, the assumption to base the toxicity on internal target lipid concentrations seems justified. However, it appears that phototoxicity of PAHs is a very toxic acute effect, with effect concentrations (EC50s) at similar levels as the lowest chronic NOECs. For PAHs this will be a reason to increase the assessment factor, possibly even to the maximum value of 5. Although phototoxicity in petroleum products as a whole will not be as extreme as for some of the individual PAHs, the occurrence of phototoxicity for petroleum products has been

demonstrated. In the target lipid model phototoxic effects were not considered at all. Therefore, the potential of petroleum products, especially those with higher amounts of PAHs, to exert phototoxicity would imply the use of an assessment factor higher than 1.

– The CTLBBs and the ACRs are not fulfilling the requirement of a log normal distribution. Although the effect cannot be quantified, it increases the uncertainty in the HC5 estimate. However, the deviation from normality for these parameters does not seem to lead to an overestimation of the relevant lower and upper 5th percentiles, for CTLBB and ACR, respectively. More relevant to the uncertainty is the way in which the distribution of the CTLBB and ACR together with the universal slope for narcosis are combined to calculcate a chronic ACR. Apart from the assumption of normal distributions, this calculation requires the individual parameters to be independent of each other. For the CTLBBs and the universal slope for narcosis this requirement is certainly not met. This hampers the validity and applicability of the equation to arrive at a chronic HC5. In principle this would imply that the HC5 derivation has to be improved by taking into account the correlation between slope and CTLBBs.

- The HC5 is QSAR based and is therefore difficult to compare with field data for example. The model has been compared with a limited number of chronic toxicity studies with petroleum compounds. The results of this exercise were rather inconclusive. This would also lead to the use of an assessment factor higher than 1.

In the target lipid model, an extrapolation constant has been chosen that corresponds to the 5% lower confidence limit of the HC5. Normally, the median estimate will be used and therefore, the applied k_z can be considered to be conservative. Nevertheless, k_z should match the number of species available. At present the total number of ACRs is determining the assessment factor and not the number of species for which an assessment factor is available. Nevertheless, the median k_z for 11 (species) is lower than the lower confidence k_z for 29 (ACRs). The chosen k_z in itself can be considered as conservative. Still, the chronic HC5 levels are higher than HC5 values derived directly from experimental data for individual substances and HC5 values derived in a comparable way from chronic toxicity data for PAHs and petroleum products. The main reasons for this discrepancy are most likely the selection of ACRs and the dependency of the parameters CTLBB and universal slope for narcosis. Before application of the model for risk assessment purposes, these aspects need some reconsideration.

In view of the shortcoming with respect to the assumption made for normal distribution, independent parameters and the numerical values, it is in our view desirable to improve the model. After this has been completed an assessment factor to the HC5 should be chosen which addresses the remaining uncertainties described above.

References

Accardi-Dey A, Gschwend PM (2002). Assessing the combined roles of natural organic matter and black carbon as sorbents in sediments. Environ Sci Technol 36: 21-29.

Ahlers J, Riedhammer C, Vogliano M, Ebert R-U, Kühne R, Schüürmann G (2006) Acute to chronic ratios in aquatic toxicity - Variation across trophic levels and relationship with chemical structure. Environ. Toxicol. Chem. 25: 2937-2945.

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.

Altenburger R, Walter H, Grote M (2004) What contributes to the combined effect of a complex mixture? Environ. Sci. Technol. 38: 6353-6362.

Altschuh J, Bruggermann R, Santl H (1999). Henry's law constants for a diverse set of organic chemicals Experimental determination and comparison of estimation methods. Chemosphere 39 (11):1871-1887.

American Petroleum Institute. 1994. Transport and fate of non-BTEX petroleum chemicals in soils and groundwater. API Publication 4593. Health and Environmental Sciences Department, Washington, DC.

Aronson D, Stewart S, Meylan W, Howard P (2005) The Calibration of Environmental Biodegradation Half-Lives For Use in Waste Water Treatment Plants. CONCAWE report letBAD04032.doc/sf, SRC Number FA429

Atkinson R (1985) Kinetics and mechanisms of the gas-phase reactions of the hydroxyl radical with organic compounds under atmospheric conditions. Chem. Rev. 85: 69-201

Bagriy EI (1989). Adamantane: Synthesis, properties, application (in Russian). Moscow: Nauka. pp. 58–123. ISBN 5-02-001382-x. http://books.google.com/?id=8RA2AAAAIAAJ&dq=isbn=502001382X as referenced in the Wikipedia entry on Adamantane; http://en.wikipedia.org/wiki/Adamantane

Belfroid AC, Sijm DTHM, Van Gestel CAM. 1996. Bioavailability and toxicokinetics of hydrophobic aromatic compounds in benthic and terrestrial invertebrates. Environ Rev 4:276–299.

Bellas J, Thor P (2007) Effects of selected PAHs on reproduction and survival of the calanoid copepod *Acartia tonsa*. Ecotoxicology 16: 465-474.

Black JA, Birge WJ, Westerman AG, Francis PC (1983) Comparative aquatic toxicology of aromatic hydrocarbons. Fundam. Appl. Toxicol. 3: 353-358.

Boethling RS, Howard PH, Beauman JA, and Larosche ME. 1995. Factors for intermedia extrapolation in biodegradability assessment. Chemosphere. 30: 741-752.

Booth AM, Sutton P, Lewis A, Lewis A, Scarlett A, Chau W, Widdows J and Rowland SJ (2007). Unresolved Complex Mixtures of Aromatic Hydrocarbons: Thousands of Overlooked Persistent, Bioaccumulative, and Toxic Contaminants in Mussels. Environ. Sci. Technol. 41, 457-464

Bucheli TD,Gustafson O (2000).Quantification of the soot-water distribution coefficient of PAHs provides mechanistic basis for enhanced sorption observations.Environ Sci Technol,34: 5144-5151.

Burgess RM, Ryba SA, Perron MM, Tien R, Thibodeau LM, Cantwell MG (2004). Sorption of 2,4'-Dichlorobiphenyl and fluoranthene to a marine sediment amended with different types of black carbon. Environ. Toxicol Chem., 23 (11), 2534-2544

Cairns MA, Nebeker AV (1982) Toxicity of acenaphthene and isophorone to early life stages of fathead minnows. Arch. Environ. Contam. Toxicol. 11: 703-707.

Calbet A, Saiz E, Barata C (2007) Lethal and sublethal effects of naphthalene and 1,2-dimethylnaphthalene on the marine copepod *Paracartia grani*. Mar. Biol. 151: 195-204.

Caldwell RS, Caldarone EM, Mallon MH (1977) Effects of a seawater-soluble fraction of Cook Inlet crude oil and its major aromatic components on larval stages of the Dungeness crab, *Cancer magister* Dana. In: Wolfe DA, ed. Fate and effects of petroleum hydrocarbons in marine ecosystems and organisms. New York: Pergamon Press. pp. 210-220.

Carreira, L. A., S. Hilal and S. W. Karickhoff, (1994) Estimation of Chemical Reactivity Parameters and Physical Properties of Organic Molecules Using SPARC (Book Chapter) Theoretical and Computational Chemistry, Quantitative Treatment of Solute/Solvent Interactions, Eds. P. Politzer and J. S. Murray, Elsevier Publishers.

CONCAWE (2009). Primary Biodegradation in seawater study. Report nrs 0822469, 0822569, 0949869

Cooney JJ, Silver SA and Beck EA (1985) Factors influencing hydrocarbon degradation in three freshwater lake. Microbial Ecol., 11 (2), 127–137.

Cornelissen G, Gustafsson O, Bucheli TD, Jonker MTO, Koelmans AA, Van Noort PCM (2005). Extensive Sorption of Organic Compounds to Black Carbon, Coal, and Kerogen in Sediments and Soils: Mechanisms and Consequences for Distribution, Bioaccumulation, and Biodegradation. Environ. Sci. Technol., 39 (18), 6881 -6895

Das N and Chandran P (2010) Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. Biotechnology Research International, 2011, 1-13

DeGraeve GM, Elder RG, Woods DC, Bergman HL (1982) Effect of naphthalene and benzene on fathead minnows and rainbow trout. Arch. Environ. Contam. Toxicol. 11: 487-490.

De Maagd PGJ (1996) Polycyclic aromatic hydrocarbons: fate and effects in the aquatic environment. Academic Thesis, Rijksuniversiteit Utrecht, The Netherlands

Diamond SA, Oris JT, Guttman SI (1995) Adaptation to fluoranthene exposure in a laboratory population of fathead minnows. Environ. Toxicol. Chem. 14: 1393-1400.

Di Toro DM, Zarba CS, Hansen DJ, Berry WJ, Swartz RC, Cowan CE, Pavlou SP, Allen HE, Thomas NA and Paquin PR (1991). Technical basis for establishing sediment quality criteria for nonionic organic chemicals by using equilibrium partitioning. Environ.Toxicol. Chem. 10(12): 1541-1583.

Di Toro DM, McGrath JA, Hansen DJ. (2000a). Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. I. Water and tissue. Environ Toxicol Chem 19:1951–1970

Di Toro DM, McGrath JA. (2000b). Technical basis for narcotic chemicals and polycyclic aromatic hydrocarbon criteria. II. Mixtures and sediments. Environ Toxicol Chem 19:1971–1982.

Di Toro DM, McGrath JA, Stubblefield WA. (2007). Predicting the toxicity of neat and weathered crude oil: Toxic potential and the toxicity of saturated mixtures. Environ Toxicol Chem 26: 24–36.

ECHA (2008). Guidance on information requirements and chemical safety assessment Chapter R.10: Characterisation of dose [concentration]-response for environment

EFDB Environmental Fate DataBase, available from SRC Inc. http://www.srcinc.com/what-we-do/efdb.aspx

EU (2008) European Union Risk Assessment Report Coal Tar Pitch, High Temperature. http://esis.jrc.ec.europa.eu/doc/existing-chemicals/risk_assessment/REPORT/pitchcoaltarreport323.pdf

Fernandez M, L'Haridon J (1992). Influence of lighting conditions on toxicity and genotoxicity of various PAH in the newt in vivo. Mutat Res 298: 31-41.

Finger SE, Little EF, Henry MG, Fairchild JF, Boyle TP (1985) Comparison of laboratory and field assessment of fluorene - Part I: Effects of fluorene on the survival, growth, reproduction, and behavior of aquatic organisms in laboratory tests. In: Boyle TP, ed. Validation and predictability of laboratory methods for assessing the fate and effects of contaminants in aquatic ecosystems. ASTM STP 865. Philedelphia, PA: American Society for Testing and Materials. pp. 120-133.

Foster KL, Mackay D, Parkerton TF, Webster E, Milford L (2005) Five-stage environmental exposure assessment strategy for mixtures: Gasoline as a case study. Environ Sci Technol 39: 2711–2718

Frenzel M, James P, Burton SK, Rowland SJ and Lappin-Scott HM (2009) Towards bioremediation of toxic unresolved complex mixtures of hydrocarbons: identification of bacteria capable of rapid degradation of alkyltetralins. J Soils Sediments, 9, 129–136

Frenzel M, Scarlett A, Rowland SJ, Galloway TS, Burton SK, Lappin-Scott HM and Booth AM (2010). Complications with remediation strategies involving the biodegradation and detoxification of recalcitrant contaminant aromatic hydrocarbons. Sci. Total Environ., 408, 4093–4101

Gala WR, Giesy JP (1992) Photo-induced toxicity of anthracene to the green alga, *Selenastrum capricornutum*. Arch. Environ. Contam. Toxicol. 23: 316-323.

Gala WR, Giesy JP (1994) Flow cytometric determination of the photoinduced toxicity of anthracene to the green alga *Selenastrum capricornutum*. Environ. Toxicol. Chem. 13: 831-840.

Gobas FAPC, Zhang X, Wells R (1993). Gastrointestinal magnification: The mechanism of biomagnification and food chain accumulation of organic chemicals. Environ.Sci.Technol. 27, 2855-2863.

Gough MA and Rowland SJ (1990) Characterization of unresolved complex mixture of hydrocarbons in petroleum. Nature, 344, 648-650

Halling-Sørensen B, Nyholm N, Baun A (1996) Algal toxicity test with volatile and hazardous compounds in air-tight test flasks with CO_2 enriched headspace. Chemosphere 32: 1513-1526.

Hannah JB, Hose JE, Landolt ML, Miller BS, Felton SP, Iwaoka WT (1982) Benzo(a)pyrene-induced morphologic and developmental abnormalities in rainbow trout. Arch. Environ. Contam. Toxicol. 11: 727-734.

Hansen DJ, DiToro DM, McGrath JA, Swartz RC, Mount DR, Spehar RL, Burgess RM, Ozretich RJ, Bell HE, Reiley MC and Linton TK (2003). Procedures for the Derivation of Equilibrium Partitioning Sediment Benchmarks (ESBs) for the Protection of Benthic Organisms: PAH Mixtures. United States Environmental Protection Agency Office of Research and Development. Washington, D.C. EPA-600-R-02-013.November.

Hatch AC, Burton Jr. GA (1998) Effects of photoinduced toxicity of fluoranthene on amphibian embryos and larvae. Environ. Toxicol. Chem. 17: 1777-1785.

Hawkins SA, Billiard SM, Tabash SP, Brown RS, Hodson PV (2002) Altering cytochrome P4501A activity affects polycyclic aromatic hydrocarbon metabolism and toxicity in rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 21: 1845-1853.

Hilal SH, SW Karickhoff and LA Carreira (2003) EPA report 600/R-03/033, March 2003, VERIFICATION AND VALIDATION OF THE SPARC MODEL

Hilal SH, LA Carreira and SW Karickhoff (2004) Prediction of the Solubility, Activity Coefficient, Gas/Liquid and Liquid/Liquid Distribution Coefficients of Organic Compounds, QSAR & Combinatorial Science, 23,709-720.

Hooftman RN, Evers-de Ruiter A (1992). The toxicity and uptake of benzo(*k*)fluoranthene using *Brachydanio rerio* in an early life stage test (draft OECD guideline). Delft, the Netherlands: TNO Environmental and Energy Research, TNO Institute of Environmental Sciences. TNO-report IMW-R 92/218.

Howard P, Meylan W, Aronson D, Stiteler W, Tunkel J, Comber M, and Parkerton TF (2005) A New biodegradation prediction model specific to petroleum hydrocarbons. Environ. Toxicol.Chem., 24 (8), 1847–1860.

Jager T (2004) Modelling Modeling ingestion as an exposure route for organic chemicals in earthworms (Oligochaeta). Ecotoxicol.Environ. Saf. 57, 30-38.

Jager T, Fleuren RHLJ, Hogendoorn EA, De Korte G (2003). Elucidating the routes of exposure for organic chemicals in the earthworm, Eisenia andrei (Oligochaeta). Environmental Science and Technology 37, 3399-3404.

Jensen TS. 1994. Petroleum hydrocarbons: Compositional changes during biodegradation and transport in unsaturated soil. PhD thesis. National Environmental Research Institute, Roskilde, Denmark.

Jonker MT, Koelmans AA. (2001). Polyoxymethylene solid-phase extraction as a partitioning method for hydrophobic organic chemicalsin sediment and soot. Environ Sci Technol 35:3742–3748.

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

Karickhoff, SW (1981) Semi-emperical estimation of sorption of hydrophobic pollutants on natural sediments and soil. Chemosphere, 10, 833-846.

Kelly BC, Ikonomou MG, Blair JD, Morin AE, Gobas FA (2007) Food web-specific biomagnification of persistent organic pollutants. Science 317: 236-239.

Kelly BC, Ikonomou MG, Blair JD, Surridge B, Hoover D, Grace R, Gobas FA (2009) Perfluoroalkyl contaminants in an Arctic marine food web: trophic magnification and wildlife exposure. Environ Sci Technol. 43(11):4037-43.

Killops SD, Al-Juboori MAHA (1990) Characterization of the unresolved complex mixture (UCM) in the gas chromatograms of biodegraded petroleums. Organic Geochemistry 15, 147-60.

Koelmans, AAM, Jonker TO, Cornelissen G, Bucheli TD, Van Noort PCM, Gustafsson O (2006). Black carbon: The reverse of its dark side. Chemosphere 63: 365-377.

Kortenkamp A, Backhaus T and Faust M (2009) State of the art report on mixture toxicity – Final Report. European Commission. Study Contract Number 070307/2007/485103/ETU/D.1.

Leppänen MT, Kukkonen JVK (1998). Relative importance of ingested sediment and pore water as bioaccumulation routes for pyrene to oligochaete (Lumbriculus variegatus, Müller): Implications for sediment toxicity testing. Environ.Sci.Technol. 32, 1503-1508.

Lohmann R, Burgess RM, Cantwell MG, Ryba SA, MacFarlane JK, and Gschwend, PM (2004) Dependency of polychlorinated biphenyl and polycyclic aromatic hydrocarbons bioaccumulation in Mya arenaria on both water column and sediment bed chemical activities. Environ. Toxicol. Chem, 23 (11), 2551-2562.

Loonen H, Muir DCG, Parsons JR, Govers HAJ (1997) Bioaccumulation of polychlorinated dibenzo-p-dioxins in sediment by oligochaetes: Influence of exposure pathway and contact time. Environ Toxicol Chem 16: 1518–1525.

Lu X, Reible DD, Fleeger JW (2004) Relative Importance of Ingested Sediment Versus Pore Water as Uptake Routes for PAHs to the Deposit-Feeding Oligochaete Ilyodrilus templetoni. Arch. Environ. Contam. Toxicol. 47, 207–214.

McGrath JA, Parkerton TF, Di Toro DM. (2004). Application of the narcosis target lipid model to algal toxicity and deriving predicted-no-effect concentrations. Environ Toxicol Chem 23: 2503–2517.

McGrath J, Parkerton T, Hellweger F, Di Toro D (2005). Validation of the narcosis target lipid model for petroleum products: Gasoline as a case study. Environ Toxicol Chem 24:2382–2394.

McGrath JA and Di Toro DM (2009) Validation of the target lipid model for toxicity assessment of residual petroleum constituents monocyclic and polycyclic hydrocarbons. Environ. Toxicol.Chem., 28 (6), 1130–1148.

Mayer LM, Chen Z, Findlay RH, Fang J, Sampson S, Self RF, Jumars PA., Quetel C, Donard OFX (1996). Bioavailability of sedimentary contaminants subject to deposit-feeder digestion. Environ.Sci.Technol. 30, 2641-2645.

Meador JP, Casillas E, Sloan CA, Varanasi U (1995a) Comparative bioaccumulation of polycyclic aromatic hydrocarbons from sediment by two infaunal organisms. Mar Ecol Prog Ser 123:107–124.

Melbye AG, Brakstad OG, Hokstad JN, Gregersen IK, Hansen BH, Booth AM, Rowland SJ and Tollefsen K (2009) Chemical and Toxicological charaterisation of an unresolved complex mixture rish biodegraded crude oil. Environ. Toxicol. Chem., 28 (9), 1815–1824.

Moles A, Bates S, Rice SD, Korn S (1981) Reduced growth in Coho salmon fry exposed to two petroleum components, toluene and naphthalene, in fresh water. Trans. Am. Fisher. Soc. 110: 430-436.

Moles A, Rice SD (1983) Effects of crude oil and naphthalene on growth, caloric content, and fat content of pink salmon juveniles in seawater. Trans. Am. Fisher. Soc. 112: 205-211.

Neff JM (2002). Polyciclic aromatic hydrocarbons in the ocean. Bioaccumulation in Marine Organisms. Elsevier, p452.

Neff JM, Stout SA, and Gunster DG. (2005). Ecological risk assessment of PAHs in sediments. Identifying sources and toxicity. Integr. Environ. Assess. Manage. 1(1):22-33.

Oris JT, Winner RW, Moore MV (1991) A four-day survival and reproduction toxicity test for *Ceriodaphnia dubia*. Environ. Toxicol. Chem. 10: 217-224.

Ott FS, Harris RP, O'Hara SCM (1978) Acute and sublethal toxicity of naphthalene and three methylated derivatives to the estuarine copepod, *Eurytemora affinis*. Mar. Environ. Res. 1: 49-58.

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.

PetroTox (2006) version 3.06 as available from CONCAWE, http://www.concawe.be/content/default.asp?PageID=619

PhysProp database, available from SRC Inc. http://www.srcinc.com/what-we-do/product.aspx?id=133

Poerschmann J, and Kopinke F (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 & Simmons KE (1998). Composition of petroleum mixtures. Total Petroleum Hydrocarbon Crietria Working Group Series. Volume 2. Amherst Scientific Publishers.

Prince RC, Parkerton, T, Lee CC (2007) The Primary Aerobic Biodegradation of Gasoline Hydrocarbons. Environ. Sci. Technol., 41, 3316-3321.

Prince RC, Haitmanek C, Lee CC (2008) The primary aerobic biodegradation of biodiesel B20. Chemosphere, 71, 1446–1451.

Raimondo S, Montague BJ, Mace G (2007) Determinants of variability in acute to chronic toxicity ratios for aquatic invertebrates and fish. Environ. Toxicol. Chem. 26: 2019-2023.

Redman A, McGrath J, Febbo E, Parkerton T, Letinski D, Connelly M, Winkelmann D and Di Toro D. (2007). Application of the Target Lipid Model for Deriving Predicted No Effect Concentrations for Wastewater Organisms. Environ. Toxicol. Chem. 26:2317–2331.

Rhodes S, Farwell A, Hewitt LM, MacKinnon M, Dixon DG (2005) The effect of dimethylated and alkylated polycyclic aromatic hydrocarbons on the embryonic development of the Japanese medaka. Ecotoxicology and Environmental Safety 60: 247-258.

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 modeling of soil sorption. Improvements and systematics of log Koc vs. log Kow correlations. Chemosphere 31:4489–4514.

Schuler LJ, Landrum PF, Lydy MJ (2004) Time-dependent toxicity of fluoranthene to freshwater invertebrates and the role of biotransformation on lethal body residues. Environ. Sci. Technol. 38: 6247-6255.

Spehar RL, Poucher S, Brooke LT, Hansen DJ, Champlin D, Cox DA (1999) Comparative toxicity of fluoranthene to freshwater and saltwater species under fluorescent light. Arch. Environ. Contam. Toxicol. 37: 496-502.

Speight, JG (1999) The Chemistry and Technology of Petroleum. 3rd Edition. Marcel Dekker, New York.

Speight JG (2002) Handbook of Petroleum Product Analysis. John Wiley & Sons.

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.

Takada H, Ishiwatari R, (1990) J. Environmental Science & Technology Vol. 24 No. 1 pp. 86-91.

Thorsen W, Cope WG and Shea D (2004) Bioavailability of PAHs: Effects of Soot Carbon and PAH Source. Environ. Sci. Technol., 38, 2029-2037.

Thursby GB, Steele RL, Kane ME (1985) Effect of organic chemicals on growth and reproduction in the marine red alga *Champia parvula*. Environ. Toxicol. Chem. 4: 797-805.

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. 2002 Jun;47(10):1049-57.

Van de Meent D (2008) Environmental fate factors and human intake fractions for exposure and risk calculation of petroleum products with the hydrocarbon block method. Reports Environmental Science no 322. Netherlands Center for Environmental Modeling. Department of Environmental Science, Faculty of Science Radboud University Nijmegen, the Netherlands.

Van de Meent D, Hollander A, Comber M, Parkerton T (2009). Environmental Fate Factors and Human Intake Fractions for Risk Assessment of Petroleum Products. Integrated Environ. Assess. Man., 6 (1), 135–144.

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

Verbruggen E, Vaes WJ, Parkerton TF, Hermens JM (2000b). 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.

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 E (2004). Environmental risk limits for mineral oil (total petroleum hydrocarbons). RIVM Report 601501021/2004. National Institute for Public Health and Environment, Bilthoven, The Netherlands.

Verbruggen EM, Beek M, Peijnenburg J, Traas TP (2008) Ecotoxicological environmental risk limits for total petroleum hydrocarbons on the basis of internal lipid concentrations. Environ Toxicol Chem. 2008 Dec;27(12):2436-48.

Verbruggen EMJ (2012). Environmental risk limits for polycyclic aromatic hydrocarbons (PAHs): for direct aquatic benthic and terrestrial toxicity. Bilthoven, The Netherlands: National Institute for Public Health and the Environment. RIVM report 6077117007.

Vinturella AE, Burgess RM, Coull BA, Thompson KM, Shine JP (2004) Importance of black carbon in distribution and bioaccumulation models of polycyclic aromatic hydrocarbons in contaminated marine sediments. Environ. Toxicol Chem. 23 (11),2578-2586

Volkering F, Breure AM (2003) Biodegrdation and general aspects of bioavailability. In Douben (ed.) PAHs: An Ecotoxicological Perspective., Wiley, 81- 98

Voparil IM, Burgess RM., Mayer LM, Tien R, Cantwell MG, Ryba S (2004). Digestive bioavailability to a deposit feeder (Arenicola marina) of polycyclic aromatic hydrocarbons associated with anthropognenic particles. Environ.Toxicol.Chem. 23, 2618-2626.

Walter H, Consolaro F, Gramatica P, Scholze M, Altenburger R (2002) Mixture toxicity of priority pollutants at no observed effect concentrations (NOECs). Ecotoxicology 11: 299-310.

Ward GS, Parrish PR, Rigby RA (1981) Early life stage toxicity test with a saltwater fish: Effects of eight chemicals on survival, growth, and development of sheepshead minnows (*Cyprinodon variegatus*). J. Toxicol. Environ. Health 8: 225-240.

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

Willows RI (1992) Optimal digestive investment: a model for filter feeders experiencing variable diets. Limnol. Oceanogr. 37, pp. 829–847.

Annex I. Excel table with all experimental and QSAR estimated data.

RIVM PetroRisk data analysis.xls