Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at Community level of

TINUVIN 123; REACTION MASS OF BIS(2,2,6,6-TETRAMETHYL-1-OCTYLOXYPIPERIDIN-4-YL)-1,10-DECANEDIOATE AND 1,8-BIS[(2,2,6,6-TETRAMETHYL-4-((2,2,6,6-TETRAMETHYL-1-OCTYLOXYPIPERIDIN-4-YL)-DECAN-1,10-DIOYL)PIPERIDIN-1-YL)OXY]OCTANE

EC number: 406-750-9
CAS number: -

CLH-O-0000004693-69-03/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

06 June 2014

Proposal for Harmonised Classification and Labelling

**Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2**

Substance Name:

Reaction mass of bis(2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-1,10-decanedioate and 1,8-bis[(2,2,6,6-tetramethyl-4-((2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-decan-1,10-dioyl)piperidin-1-yl)oxy]octane

EC Number: 406-750-9

CAS Number:

Index Number: 607-331-00-5

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Dossier prepared by:
Industry in accordance with Article 37(6) of CLP Regulation

Version number: 2.0 (post Accordance Check)

Date: November 2013

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Part A.

Part B.

Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 SUBSTANCE

Table 1: Substance identity

Substance name:	***Reaction mass of bis(2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-1,10-decanedioate and 1,8-bis[(2,2,6,6-tetramethyl-4-((2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-decan-1,10-dioyl)piperidin-1-yl)oxy]octane***
EC number:	***406-750-9***
CAS number:	
Annex VI Index number:	***607-331-00-5***
Degree of purity / Impurities:	***The composition of the substance is considered confidential and therefore included in the IU5-dossier only.***

1.2 HARMONISED CLASSIFICATION AND LABELLING PROPOSAL

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation	**Directive 67/548/EEC (Dangerous Substances Directive; DSD)**
Current entry in Annex VI, CLP Regulation	Aquatic Chronic 4; H413	R53
Current proposal for consideration by RAC	Removal: Aquatic Chronic 4; H413	Removal: R53
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	No classification	No classification

1.3 PROPOSED HARMONISED CLASSIFICATION AND LABELLING BASED ON CLP REGULATION AND/OR DSD CRITERIA

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	none		none	conclusive but not sufficient for classification
2.2.	Flammable gases	none		none	conclusive but not sufficient for classification
2.3.	Flammable aerosols	none		none	conclusive but not sufficient for classification
2.4.	Oxidising gases	none		none	conclusive but not sufficient for classification
2.5.	Gases under pressure	none		none	conclusive but not sufficient for classification
2.6.	Flammable liquids	none		none	conclusive but not sufficient for classification
2.7.	Flammable solids	none		none	conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	none		none	conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	none		none	conclusive but not sufficient for classification
2.10.	Pyrophoric solids	none		none	conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	none		none	conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	none		none	conclusive but not sufficient for classification
2.13.	Oxidising liquids	none		none	conclusive but not sufficient for classification
2.14.	Oxidising solids	none		none	conclusive but not sufficient for classification
2.15.	Organic peroxides	none		none	conclusive but not sufficient for classification
2.16.	Substance and mixtures corrosive to metals	none		none	conclusive but not sufficient for classification

3.1.	Acute toxicity - oral	none		none	conclusive but not sufficient for classification
	Acute toxicity - dermal	none		none	conclusive but not sufficient for classification
	Acute toxicity - inhalation	none		none	data lacking
3.2.	Skin corrosion / irritation	none		none	conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	none		none	conclusive but not sufficient for classification
3.4.	Respiratory sensitisation	none		none	data lacking
3.4.	Skin sensitisation	none		none	conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	none		none	data lacking
3.6.	Carcinogenicity	none		none	data lacking
3.7.	Reproductive toxicity	none		none	data lacking
3.8.	Specific target organ toxicity –single exposure	none		none	data lacking
3.9.	Specific target organ toxicity – repeated exposure	none		none	data lacking
3.10.	Aspiration hazard	none		none	data lacking
4.1.	Hazardous to the aquatic environment	No classification	--	Aquatic Chronic 4	--
5.1.	Hazardous to the ozone layer				conclusive but not sufficient for classification

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling: Signal word: no signal word
Hazard statements: no hazard statements
Precautionary statements: no precautionary statements

Proposed notes assigned to an entry: none

Table 4: Proposed classification according to DSD

Hazardous property	Proposed classification	Proposed SCLs	Current classification [1)]	Reason for no classification [2)]
Explosiveness	none		none	conclusive but not sufficient for classification
Oxidising properties	none		none	conclusive but not sufficient for classification
Flammability	none		none	conclusive but not sufficient for classification
Other physico-chemical properties	none		none	conclusive but not sufficient for classification
Thermal stability	none		none	conclusive but not sufficient for classification
Acute toxicity	none		none	conclusive but not sufficient for classification
Acute toxicity – irreversible damage after single exposure	none		none	conclusive but not sufficient for classification
Repeated dose toxicity	none		none	conclusive but not sufficient for classification
Irritation / Corrosion	none		none	conclusive but not sufficient for classification
Sensitisation	none		none	conclusive but not sufficient for classification
Carcinogenicity	none		none	data lacking
Mutagenicity – Genetic toxicity	none		none	conclusive but not sufficient for classification
Toxicity to reproduction – fertility	none		none	data lacking
Toxicity to reproduction – development	none		none	data lacking
Toxicity to reproduction – breastfed babies. Effects on or via lactation	none		none	data lacking
Environment	No classification	--	R53 – May cause long-term adverse effects in the aquatic environment	--

[1)] Including SCLs
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:	Indication of danger:	no indication of danger
	R-phrases:	no R-phrases
	S-phrases:	no S-phrases

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the Previous Classification and Labelling

Because of the following data the substance has been classified as R 53 and added to Annex I of Directive 67/548/EEC in 2001 by the 28.ATP:

The substance has a very low water solubility (< 0.046 mg/L) and shows no toxic effect in the range of water solubility in acute aquatic studies on fish, daphnia and algae. Furthermore, the substance is not readily biodegradable (approx. 20% degradation after 28 days). The assessment of potential bioaccumulative properties of the substance was based on a calculated log Pow>>10.

In February 2005 a study on bioconcentration according to OECD 305 C was submitted (BCF = 32 - 47). In the follow-up period to the TC C+L Meeting held in April 2006 the declassification was confirmed.

For the purpose of this CLH proposal all registration dossiers available in REACH-IT in August 2013 have been considered by the German CA. There are no other studies available, which are relevant for environmental classification and labelling.

2.2 Short Summary of the Scientific Justification for the CLH Proposal

A study on aquatic bioaccumulation according to OECD Guideline 305 C was performed. This study revealed a measured bioconcentration factor (BCF) of < 100 respectively 500 (32-46 for the upper concentration of 0.025 mg/L and 43-47 for the lower concentration of 0.0025 mg/L, respectively). For details please refer to Part B of this document.

According to Table 4.1.0 (“Classification categories for hazardous to the aquatic environment”) of Regulation (EC) No 1272/2008, classification criteria for Aquatic Chronic 4 include

(1) poorly soluble substances for which no acute toxicity is recorded at levels up to the water solubility

(2) and which are not rapidly degradable

(3) and have an experimentally determined BCF ≥ 500 (or, if absent, a log Kow ≥ 4)

With respect to the findings of the BCF study mentioned above, criterion (3) is clearly not fulfilled. Therefore, it appears appropriate to declassify the substance for environmental hazards.

2.3 Current Harmonised Classification and Labelling

2.3.1 Current Classification And Labelling in ANNEX VI, Table 3.1 in the CLP Regulation

- Aquatic Chronic 4 – H 413

2.3.2 Current classification and labelling in ANNEX VI, Table 3.2 in the CLP Regulation

- R53

2.4 Current self-classification and labelling

2.4.1 Current self-classification and labelling based on the CLP Regulation criteria

Table 5: Notified classification and labelling for Tinuvin 123 according to ECHAs C&L Inventory (query from April 2013)

Classification		Labelling			Specific Concentration limits, M-Factors	Numbers of Notifiers
Hazard Class and Category Code(s)	Hazard Statement Code(s)	Hazard Statement Code(s)	Supplementary Hazard Statement Code(s)	Pictograms, Signal Word Code		
Aquatic Chronic 4	H413	H413				262
Not classified						9

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

Considering all available information the existing legal classification with R53 (according to DSD) and Aquatic Chronic 4 (according to CLP) is not appropriate (see chapter 2.1).

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and Other Identifiers of the Substance

Table 6: Substance identity

EC number:	406-750-9
EC name:	A mixture of: bis(2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-1,10-decanedioate; 1,8-bis[(2,2,6,6-tetramethyl-4-((2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-decan-1,10-dioyl)piperidin-1-yl)oxy]octane
CAS number:	-
CAS name:	-
IUPAC name:	Reaction mass of bis(2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-1,10-decanedioate and 1,8-bis[(2,2,6,6-tetramethyl-4-((2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-decan-1,10-dioyl)piperidin-1-yl)oxy]octane
CLP Annex VI Index number:	607-331-00-5
Molecular formula:	$C_{44}\ H_{84}\ N_2\ O_6 + C_{80}\ H_{150}\ N_4\ O_{12}$
Molecular weight range:	2097.26

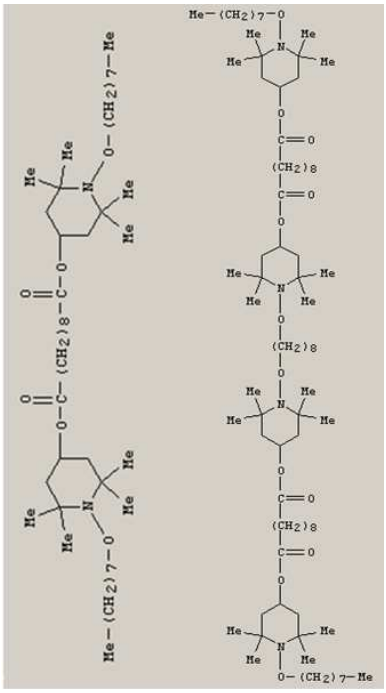
Tinuvin 123 consists of two main components:

- Decanedioic acid, 1,10-bis[2,2,6,6-tetramethyl-1-(octyloxy)-4-piperidinyl] ester
  [referred to as “compound 1” in the QSAR estimations that are part of section 5.3 AQUATIC BIOACCUMULATION]

- Decanedioic acid, 1,8-octanediylbis(oxy(2,2,6,6-tetramethyl-1,4-piperidinediyl)) bis(2,2,6,6-tetramethyl-1-(octyloxy)-4-piperidinyl)ester
  (Dimer of main constituent)
  [referred to as “compound 2” in the QSAR estimations that are part of section 5.3 AQUATIC BIOACCUMULATION]

Structural formula:

A mixture of: bis(2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl) -1,10-decanedioate; 1,8-bis[(2,2,6,6-tetramethyl-4-(2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl) -decan-1,10-dioyl) piperidin-1-yl) oxy]octane



1.2 Composition of the Substance

The composition of the substance is considered confidential and therefore included in the IUCLID-dossier and confidential annex only.

1.2.1 Composition of Test Material

The composition of the substance is considered confidential and therefore included in the IUCLID-dossier and confidential annex only.

1.3 Physico-Chemical Properties

Except for information on water solubility, physico-chemical properties are not relevant for the purpose of this CLH report. Therefore, water solubility is the only endpoint covered hereunder.

Table 7: Summary of relevant information on physico-chemical properties

Property	Value	Reference	Comment (e.g. measured or estimated)
State of the substance at 20°C and 101,3 kPa	clear pale yellow liquid	Ciba-Geigy Ltd. (1990)	measured (GLP-study)
Melting/freezing point	-50.5 °C (glassy state)	Ciba-Geigy Ltd. (1990)	measured (GLP-study)
Boiling point	decomposed before boiling (decomposition at >=234°C)	Ciba-Geigy Ltd. (1992)	measured (GLP-study)
Relative density	971.7kg/m³ at 20°C	Ciba-Geigy Ltd. (1989)	measured (GLP-study)
Vapour pressure	0.00036 Pa at 25 °C (extrapolated)	Ciba-Geigy Ltd. (1990)	measured (extrapolated)
Surface tension	54.7 - 59.8 mN/m at 20 °C (Filtrates of 10g/L emulsions)	Ciba-Geigy Ltd. (1989)	measured (GLP-study)
Water solubility	<0.046 mg/L	RCC Ltd. (2002)	Derived from the analytically determined concentration that was measured in the guideline study on D. magna
Partition coefficient n-octanol/water	log Pow= >>10 (calculated)	Ciba-Geigy Ltd. (1990)	calculated
Flash point	95 °C at 983 mbar	Ciba-Geigy Ltd. (1990)	measured
Flammability	- The flammability of a liquid is deduced from flash point and boiling point. - The substance has no pyrophoric properties and does not liberate flammable gases on contact with water.		Expert judgement
Explosive properties	non explosive	Ciba-Geigy Ltd. (1990)	measured
Self-ignition temperature	280 °C at 992 hPa	Ciba-Geigy Ltd. (1990)	measured
Oxidising properties	non-oxidising	Swiss Institute for Safety and Security (2010)	measured

Granulometry	The test substance is a liquid and is marketed or used in a non solid form.		Expert judgement
Stability in organic solvents and identity of relevant degradation products	not applicable	The stability of the test substance is not considered to be critical	Expert judgement
Dissociation constant	not applicable	The substance does not contain any ionic structure.	Expert judgement
Viscosity	2900-3100 mPa.s at 20 °C 590-620 mPa.s at 40 °C	Ciba-Geigy Ltd. (1989)	measured (GLP-study)

2 MANUFACTURE AND USES

Not relevant for the purpose of this dossier.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Not classified for physico-chemical properties.

4 HUMAN HEALTH HAZARD ASSESSMENT

Not classified for human health hazards.

5 ENVIRONMENTAL HAZARD ASSESSMENT

5.1 Degradation

Table 8: Summary of relevant information on biodegradation

Method	Results	Remarks	Reference
Test type: ready biodegradability activated sludge, domestic, non-adapted OECD 301B	not readily biodegradable % Degradation of test substance: 19 after 28 d (CO2 evolution) (20 mg/l test substance) 21 after 28 d (CO2 evolution) (11.3 mg/l test substance)	2 (reliable with restrictions) key study experimental result **Test material (EC name): A mixture of: bis(2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-1,10-decanedioate; 1,8-bis[(2,2,6,6-tetramethyl-4-((2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-decan-1,10-dioyl)piperidin-1-yl)oxy]octane**	Ciba-Geigy Ltd. (1989a)

Summary And Discussion Of Degradation

A guideline study performed according to OECD 301B determined the CO_2 evolution within 28 days. The test detected a degradation rate of ca. 20 %. The substance is poorly biodegradable.

5.2 Environmental Distribution

5.2.1 Adsorption/Desorption

Method	Results	Remarks	Reference
Study type: adsorption (soil) Calculated Calculated using KOCWIN Program (v2.00)	Adsorption coefficient: Koc: 8831000000 at 25 °C log Koc: 9.95 at 25 °C	2 (reliable with restrictions) key study estimated by calculation **Test material (Common name): Tinuvin 123, compound 1**	Department of Product Safety (2013)
Study type: adsorption (soil) Calculated Calculated using KOCWIN Program (v2.00)	Adsorption coefficient: Koc: 10000000000 at 25 °C log Koc: 19.85 at 25 °C	2 (reliable with restrictions) key study estimated by calculation **Test material (Common name): Tinuvin 123, compound 2**	Department of Product Safety (2013)

Experimental data are not available. A study on the adsorption of the substance was not accomplishable due to the physico-chemical properties of the substance. However, the molecular structure and physio-chemical properties of the substance indicate that an adsorption of the substance to organic surfaces can be expected. This assumption is supported by calculated log KOC values of 9.95 for compound 1 and 19.85 for compound 2 of Tinuvin 123, respectively (KOCWIN Program (v2.00)).

5.2.2 Volatilisation

Method	Results	Remarks	Reference
Calculated using SRC HENRYWIN v3.20 (Bond estimation method)	Henry's Law constant H: 0.0000035 Pa m³/mol at 25 °C (3.50E-006 Pa-m3/mole)	2 (reliable with restrictions) key study estimated by calculation **Test material (Common name): Tinuvin 123, compound 1**	BASF SE (2013a)
Calculated using SRC HENRYWIN v3.20 (Bond estimation method)	Henry's Law constant H: 0 Pa m³/mol at 25 °C (2.25E-016 Pa-m3/mole)	2 (reliable with restrictions) key study estimated by calculation **Test material (Common name): Tinuvin 123, compound 2**	BASF SE (2013a)

There are no experimental data on the volatilisation of Tinuvin 123 available. Therefore, the potential to evaporate into the atmosphere from the water surface was estimated using EpiSuites HENRYWIN (v3.20) software.

For compound 1 of Tinuvin 123, a Henry´s Law Constant (@25°C) of 3.50E-006 Pa-m3/mole was predicted. An estimation for compound 2 resulted in a Henry´s Law Constant (@25°C) of 2.25E-016 Pa-m3/mole.

Based on these calculations, Tinuvin 123 is not expected to evaporate into the atmosphere from the water surface.

5.2.3 Distribution Modelling

Method	Results	Remarks	Reference
Media: air - biota - sediment(s) - soil - water Calculation according to Mackay, Level I Calculation programme: Level I Version 3.0 Input data: CHEMICAL PARAMETERS Chemical Type 1 Molar Mass 737 g/mol Data Temperature 25 °C 298,15 K Water Solubility 7,37E-03 g/m³ 1,00E-05 mol/m³ Vapour Pressure 1,29E-13 Pa The Vapour Pressure required is that of the chemical in the state at the data temperature. For solids, the cooled liquid vapour pressure is also calculated. Melting Point 292 °C 565,15 K Fugacity Ratio 2,29E-03 Sub-cooled Liquid Vapour Pressure 5,64E-11 Pa Henry's Law Constant 1,29E-08 Pa.m³/mol Log Kow 12,0	Percent distribution in media: Air (%): 0 Water (%): 0.00000011 Soil (%): 97.8 Sediment (%): 2.17 Susp. sediment (%): 0.0679 Biota (%): 0.00552 Aerosol (%): 0.000000001	2 (reliable with restrictions) key study estimated by calculation **Test material (Common name): Tinuvin 123, compound 1**	BASF SE (2013b)
Media: air - biota - sediment(s) - soil - water Calculation according to Mackay, Level I Calculation programme: Level I Version 3.0 Input data: CHEMICAL PARAMETERS	Percent distribution in media: Air (%): 0 Water (%): 0.00000492 Soil (%): 0.00000387 Sediment (%): 96.7 Susp. sediment (%): 3.02	2 (reliable with restrictions) key study estimated by calculation **Test material (Common name): Tinuvin 123,**	BASF SE (2013b)

Method	Results	Remarks	Reference
Chemical Type 1 Molar Mass 1360 g/mol Data Temperature 25 °C 298,15 K Water Solubility 0,0130 g/m³ 9,56E-06 mol/m³ Vapour Pressure 4,00E-23 Pa The Vapour Pressure required is that of the chemical in the state at the data temperature. For solids, the cooled liquid vapour pressure is also calculated. Melting Point 349 °C 622,15 K Fugacity Ratio 6,24E-04 Sub-cooled Liquid Vapour Pressure 6,41E-20 Pa Henry's Law Constant 4,18E-18 Pa.m³/mol Log Kow 12,0	Biota (%): 0.246 Aerosol (%): 0.000000008	**compound 2**	

According to Mackay Level I calculations (v3.00), the compound 1 of Tinuvin 123 will preferentially distribute into the soil (97.8%) and sediment (2.17%). Compound 2 of Tinuvin 123 is expected to preferentially distribute into sediment (96.7%) and suspended particles (3.0%).

5.3 Aquatic Bioaccumulation

Table 9: Summary of relevant information on aquatic bioaccumulation

Method	Results	Remarks	Reference
Cyprinuscarpio aqueous (freshwater) flow-through Total uptake duration: 8 wk Total depuration duration: h OECD Guideline 305 C (Bioaccumulation: Test for the Degree of Bioconcentration in Fish)	BCF: 32 — 46 (at 0.025 mg/l) BCF: 43 — 47 (0.0025 mg/l)	2 (reliable with restrictions) key study experimental result **Test material (EC name): A mixture of: bis(2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-1,10-decanedioate; 1,8-bis[(2,2,6,6-tetramethyl-4-((2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-decan-1,10-dioyl) piperidin-1-yl)oxy]octane**	Ciba-Geigy Japan Ltd. (1996)

Cyprinuscarpio aqueous (freshwater) flow-through Total uptake duration: 8 wk Test methods conform to the guidelines for " Method for Testing the Degree of Accumulation of Chemical Substances in Fish Body " published in the official gazette of EA (Environmental Agency) 49 KANPOGYO No.5 ; MHW (Ministry of Health and Welfare) 49 YAKUHATSU No.615 ; MITI (Ministry of International Trade & Industry) 49 KIKYOKU No.392 .	BCF: < 4.6 (at 1 mg/l) BCF: 4.5 — < 35 (at 0.1 mg/l)	2 (reliable with restrictions) supporting study experimental result **Test material (EC name): A mixture of: bis(2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-1,10-decanedioate; 1,8-bis[(2,2,6,6-tetramethyl-4-((2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-decan-1,10-dioyl) piperidin-1-yl)oxy]octane**	Ciba-Geigy japan Ltd. (1992)
Details on estimation of bioconcentration: BASIS INFORMATION - Measured/calculated logPow: calculated BASIS FOR CALCULATION OF BCF - Estimation software: BCF base-line model v02.05 of OASIS CATALOGIC v5.11.2 SMILES codes used for calculation were: - compound 1: CCCCCCCCON1C(CC(CC1(C)C)OC(=O)CCCCCCCCC(=O)OC2CC(N(C(C2)(C)C)OCCCCCCCC)(C)C)(C)C - compound 2: CCCCCCCCON1C(CC(CC1(C)C)OC(=O)CCCCCCCCC(=O)OC2CC(N(C(C2)(C)C)OCCCCCCCCON3C(CC(CC3(C)C)OC(=O)CCCCCCCCC(=O)OC4CC(N(C(C4)(C)C)OCCCCCCCC)(C)C)(C)C)(C)C)(C)C Calculated with Catalogic v5.11.2 BCF base-line model v02.05.	BCF: 7.43 (log BCF: 0.8710, all mitigating factors applied (result is identical for both components of Tinuvin 123))	3 (not reliable) weight of evidence estimated by calculation substance not in applicability domain, but prediction assumed to be reasonable **Test material (EC name): A mixture of: bis(2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-1,10-decanedioate; 1,8-bis[(2,2,6,6-tetramethyl-4-((2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-decan-1,10-dioyl)piperidin-1-yl)oxy]octane**	BASF SE (2013a) Dimitrov S, Dimitrova N, Parkerton T, Comver M, Bonnell M, Mekenyan O (2005)
fish Details on estimation of bioconcentration: BASIS FOR CALCULATION OF BCF - Estimation software: US EPA T.E.S.T. v4.0.1 Applied estimation methods: - Hierarchical method : The toxicity	BCF: 2.68 (method: consensus, result for compound 1) log BCF: 0.43 (method: consensus, result for compound 1) BCF: 1.43 (method: consensus, result for	2 (reliable with restrictions) weight of evidence (Q)SAR Substance in applicability domain **Test material (EC name): A mixture of: bis(2,2,6,6-**	BASF SE (2013b)

for a given query compound is estimated using the weighted average of the predictions from several different cluster models. - FDA method : The prediction for each test chemical is made using a new model that is fit to the chemicals that are most similar to the test compound. Each model is generated at runtime. - Single model method : Predictions are made using a multilinear regression model that is fit to the training set (using molecular descriptors as independent variables). - Group contribution method : Predictions are made using a multilinear regression model that is fit to the training set (using molecular fragment counts as independent variables). - Nearest neighbor method : The predicted toxicity is estimated by taking an average of the 3 chemicals in the training set that are most similar to the test chemical. - Consensus method : The predicted toxicity is estimated by taking an average of the predicted toxicities from the above QSAR methods (provided the predictions are within the respective applicability domains; recommended method by T.E.S.T. for providing the most accurate predictions). T.E.S.T. is a toxicity estimation software tool. The program requires only the molecular structure of the test item, all other molecular descriptors which are required to estimate the toxicity are calculated within the tool itself. The molecular descriptors describe physical characteristics of the molecule (e.g. E-state values and E-state counts, constitutional descriptors, topological descriptors, walk and path counts, connectivity, information content, 2d autocorrelation, Burden eigenvalue, molecular property (such as the octanol-water partition coefficient), Kappa, hydrogen bond acceptor/donor counts, molecular distance edge, and molecular fragment counts). Each of the available methods	compound 2) log BCF: 0.15 (method: consensus, result for compound 2)	**tetramethyl-1-octyloxypiperidin-4-yl)-1,10-decanedioate; 1,8-bis[(2,2,6,6-tetramethyl-4-((2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-decan-1,10-dioyl)piperidin-1-yl)oxy]octane**	

uses a different set of these descriptors to estimate the toxicity. The bioaccumulation factor (BCF) was estimated using several available methods: hierarchical method; FDA method, single model method; group contribution method; nearest neighbor method; consensus method. The methods were validated using statistical external validation using separate training and test data sets. The experimental data set was obtained from several different databases (Dimitrov et al., 2005; Arnot and Gobas, 2006; EURAS; Zhao, 2008). From the available data set containing 643 chemicals salts, mixtures and ambiguous compounds were removed. The final data set contained 598 chemicals. References: - Dimitrov, S., N. Dimitrova, T. Parkerton, M. Combers, M. Bonnell, and O. Mekenyan. 2005. Base-line model for identifying the bioaccumulation potential of chemicals. SAR and QSAR in Environmental Research 16:531-554. - Arnot, J.A., and F.A.P.C. Gobas. 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ. Rev. 14:257-297. - EURAS. Establishing a bioconcentration factor (BCF) Gold Standard Database. EURAS [cited 5/20/09]. Available from http://www.euras.be/eng/project.asp?ProjectId=92. - Zhao, C.; Boriani, E.; Chana, A.; Roncaglioni, A.; Benfenati, E. 2008. A new hybrid system of QSAR models for predicting bioconcentration factors (BCF). Chemosphere 73:1701-1707.			
calculation Details on estimation of bioconcentration: BASIS INFORMATION - Measured/calculated logPow:	BCF: 3.651 L/kg (log BCF: 0.562, result for compound 1) BAF: 1.55 L/kg (log BAF: 0.19; Arnot-	3 (not reliable) weight of evidence (Q)SAR Not in applicability domain, but	BASF SE (2013c)

calculated BASIS FOR CALCULATION OF BCF - Estimation software: BCFBAF Program (v3.01) (part of EPI Suite v4.10) - Result based on # compound 1: calculated log Pow of: 14.27 (KOWWIN Program (v1.68)) # compound 2: calculated log Pow of: 24.27 (KOWWIN Program (v1.68)) Calculated with SRC BCFBAF v3.01	Gobas BAF method (including biotransformation rate estimates; upper trophic level), result for compound 1) BCF: 3.162 L/kg (Minimum Log BCF of 0.50 applied when Log Kow > 7, result for compound 1) BAF: 0.893 L/kg (log BAF: -0.05; Arnot-Gobas BAF method (including biotransformation rate estimates; upper trophic level), result for compound 2)	results considered reasonable **Test material (EC name): A mixture of: bis(2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-1,10-decanedioate; 1,8-bis[(2,2,6,6-tetramethyl-4-((2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-decan-1,10-dioyl)piperidin-1-yl)oxy]octane**	
Details on estimation of bioconcentration: BASIS INFORMATION - Measured/calculated logPow: calculated BASIS FOR CALCULATION OF BCF Result for compound 1 based on calculated log Pow of: 14.27 (estimated by KOWWIN Program (v1.68)) - Result for compound 2 based on calculated log Pow of: 24.27 (estimated by KOWWIN Program (v1.68)) Comparison of different published QSAR models for BCF estimation on the basis of log Kow	log BCF: >= -12.52 — <= 13.4 (Results for compound 1. The log Kow of the substance was not within the range of any of the models.) log BCF: >= -78.54 — <= 569.81 (Results for compound 2. The log Kow of the substance was not within the range of any of the models.)	3 (not reliable with restrictions) weight of evidence estimated by calculation not in applicability domain **Test material (EC name): A mixture of: bis(2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-1,10-decanedioate; 1,8-bis[(2,2,6,6-tetramethyl-4-((2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-decan-1,10-dioyl)piperidin-1-yl)oxy]octane**	Müller M, Nendza M (2011)
Details on estimation of bioconcentration: BASIS INFORMATION - Measured/calculated logPow: calculated BASIS FOR CALCULATION OF BCF - Estimation software: VEGA CAESAR v 2.1.8 SMILES codes used for calculation	log BCF: 0.09 (VEGA (CAESAR, version 2.1.11), result for compound 1) BCF: 1 L/kg (VEGA (CAESAR, version 2.1.11), result for compound 1) log BCF: 0.5 (VEGA (MEYLAN, version 1.0.0), same result	3 (not reliable) weight of evidence estimated by calculation not in applicability domain **Test material (EC name): A mixture of: bis(2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl)-1,10-decanedioate; 1,8-bis[(2,2,6,6-tetramethyl-4-((2,2,6,6-**	BASF SE (2013d) Zhao, C., Boriani, E., Chana, A., Roncaglioni,A., Benfenati, E (2008) Lombardo A, Roncaglioni A, Boriani E, Milan C, Benfenati E. (2010) Meylan W.M., Howard P.H.,

were: - compound 1: CCCCCCCCON1C(CC(CC1(C)C)OC(=O)CCCCCCCCC(=O)OC2CC(N(C(C2)(C)C)OCCCCCCCC)(C)C)(C)C - compound 2: CCCCCCCCON1C(CC(CC1(C)C)OC(=O)CCCCCCCCC(=O)OC2CC(N(C(C2)(C)C)OCCCCCCCCCON3C(CC(CC3(C)C)OC(=O)CCCCCCCCC(=O)OC4CC(N(C(C4)(C)C)OCCCCCCCC)(C)C)(C)C)(C)C)(C)C	for compound 1 and 2) BCF: 3 (VEGA (MEYLAN, version 1.0.0), same result for compound 1 and 2) log BCF: 1.19 (VEGA (Read-across, version 1.0.0), result for compound 1) BCF: 82 (VEGA (Read-across, version 1.0.0), result for compound 1) log BCF: 0.12 (VEGA (CAESAR, version 2.1.11), result for compound 2) BCF: 7.43 (VEGA (CAESAR, version 2.1.11), result for compound 2) log BCF: 2.08 (VEGA (Read-across, version 1.0.0), result for compound 2) BCF: 121 (VEGA (Read-across, version 1.0.0), result for compound 2)	**tetramethyl-1-octyloxypiperidin-4-yl)-decan-1,10-dioyl)piperidin-1-yl)oxy]octane**	Boethling R.S. et al. (1999) VEGA

5.3.1 Aquatic Bioaccumulation

In a weight of evidence approach, an OECD 305C as well as several QSAR models were used to assess the potential of Tinuvin 123 to bioaccumulate in organisms.

In a GLP guideline study conducted in compliance with OECD 305C, the test fish (Cyprinus carpio) were continously exposed to concentrations of 0.025 mg/l and 0.0025 mg/l, respectively, of ^{14}C-labeled test material. A dispersant (HCO-30) was used to prepare the test solutions. Concentration of HCO-30 in final test solutions at different concentrations and in the control was 0.025 mg/L. Test temperature was 25 ± 2 °C, concentration of dissolved oxygen during the exposure period was > 6.3 mg/L.
Concentrations of the test substance in water and fish body were measured using a liquid scintillation counter. Results for the exposure concentrations in water were as follows:

- High concentration (0.025 mg/L nominal)
  0.0246 mg/L (2 weeks)
  0.0242 mg/L (4 weeks)
  0.0243 mg/L (6 weeks)
  0.0244 mg/L (8 weeks)

- Low concentration (0.0025 mg/L nominal)
  0.00269 mg/L (2 weeks)
  0.00265 mg/L (4 weeks)
  0.00262 mg/L (6 weeks)
  0.00260 mg/L (8 weeks)

The test was terminated after 8 weeks of exposure. No information is provided in the report about depuration duration. For test fish exposed to 0.025 mg/l, a BCF of 32 - 46 was determined, whereas at the test concentration of 0.0025 mg/l, a BCF of 43 – 47 was observed.
A supporting study was performed with Cyprinuscarpio according to "Study Methods Concerning New Chemical Substances: The Test on the Degree of Bioconcentration in Fish and Shellfish (Kanpogyo No.5, Yakuhatsu No.615, 49-Kikyoku No.392, 1974)" which is equivalent to OECD Guideline 305 C. The fish were exposed to concentrations of 1 mg/l and 0.1 mg/l for a test period of 8 weeks in a flow-through system. A BCF < 4.6 was determined for the test concentration 1 mg/l. At a concentration of 0.1 mg/l the BCF ranged from 4.5 - < 35.

To further contribute to the weight of evidence, a series of QSAR estimations were performed to estimate the bioaccumation potential of Tinuvin 123. SMILES codes of the two compounds of Tinuvin 123 were used to calculate the following log KOW values:

	compound 1	compound 2
SMILES	CCCCCCCCON1C(CC(CC1(C)C)OC(=O)CCCCCCCCC(=O)OC2CC(N(C(C2)(C)C)OCCCCCCCC)(C)C)(C)C	CCCCCCCCON1C(CC(CC1(C)C)OC(=O)CCCCCCCCC(=O)OC2CC(N(C(C2)(C)C)OCCCCCCCCCON3C(CC(CC3(C)C)OC(=O)CCCCCCCCC(=O)OC4CC(N(C(C4)(C)C)OCCCCCCCC)(C)C)(C)C)(C)C)(C)C
Log KOW KOWWIN Program (v1.68)	14.27	24.27
Log D SPARC	13.45	Input to model did not return meaningful result
Log KOW Catalogic	14.269	24.267

SMILES codes and/or estimated log KOW values were used as input parameters for several tools to estimate the bioaccumulation potential of Tinuvin 123. Only one of the predictions made is within the applicability domain of the respective model and hence, all other predictions were assigned to be not reliable. However, as explained in the following sections, their results are still considered meaningful, an assumption which is also confirmed by their agreement with the experimentally observed values and by their agreement with each other.

Consequently, these results are considered as useful information to support the overall weight of evidence, which is still mainly based on the experimentally measured values.

For details on the models used please see information in Annex I of this dossier.

Comparative analysis of estimated and measured BCF data (UBA models: Müller & Nendza, 2011)

Check for OECD Principles for (Q)SAR validation

defined endpoint	Yes (see Annex I for details)
unambiguous algorithm	Yes (see Annex I for details)
defined domain of applicability	Yes (see Annex I for details)
appropriate measures of goodness-of-fit, robustness and predictivity	Yes (see Annex I for details)
mechanistic interpretation, if possible	Not applicable

A collection of models taking into account the log KOW alone (see UBA models: Müller & Nendza, 2011) revealed values for estimated log BCFs ranging from -12.52 to 13.40 for compound 1 and from -78.54 to 569.81 for compound 2, respectively. It is important to note that neither the log KOW of compound 1 nor compound 2 is within the appropriate range of any of the single models that are part of the compilation. Furthermore, the wide variation in the results for both compounds suggests a rather low reliability of the numbers received. Hence, the estimations provided by these models are considered a weak contribution to the assessment of potential for bioaccumulation of the substance.

CATALOGIC v5.11.2; BCF base-line model v02.05

Check for OECD Principles for (Q)SAR validation

defined endpoint	Yes (see Annex I for details)
unambiguous algorithm	Yes (see Annex I for details)
defined domain of applicability	Yes (see Annex I for details)
appropriate measures of goodness-of-fit, robustness and predictivity	Yes (see Annex I for details)
mechanistic interpretation, if possible	Yes (see Dimitrov et al., 2005)

Since bioaccumulation is also influenced by other factors than the log KOW, the CATALOGIC v5.11.2 BCF model takes several mitigating factors into consideration when deriving the BCF. According to the output, the most important mitigating factor of the two Tinuvin 123 compounds is the low water solubility. In total – and taking mitigating factors into account – the BCF was estimated to be 7.43 for both compounds.

Molecular dimensions as indicators for limited bioconcentration, i.e. average maximum diameter were assessed as well. According to ECHA's Guidance on Information Requirements and Chemical Safety Assessment, R.11: PBT Assessment, the capability of crossing biological membranes is hindered if the average maximum diameter is > 1.7 nm. The compounds of Tinuvin 123 have a minimum DiamMax of 18.6 and 28.5 nm, respectively. This is more than tenfold above the threshold given in R.11, therefore providing strong evidence for a very limited ability of the compounds of Tinuvin 123 to enter cells.

Model data

	Compound 1	Compound 2
Model domain similarity		
Parametric domain	In domain	Out of domain
Structural domain	80.77% correct 0.0000 incorrect 19.23% unknown	79.17% correct 0.0000 incorrect 20.83% unknown
Mechanistic domain	In domain	In domain
Effects of mitigating factors on BCF		
Acids	0.0000	0.0000
Metabolism	0.0000	0.0000
Phenols	0.0000	0.0000
Size	0.0000	0.0000
Water solubility	0.0904	0.0904
Molecular dimensions		
DiamMax-Min [Å]	186.187	285.184
DiamMax-Max [Å]	477.111	806.276
Estimation		
Log BCF	0.8710	0.8710
BCF	7.43	7.43

According to the model data, compound 2 is out of the parametric domain. Its log KOW (24.267) exceeds the domain (upper threshold = 16.1) and also the molecular weight of compound 2 (1360.11) is higher than the boundary of the domain (upper threshold = 1132). However, a very high log KOW as well as a very high molecular weight does support the assumption of a reduced bioavailability. Very bulky molecules will less easily pass the cell membranes and of course the molecular weight contributes to size. According to ECHA's Guidance on Information Requirements and Chemical Safety Assessment, R.11: PBT Assessment, a molecular weight higher than 1100 g/mol is indicative of a limited bioavailability. Moreover, also very hydrophobic chemicals are known to show reduced uptake into cells. According to R.11, the aquatic BCF of a substance is probably lowered if the calculated log Kow is higher than 10. This is clearly the case for compound 2 having a log KOW of 24.267. Therefore, although the parametric domain is not met by compound 2, the predicted – low – BCF value provided by the model is still considered a meaningful result substantiating the weight of evidence.

With regard to structural domain, the atom centered fragments (ACF) of both compounds are largely presented in the training chemicals of the model (approx. 80%). It has to be emphasized that both compounds do not contain any structural elements that are considered incorrect by the model. The remaining parts of the molecules consist of ACFs unknown to the model (approx. 20%). This is not considered to significantly impact the basic result provided by the model. The prediction is clearly indicating a very low potential for accumulation (BCF < 8) and is based on the major share of the ACFs of the molecule(s). Even if the remaining, minor part(s) are assumed to influence the outcome of the estimation – an assumption for which there is no evidence at all – it is highly unlikely that the general statement would change substantially. Therefore, the prediction provided by the model is considered a reasonable contribution to assess the bioaccumulation potential of the compounds of the mixture.

Taking the available information into account and recognizing the restrictions described above, the result of the estimation provided by the BCF base-line model v02.05 is considered providing reasonable information to add to the weight of evidence approach for potential accumulation of EC 406-750-9.

US EPA T.E.S.T. V4.0.1

Check for OECD Principles for (Q)SAR validation

defined endpoint	Yes (see Annex I for details)
unambiguous algorithm	Yes (see Annex I for details)
defined domain of applicability	Yes (see Annex I for details)
appropriate measures of goodness-of-fit, robustness and predictivity	Yes (see Annex I for details)
mechanistic interpretation, if possible	Not applicable

The US EPA TEST package calculates the BCF on different sets of molecular descriptors. According to this strategy, the compounds yielded BCF values of 2.68 and 1.43 using the consensus method.

Model data

	Compound 1	Compound 2
Predicted Bioaccumulation factor from Consensus method		
Bioaccumulation factor Log10	0.43	0.15
Bioaccumulation factor	2.68	1.43
Individual Predictions [Log10]		
Hierarchical clustering	N/A	N/A
Single model	N/A	N/A
Group contribution	N/A	N/A
FDA	0.46	-0.17
Nearest neighbor	0.40	0.48

(For comprehensive results for compound 1 and 2 using T.E.S.T see Annex I)

The Toxicity Estimation Software Tool (T.E.S.T.) has been developed to allow users to easily estimate toxicity and/or bioaccumulation using a variety of QSAR methodologies. Before any model can be used to make a prediction for a test chemical, it must be determined whether the test chemical falls within the domain of applicability for the model. The applicability domain is defined using several different constraints, e.g. the model ellipsoid constraint, the Rmax constraint, or the fragment constraint (for details see Annex I). The domain check is automatically implemented into the tool, i.e. the individual methods return a value only, when the tested substance is in domain. As apparent from the table above, the models hierarchical clustering, single model and group contribution did not deliver a result for compound 1 and 2, respectively. However, the methods FDA and nearest neighbour were able to provide predictions for the bioaccumulation factor. For the reasons stated above these values – and consequently also the result from the consensus method, which is derived by taking an average of the predicted values from the above QSAR methods – are considered reliable and valid with restrictions.

BCFBAF v3.01 model (part of EPI Suite v4.10)

Check for OECD Principles for (Q)SAR validation

defined endpoint	Yes (see Annex I for details)
unambiguous algorithm	Yes (see Annex I for details)
defined domain of applicability	Yes (see Annex I for details)
appropriate measures of goodness-of-fit, robustness and predictivity	Yes (see Annex I for details)
mechanistic interpretation, if possible	Not applicable

According to the BCFBAF v3.01 model of EPI Suite v4.10, the BCF for compound 1 is 3.651 and 3.162 for compound 2, respectively.

Model data

	Compound 1	Compound 2
Model domain similarity		
Currently there is no universally accepted definition of model domain. However, users may wish to consider the possibility that bioconcentration factor estimates are less accurate for compounds outside the MW and logKow ranges of the training set compounds		
Molecular weight (68.08 – 959.17)	accurate (737.17)	less accurate (1360.11)
Log Kow (-6.50 – 11.26)	less accurate (14.27)	less accurate (24.27)
Correction factor	No Applicable Correction Factors	No Applicable Correction Factors
Estimation		
Log BCF	0.562 (based on molecular weight)	Minimum Log BCF of 0.50 applied when Log Kow > 7
BCF	3.651	3.162

Compound 1 has a molecular weight within the appropriate range, but a log Kow exceeding the upper threshold of the training set compounds. As a consequence, the prediction of log BCF for compound 1 is based on molecular weight alone. Hence, this result is of restricted validity, but still considered a reasonable contribution to the assessment of potential bioaccumulation of the substance.

According to the original methodology developed by Meylan et al for the US EPA, estimates of log BCF from the QSAR estimation equation derived for substances with a Log Kow > 7.0 must be truncated at 0.5 (i.e., the equation used is log BCF = 0.5), because negative values are otherwise resulting for substances with a high log Kow. This is clearly the case for compound 2. Therefore – although the properties of compound 2 are not within the MW and logKow ranges of the training set compounds, respectively – the prediction derived for substance 2 appears appropriate in the context of a weight of evidence approach.

VEGA BCF models

Check for OECD Principles for (Q)SAR validation

defined endpoint	Yes (see Annex I for details)
unambiguous algorithm	Yes (see Annex I for details)
defined domain of applicability	Yes (see Annex I for details)
appropriate measures of goodness-of-fit, robustness and predictivity	Yes (see Annex I for details)
mechanistic interpretation, if possible	Not applicable

Model data

	Compound 1	Compound 2
BCF model (CAESAR) (version 2.1.11)		
Prediction [log(L/kg)]	0.09	-0.12
Prediction [L/kg]	1	0.75
Conclusion	Compound is non-bioaccumulative	Compound is non-bioaccumulative
Reliability	low	low
BCF model (Meylan) (version 1.0.0)		
Prediction [log(L/kg)]	0.5	0.5
Prediction [L/kg]	3	3
Conclusion	Compound is non-bioaccumulative	Compound is non-bioaccumulative
Reliability	low	low
BCF Read-Across (version 1.0.0)		
Prediction [log(L/kg)]	1.91	2.08
Prediction [L/kg]	82	121
Conclusion	Compound is non-bioaccumulative	Compound is non-bioaccumulative
Reliability	low	low

(For comprehensive results for compound 1 and 2 using VEGA see detailed reports attached to the concurrent entries in the IUCLID 5 dossier, section 5.3.1)

The VEGA BCF models (CAESAR; v2.1.11, Meylan v1.0.0, Read-Across v1.0.0) predict BCFs for the two compounds in the range of 0.75 to 121, i.e. estimating a low potential for bioaccumulation. However the reliabilities of the calculations are low as the two molecules do not fall within the domain of applicability. As a consequence, these values are considered of low reliability and therefore of minor importance. Nevertheless, the results of the VEGA models still are supporting the assumption of a low potential for bioaccumulation of Tinuvin 123.

Overview table

Experimental result [Ciba-Geigy Japan Ltd. (1996), 5B615G, 1996-10-17]		
	@ 0.025 mg/L	@ 0.0025 mg/L
BCF	32 – 46	43 – 47

	Compound 1	Compound 2
UBA models: Müller & Nendza, 2011		
logBCF	-12.52 to 13.40	-78.54 to 569.81
BCF base-line model v02.05		
BCF	7.43	7.43
US EPA T.E.S.T. V4.0.1		
BCF	2.68	1.43
BCFBAF v3.01 model		
BCF	3.651	3.162
BCF model (CAESAR) (version 2.1.11)		
BCF	1	0.75
BCF model (Meylan) (version 1.0.0)		
BCF	3	3
BCF Read-Across (version 1.0.0)		
BCF	82	121

Major evidence is derived from an experimental study on bioaccumulation in fish that yields BCF values ranging from 32 to 47. Additionally, several QSAR results are available, all of which confirm the low BCF values observed. Although the individual QSAR results are less reliable than the experimentally measured BCF values, they still contribute to the overall weight of evidence and support the conclusion that Tinuvin 123 shows only low bioaccumulation. Both experimental and calculated values are significantly lower than the trigger value of 500.

Summary and Discussion of Aquatic Bioaccumulation

A weight-of-evidence approach using experimentally determined BCF values of max. 47, several QSAR estimations and taking molecular dimensions of Tinuvin 123 into account, demonstrates that the substance does not significantly accumulate in organisms.

5.4 Aquatic toxicity

Short-term toxicity to aquatic organisms

Data on the acute toxicity are available for three trophic levels of the aquatic environment.
In a guideline study (OECD 203) using Brachydanio rerio, a LC50 > 58 mg/l based on analytically determined test concentrations was detected (Ciba-Geigy Ltd. (1990)).
A water accommodated fraction using 100 mg/l loading rate was tested in an OECD 202 study with Daphnia magna. No effect in the range of the water solubility of the test substance was observed at test termination after 48 hours (RCC Ltd. (2002)).
In a study according to directive 92/69/EWG, C.3 EEC, the toxicity of the substance to Scenedesmus subspicatus was investigated. An E_rC50 (72 h):> 2 mg/L was derived (Ciba-Geigy Ltd. (1995)).

Long-Term Toxicity To Aquatic Organisms

No information is available regarding the long-term toxicity of the substance.

Summary And Discussion Of Aquatic Toxicity

All tests demonstrated no toxic effects related to the test substance within the range of its water solubility(< 0.046 mg/L).

5.5 Comparison With Criteria For Environmental Hazards (Sections 5.1 – 5.4)

According to table 4.1.0 (“Classification categories for hazardous to the aquatic environment”) of Regulation (EC) No 1272/2008, classification criteria for Chronic Category 4 include

(1) poorly soluble substances for which no acute toxicity is recorded at levels up to the water solubility

=> water solubility < 0.046 mg/L
=> no acute toxicity is recorded at levels up to the water solubility

=> criterion fullfilled

(2) and which are not rapidly degradable

=> max. 21% after 28 d

=> criterion fullfilled

(3) and have an experimentally determined BCF ≥ 500 (or, if absent, a log Kow ≥ 4)

=> BCF < 47

=> *criterion not fullfilled*

5.6 Conclusions On Classification And Labelling For Environmental Hazards (Sections 5.1 – 5.4)

Dangerous Substance Directive (67/548/EEC):

The available studies are considered reliable and suitable for classification purposes under 67/548/EEC. As a result the substance is considered **not** to be classified for environmental hazards (R53) under Directive 67/548/EEC.

Classification, Labeling, and Packaging Regulation (EC) No. 1272/2008:

The available experimental test data are reliable and suitable for classification purposes under Regulation 1272/2008. As a result the substance is considered **not** to be classified for environmental hazards under Regulation (EC) No. 1272/2008.

RAC evaluation of environmental hazards
Summary of the Dossier submitter's proposal
The proposal was drafted by BASF SE and submitted by BAuA Germany according to CLP article 37(6). The reaction mass of bis (2,2,6,6 -tetramethyl-1-octyloxypiperidin-4-yl) -1,10-decanedioate and 1,8-bis [(2,2,6,6-tetramethyl-4- ((2,2,6,6-tetramethyl-1-octyloxypiperidin-4-yl) -decan-1,10-dioyl) piperidin-1-yl) oxy]octane (Tinuvin 123) was classified as R53 and added to Annex I of Directive 67/548/EEC in 2001 by the 28th ATP, based on the following data. The substance has very low water solubility (< 0.046 mg/L) and shows no toxic effects within the water solubility range in acute aquatic studies on fish, daphnia and algae. Furthermore, the substance is not readily biodegradable (approx. 20% degradation after 28 days). The assessment of potential bioaccumulative properties of the substance was based on a calculated log Kow >> 10. However, a new GLP study on bioconcentration (Ciba-Geigy Japan Ltd., 1996) conducted in compliance with OECD TG 305 C resulted in a BCF = 32 - 47. Based on the results of this study, in the follow-up period to the TC C&L Meeting held in April 2006 the

declassification was confirmed.

In this study, the test fish (*Cyprinus carpio*) were continously exposed to concentrations of 0.025 mg/L and 0.0025 mg/L of ^{14}C-labeled test material (Tinuvin 123). Concentrations of the test substance in water and fish body were measured using a liquid scintillation counter. The test concentrations were measured every 2 weeks and remained in the range 0.0242 to 0.0246 mg/L and 0.00260 to 0.00269 mg/L for the high and low concentration solutions, respectively. A dispersant (HCO-30) was used to prepare the test solutions. The concentration of HCO-30 in the final test solutions at different concentrations of test material and in the control was 0.025 mg/L. The test temperature was 25 ± 2 °C and the concentration of dissolved oxygen during the exposure period was > 6.3 mg/L.

The test was terminated after 8 weeks of exposure. For test fish exposed to 0.025 mg/l, a BCF = 32 - 46 was determined, whereas at the test concentration of 0.0025 mg/L, a BCF = 43 – 47 was observed.

A supporting study (Ciba-Geigy Japan Ltd., 1992) was performed with *Cyprinus carpio* according to a method equivalent to OECD TG 305C (see the background document for more details). The fish were exposed to concentrations of 1 mg/L and 0.1 mg/L for a test period of 8 weeks in a flow-through system. A BCF < 4.6 was determined for the test concentration of 1 mg/L. At a concentration of 0.1 mg/L the BCF ranged from 4.5 to < 35.

Based on the study on bioconcentration of the substance according to OECD TG 305 C, the weight of evidence of another supporting bioconcentration study and the results of supporting QSAR estimations, the dossier submitter proposed to delete the existing classification in CLP (Regulation (EC) No. 1272/2008) of Tinuvin 123 as Aquatic Chronic 4; H413, since the substance does not meet the criteria for aquatic chronic classification .

Comments received during public consultation

Two MSCAs supported the no classification proposal for aquatic chronic toxicity. No comments opposing the proposal were received.

Assessment and comparison with the classification criteria

According to Table 4.1.0 ("Classification categories for hazardous to the aquatic environment") of the CLP Regulation, classification criteria for Aquatic Chronic 4 includes:

(1) poorly soluble substances for which no acute toxicity is recorded at levels up to the water solubility

(2) and which are not rapidly degradable

(3) and have an experimentally determined BCF ≥ 500 (or, if absent, a log Kow ≥ 4)

Tinuvin 123 fulfils criteria (1) and (2), but with respect to the findings of the BCF study (as described above), criterion (3) is clearly not fulfilled. Therefore, it is considered appropriate to declassify the substance for environmental hazards.

In conclusion, RAC recommends that Tinuvin 123 should not be classified as aquatic chronic according to CLP (Regulation (EC) No. 1272/2008).

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Not applicable

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8 ANNEX I: COMPILATION OF INFORMATION ON QSAR MODELS

8.1 Comparative Analysis Of Estimated And Measured Bcf Data (Uba Models: Müller & Nendza, 2011)

QSAR-models for BCF estimation compiled are

1) Veith et al. 1979

log BCF = 0.85 log KOW - 0.70
n = 55, r = 0.95, species: Pimephales promelas, chemicals: heterogeneous dataset
range of log KOW: 1 – 7.05

This model is recommended in the Technical Guidance Documents on Risk Assessment, part II, (TGD equation 74) for substances with log KOW between 0 and 6 (for a detailed discussion of this QSAR, see 2.2.1.1.).

2) Connell and Hawker, 1988

log BCF = (6.9*10-3)*log KOW4 - 0.185 log KOW3 + 1.55 log KOW2 - 4.18 log KOW + 4.79
n=45, species: fish (various), chemicals: heterogeneous dataset
range of log KOW: 2.6 – 9.8
3) European Communities, 2003
log BCF = -0.20 log KOW2 + 2.74 log KOW - 4.72
n = 43, r = 0.883, species: fish (various), chemicals: heterogeneous dataset

This model is recommended in the Technical Guidance Documents on Risk Assessment, part II, (TGD) for substances with log KOW > 6. The model (TGD equation 75) is based on data from: Connell, D.W., Hawker, D.W. "Use of Polynomial Expressions to describe the Bioconcentration of Hydrophobic Chemicals by Fish", Ecotox. Environ. Saf. 16, 242 - 257,1988.
range of log KOW: 2.6 – 9.8

3) European Communities, 2003

log BCF = -0.20 log KOW2 + 2.74 log KOW - 4.72
n = 43, r = 0.883, species: fish (various), chemicals: heterogeneous dataset

This model is recommended in the Technical Guidance Documents on Risk Assessment, part II, (TGD) for substances with log KOW > 6. The model (TGD equation 75) is based on data from: Connell, D.W., Hawker, D.W. "Use of Polynomial Expressions to describe the Bioconcentration of Hydrophobic Chemicals by Fish", Ecotox. Environ. Saf. 16, 242 - 257,1988.
range of log KOW: 2.6 – 9.8

4) Nendza, 1991

log BCF = 0.99 log KOW - 1.47*log(4.97*10-8*KOW + 1) + 0.0135
n = 132, species: fish (various), chemicals: heterogeneous dataset
range of log KOW: 1 – 11

The "worst case"-bilinear model calculates the maximum bioaccumulation potential to be expected for compounds. The model has not been derived by regression, therefore, neither statistical parameters nor confidence intervals are available.

5) Mackay, 1982

log BCF = log KOW - 1.32
n = 44, r = 0.95, s = 0.25, species: fish (various), chemicals: heterogeneous dataset, mainly chlorinated hydrocarbons
range of log KOW: 1 – 7.1

6) Veith et al. 1983 log BCF = 0.79 log KOW - 0.40

n=122, r=0.927, s=0.49, species: fish (various), chemicals: heterogeneous dataset, mainly halogenated compounds
range of log KOW: 1 – 6.9

7) Bintein et al. 1993

log BCF = 0.91 log KOW - 1.975*log(6.8*10-7*KOW + 1) - 0.786
n = 154, r = 0.95, s = 0.347, species: fish (various), chemicals: heterogeneous dataset

This model is recommended by the authors for compounds with log KOW > 6.
range of log KOW: 1.2 – 8.5

8) Schüürmann and Klein, 1988

log BCF = 0.75 log KOW - 0.32
n = 32, r = 0.87, s = 0.54, species: fish (various), chemicals: heterogeneous dataset, mainly chlorinated and polycyclic hydrocarbons
range of log KOW: 1.8 – 6.5

9) Köneman and van Leeuwen, 1980

log BCF = 3.41 log KOW - 0.264 log KOW^2 - 5.513
n = 6, r = 0.999, s = 0.039, species: Poecilia reticulata, chemicals: chlorobenzenes
range of log KOW: 3.5 – 6.4

This model is based on 6 compounds from the same compound class. However, the model should be applicable for similar organic compounds (small, inert molecules, not degrading) within the range of applicability (log KOW between 3.5 and 6.4).

10) Lu et al. 1999

log BCF = 0.9 log KOW - 0.8
n = 80, r = 0.944, species: various fish, chemicals: diverse non-polar chemicals
range of log KOW: 1 – 7.1

11) Escuder-Gilabert et al. 2001

log BCF = 0.74 log KOW + 0.8
n = 66, r = 0.917, species: various fish, chemicals: diverse
range of log KOW: 0.3 – 5.8

12) Neely et al. 1974

log BCF = 0.54 log KOW + 0.12
n = 8, r = 0.949, species: Salmo gairdneri, chemicals: halogenated aromatics
range of log KOW: 2.6 – 7.6

13) Zok et al. 1991

log BCF = 0.67 log KOW - 0.18
n = 9, r = 0.934, species: Brachydanio rerio, chemicals: substituted anilines
range of log KOW: 0.9 – 2.8

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8.2 Bcf base-line model v02.05

8.2.1 Endpoint

BCF base-line model predicts bioconcentration factor (BCF, l/kg wet) in fish. Model accounts for a number of mitigating factors, such as molecular size, metabolism of parent chemical, water solubility and ionization.

8.2.2 Data

The training set of the model consists of 705 chemicals and is a compilation of three databases:

- 393 chemicals extracted from Biodegradation and Bioaccumulation Data of Existing Chemicals Based on the CSCL Japan (MITI database) [1].
- 167 chemicals tested by National Institute of Technology and Evaluation of Japan (NITE) using the same fish (*Cyprinos carpio*) [2].
- 145 *BCF* values extrapolated from dietary bioaccumulation experiments with salmonids [3].

MITI and NITE BCF data derived at the lowest concentration exposure have been used in the model development. All experimental data meet the OECD 305 protocol criteria and were generated based on the concentration of the parent chemicals only and not on the total amount of parent and metabolites (e.g., the total radioactivity).

Another training database of documented fish and rat liver transformation maps for 433 organic compounds and expert knowledge was used to determine the principal transformations and to train the system to simulate the fish liver metabolism chemicals. The documented pathways were collected from scientific papers, monographs and databases accessible over the Internet.

8.2.3 Model

The BCF base-line model consists of two major components: a model for predicting the maximum potential for bioaccumulation (*log BCFmax*) based solely on chemicals' lipophilicity and a set of mitigating factors that account for the reduction of the bioaccumulation potential of chemicals based on chemical (molecular size, ionization and water solubility) and organism (metabolism) dependent factors. Mathematical formulation of the model is:

$$\log BCF = \log\left(\prod_i F_i \frac{{K_{OW}}^n}{(aK_{OW}+1)^{2n}} + F_W.F_{WS}\right)$$

where *Kow* is octanol-water partition coefficient, *Fi* stands for the set of mitigating factors: metabolism, molecular size, ionization, *FWS* is water solubility factor, *Fw* is the organism water content. Further details on the mathematical formalism of the model can be reviewed in [4, 5]

8.2.4 Domain

The stepwise approach [6] was used to define the applicability domain of the model. It consists of the following sub-domain levels:

- General parametric requirements – includes ranges of variation log K_{OW} and *MW*,
- Structural domain – based on atom-centered fragments (ACFs),
- Mechanistic domain – identifies the mode of bioaccumulation of chemicals (partitioning in the organism lipids or binding to proteins).

A chemical is considered *In Domain* if its *log KOW* and *MW* are within the specified ranges (MW ranging from 16 to 1132 and log KOW in the range of -3.9 and 16.1, respectively, according to [5]), its ACFs are presented in the training chemicals and if the mode of bioaccumulation is driven by the lipophilicity only. The information implemented in the applicability domain is extracted from the correctly predicted training chemicals used to build the model and in this respect, the applicability domain determines practically the interpolation space of the model.

8.2.5 Performance

The goodness of fit evaluated by the squared coefficient of correlation is R^2 = 0.85. The model correctly classified 84% of experimentally bioaccumulative and 99% of experimentally not bioaccumulative training chemicals.

8.2.6 Reporting

The model provides results for:

- *log BCF* of organic chemicals corrected with mitigating factors, (l/kg. wet),
- *log* BCF_{max} ,
- Range of variation of maximum diameter for energetically stable conformers,
- Whole body primary biotransformation half-lives (*HL*) for organic chemicals in fish, days,
- Metabolic biotransformation rate constant K_M (d^{-1}),
- Effect of mitigating factors,
- Applicability domain details.

8.2.7 References

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8.3 US EPA T.E.S.T. V4.0.1

8.3.1 Qsar methodology

T.E.S.T allows you to estimate toxicity values using several different advanced Quantitative Structure Activity Relationship (QSAR) methodologies (Martin et al. 2008):

- **Hierarchical method:** The toxicity for a given query compound is estimated using the weighted average of the predictions from several different models. The different models are obtained by using Ward's method to divide the training set into a series of structurally similar clusters. A genetic algorithm based technique is used to generate models for each cluster. The models are generated prior to runtime.
- **FDA method**: The prediction for each test chemical is made using a new model that is fit to the chemicals that are most similar to the test compound. Each model is generated at runtime.
- **Single model method**: Predictions are made using a multilinear regression model that is fit to the training set (using molecular descriptors as independent variables) using a genetic algorithm based approach. The regression model is generated prior to runtime.
- **Group contribution method**: Predictions are made using a multilinear regression model that is fit to the training set (using molecular fragment counts as independent variables). The regression model is generated prior to runtime.
- **Nearest neighbor method**: The predicted toxicity is estimated by taking an average of the 3 chemicals in the training set that are most similar to the test chemical.
- **Consensus method**: The predicted toxicity is estimated by taking an average of the predicted toxicities from the above QSAR methods (provided the predictions are within the respective applicability domains).
- **Random forest method:** The predicted toxicity is estimated using a decision tree which bins a chemical into a certain toxicity score (i.e. positive or negative developmental toxicity) using a set of molecular descriptors as decision variables. *The random forest method is currently only available for the developmental toxicity endpoint*. The random forest models for the developmental toxicity endpoint were developed by researchers at Mario Negri Institute for Pharmacological Research as part of the CAESAR project (CAESAR 2009).

T.E.S.T provides multiple prediction methodologies so that one can have greater confidence in the predicted toxicities (assuming the predicted toxicities are fairly similar from different methods). In addition some researchers may have more confidence in particular QSAR approaches based on personal experience. The QSAR methodologies above are described in more detail in the Theory section.

The different QSAR methods have different advantages and disadvantages:

Method	Advantages	Disadvantages
Hierarchical	• Can produce more reliable predictions since predictions are made from multiple models	• Cannot provide external estimates of toxicity for compounds in the training set
Single model	• Single transparent model can be easily viewed/exported • The model does not need to rely on clustering the chemicals correctly	• Since the model is fit to the entire dataset it may incorrectly predict the trends in toxicity for certain chemical classes • Cannot provide external estimates of toxicity for compounds in the training set
Group contribution	• Single transparent model can be easily viewed/exported • Estimates of toxicity can be made without using a computer program	• The model doesn't correct for the interactions of adjacent fragments • Since the model is fit to the entire dataset it may incorrectly predict the trends in toxicity for certain chemical classes • Cannot provide external estimates of toxicity for compounds in the training set
FDA	• Can generate a new model based the closest analogs to the test compound • Always provides an external prediction of toxicity	• Predictions sometimes take longer since it has to generate a new model each time
Nearest neighbor	• Provides a quick estimate of toxicity • Allows one to determine structural analogs for a given test compound • Always provide an external prediction of toxicity	• It does not use a QSAR model to correlate the differences between the test compound and the nearest neighbors • Was shown to achieve the worst prediction results during external validation
Consensus	• Was shown to achieve the best prediction results during external validation	• Cannot provide external estimates of toxicity for compounds in the training set

8.3.2 Theory

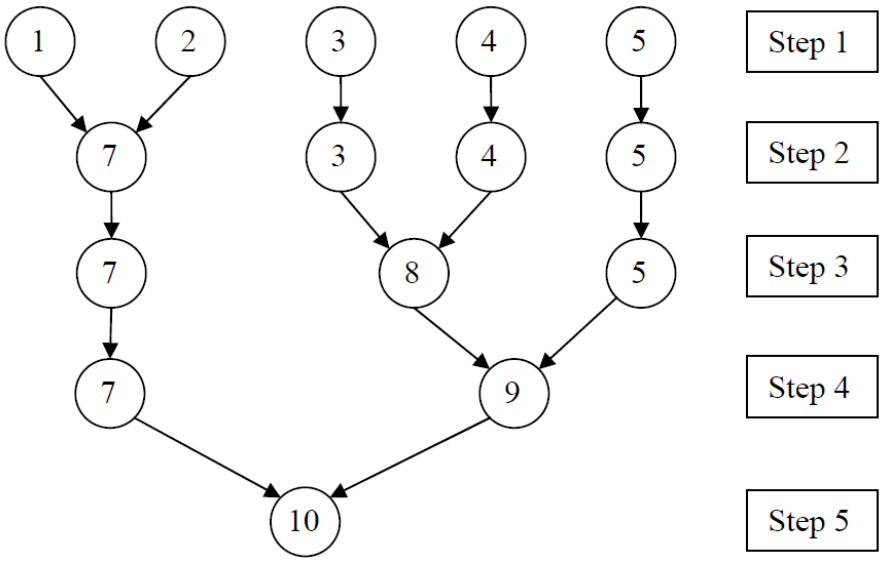
Molecular Descriptors

Molecular descriptors are physical characteristics of the structure of chemicals such as the molecular weight or the number of benzene rings. The overall pool of descriptors in the software contains 797 2-dimensional descriptors. The descriptors include the following classes of descriptors: E-state values and E-state counts, constitutional descriptors, topological descriptors, walk and path counts, connectivity, information content, 2d autocorrelation, Burden eigenvalue, molecular property (such as the octanol-water partition coefficient), Kappa, hydrogen bond acceptor/donor counts, molecular distance edge, and molecular fragment counts.

The descriptors were calculated using computer code written in Java. The basis of the molecular calculations was the Chemistry Development Kit (Steinbeck et al. 2003). The Chemistry Development Kit (CDK) is a Java library for structural chemo- and bioinformatics which is available at the following link: http://sourceforge.net/projects/cdk. The descriptor values were validated using MDL QSAR (Elsevier MDL 2006), Dragon (Talete 2006), and Molconn-z (Edusoft-LC 2006). The descriptor values were generally in good agreement (aside from small differences in the descriptor definitions for descriptors such as the number of hydrogen bond acceptors).

Hierarchical Clustering

The hierarchical clustering method utilizes a variation of Ward's Method (Romesburg1984) to produce a series of clusters from the training set. Clusters are subsets of chemicals from the overall set which possess similar properties. An example of a hierarchical clustering for a hypothetical training set with five chemicals is as follows:



For a training set of *n* chemicals, initially there will be n clusters (each cluster contains one chemical). The overall variance in the system at a given step l is defined to be the sum of the variances of the individual clusters:

$$V(l) \equiv \sum_{k=1}^{m} v(k,l) \tag{1}$$

where *v(k, l)* is the variance (in terms of the molecular descriptors) for cluster *k* at step *l*:

$$v(k,l) \equiv \sum_{i=1}^{n_k} \sum_{j=1}^{d} \left(x_{ij} - C_j\right)^2 \tag{2}$$

where n_k is the number of chemicals in the *k*th cluster, *d* is the number of descriptors in the overall descriptor pool, x_{ij} is the normalized descriptor *j* for chemical *i*, and C_j is the centroid or average value for descriptor *j* for cluster *k*:

$$C_j = \frac{1}{n_k} \sum_{i=1}^{n_k} x_{ij} \tag{3}$$

Each step of the method adds two of the clusters together into one cluster so that the increase in variance over all clusters in the system is minimized:

$$\min \Delta V(l+1) \equiv V(l+1) - V(l) = v(k', l+1) - v(k_1, l) - v(k_2, l) \tag{4}$$

where clusters k_1 and k_2 join together at step *l* to make cluster *k'* at step *l + 1*. The process of combining clusters continues until all of the chemicals are lumped into a single cluster.

After the clustering is complete, each cluster is analyzed to determine if an acceptable QSAR can be developed. Each cluster undergoes evaluation using a genetic algorithm technique to determine an optimal descriptor set for characterizing the toxicity values of the chemicals within that cluster. The maximum number of descriptors allowed for a given cluster will be nk / 5 since the recommended ratio of compounds to variables should be at least 5 (Eriksson et al. 2003; Topliss and Edwards 1979) for reasonably small probability for chance correlations. The genetic algorithm used in this study was taken from the Weka statistical package, version 3.5.1 (The University of Waikato 2007; Witten 2005).

The genetic algorithm is used to maximize the adjusted 5 fold leave many out cross validation coefficient ($q^2_{adj,\,LMO}$):

$$q^2_{adj.LMO} = 1 - \left[\frac{\sum_{i=1}^{n_k} \left(\hat{y}_i - y_{\exp,i}\right)^2 / (n_k - p - 1)}{\sum_{i=1}^{n_k} \left(y_{\exp,i} - \bar{y}_{\exp}\right)^2 / (n_k - 1)} \right] \tag{5}$$

where $\hat{y}_i$ and $y_{exp,i}$ are the predicted and experimental toxicity values for chemical *i*, y_{exp} is the average experimental toxicity for the chemicals in the cluster, and *p* is the number of parameters in the model. The predicted toxicity values are calculated by dividing the dataset into five folds (a fold is a subset of the training set). The toxicities of the chemicals in each fold ($\hat{y}_i$) are predicted using a multiple linear regression model fit to the chemicals in the other folds. The five fold q^2 was used instead of the traditional q^2 LOO (leave one out) inside the genetic algorithm because it yields a significant degree of computational savings for large cluster sizes.The $n_k - p - 1$ term penalizes models that include extra parameters that do not significantly increase the predictive power of the model (by decreasing the value of $q^2_{adj,\,LMO}$).

During the optimization process the models are also checked for outliers. A chemical is determined to be an outlier if at least two statistical tests (e.g., DFFITS, leverage, Cook's distance, and covariance ratio) indicate that the chemical represents an influential data point and if the chemical represents an outlier in terms of the studentized deleted residual (Kutner 2004). If a chemical is determined to be an outlier, the chemical is deleted from the cluster and the genetic algorithm descriptor selection is repeated. The process of model building via the genetic algorithm and outlier removal is repeated until no outliers are detected in the optimized model. *For binary endpoints such as Ames mutagenicity, outliers were not removed since this had the potential to produce clusters with all positive or all negative chemicals. In addition the outlier statistical tests described above may not apply to binary endpoints.*

Once the iteration for the optimum model has been completed, the q^2 LOO value for themodel is calculated. If the q^2 LOO is greater than or equal to 0.5, the model is considered to be valid (see pg 67 of (Eriksson et al. 2001)). If the q^2 LOO is less than 0.5, the model from the cluster is not used to make predictions for test compounds. For binary endpoints, the validity of a model is determined from the concordance LOO instead of q^2 LOO. Concordance is the fraction of all compounds that are predicted correctly (i.e. experimentally active compounds that are predicted to be active and experimentally inactive compounds that are predicted to be inactive). If the concordance LOO is greater than or equal to 0.8, the model is considered to be valid. In addition both the leave one out sensitivity and specificity must be at least 0.5 to avoid using models which are heavily biased to predict either active or inactive scores. Sensitivity is the fraction of experimentally active compounds that are predicted to be active. Specificity is the fraction of experimentally inactive compounds that are predicted to be inactive.

The predicted toxicity ($\hat{y}$) for a test chemical is given by the weighted average for all the valid predictions (Wikipedia.org 2008):

$$\hat{y} = \frac{\sum_{j=1}^{nvc} w_j \hat{y}_j}{\sum_{j=1}^{\#valid\ clusters} w_j} \qquad (6)$$

where $\hat{y}_j$ and w_j are prediction and weight for the *j*th model and *nvc* is the number of valid cluster model predictions. If the mean toxicity is given by the maximum likelihood estimator of the mean of the probability distributions, the weight values are given by (Wikipedia.org 2008)

$$w_j = \frac{1}{se_j^{\,2}} \qquad (7)$$

where se_j is the standard error for the jth prediction given by

$$se_j = \sqrt{\sigma_j^2 (1 + h_{00})} \tag{8}$$

where σ_j^2 is given by

$$\sigma_j^2 = \frac{\sum_{i=1}^{n_j} (\hat{y}_i - y_{\exp,i})^2}{n_j - p_j - 1} \tag{9}$$

where n_j is the number of chemicals in cluster model j and p_j is the number of model parameters for model j. h_{00}, the leverage for the test chemical, is given by

$$h_{00} = X_o^T (X^T X)^{-1} X_0 \tag{10}$$

where X_0 is the vector of model descriptor values for the test compound. *For binary endpoints such as Ames mutagenicity, the predictions were made using equal weighting of the individual predictions (i.e. wj = 1 in equation 6) since weighting by the standard error (see equation 7) did not improve the external prediction accuracy.*

The square of the standard deviation for the prediction from multiple models (σ_μ^2) can be approximated as

$$\sigma_\mu^2 = \frac{\overline{\sigma^2}}{nvc} = \left(1/nvc\right) \frac{\sum_{j=1}^{nvc} w_j se_j^2}{\sum_{j=1}^{nvc} w_j} = \left(1/nvc\right) \frac{\sum_{j=1}^{nvc} \left(1/se_j^2\right) se_j^2}{\sum_{j=1}^{nvc} \left(1/se_j^2\right)} = \frac{1}{\sum_{j=1}^{nvc} \left(1/se_j^2\right)} \tag{11}$$

The uncertainty (uˆ) in the overall prediction for the test chemical is given by

$$\hat{u} = t_{1-\alpha/2,nvc} \sigma_\mu = t_{1-\alpha/2,nvc-1} \sqrt{1 / \sum_{j=1}^{nvc} \frac{1}{se_j^2}} \tag{12}$$

where t is the t-statistic, $\alpha = 0.1$ (90% confidence interval), and se_j is the standard error for the jth prediction. The prediction interval is obtained by adding and subtracting the uncertainty from the predicted toxicity:

$$\hat{y} - \hat{u} \le Toxicity \le y + \hat{u} \tag{13}$$

The prediction interval indicates that one is 90% confident that the actual toxicity is between $\hat{y} - \hat{u}$ and $\hat{y} + \hat{u}$.

The prediction uncertainty for a given cluster model is given by (Montgomery 1982)

$$u_j = t_{1-\alpha/2,\, n_j - p - 1} \sqrt{\sigma^2 (1 + h_{00})} \qquad (14)$$

The uncertainty is a function of the quality of the regression model (from the σ^2parameter) and the distance (in the descriptor space of the model) between the test chemical and the chemicals in the cluster used to build the model (from the h_{00} parameter).

Before any cluster model can be used to make a prediction for a test chemical, it must be determined whether the test chemical falls within the domain of applicability for the model. The applicability domain is defined using several different constraints. The first constraint, the model ellipsoid constraint, checks if the test chemical is within the multidimensional ellipsoid defined by the ranges of descriptor values for the chemicals in the cluster (for the descriptors appearing the cluster model). The model ellipsoid constraint is satisfied if the leverage of the test compound (h_{00}) is less than the maximum leverage value for all the compounds used in the model (Montgomery 1982). The second constraint, the Rmax constraint, checks if the distance from the test chemical to the centroid of the cluster is less than the maximum distance for any chemical in the cluster to the cluster centroid. The distance is defined in terms of the entire pool of descriptors (instead of just the descriptors appearing in the model):

$$distance_i = \sum_{j=1}^{d} \left(x_{ij} - C_j \right)^2 \qquad (15)$$

where $distance_i$ is the distance of chemical *i* to the centroid of the cluster.

The last constraint, the fragment constraint, is that the compounds in the cluster have to have at least one example of each of the fragments contained in the test chemical. For example if one was trying to make a prediction for ethanol, the cluster must contain at least one compound with a methyl fragment (-CH3 [aliphatic attach]), one compound with a methylene fragment (-CH2 [aliphatic attach]), and one compound with a hydroxyl fragment (-OH [aliphatic attach]). This constraint was added to avoid situations where a chemical might have a similar backbone structure to the chemicals in a given cluster but has a different functional group attached. For example if a given cluster contained only short-chained aliphatic amines one wouldn't want to use it to predict the toxicity of ethanol. If a chemical contains a fragment that is not present in the training set, the toxicity cannot be predicted. The fragment constraint can be removed by checking the **Relax fragment constraint checkbox**. *For binary endpoints such as Ames mutagenicity, the fragment constraint was not employed since it did not improve the external prediction accuracy and decreased the prediction coverage.*

In the current version of the software, the predictions are made using the *closest cluster from each step* in the hierarchical clustering (in terms of the distance of the chemical to the centroid of the cluster defined above). The rationale behind this approach is that one would like to follow the hierarchical clustering process, selecting the best model from each step. In order for the prediction from the model to be used it must be statistically valid and meet the constraints defined above. If the closest cluster for a given step does not have a statistically valid model (or violates any of the constraints), no prediction is used from that step. If the closest cluster for a given step in the clustering process is the same as the closest cluster from a previous step it is not used again in the prediction of toxicity.

FDA Method

The FDA (Food and Drug Administration) method is based on the work of Contrera and coworkers (Contrera et al. 2003). In this method, predictions for each test chemical are made using a unique cluster (constructed at runtime) which contains structurally similar chemicals selected from the overall training set. This is in contrast to the Hierarchical method, where the predictions are made using one or more clusters that were constructed a priori using Ward's method.

Contrera and coworkers constructed the training cluster by selecting 15-20 chemicals which had at least a cosine similarity coefficient of 75% with the test chemical. The cosine similarity coefficient, $SC_{i,k}$, is given by

$$SC_{i,k} = \frac{\sum_{j=1}^{\#descriptors} x_{ij} x_{kj}}{\sqrt{\sum_{j=1}^{\#descriptors} x_{ij}^{2} \cdot \sum_{j=1}^{\#descriptors} x_{kj}^{2}}} \qquad (16)$$

where x_{ij} is the value of the jth normalized descriptor for chemical *i* (normalized with respect to all the chemicals in the original training set) and x_{kj} is the value of the jth descriptor for chemical *k*. A multiple linear regression model is then built for the new cluster using a genetic algorithm and the toxicity is predicted. The advantage of this method is that the training cluster is tailored to fit the test chemical. In addition the test chemical is never present in the cluster model, which allows one to make external predictions for training set chemicals. The disadvantage of this method is that a new model has to be generated at runtime (which takes somewhat longer than computing the toxicity from preexisting models).

In this version of the software, clusters are constructed using the thirty most similar chemicals from the training set in terms of the cosine similarity coefficient. However, a minimum similarity coefficient of 75% is not required for membership in the training cluster. Previously it was determined that this constraint did not increase the predictive performance of the methodology (Martin et al. 2008). For a prediction to be valid, the cluster must not violate the model ellipsoid and fragment constraints described above. In addition, the predicted toxicity value must be within the range of experimental toxicity values for the chemicals used to build the model. This additional constraint was added to avoid potentially erroneous predictions.

However this constraint was not utilized for binary toxicity endpoints such as Ames mutagenicity since predicted values less than 0 or greater than 1 do not invalidate the prediction result.

Again for a cluster to have a valid predictive model, the LOO q^2 must be at least 0.5. If the model for the cluster is invalid or the prediction violates one of the constraints, the cluster size is increased incrementally (up to a maximum of 75 chemicals) until a valid prediction can be made. If a prediction cannot be made using a cluster with 75 chemicals, no prediction is made.

Single model

In the single model approach, a single multiple linear regression model is fit to the entire training set. The model is generated using techniques and constraints similar to those for the hierarchical

method (except that the training cluster contains the entire training set). The advantage of this approach is that a simple transparent model can be developed which does not rely on clustering the chemicals correctly. The disadvantage of this approach is that sometimes an overall model cannot correctly correlate the toxicity for every chemical class (Benigni and Richard 1996). For example the single model might be able to correctly describe the trend of linearly increasing toxicity for a series of normal alcohols (i.e. 1-propanol, 1-butanol,1-pentanol, …) but it may incorrectly describe the trend for a series of normal acids (i.e. propanoic acid, butanoic acid, pentanoic acid, …) which does not increase linearly.

Group contribution

The group contribution approach is based on the group contribution approach of Martin and Young (Martin and Young 2001). Fragment counts (such as the number of methyl and hydroxyl groups in a compound) are used to fit a multiple linear regression model to the entire data set. A genetic algorithm approach is not used to reduce the number of parameters in the model since the approach tries to characterize the contribution from all the fragments appearing in the training set. The only constraint on the fragments appearing in the final model is that there must be at least three molecules in the training set that contain each fragment. If a fragment appears less than three times in the training set, it is deleted from the list of fragments and all the chemicals containing this fragment are removed from the training set. After the multiple linear regression is performed, the model is checked for outliers. If any outliers are detected, they are removed and the regression is performed again. The process is repeated until no more outliers are found. Similar to the hierarchical methodology, predictions are made using the model ellipse and fragment constraints.

The advantage of this approach is a single transparent model can be developed whose descriptors can be determined from visual inspection of the molecular structure of the test compound. The disadvantage of this approach is that it assumes that the contribution of each fragment does not depend on the presence of nearby fragments in the molecule.

Nearest neighbor

In the nearest neighbor approach, the predicted toxicity is simply the average of the toxicities of the three most similar chemicals (structural analogs) in the training set. In order to make a prediction, each of the structural analogs must exceed a certain minimum cosine similarity coefficient (SC*min*). SC*min* was set at 0.5 so that the prediction coverage was similar to the other QSAR methods (Martin et al. 2008). The nearest neighbor method provides a quick external estimate of toxicity (the test chemical is never present in the selected set of analogs). The disadvantage of the nearest neighbor method is that the structural differences between the test chemical and its structural analogs are not accounted for.

Consensus

In the consensus method, the predicted toxicity is simply the average of the predicted toxicities from the other QSAR methodologies (taking into account the applicability domain of each method)(Zhu et al. 2008). If only a single QSAR methodology can make a prediction, the predicted value is deemed unreliable and not used. This method typically provides the highest prediction

accuracy since errant predictions are dampened by the predictions from the other methods. In addition this method provides the highest prediction coverage because several methods with slightly different applicability domains are used to make a prediction.

8.3.3 Validation Methods

Statistical external validation

The predictive ability of each of the QSAR methodologies was evaluated using statistical external validation (Gramatica and Pilutti 2004). In version 2.0 of the TEST software, the data set was divided into training and test sets using the Kennard-Stone rational design algorithm (Bourguignon et al. 1994a; Bourguignon et al. 1994b; Kennard and Stone 1969; Snarey et al.1997). Starting in version 3.0, random selection was used to develop the training and test sets because it was felt that using Kennard-Stone method yields an overly optimistic estimate of predictive ability (because the test compounds are always within the model calibration domain). *For the developmental toxicity endpoint, however, the training and test sets were taken from the datasets used in CAESAR* (CAESAR 2009). This was done so that the CAESAR random forest model could be incorporated into the TEST software.

A QSAR model has acceptable predictive power if the following conditions are satisfied (Golbraikh et al. 2003):

$$q^2 > 0.5; \tag{17}$$

$$R^2 > 0.6; \tag{18}$$

$$\frac{\left(R^2 - R_o^2\right)}{R^2} < 0.1 \text{ and } 0.85 \le k \le 1.15 \tag{19}$$

where q^2 is the leave one out correlation coefficient for the training set, R^2 is correlation coefficient between the observed and predicted toxicities for the test set, R_o^2 is correlation coefficient between the observed and predicted toxicities for the test set with the Y-intercept set to zero (where the regression line is given by Y=kX).

The prediction accuracy will be evaluated in terms of equations 18 and 19. In addition the accuracy will be evaluated in terms of the RMSE (root mean square error), and the MAE (mean absolute error) for the test set. It has been demonstrated that q2 (the leave one out correlation coefficient for the training set) is not correlated with R2 for the test set (Golbraikh and Tropsha 2002). The prediction coverage (fraction of chemicals predicted) must also be considered because the prediction accuracy (in terms of R^2 and RMSE) can sometimes be improved at the sacrifice of the prediction coverage.

For binary (active/inactive) toxicity endpoints such as developmental toxicity, the prediction accuracy is evaluated in terms of the fraction of compounds that are predicted accurately. The prediction accuracy is evaluated in terms of three different statistics: concordance, sensitivity, and specificity. Concordance is the fraction of all compounds that are predicted correctly (i.e. experimentally active compounds that are predicted to be active and experimentally inactive compounds that are predicted to be inactive). Sensitivity is the fraction of experimentally active compounds that are predicted to be active. Specificity is the fraction of experimentally inactive compounds that are predicted to be inactive.

8.3.4 Experimental Data Sets

Bioconcentration factor data set

The bioconcentration factor BCF is defined as the ratio of the chemical concentration in biota as a result of absorption via the respiratory surface to that in water at steady state (Hamelink 1977). Data was compiled from several different databases (Dimitrov et al. 2005; Arnot and Gobas 2006; EURAS ; Zhao 2008). The final dataset consists of 676 chemicals (after removing salts, mixtures, and ambiguous compounds). The modeled endpoint was the Log10(BCF).

8.3.5 Validation Results

Bioaccumulation factor (BCF)

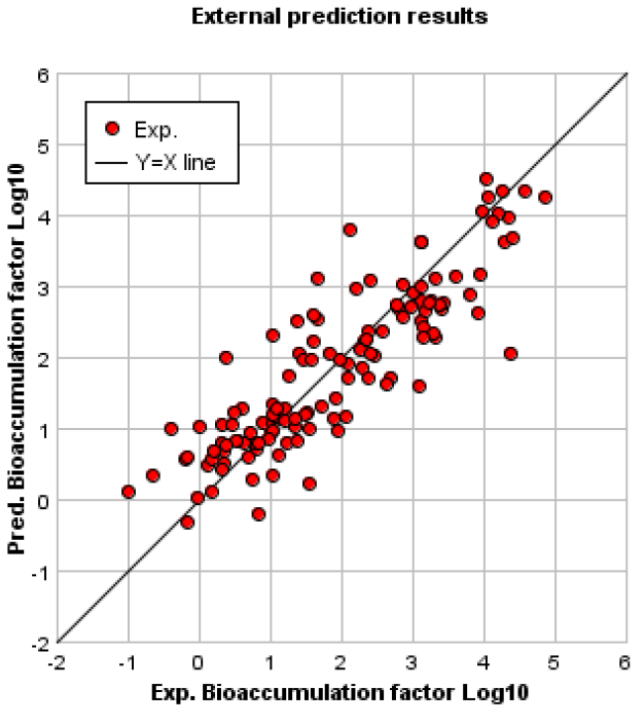
– Statistical External Validation –

The prediction results for the BCF endpoint were as follows:

Method	R^2	$\frac{R^2 - R_0^2}{R^2}$	k	RMSE	MAE	Coverage
Hierarchical	0.734	0.019	0.888	0.712	0.541	0.926
Single Model	0.742	0.083	0.901	0.684	0.543	0.926
FDA	0.705	0.036	0.905	0.746	0.571	0.911
Group Contribution	0.675	0.187	0.888	0.760	0.622	0.874
Nearest neighbor	0.609	0.100	0.931	0.884	0.604	0.948
Consensus	0.760	0.066	0.900	0.661	0.513	0.926

Again the consensus method yielded the best results if one considers both prediction accuracy and coverage.

The prediction results for the consensus method are given by:

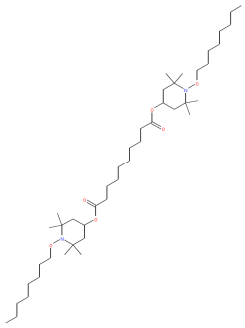
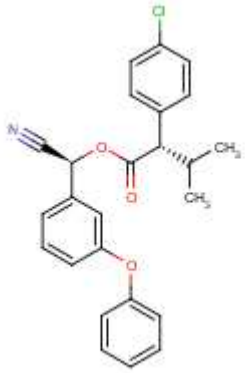


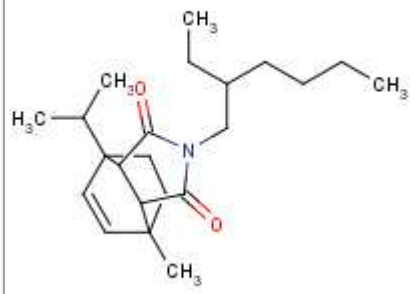
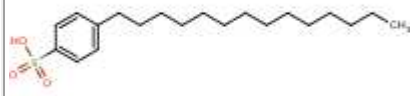
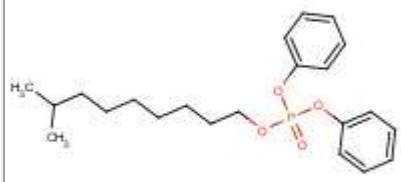
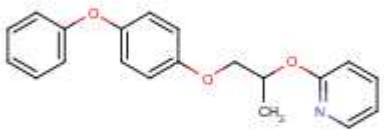
The BCFBAF module (v. 3.00) of US EPA's EPI Suite software package (USEPA 2009) yielded an R^2 value of 0.766 and MAE of 0.50 (for the same chemicals that were able to be predicted by the consensus method). Thus the predictions for the consensus method are comparable to those from EPI Suite. However, this may not be a fair comparison since some of the chemicals in the prediction set may have appeared in the training set for the BCF model in EPI Suite.

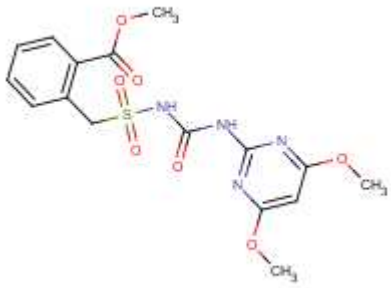
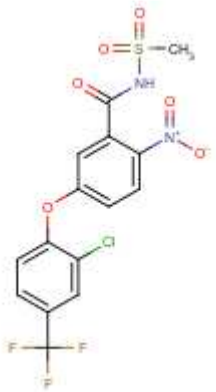
8.3.6 Comprehensive Results For Compound 1 And 2 Using T.E.S.T

Compound 1

Predictions for the test chemical and for the most similar chemicals in the external test set

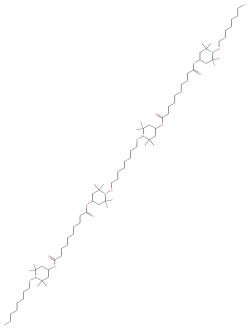
CAS	Structure	Similarity Coefficient	Experimental value Log10	Predicted value Log10
Compound 1 (test chemical)			N/A	0.43
4051-66-5		0,78	1.48	1.21
66230-04-4		0,68	3.17	2.01

CAS	Structure	Similarity Coefficient	Experimental value Log10	Predicted value Log10
13358-11-7		0.63	3.16	1.71
47377-16-2		0.63	0.52	1.80
29761-21-5		0.62	2.63	2.01
95737-68-1		0.56	3.14	2.16

CAS	Structure	Similarity Coefficient	Experimental value Log10	Predicted value Log10
83055-99-6		0.53	0.20	0.64
72178-02-0		0.52	0.78	1.46

Compound 2

Predictions for the test chemical and for the most similar chemicals in the external test set

CAS	Structure	Similarity Coefficient	Experimental value Log10	Predicted value Log10
Compund 2 (test chemical)			N/A	0.15
4051-66-5		0.64	1.48	1.21

8.3.7 References

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8.4 BCFBAF Program (V3.01) (part of EPI Suite V4.10)

8.4.1 Estimation Methodology

The original estimation methodology used by the original BCFWIN program is described in a document prepared for the U.S. Environmental Protection Agency (Meylan et al., 1997). The estimation methodology was then published in journal article (Meylan et al, 1999).

The BCFBAF Program updates the BCF estimation methodology of the BCFWIN program by using an updated and better evaluated BCF database for selecting training and validation datasets. The exact same regression methodology used to derive the original BCFWIN method was used to derive the BCFBAF method for estimating BCF.

Experimental BCF Data

The measured BCF values used in the revised regressions were selected from a quality reviewed BCF database (Arnot and Gobas, 2006); details of the data quality review methods are described in Arnot and Gobas (2006). Single BCF values were selected for each compound (median values were generally selected for compounds with multiple values).

The BCF values selected for the BCFBAF training and validation datasets are available in Appendix G and via Internet download at:

http://esc.syrres.com/interkow/EpiSuiteData.htm ... A substructure searchable version of the data can be downloaded at: http://esc.syrres.com/interkow/EpiSuiteData_ISIS_SDF.htm

Estimation Methodology

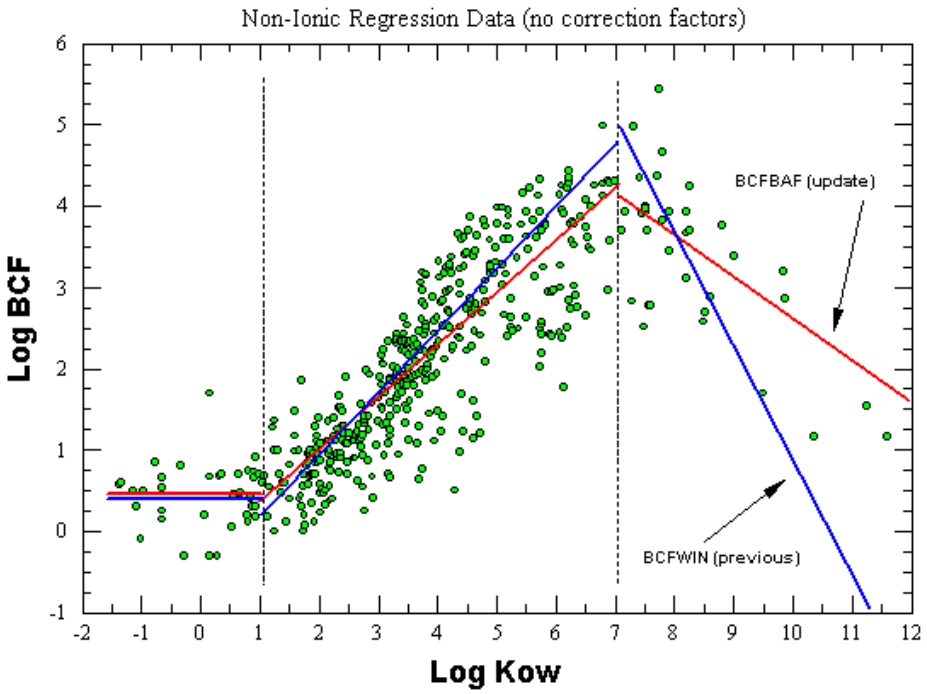
The following is a brief summary of the estimation methodology :

The BCFBAF method classifies a compound as either ionic or non-ionic. Ionic compounds include carboxylic acids, sulfonic acids and salts of sulfonic acids, and charged nitrogen compounds (nitrogen with a +5 valence such as quaternary ammonium compounds). All other compounds are classified as non-ionic.

Training Dataset Included:
466 Non-Ionic Compounds
61 Ionic Compounds (carboxylic acids, sulfonic acids, quats)

Methodology for Non-Ionic was to separate compounds into three divisions by Log Kow value as follows:
Log Kow < 1.0
Log Kow 1.0 to 7.0
Log Kow > 7.0

The following graph of the raw data illustrates the divisions and the comparison of the new BCFBAF regression lines to the previous BCFWIN regression lines:



For each division, a "best-fit" straight line was derived by common statistical regression methodology. The graph does not adjust individual data points with correction factors derived for BCFBAF. The regression methodology includes derivation of correction factors based on specific structural features. Appendix E lists all correction factors used by BCFBAF (with a comparison to BCFWIN). Non-ionic compounds are predicted by the following relationships:

<u>For Log Kow 1.0 to 7.0 the derived QSAR estimation equation is :</u>

$$\text{Log BCF} = 0.6598 \text{ Log Kow} - 0.333 + \Sigma \text{ correction factors}$$

(n = 396, r^2 = 0.792, Q^2 = 0.78, std dev = 0.511, avg dev = 0.395)

The previous BCFWIN equation:

$$\text{Log BCF} = 0.77 \text{ Log Kow} - 0.70 + \Sigma \text{ correction factors}$$

<u>For Log Kow > 7.0 the derived QSAR estimation equation is :</u>

$$\text{Log BCF} = -0.49 \text{ Log Kow} + 7.554 + \Sigma \text{ correction factors}$$

(n = 35, r^2 = 0.634, Q^2 = 0.57, std dev = 0.538, avg dev = 0.396)

The previous BCFWIN equation:

$$\text{Log BCF} = -1.37 \text{ Log Kow} + 14.4 + \Sigma \text{ correction factors}$$

Certain super-hydrophobic chemicals (Log Kow >7.0) selected from the empirical database had reported BCF values with measured water concentrations that exceed water solubility limits. These BCF values were corrected based on estimates of water solubility limits (Arnot and Gobas, 2006).

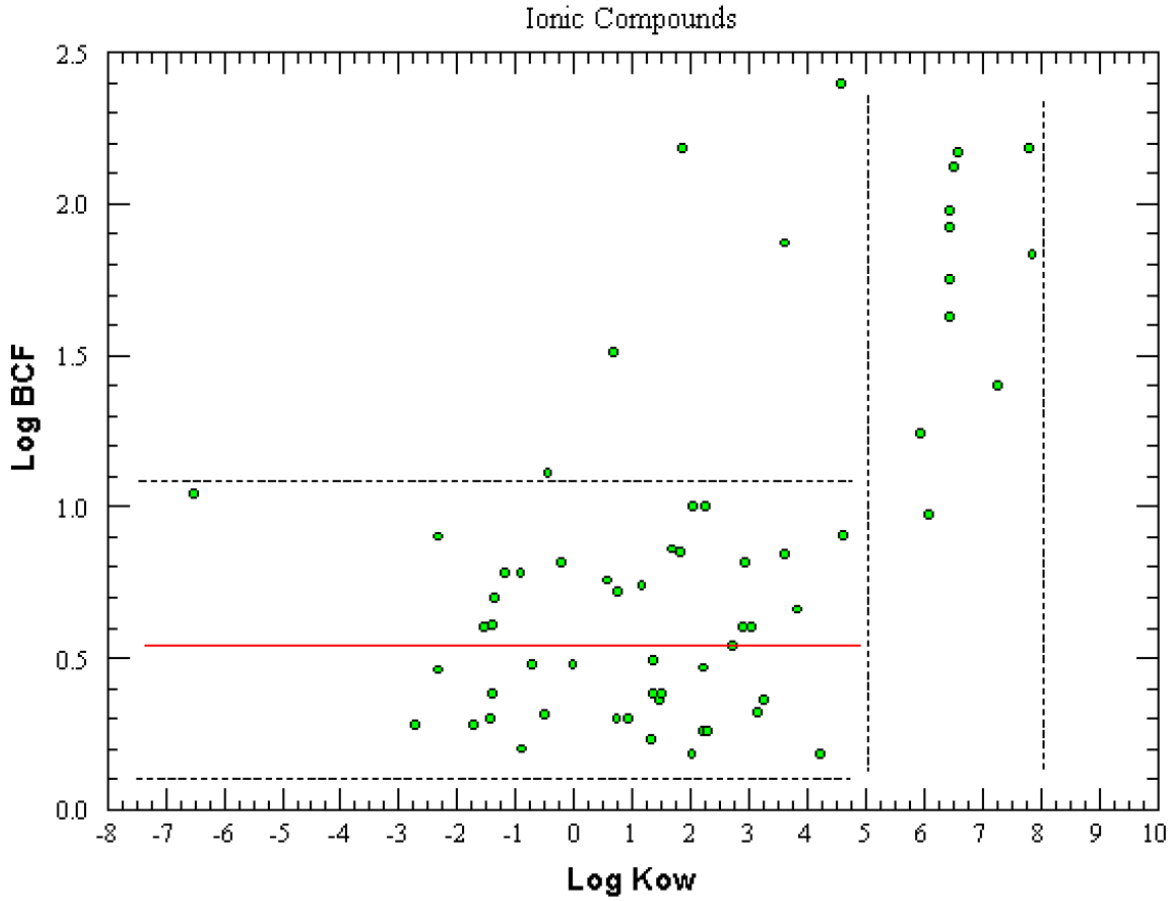
For Log Kow < 1.0 the derived QSAR estimation equation is :

All compounds with a log Kow of less than 1.0 are assigned an estimated log BCF of 0.50 (same as in BCFWIN).

Ionic compounds are predicted as follows:

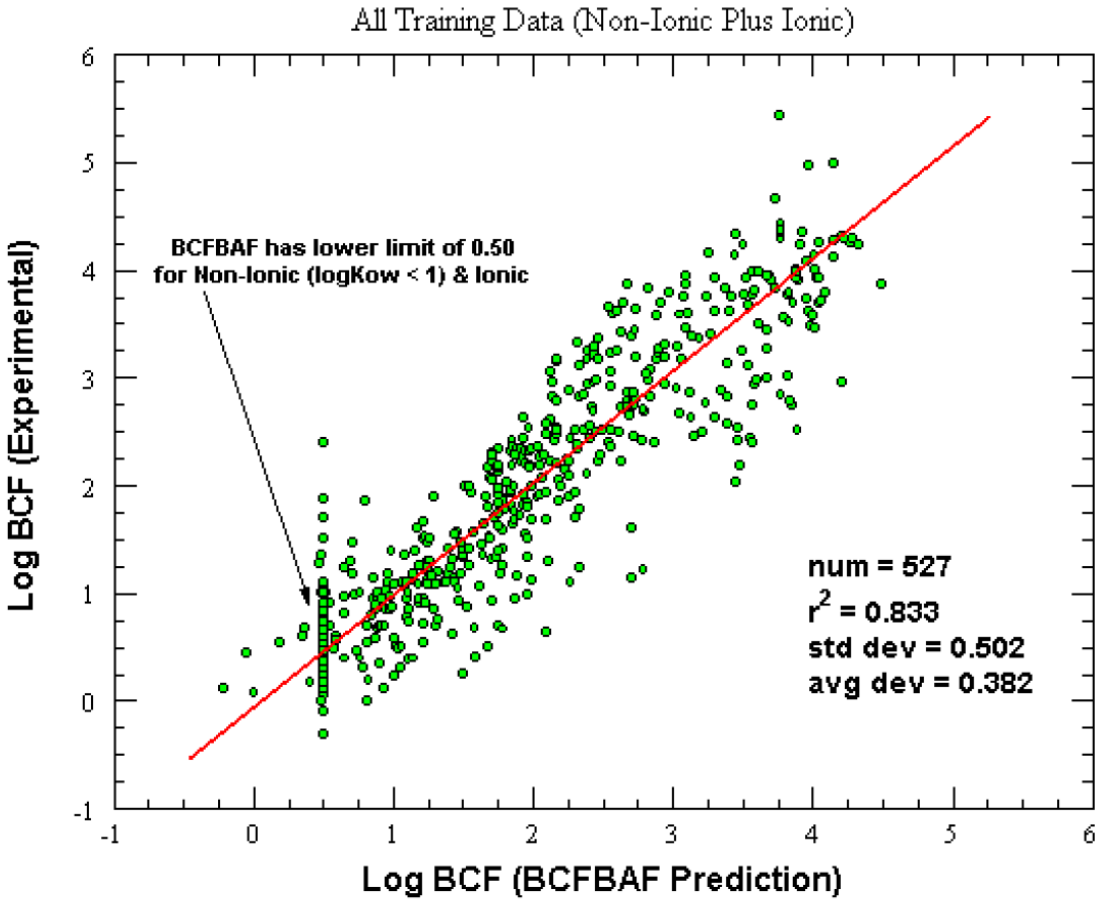
log BCF = 0.50 (log Kow < 5.0)
log BCF = 1.00 (log Kow 5.0 to 6.0)
log BCF = 1.75 (log Kow 6.0 to 8.0)
log BCF = 1.00 (log Kow 8.0 to 9.0)
log BCF = 0.50 (log Kow > 9.0)

The graph of Ionic Compounds versus Log Kow :

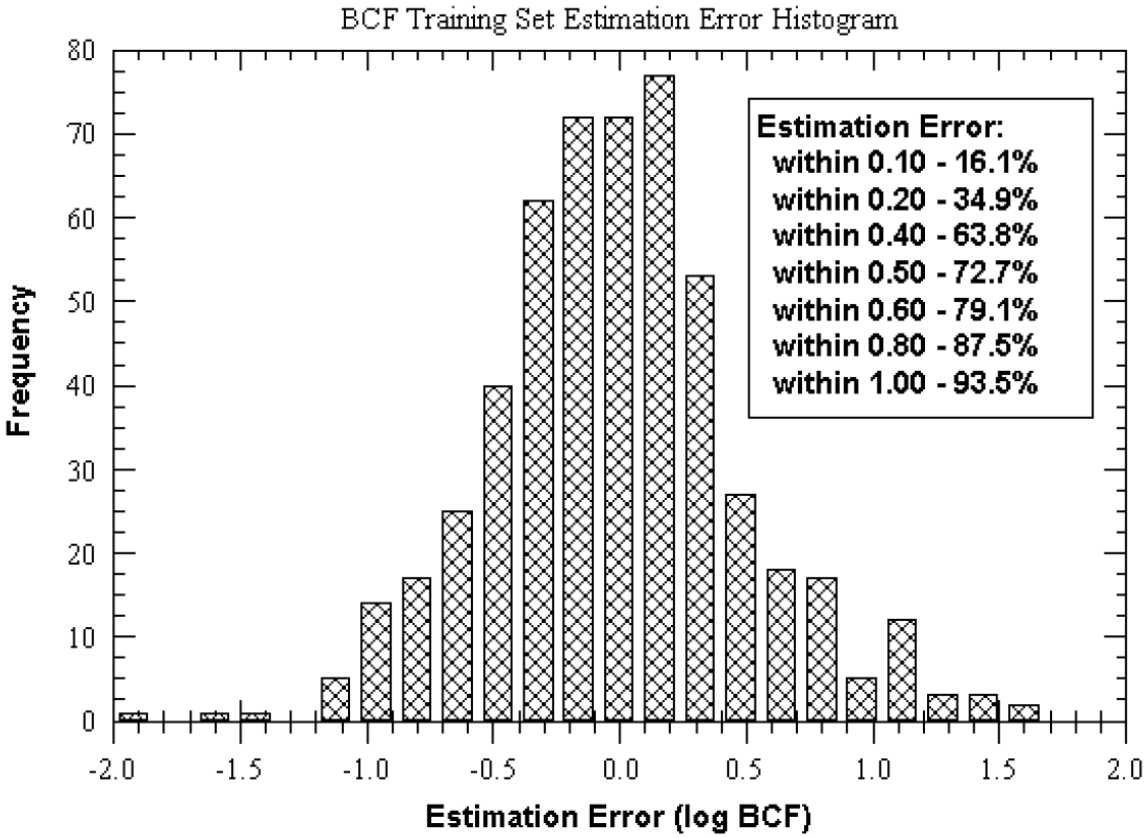


Metals (tin and mercury), long chain alkyls and aromatic azo compounds require special treatment.

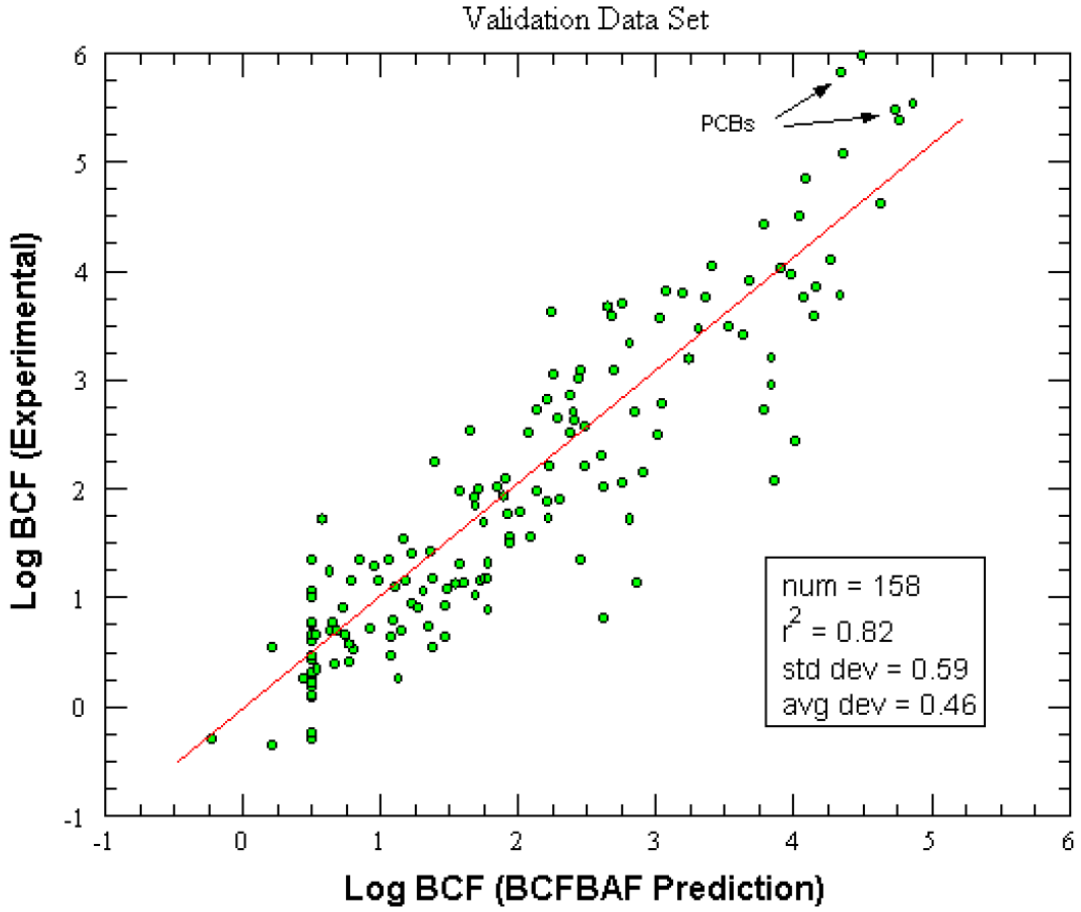
*Estimation Accuracy*Accuracy of the Training Set:



Error Histogram for the Training Set:



Accuracy of the Validation Set:



Estimation Domain

Appendix E gives for each correction factor the maximum number of instances of that factor in any of the 527 training set compounds (the minimum number of instances is of course zero, since not all compounds had every correction factor). The minimum and maximum values for molecular weight and logKow are listed below. Currently there is no universally accepted definition of model domain. However, users may wish to consider the possibility that bioconcentration factor estimates are less accurate for compounds outside the MW and logKow ranges of the training set compounds, and/or that have more instances of a given correction factor than the maximum for all training set compounds. It is also possible that a compound may have a functional group(s) or other structural features not represented in the training set, and for which no fragment coefficient was developed; and that a compound has none of the fragments in the model's fragment library. In the latter case, predictions are based on molecular weight alone. These points should be taken into consideration when interpreting model results.

– Training Set (527 Compounds) –

Molecular Weight:
Minimum MW: 68.08 (Furan)
Maximum MW: 991.80 Ionic: (2,7-Naphthalenedisulfonic acid, 4-amino-5-hydroxy-3,6- bis[[4-[[2-(sulfooxy)ethyl]sulfonyl]phenyl]azo]-, tetrasodium salt)
Maximum MW: 959.17 Non-Ionic: (Benzene, 1,1 -oxybis[2,3,4,5,6-pentabromo-)
Average MW: 244.00

Log Kow:
Minimum LogKow: -6.50 Ionic: (2,7-Naphthalenedisulfonic acid, 4-amino-5-hydroxy-3,6-bis[[4-[[2- (sulfooxy)ethyl]sulfonyl]phenyl]azo]-, tetrasodium salt)
Minimum LogKow: -1.37 Non-Ionic: (1,3,5-Triazine-2,4,6-triamine)
Maximum LogKow: 11.26 (Benzenamine, ar-octyl-N-(octylphenyl)-)

8.5 VEGA

8.5.1 VEGA CAESAR BCF Model Version 2.1.13

Introduction

The model provides a quantitative prediction of bioconcentration factor (BCF) in fish, given in log(L/kg). It is implemented inside the VEGA online platform, accessible at:http://www.vega-qsar.eu/ The model extends the original CAESAR model, freely available at: http://www.caesar-project.eu/software/

Model details

Two models, Model A and Model B, have been used to build hybrid model, Model C. In the proposed approach, the outputs of the individual models (Model A and B) were used as inputs of the hybrid model. Model A was developed by Radial Basis Function Neural Networks (RBFNN) using an heuristic method to select the optimal descriptors; Model B was developed by RBFNN using genetic algorithm for the descriptors selection. RBFNN was used with a Matlab function for building the models. An in-house software made as a PC-Windows Excel macro was used to combine Models A and B within the Model C. Model A used an heuristic method to select the optimal descriptors and Model B used genetic algorithm for the descriptors selection. Full reference and details of the used formulas can be found in:

Zhao, C., Boriani, E., Chana, A., Roncaglioni,A., Benfenati, E. A new hybrid system of QSAR models for predicting bioconcentration factors (BCF). Chemosphere (2008), 73, 1701-1707.

Lombardo A, Roncaglioni A, Boriani E, Milan C, Benfenati E. Assessment and validation of the CAESAR predictive model for bioconcentration factor (BCF) in fish. Chemistry Central Journal (2010), 4 (Suppl 1).

The descriptors used are the following:

- Moriguchi octanol-water partition coefficient (MlogP).

- Moran autocorrelation of lag 5, weighted by atomic van der Waals volumes (MATS5V): molecular descriptor calculated from the molecular graph by summing the products of atom weights of the terminal atoms of all paths of the considered path length (the lag).

- Number of chlorine atoms (Cl-089), Cl attached to carbon (sp2).

- Second highest eigenvalue of Burden matrix, weighted by atomic polarizabilities (BEHp2).

- Geary autocorrelation of lag 5, weighted by atomic van der Waals volumes (GATS5V): molecular descriptor calculated from the molecular graph by summing the products of atom weights of the terminal atoms of all paths of the considered path length (the lag).

- Solvation connectivity index chi-0 (XOSolv): molecular descriptor designed for modeling solvation entropy and describing dispersion interactions in solution.

- Sum of all -Cl groups E-state values in molecule (SsCl).

- Absolute eigenvalues sum from electronegativity weighted distance matrix (Aeige).

The descriptors were calculated, in the original CAESAR version, by means of dragonX software and are now entirely calculated by an in-house software module in which they are implemented as described in: R. Todeschini and V. Consonni, Molecular Descriptors for Chemoinformatics, Wiley-VCH, 2009.

Applicability Domain

The applicability domain of predictions is assessed using an Applicability Domain Index (ADI) that has values from 0 (worst case) to 1 (best case). The ADI is calculated by grouping several other indices, each one taking into account a particular issue of the applicability domain. Most of the indices are based on the calculation of the most similar compounds found in the training and test set of the model, calculated by a similarity index that consider molecule's fingerprint and structural aspects (count of atoms, rings and relevant fragments). Note that when the experimental value for the given compound is found, the Applicability Domain indices are calculated only considering this value, without taking into account the first n similar compounds.

For each index, including the final ADI, three intervals for its values are defined, such that the first interval corresponds to a positive evaluation, the second one corresponds to a suspicious evaluation and the last one corresponds to a negative evaluation.

Following, all applicability domain components are reported along with their explanation and the intervals used.

- Similar molecules with known experimental value. This index takes into account how similar are the first two most similar compounds found. Values near 1 mean that the predicted compound is well represented in the dataset used to build the model, otherwise the prediction could be an extrapolation. Defined intervals are:

1 >= index > 0.9	strongly similar compounds with known experimental value in the training set have been found
0.9 >= index > 0.75	only moderately similar compounds with known experimental value in the training set have been found
index <= 0.75	no similar compounds with known experimental value in the training set have been found

- Accuracy (average error) of prediction for similar molecules. This index takes into account the error in prediction for the two most similar compounds found. Values near 0 mean that the predicted compounds falls in an area of the model's space where the model gives reliable predictions, otherwise the greater is the value, the worse the model behaves. Defined intervals are:

index < 0.5	accuracy of prediction for similar molecules found in the training set is
0.5 <= index <= 1.0	accuracy of prediction for similar molecules found in the training set is not optimal
index > 1.0	accuracy of prediction for similar molecules found in the training set is not adequate

- Concordance with similar molecules (average difference between target compound prediction and experimental values of similar molecules) . This index takes into account the difference between the predicted value and the experimental values of the two most similar compounds. Values near 0 mean that the prediction made agrees with the experimental values found in the model's space, thus the prediction is reliable. Defined intervals are:

index < 0.5	similar molecules found in the training set have experimental values that
0.5 <= index <= 1.0	similar molecules found in the training set have experimental values that slightly disagree with the target compound predicted value
index > 1.0	similar molecules found in the training set have experimental values that completely disagree with the target compound predicted value

- Maximum error of prediction among similar molecules. This index takes into account the maximum error in prediction among the two most similar compounds. Values near 0 means that the predicted compounds falls in an area of the model's space where the model gives reliable predictions without any outlier value. Defined intervals are:

index < 0.5	the maximum error in prediction of similar molecules found in the training set has a low value, considering the experimental variability
0.5 <= index < 1.0	the maximum error in prediction of similar molecules found in the training set has a moderate value, considering the experimental variability
index >= 1.0	the maximum error in prediction of similar molecules found in the training set has a high value, considering the experimental variability

- Atom Centered Fragments similarity check. This index takes into account the presence of one or more fragments that aren't found in the training set, or that are rare fragments. First order atom centered fragments from all molecules in the training set are calculated, then compared with the first order atom centered fragments from the predicted compound; then the index is calculated as following: a first index RARE takes into account rare fragments (those who occur less than three times in the training set), having value of 1 if no such fragments are found, 0.85 if up to 2 fragments are found, 0.7 if more than 2 fragments are found; a second index NOTFOUND takes into account not found fragments, having value of 1 if no such fragments are found, 0.6 if a fragments is found, 0.4 if more than 1 fragment is found. Then, the final index is given as the product RARE * NOTFOUND. Defined intervals are:

index = 1	all atom centered fragment of the compound have been found in the compounds of the training set
1 > index >= 0.7	some atom centered fragment of the compound have not been found in the compounds of the training set or are rare fragments
index < 0.7	a prominent number of atom centered fragments of the compound have not been found in the compounds of the training set or are rare

- Descriptors noise sensitivity analysis. This index checks whether the predicted compound falls in a reliable and stable descriptors space or not. A sequence of random scrambling (noise) is applied to the descriptors calculated for the considered compound, and it is checked if the perturbation of descriptors lead to a significant change in the prediction; if the studied descriptors space is stable, these changes should be of little entity. After a large number of such random scrambling, a final index is calculated. Defined intervals are:

1 >= index > 0.8	predictions has a good response to noise scrambling, thus shows a good reliability
0.8 >= index > 0.5	predictions has a not so good response to noise scrambling, thus shows an uncertain reliability
index <= 0.5	predictions has a bad response to noise scrambling, thus shows a low

- Model descriptors range check. This index checks if the descriptors calculated for the predicted compound are inside the range of descriptors of the training and test set. The index has value 1 if all descriptors are inside the range, 0 if at least one descriptor is out of the range. Defined intervals are:

index = 1	descriptors for this compound have values inside the descriptor range of the compounds of the training set
index = 0	descriptors for this compound have values outside the descriptor range of the compounds of the training set

- Global AD Index. The final global index takes into account all the previous indices, in order to give a general global assessment on the applicability domain for the predicted compound. Defined intervals are:

1 >= index > 0.85	predicted substance is into the Applicability Domain of the model
0.85 >= index > 0.75	predicted substance could be out of the Applicability Domain of the model
index <= 0.75	predicted substance is out of the the Applicability Domain of the model

Structural Alerts for outliers

The model implements the detection of a set of Structural Alerts that have been found only in compounds that are outlier (labeled as SO). When such SO are found, a warning in the final assessment is given, and the results should be carefully checked. The SO for outlier compounds are the following:

- SO 01: 6 Cl atoms in the molecule

- SO 02: 2 t-butyl linked to aromatic

- SO 03: Si atom in the molecule

- SO 04: Sn atom in the molecule

- SO 05: O linked to aromatic and 3 Br/Cl linked to aromatic

- SO 06: Azo group liked to aromatic

- SO 07: 3 Nitro-groups linked to aromatic

- SO 08: Peroxide

- SO 09: Phosphinothioyl-oxy-imino

- SO 010: 10 F atoms in the molecule

- SO 011: Phosphorodithioate

Other Structural Alerts

Other relevant Structural Alerts have been studied and proposed for reasoning, each one is related to a class of chemicals that have a particular BCF behavior (they are labeled as SR). The relevant SR are the following, given with the full explanation of the behavior they are bound to:

- SR 01: O=Cc1ccccc1 moiety; this SA has been found only in non-bioaccumulative compounds (24 chemicals), even when the logP value was higher than 3.

- SR 02: Carbonyl residue; this SA has been found to be present in a very large (112) number of non- bioaccumulative compounds, even when the logP value was higher than 3.

- SR 03: O-P=O residue; this SA has been found only in non-bioaccumulative compounds (45 chemicals), even when the logP value was higher than 3.

- SR 04: Thiobenzene residue; this SA has been found only in non-bioaccumulative compounds (39 chemicals), even when the logP value was higher than 3.

- SR 05: Tertiary amine; this SA has been found to be present in a large number of non-bioaccumulative compounds (28), even when the logP value was higher than 3.

- SR 06: Triazole ring; this SA has been found to be present in a number of non- bioaccumulative compounds (16), even when the logP value was higher than 3.

- SR 07: Clc1ccccc1c1ccc(Cl)cc1 moiety; this SA has been found only in bioaccumulative compounds

(15 chemicals). The high lipophylicity of this moiety increases the bioaccumulative behavior.

- SR 08: C1cc(Oc2ccccc2)ccc1Cl; this SA has been found only in bioaccumulative compounds (9 chemicals). The high lipophylicity of this moiety increases the bioaccumulative behavior.

- SR 09: Clc1cc(c2ccccc2)c(Cl)cc1; this SA has been found only in bioaccumulative compounds (15 chemicals). The high lipophylicity of this moiety increases the bioaccumulative behavior.

Furthermore, another set of Structural Alerts for polar groups (labeled as PG) is used for reasoning purpose: usually, the presence of one or more polar groups is related to high hydrophilicity. These SAs have been divided into 3 groups, starting from more relavant (under the aspect of polarity); they are searched in a progressive way, so that if some SAs of the first group are found, no more groups are searched, otherwise the reasearch proceed with the second group, and so on. The group are the following:

First group:

- PG 01: COOH group.

- PG 02: SO3H group.

- PG 03: PO3 group.

- PG 04: PO2S group.

- PG 05: POS2 group.

Second group:

- PG 06: OH group.

- PG 07: NH2 group.

- PG 08: CS2 group.

Third group:

- PG 09: >C=O group

Model statistics

Following, statistics obtained applying the model to its original dataset:

- Training set: n = 378; R^2 = 0.82; RMSE = 0.58
- Test set: n = 95; R^2 = 0.78; RMSE = 0.62

Furthermore, the statistics for the test set considering the Applicability Domain (AD) index is here reported; the AD index is used, as in the final model's assessment, in order to divide results in three groups (into AD, possibly out of AD, out of AD), showing that compounds considered into AD have better performance than the others:

- Test set with AD index greater than 0.85 (compounds into the AD):
  n = 35; R2 = 0.87; RMSE = 0.52
- Test set with AD index between 0.85 and 0.7 (compounds could be out of AD):
  n = 29; R2 = 0.77; RMSE = 0.61
- Test set with AD index lower than 0.7 (compounds out of the AD):
  n = 31; R2 = 0.67; RMSE = 0.72

Model output

Results given as text file consist of a plain-text tabbed file (easily importable and processable by any spreadsheet software) containing in each row all the information about the prediction of a molecule. Note that if some problems were encountered while processing the molecule structure, some warning are reported in the last field (Remarks).

Results given as PDF file consists of a document containing all the information about the prediction. For each molecule, results are organized in sections with the following order:

1 – Prediction summary

Here is reported a depiction of the compound and the final assessment of the prediction (i.e. the prediction made together with the analysis of the applicability domain). Following, all information related to the prediction are reported (the predicted values of the two sub-models, the calculated logP). The prediction and the experimental value (if available) are given in log(L/kg), the same prediction expressed in L/kg is also provided. Note that if some problems were encountered while processing the molecule structure, some warning are reported in the last field (Remarks).

A graphical representation of the evaluation of the prediction and of its reliability is also provided, using the following elements:


Compound is non-bioaccumulative, logBCF value is less than 2.7


Compound could be bioaccumulative, logBCF value is more than 2.7 and less than 3.3


Compound is bioaccumulative, logBCF value is more than 3.3


Prediction has low reliability (compound out of the AD)


Prediction has moderate reliability (compound could be out of the AD)


Prediction has high reliability (compound into the AD)

2 – Possible use and uncertainty

Here is reported a classification for two relevant thresholds (3.3 and 3.7 log units). To the given prediction is associated a conservative interval, if this adjusted value falls under the given threshold the compound can be safely classified under the threshold. Intervals are determined on the basis of the AD index value, for each threshold the original BCF dataset has been studied and each interval defined as the minimum value to be added to the prediction in order to obtain no false negative classification. Values lower than 0.5 log units have been set to 0.5, which is estimated as the experimental variability of data. If compound is outside the applicability domain, no confidence interval is available. In the following these intervals are reported:

	1.0 <= ADI < 0.85	0.85 <= ADI < 0.75	ADI < 0.75
For 3.3 threshold	0.5 log units	0.7 log units	n.a
For 3.7 threshold	0.5 log units	0.5 log units	n.a

3.1 – Applicability Domain: Similar compounds, with predicted and experimental values

Here it is reported the list of the six most similar compounds found in the training and test set of the model, along with their depiction and relevant information (mainly experimental value and predicted value).

3.2 – Applicability Domain: Measured Applicability Domain scores

Here it is reported the list of all Applicability Domain scores, starting with the global Applicability Domain Index (ADI). Note that the final assessment on prediction reliability is given on the basis of the value of the ADI. For each index, it is reported its value and a brief explanation of the meaning of that value.

4.1 – Reasoning: Relevant chemical fragments and moieties

If some relevant fragments are found (see section 1.4 and 1.5 of this guide), they are reported here (one for each page) with a brief explanation of their meaning and the list of the three most similar compounds that contain the same fragment. Note that if no relevant fragments are found, this section is not shown.

4.2 – Reasoning: Analysis of molecular descriptors

Here it is reported an analysis on the most relevant descriptor for the BCF model, LogP, made of two charts. The first one is a scatter plot of MLogP against response values for all compounds of the training set, and the MLogP value against the predicted value for the studied compound. The second one is a scatter plot of MLogP against response values only for the three most similar compounds in the training set where red dot is the value of the studied compound, black outlined circles represents experimental values of compounds from training set, black dots represents predicted value of the same compound; the size of the circle is proportional to the similarity to the studied compound.

8.5.2 VEGA BCF MEYLAN Model Version 1.0.2

Introduction

The model provides a quantitative prediction of bioconcentration factor (BCF) in fish, given in log(L/kg). It is implemented inside the VEGA online platform, accessible at:http://www.vega-qsar.eu/ The model implements the Meylan model, as described in EPI Suite BCFBAF module: http://www.epa.gov/oppt/exposure/pubs/episuite.htm

Model details

The model is based on the method proposed by Meylan et al (Meylan W.M., Howard P.H., Boethling R.S. et al. Improved Method for Estimating Bioconcentration / Bioaccumulation Factor from Octanol/Water Partition Coefficient. 1999, Environ. Toxicol. Chem. 18(4): 664-672) et implemented in the EPI Suite BCFBAF module (http://www.epa.gov/oppt/exposure/pubs/episuite.htm). The model provides a BCF prediction based on different regression equations or fixed values, selected on the basis of an initial classification between ionic and non-ionic compounds, and on the value of the predicted logP value.

For the purpose of the model, ionic compounds include carboxylic acids, sulfonic acids and salts of sulfonic acids, and charged nitrogen compounds (nitrogen with a +5 valence such as quaternary ammonium compounds). All other compounds are classified as non-ionic. The logP prediction is provided by the VEGA logP model.

The original dataset from EPI Suite has been taken, then processed and cleared from duplicates and compounds provided with structure that had problems. The final dataset has 662 compounds.

Applicability Domain

The applicability domain of predictions is assessed using an Applicability Domain Index (ADI) that has values from 0 (worst case) to 1 (best case). The ADI is calculated by grouping several other indices, each one taking into account a particular issue of the applicability domain. Most of the indices are based on the calculation of the most similar compounds found in the training and test set of the model, calculated by a similarity index that consider molecule's fingerprint and structural aspects (count of atoms, rings and relevant fragments). Note that when the experimental value for the given compound is found, the Applicability Domain indices are calculated only considering this value, without taking into account the first n similar compounds.

For each index, including the final ADI, three intervals for its values are defined, such that the first interval corresponds to a positive evaluation, the second one corresponds to a suspicious evaluation and the last one corresponds to a negative evaluation.

Following, all applicability domain components are reported along with their explanation and the intervals used.

- Similar molecules with known experimental value. This index takes into account how similar are the first two most similar compounds found. Values near 1 mean that the predicted compound is well represented in the dataset used to build the model, otherwise the prediction could be an extrapolation. Defined intervals are:

1 >= index > 0.9	strongly similar compounds with known experimental value in the training set have been found
0.9 >= index > 0.75	only moderately similar compounds with known experimental value in the training set have been found
index <= 0.75	no similar compounds with known experimental value in the training set have been found

- Accuracy (average error) of prediction for similar molecules. This index takes into account the error in prediction for the two most similar compounds found. Values near 0 mean that the predicted compounds falls in an area of the model's space where the model gives reliable predictions, otherwise the greater is the value, the worse the model behaves. Defined intervals are:

index < 0.5	accuracy of prediction for similar molecules found in the training set is
0.5 <= index <= 1.0	accuracy of prediction for similar molecules found in the training set is not optimal
index > 1.0	accuracy of prediction for similar molecules found in the training set is not adequate

- Concordance with similar molecules (average difference between target compound prediction and experimental values of similar molecules) . This index takes into account the difference between the predicted value and the experimental values of the two most similar compounds. Values near 0 mean that the prediction made agrees with the experimental values found in the model's space, thus the prediction is reliable. Defined intervals are:

index < 0.5	similar molecules found in the training set have experimental values that agree with the target compound predicted value
0.5 <= index <= 1.0	similar molecules found in the training set have experimental values that slightly disagree with the target compound predicted value
index > 1.0	similar molecules found in the training set have experimental values that completely disagree with the target compound predicted value

- Maximum error of prediction among similar molecules. This index takes into account the maximum error in prediction among the two most similar compounds. Values near 0 means that the predicted compounds falls in an area of the model's space where the model gives reliable predictions without any outlier value. Defined intervals are:

index < 0.5	the maximum error in prediction of similar molecules found in the training set has a low value, considering the experimental variability
0.5 <= index <= 1.0	the maximum error in prediction of similar molecules found in the training set has a moderate value, considering the experimental variability
index > 1.0	the maximum error in prediction of similar molecules found in the training set has a high value, considering the experimental variability

- LogP reliability. This index takes into account the reliability of the logP value used in the model. Note that the Meylan BCF model is strongly based on the logP prediction of the compound, thus this index is highly relevant for the assessment of the final prediction. The reliability of the logP value comes from the assessment of the VEGA LogP model (that provides the used logP value), which is also provided in the “Prediction summary” section of the report. Defined intervals are:

index = 1	reliability of logP value used by the model is good
index = 0.7	reliability of logP value used by the model is not optimal
index = 0	reliability of logP value used by the model is not adequate

- Model descriptors range check. This index checks if the descriptors calculated for the predicted compound are inside the range of descriptors of the training and test set. The index has value 1 if all descriptors are inside the range, 0 if at least one descriptor is out of the range. Defined intervals are:

index = 1	descriptors for this compound have values inside the descriptor range of the compounds of the training set
index = 0	descriptors for this compound have values outside the descriptor range of the compounds of the training set

- Global AD Index. The final global index takes into account all the previous indices, in order to give a general global assessment on the applicability domain for the predicted compound. Defined intervals are:

1 >= index > 0.85	predicted substance is into the Applicability Domain of the model
0.85 >= index > 0.75	predicted substance could be out of the Applicability Domain of the model
index <= 0.75	predicted substance is out of the the Applicability Domain of the model

Model statistics

Following, statistics obtained applying the model to its original dataset:

- Training set: n = 516; R^2 = 0.80; RMSE = 0.55
- Test set: n =146; R^2 = 0.79; RMSE = 0.66

Furthermore, the statistics for the test set considering the Applicability Domain (AD) index is here reported; the AD index is used, as in the final model's assessment, in order to divide results in three groups (into AD, possibly out of AD, out of AD), showing that compounds considered into AD have better performance than the others:

- Test set with AD index greater than 0.85 (compounds into the AD):
  n = 36; R^2 = 0.91; RMSE = 0.45
- Test set with AD index between 0.85 and 0.7 (compounds could be out of AD):
  n = 58; R^2 = 0.79; RMSE = 0.53
- Test set with AD index lower than 0.7 (compounds out of the AD):
  n = 52; R^2 = 0.74; RMSE = 0.87

Model output

Results given as text file consist of a plain-text tabbed file (easily importable and processable by any spreadsheet software) containing in each row all the information about the prediction of a molecule. Note that if some problems were encountered while processing the molecule structure, some warning are reported in the last field (Remarks).

Results given as PDF file consists of a document containing all the information about the prediction. For each molecule, results are organized in sections with the following order:

1 – Prediction summary

Here is reported a depiction of the compound and the final assessment of the prediction (i.e. the prediction made together with the analysis of the applicability domain). Following, all information related to the prediction are reported (the calculated logP, the reliability of the calculated logP, the classification of the given compound as ionic or non-ionic). The prediction and the experimental value (if available) are given in log(L/kg), the same prediction expressed in L/kg is also provided. Note that if some problems were encountered while processing the molecule structure, some warning are reported in the last field (Remarks).

A graphical representation of the evaluation of the prediction and of its reliability is also provided, using the following elements:


Compound is non-bioaccumulative, logBCF value is less than 2.7


Compound could be bioaccumulative, logBCF value is more than 2.7 and less than 3.3


Compound is bioaccumulative, logBCF value is more than 3.3


Prediction has low reliability (compound out of the AD)


Prediction has moderate reliability (compound could be out of the AD)


Prediction has high reliability (compound into the AD)

3.1 – Applicability Domain: Similar compounds, with predicted and experimental values

Here it is reported the list of the six most similar compounds found in the training and test set of the model, along with their depiction and relevant information (mainly experimental value and
predicted value).

3.2 – Applicability Domain: Measured Applicability Domain scores

Here it is reported the list of all Applicability Domain scores, starting with the global Applicability Domain Index (ADI). Note that the final assessment on prediction reliability is given on the basis of the value of the ADI. For each index, it is reported its value and a brief explanation of the meaning of that value.

4.2 – Reasoning: Analysis of molecular descriptors

Here it is reported an analysis on the fundamental descriptor for the BCF model, LogP, made of two charts. The first one is a scatter plot of LogP against response values for all compounds of the training set, and the LogP value against the predicted value for the studied compound. The second one is a scatter plot of LogP against response values only for the three most similar compounds in the training set where red dot is the value of the studied compound, black outlined circles represents experimental values of compounds from training set, black dots represents predicted value of the same compound; the size of the circle is proportional to the similarity to the studied compound

8.5.3 VEGA BCF Read-Across Version 1.0.2

Introduction

The model performs a read-across and provides a quantitative prediction of bioconcentration factor (BCF) in fish, given in log(L/kg). It is implemented inside the VEGA online platform, accessible at: http://www.vega-qsar.eu/

Model details

The model performs a read-across on a dataset of 860 chemicals. This dataset has been made by Istituto di Ricerche Farmacologiche Mario Negri, merging experimental data from several reliable sources, including the original dataset of the CAESAR BCF model (note that experimental values may differ from the ones in the CAESAR BCF dataset, as this new dataset has been built including more sources). The read-across is based on the similarity index developed inside the VEGA platform; the index takes into account several structural aspects of the compounds, such as their fingerprint, the number of atoms, of cycles, of heteroatoms, of halogen atoms, and of particular fragments (such as nitro groups). The index value ranges from 1 (maximum similarity) to 0. On the basis of this structural similarity index, the three compounds from the dataset resulting most similar to the chemical to be predicted are taken into account: the estimated BCF value is calculated as the weighted average value of the experimental values of the three selected compounds, using their similarity values as weight.

Applicability Domain

The applicability domain of predictions is assessed using an Applicability Domain Index (ADI) that has values from 0 (worst case) to 1 (best case). The ADI is calculated by grouping several other indices, each one taking into account a particular issue of the applicability domain. For each index, including the final ADI, two intervals for its values are defined, such that the first interval corresponds to a positive evaluation, and the second one corresponds to a negative evaluation.

Following, all applicability domain components are reported along with their explanation:

- Highest similarity found for similar compounds. This index takes into account the maximum value of similarity among the three most similar compounds found. Values higher than 0.7 mean that at least one compound with a good structural similarity with the chemical to be predicted has been found. Values lower than 0.7 mean that no remarkably similar compounds have been found, and the read- across could be not reliable. Defined intervals are:

index >= 0.85	the highest similarity value found for similar compounds is adequate for a reliable read-across
index < 0.85	the highest similarity value found for similar compounds is not adequate for a reliable read-across

- Lowest similarity found for similar compounds. This index takes into account the minimum value of similarity among the three most similar compounds found. Values higher than 0.6 mean that also the least similar among the three compounds has an acceptable structural similarity with the chemical to be predicted. Values lower than 0.6 mean that the read-across could be not reliable. Defined intervals are:

index >= 0.7	the lowest similarity value found for similar compounds is adequate for a reliable read-across
index < 0.7	the lowest similarity value found for similar compounds is not adequate for a reliable read-across

- Global AD Index. The final global index takes into account the previous indices, in order to give a general global assessment on the applicability domain for the predicted compound. If at least one of the previous indices has a negative evaluation, the final global index will result in an assessment of unreliability; if all indices have positive evaluation, then the global index will result in an assessment of reliability. In both cases, the global index value is calculated as the average value of the similarity index for the three compounds taken into account for the read-across.

Model statistics

Following, statistics obtained applying the read-across prediction to its original dataset, with a leave- one-out approach (read-across for each compound has been performed on the whole dataset without the compound itself)

- n = 860; R^2 = 0.63; RMSE = 0.81

Furthermore, the statistics considering the Applicability Domain (AD) index is here reported. The AD index is used to choose only the results that are considered fully reliable predictions (614 over 860 compounds), showing that this subset of compounds has better performance:

- n = 614; R^2 = 0.73; RMSE = 0.69

Model output

Results given as text file consist of a plain-text tabbed file (easily importable and processable by any spreadsheet software) containing in each row all the information about the prediction of a molecule. Note that if some problems were encountered while processing the molecule structure, some warning are reported in the last field (Remarks).

Results given as PDF file consists of a document containing all the information about the prediction. For each molecule, results are organized in sections with the following order:

1 – Prediction summary

Here is reported a depiction of the compound and the final assessment of the prediction (i.e. the prediction made together with the analysis of the applicability domain). Following, all information related to the prediction are reported (the logP value, calculated with two different descriptors: MLogP and ALogP). The prediction and the experimental value (if available) are

given in log(L/kg), the same prediction expressed in L/kg is also provided. Note that if some problems were encountered while processing the molecule structure, some warning are reported in the last field (Remarks).
A graphical representation of the evaluation of the prediction and of its reliability is also provided, using the following elements:


Compound is non-bioaccumulative, logBCF value is less than 2.7


Compound could be bioaccumulative, logBCF value is more than 2.7 and less than 3.3


Compound is bioaccumulative, logBCF value is more than 3.3


Prediction has low reliability (compound out of the AD)


Prediction has moderate reliability (compound could be out of the AD)


Prediction has high reliability (compound into the AD)

3.1 – Applicability Domain: Similar compounds, with predicted and experimental values

Here it is reported the list of the six most similar compounds found in the training and test set of the model, along with their depiction and relevant information (mainly experimental value and
predicted value). Note that the first three compounds shown are the molecules used for the read- across.

3.2 – Applicability Domain: Measured Applicability Domain scores

Here it is reported the list of all Applicability Domain scores, starting with the global Applicability Domain Index (ADI). Note that the final assessment on prediction reliability is given on the basis of the value of the ADI. For each index, it is reported its value and a brief explanation of the meaning of that value