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CASE STUDY: ASSESSMENT OF AN EXTENDED CHEMICAL CATEGORY, THE SHORT-CHAIN METHACRYLATES, TARGETED ON BIOACCUMULATION

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FOREWORD

In November 2010, the Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology agreed to a new Cooperative Chemicals Assessment Programme, replacing the OECD HPV Chemicals Programme completed in 2010. One of the focus points of the new Cooperative Chemicals Assessment Programme is the application of integrated approaches to testing and assessment, for instance through the use of chemical grouping approaches, including the extension of already assessed chemical categories. Furthermore, the new programme aims at improving the expertise in and regulatory use of (Q)SAR methodologies in general.

Existing chemical categories already assessed in the OECD HPV Chemicals Programme may sometimes be extended to chemicals not originally sponsored, but falling within the applicability domain of a defined chemical category. It may thus sometimes be possible to extend predictions for a defined endpoint, based on similar structure, functional group, physical chemical properties, of a larger group of chemicals beyond original members of a category, without necessarily performing extensive additional animal testing.

This case study intends to illustrate this concept for the short chain methacrylates, originally composed of four chemicals, for the bioaccumulation endpoint. The OECD QSAR Toolbox identified about 160 chemicals potentially falling within the applicability domain of the original category, and the hypothesis tested in this case study is the possibility to predict the bioaccumulation potential for untested members of the category. The document was prepared by the European Chemicals Agency (ECHA) and discussed at the OECD SIDS Initial Assessment Meeting in October 2010. The document reviews results of predictions made using various models and discusses the level of uncertainty and factors contributing to uncertainty.

This document is published under the responsibility of the Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology of the OECD.

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CASE STUDY: ASSESSMENT OF AN EXTENDED CHEMICAL CATEGORY, THE SHORT-CHAIN METHACRYLATES, TARGETED ON BIOACCUMULATION

Summary

The purpose of this study was to evaluate the possibility to extend the short chain methacrylates category containing four members, already agreed as an OECD chemical category (published assessment profile available at:

http://webnet.oecd.org/HPV/UI/handler.axd?id=2b726a59-b02c-4787-aa8e-125651bc5335), with medium chain analogues (C2-C8), at the restriction that no additional functional groups are included, and all esters are aliphatic and saturated in the alcohol part of the esters, while the acid part does not vary. The structures of 160 methacrylates had been provided by OECD. ECHA has chosen the endpoint bioaccumulation for this exercise.

The (Q)SAR Application Toolbox did not reveal any difference between the 160 methacrylates, except for the branching (BioWin MITI fragments). Only one experimental data point on bioaccumulation could be retrieved for the evaluated series, which did not provide enough evidence to justify any hypothesis for the bioaccumulation endpoint and for the selected methacrylate series.

(Q)SAR models (BCF base-line model (OASIS), EpiSuite BCFBAF (US EPA), CAESAR) were used to provide supporting information for the 160 methacrylates. Based on the range of the octanol-water partitioning coefficients of the 160 methacrylates (logKow = 1.77 - 4.71 (calculated)), a bioaccumulation potential for a part of the group would be expected. The one experimental study was conducted for 2-Ethylhexyl Methacrylate (2-EHMA). Although the logKow of this chemical is at the higher end of the range of logKow values (value), it still shows very limited bioaccumulation with a BCF of 37. Most likely a rapid metabolisation in fish is the reason for the low bioaccumulation potential. Models based only on logKow are therefore expected to overestimate the bioaccumulation for those methacrylates with similar metabolisation.

All the predictions generated were inside the parametric domain of the models. The models did, however, not contain methacrylates in their training sets, although there were reasonably good structural similarities with the compounds in the training set. The BCF base-line model was updated by including the experimental value for 2-Ethylhexyl Methacrylate (2-EHMA) in the training set. Carboxylesterase mediated hydrolisation of the ester bond of methacrylates was specified to account for metabolisation.

All three models indicate a limited bioaccumulation potential for the 160 methacrylates with predicted BCFs below 400 L/kg wet body weight, if the current version of the OASIS BCF baseline model is considered as not appropriate for this series of methacrylates. The reason for not considering that model, which appears also a worst-case scenario model, is the indication that most of the methacrylates can undergo more or less rapid metabolism *in vivo*. Since metabolism is a mitigating factor for bioaccumulation, the real bioconcentration is expected to be lower than estimated in a worst case scenario. Otherwise, there is a good consensus between three other models (EpiSuite BCFBAF v 3.0, CAESAR and OASIS model with mitigating factors) that do not predict a BCF higher than 400 L/kg wet body weight. Uncertainty remains because there is only one experimental value available validating the predictions. 2-

EHMA is the only methacrylate for which a reliable experimental BCF value is available; it has a logKow value in the upper range of the 160 short chain alkyl methacrylates and it is branched and would therefore be expected to exhibit a higher bioaccumulation potential compared to other (non branched) methacrylates in the group. The BCF however is low, probably due to carboxylesterase mediated metabolisation. However, there are indications that other branched isomers may be less susceptible to carboxylesterase mediated biotransformation. In order to justify the category regarding the endpoint bioconcentration, further experimental BCF data would be needed.

If new tests are carried out for methacrylates, representatives should be chosen which allow conclusions for the whole category, e.g. demonstration that a trend holds between the proposed members, using an acceptable number of experimental data points, investigation of the borders of the series for interpolations.

As a conclusion, even if there remains uncertainty to conclude on the extended methacrylate group for bioconcentration on its own, the findings could still be useful for the assessment of the PBT potential of short chain alkyl methacrylates taking into account especially persistency in the environment and regulatory threshold levels triggering risk management measures.

The design of the exercise demonstrated that it could be possible to group a large number of substances from various inventories of Member Countries on simple principles like a common functional group and then explore if the categorisation is justified and data gap filling possible. Therefore, the effort to find more of such groups is encouraged. The exercise to explore similarity of a group with the Toolbox and (Q)SAR models provided useful knowledge on their capabilities, the applicability domain, and documentation.

Introduction

The structure of 160 methacrylates has been provided by OECD. The purpose of the study is to evaluate the possibility to extend the short chain methacrylates category[1], consisting of four members and already agreed by OECD, with medium chain analogues (C2-C8), at the restriction that no additional functional groups are included, and all esters are aliphatic and saturated in the alcohol part of the esters, while the acid part does not vary.

The following assumptions were made for this study:

- The substances are pure.
- The potential degradation products are not considered separately since they are not expected to affect the grouping (the degradation products of the methacrylates will make a similar series of alcohols).
- Volatility, sorption/desorption and hydrolysis do not affect BCF since in laboratory conditions the concentration of the substances can be maintained despite of possible abiotic losses.
- The bioaccumulation is assessed on its own, without connection to persistency and toxicity.

The following tasks were formulated at the beginning of the study:

1. Find references (experimental bioaccumulation data).
2. Evaluate category hypothesis and justification, including borders.
3. Investigate factors that might influence the bioaccumulation (e.g. branching, size and metabolism).
4. Select models for prediction the bioaccumulation potential and assess their scientific validity
5. Assess the reliability and adequacy of predictions by these models
6. Check if log P reflects branching (estimated vs, experimental values).
7. Compare different model predictions for predicting the bioaccumulation of short chain methacrylates.

Substances looked at for extending the category:

160 substances listed in the OECD document ENV/JM/HA(2009)11 – Elaboration of targeted chemical categories: outline of concept. See also Annex 3

Search for further experimental data:

ECHA registration database

[1] http://webnet.oecd.org/hpv/UI/ChemGroup.aspx#ctl00_ContentPlaceHolder1_53-header

Assessment of mitigating factors:

According to REACH guidance documents (Guidance on information requirements and chemical safety assessment; Chapter R.7c: Endpoint specific guidance; Chapter R R.7.10 Bioconcentration and bioaccumulation; long-term toxicity to birds)

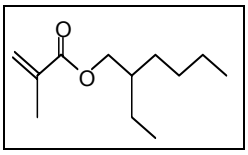
BCF models used:

- BCF base-line model v.01.03 (in OASIS proprietary software CATALOGIC and POPs profiler)
- EpiSuite BCFBAF v3.00
- CAESAR v1.1
- EpiSuite BIOWIN version 4.10

Results and Discussion

I. Availability of experimental data

One bioconcentration test with fish is available for one of the members of the original category on short chain alkyl methacrylates, namely 2-Ethylhexyl Methacrylate (2-EHMA); CAS 688-84-6;



The BCF is 37; logKow : 4.95 – 5.59; as summary on the study was given in the SIDS report (Annex I). The study is reliable without restrictions.

A search in the ECHA registration database and other public databases and data sources did not reveal further test results on bioconcentration for methacrylates.

II. Category hypothesis and justification

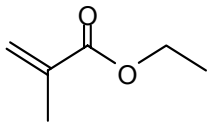
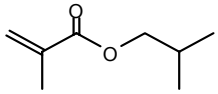
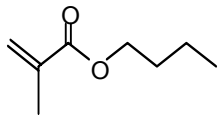
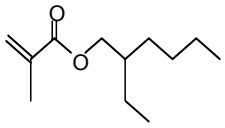
Original OECD category on short chain alkyl methacrylates:

The original OECD category is based on following justification in regards to bioconcentration.

"Based on the Log K_{ow} values of the short chain methacrylate esters the potential for bioaccumulation is for MMA, EMA, i-BMA and n-BMA. In a bioconcentration test with fish, 2-EHMA showed a low bioconcentration factor of 37. Elimination from the aquatic compartment is expected to be rapid, evaporation and biodegradation being the main processes involved."

Low priority is given for further work on the category:

"…However, the chemicals in this category are of low priority for further work because of their rapid biodegradation and their limited potential for bioaccumulation."

CAS No.	97-63-2	97-86-9	97-88-1	688-84-6
Chemical Name	Ethyl Methacrylate (EMA)	Iso-Butyl Methacrylate (i-BMA)	n-Butyl Methacrylate (n-BMA)	2-Ethylhexyl Methacrylate (2-EHMA)
Structural Formula				

Extension of the category to 160 potential members:

The QSAR Toolbox profiling was applied to all the 160 methacrylate analogues. The result was analysed in 3 aspects:

- Profiling result which may be relevant for bioaccumulation
- Profiling result which are probably not relevant for bioaccumulation
- Profiling result which confirm the structure of selected substances

These are summarised in Table 1. Based on the rules implemented in the Toolbox, there is no reason (apart from branching) to consider the 160 analogues belonging to different groups/categories. The correct application of the criteria, formulated at the selection of the analogues, was confirmed. The Toolbox also calculates a number of 2D and 3D parameters that can be exported and evaluated externally.

Table 1. Properties, common for all 160 methacrylates, as calculated by the Toolbox v 1.1.02:

Toolbox Profiler	**Result**
Relevant for bioaccumulation properties	
BioWin MITI fragments	**Several branching patterns!**
Lipinski Rule	Molecule satisfies the rule of 5, (bioavailable)
EcoSAR Classification	Methacrylates
US EPA Categorization	Acrylates/Methacrylates, Esters, Esters (Acute toxicity)
OASIS Acute Toxicity MOA OECD categorization	Reactive unspecified Short chain alkyl methacrylates esters

Protein Binding	Michael-type nucleophilic addition
Verhaar scheme	Class 5 (Not possible to classify according to these rules)
Not relevant for the bioaccumulation properties	
Benigni/Bossa rulebase	No structural alert identified
ER-binding	Non-cyclic structure
BfR rulebase for eye irritation/corrosion	Group C (C,H,O)
BfR rulebase for skin irritation/corrosion	Group C (C,H,O),AcrylicAndMethacrylicEsters
DNA Binding	No Binding
Cramer rules	Low (Class I)
Confirming the structure	
Groups of elements	Non-Metals
Number of aromatic bonds	0
Number of cyclic bonds	0
Number of double bonds	2
Number of rings	0
Superfragment profiling	No superfragment

III. (Q)SAR models

In the absence of experimental data, following (Q)SAR models were applied:

i. BCF base-line model

The base-line modeling concept is based on the assumption of a maximum bioconcentration factor with mitigating factors (acids, metabolism, phenols, size, water solubility) that reduce the BCF.

Endpoint: Bioconcentration, BCF fish; dependent variable: logBCF

Model: BCF base-line model; version v.01.03 (in OASIS CATALOGIC and POPs profiler)

QMRF: not available

Input for predictions: SMILES (Annex 3)

Applicability domain:

Parametric domain: All 160 methacrylates fall within parametric domain (logKow_min: -4.0; logKow_max: 16.1; molecular weight_min: 16; molecular weight_max: 943).

Structural domain: None of the 160 methacrylates contains incorrect fragments, but many fragments are unknown (correct fragments: 12.5% - 50%; incorrect fragments: 0%; unknown fragments: 50% - 87.5%).

Prediction for 2-EHMA:

logBCF (corrected for mitigating factors size and metabolisation) = 3.0367 (BCF = 1088). 2-EHMA is within the parametric domain (calculated logKow = 4.639; molecular weight = 198 and contains 50% correct and 50% unknown fragments. Compared to the experimental vale for 2-EHMA, the BCFmax including mitigating factors gives a much higher value, indicating that methacrylates are outside the applicability domain of the model.

Results for the 160 potential members of the category:

The log BCFmax values range from 1.6 - 4.0. The log BCFmax values corrected for mitigating factors range from 1.2 – 3.1 (BCFmax corrected for mitigating factors (size and metabolisation) are between ca. 20-1300). Mitigating factors metabolism and size reduce the BCF values with this model, whereas the other factors acids, phenols and water solubility do not contribute to the reduction of the BCF values.

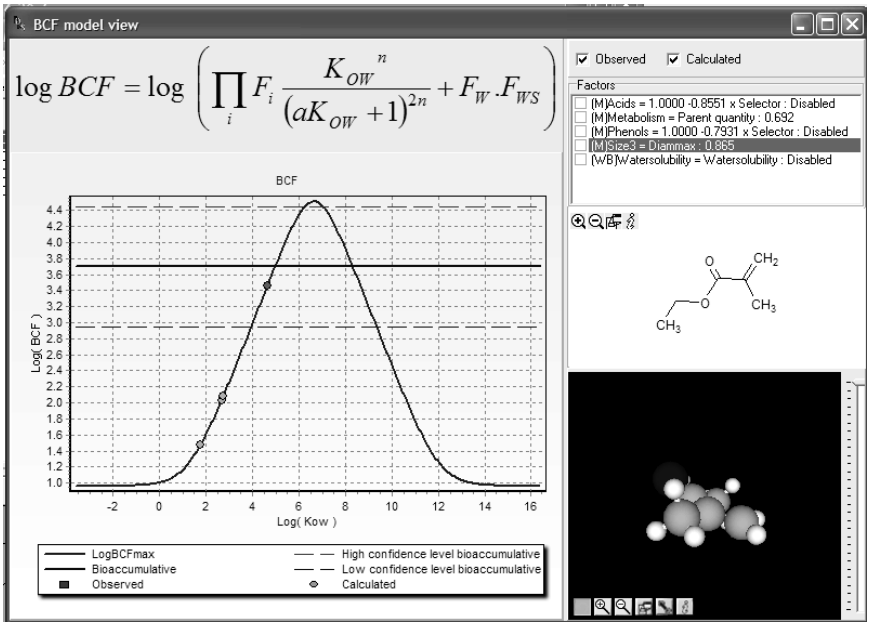


Figure 1. logBCFmax (4 initial category members).

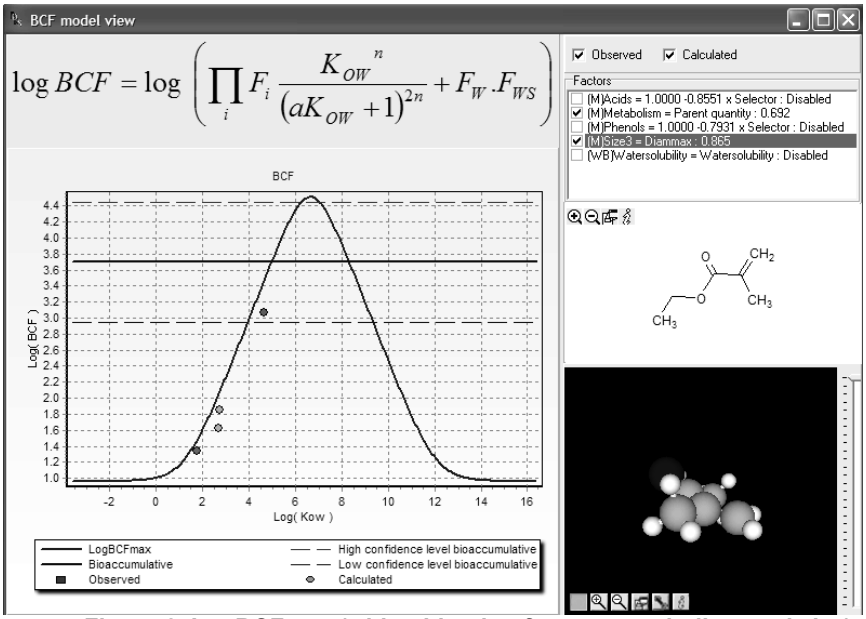


Figure 2. LogBCFmax (with mitigating factors metabolism and size): (4 initial category members)

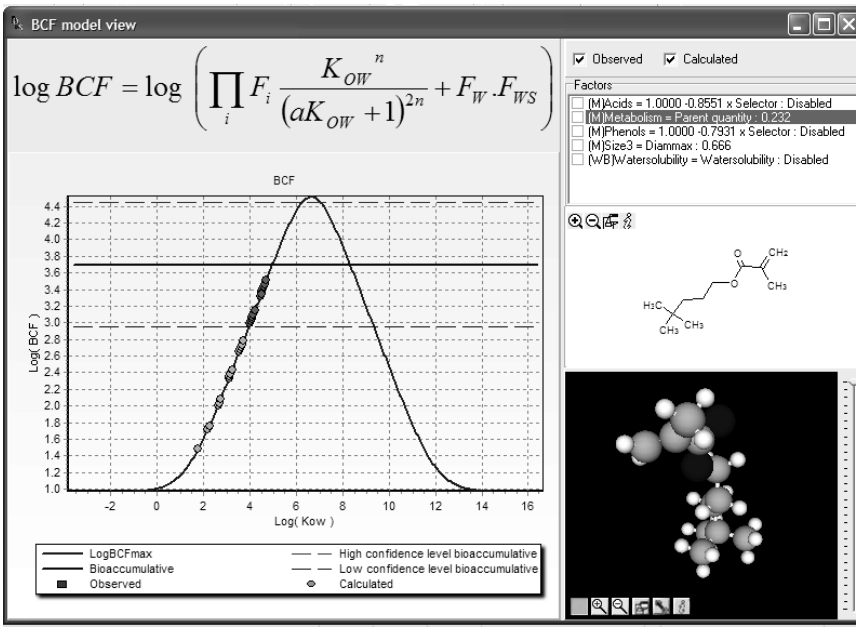


Figure 3. logBCFmax (160 substances)

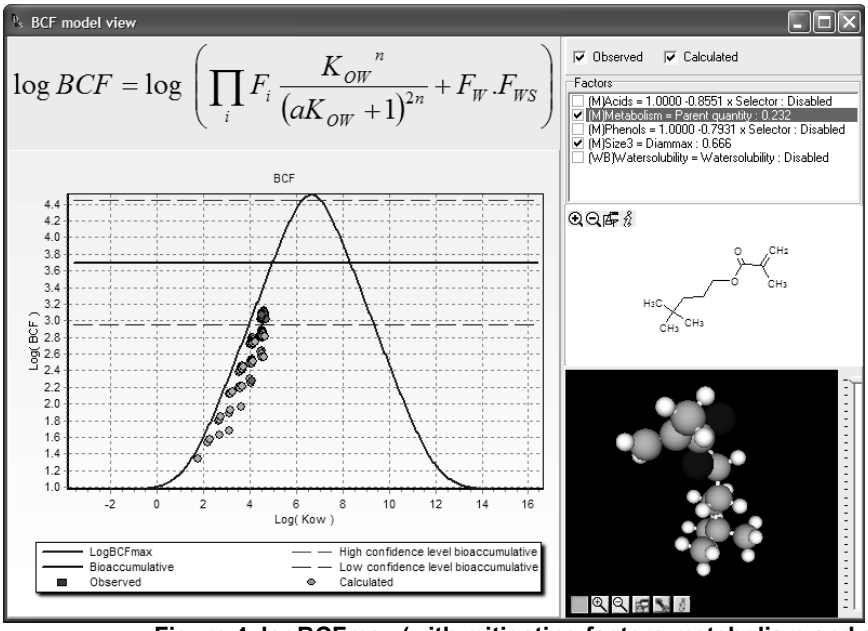


Figure 4. logBCFmax (with mitigating factors metabolism and size) (160 substances)

BCF base-line model – updated by OASIS

We asked Prof. Ovanes Mekenyan (developer of the OASIS software) to look into the predictions and the applicability domain for methacrylates in order to understand better why the BCF predictions for methacrylates are high when mitigating factors are taken into account. Indeed, methacrylates were not part of the training set, and therefore out of the applicability domain of the model. As described in the response from OASIS (see Annex 4), the only first step of metabolism is most likely hydrolysis of the ester group, which is very rapid. In an updated version of the base line model, this reaction was added to the software and the new prediction for 2-EHMA gives a BCF of 32 (logBCF = 1.51).

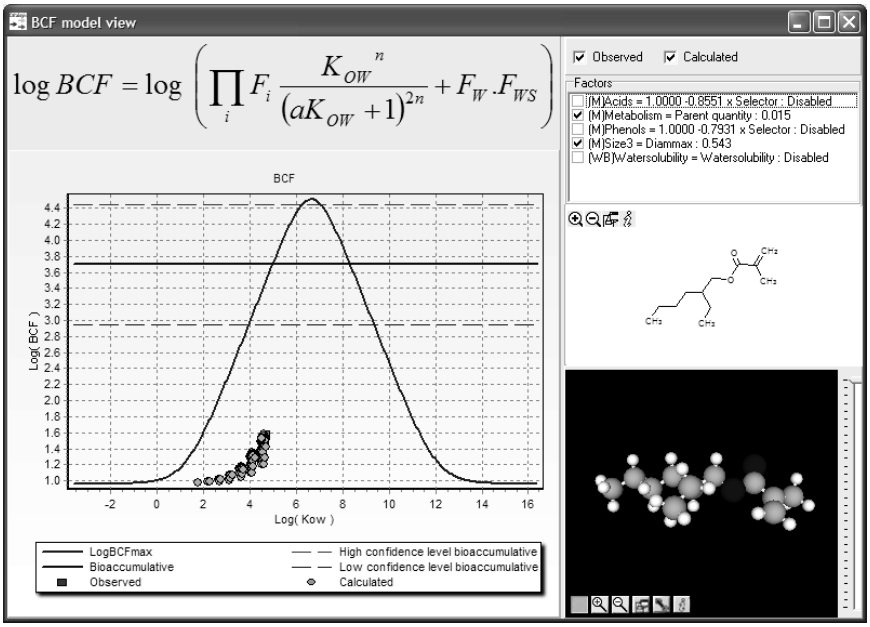


Figure 5. logBCFmax (with mitigating factors metabolism and size) (160 substances) – updated software taking into account methacrylate in the training set and its likely metabolisation.

Applicability domain:

Structural domain (updated model): 26 are in domain, 134 out of the domain. Under the assumption that the "tail" of methacrylates will not affect significantly hydrolysis of the methacrylic group, all 160 chemicals are within the structural domain.

Prediction for 2-EHMA (updated model):

logBCF (corrected for mitigating factors) = 1.51 (BCF (corrected for mitigating factors) = 32). The prediction and the experimental value are in line with the updated model.

Results for the 160 potential members of the category (updated model):

The log BCFmax values corrected for mitigating factors (metabolism and size) range from 1.0 – 1.6. (BCFmax corrected: 9-38).

ii. EpiSuite BCFBAF model

The BCFBAF method classifies a compound as either ionic or non-ionic. For each division, a "best-fit" straight line was derived by common statistical regression methodology. The regression methodology includes derivation of correction factors based on specific structural features. (see also Annex 3)

Endpoint: Bioconcentration, BCF fish; dependent variables: logBCF, BCF, logBAF, BAF

Model: BCFBAF v.3.00

QMRF: not available

Input for predictions: SMILES (Annex 3)

Applicability domain:

Parametric domain: All 160 methacrylates fall within the parametric domain of the training set (logKow _min: -1.37; logKow_max: 11.26; molecular weight_min: 68; molecular weight_max: 959.

Structural domain: The training set does not contain methylacrylates. However, esters in general are included. An external tool (Domain manager from LMC; version 1.02) was applied to check the similarity between of the BCFBAF training set chemicals and the set of 160 methacrylates. Using a similarity index (Tanimoto with atom pairs, default program options, similarity cut-off at 80%), it was found that only about 40% of the 160 substances overlap with the BCFBAF training set. If the structural domain is defined by the number of fragments present in the training set, all the structures have at least 70 % of their fragments represented in the training set, and 90 of them (56%) have more than 90% of the fragments represented in the training set.

Since the BCFBAF model uses also an estimation of the metabolisation rate constant (k_M) as part of the BCF calculation, the domain manager was also used to determine whether the k_M predictions were within domain. The results are similar to those obtained for the BCF training set: all the structures have at least 70 % of their fragments represented in the training set, and 79 of them (49%) have more than 90% of the fragments represented in the training set. It is unclear to what extent this deviation from the structural domain affects the validity of the predictions.

Prediction for 2-EHMA: logBCF = 2.66 (BCF=460 L/kg wet-wt). Estimated log BCF (including biotransformation rate estimates; Arnot-Gobas BCF & BAF methods) (mid trophic)) = 2.220 (BCF = 166.1 L/kg wet-wt))

2-EHMA is within the parametric domain (experimental logKow=4.54; molecular weight = 198).

The experimental vale for 2-EHMA and the BCF estimate including the biotransformation rate are in the same range.

Results for the four original and 160 potential new members of the category:

Table 2. Results for the 4 initial category members of the category

log BCF values: 1.5 - 3.5;

Ester	Molecular weight	Octanol-water partition coefficient (log_{10} and 20/25ºC)	BCF (EpiSuite; regression based estimate)	BCF (EpiSuite; Arnot Gobas; mid trophic level)
EMA	114.12	1.94 (exp)	9	5
i-BMA	142.2	2.66 (exp)	26	19
n-BMA	142.2	2.88 (exp)	37	27
2-EHMA	198	4.54 (exp)	460	166

Table 3. Results for the 160 potential members of the category

	Training set	Estimation set
MW (non-ionic)	68.08 ÷ 244.0	114.0 ÷198.0
Water solubility (c) log(1/mol/L) Epi		1.69 ÷ 4.67
logKow (non-ionic)	-1.37 ÷ 11.26	1.77 ÷ 4.7
Log BCF (est) log(L/kg wet) Epi		0.71 ÷ 2.38 (mid trophic level 0.95 ÷ 2.7 (regression based estimate)

iii. CAESAR for BCF

Endpoint: Bioconcentration, BCF fish; dependent variables: log BCF

Model: CAESAR for BCF v.1.1

Computer Assisted Evaluation of industrial chemical Substances According to Regulations (CAESAR) – EC funded project (http://www.caesar-project.eu/software/)

QMRF: not available

Input for predictions: SMILES (Annex 3)

Applicability domain:

Parametric domain: The log BCF from the training set ranges from −1.00 to 4.85, with a logKow from −4.3 to 12.7 and molecular weights from 68 to 943. Therefore, all 160 methacrylates fall within the parametric domain of the training set.

Structural domain: In the training set of the model there was not any molecule with the fragment methacrylate. Therefore the performance of the model for this type of molecules has not been assessed, and the reliability of these predictions could be questioned. The methacrylates do not fall within any of the groups for which the model has been found to perform poorly. The model also provides the structural similarity of the closest analogue in the training set. For the 160 new members, the structural similarity ranged between 0.65 and 0.732.

Results for the 4 original members of the category

The model seems to perform well based on the only experimental result available for the original members of the category. The experimental BCF for 2-EHMA is 37 (log BCF=1.56), whereas the result calculated with Caesar is 67 (log BCF=1.83). This result falls within the normal error of the Caesar model (around 0.5 log units), which seems to support the validity of the predictions for this model.

Table 4. Calculated values for the 4 original members of the category:

Ester	BCF	logBCF	Structural similarity to the closest analogue
EMA	4	0.57	0.654
i-BMA	7	0.84	0.639
n-BMA	9	0.94	0.666
2-EHMA	67	1.83	0.732

Results for the160 members of the category

The values for BCF and log BCF calculated with Caesar show that none of the substances investigated is highly bioaccumulative. The values for BCF ranged between 4 and 365 (logBCF= 0.57 to 2.56). All the BCFs calculated with Caesar were consistently lower for all the substances than the ones calculated using EpiSuite BCFBAF model and OASIS BCF baseline model.

IV. Factors influencing bioaccumulation and bioaccumulation predictions

i. Size

There are cut-offs suggested for the size of a substance (e.g. molecular length and cross-sectional diameter) above which a substance is considered to have a low probability to pass the biological membrane. None of the suggested 160 analogues exceeds these limits. The size has influence on the prediction as one of the mitigating factors in the BCF base-line model from OASIS, but the impact is not significant.

ii. Stability - Abiotic degradation

Hydrolysis does not seem to apply for longer chain analogues due to the stabilising effect of the hydrocarbon chains of the alcohols on the ester bond. For example, based on HydroWin prediction, the hydrolysis half-life even of methyl methacrylate itself at neutral pH is predicted in the range of years (6.4 years) and the hydrolysis rate for the analogues is predicted even lower (the Kb predictions to be checked by expert).

iii. Metabolism

Metabolism of methacrylates has been discussed for the original OECD category on short chain alkyl methacrylates in relation to human health hazard assessment. The SIAP conclusions for short chain alkyl methacryles state[2]:

"These esters are rapidly metabolized to methacrylic acid (CAS 79-41-4) and the structurally corresponding alcohol by non-specific carboxylesterases in several tissues. Methyl methacrylate (MMA) (CAS 80-62-6), the C1 ester, is the largest volume methacrylate ester that has been studied extensively and reviewed in the OECD HPV Chemicals Program. As such, MMA provides a robust reference chemical for this category."

An EU risk assessment for methyl methacrylate[3] is available and the authors come to the conclusion that:

"… methyl methacrylate is metabolised via physiological pathways and enters into the citric acid cycle via methylmalonyl-CoA and succinyl-CoA, which is a part of the valine pathway."

There is evidence that methacrylate ester hydrolyses in rats and humans to MAA and the structurally corresponding alcohol, mediated by carboxylesterase. Jones (2002)[4] investigated pharmacokinetics and toxicity of methacrylate esters and states:

"MMA follows this pathway in vivo and it is likely that other esters within the series will also be metabolised in this way. Branched esters may undergo rates of hydrolysis that are slower than the straight-chained equivalent, due to the steric hindrance of the bulky side chain."

In Jones (2002) it is described that n-butyl methacrylate is metabolised more rapidly than its branched chained isomer iso-butyl methacrylate (measured in whole rat blood and whole human blood). The branching had not the same effect on 2-EHMA, which was hydrolysed at almost exactly the same rate as octyl methacrylate. This observation was associated with the remoteness of the branching for 2-EHMA. Studies with rat liver microsomes and human liver microsomes have shown that transformation rates were lower for branched chained esters compared to their straight-chained isomers.

It could be concluded that 2-EHMA does not necessarily serve as a worst case substance for biotransformation in fish among the 160 short chain alkyl methacrylates, although the molecular weight is in the upper range of the category. Other members of the category may be more resistant towards enzymatic hydrolysis due to the branching.

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[2] SIAP conclusions on short chain alkyl methacrylates: http://webnet.oecd.org/hpv/UI/handler.axd?id=4b9ff16d-f39c-42fa-b65b-f35d48f4539d

[3] EU risk assessment for methyl methacrylate (http://ecb.jrc.ec.europa.eu/DOCUMENTS/Existing-Chemicals/RISK_ASSESSMENT/REPORT/methylmethacrylatereport024.pdf

[4] Jones, O. 2002. *Using physiologically-based pharmacokinetic modelling to predict the pharmacokinetics and toxicity of methacrylate esters*. A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy.

iv. Biotic degradation and degradation products

Biotic degradation of methacrylates does not directly link to metabolisation of methacrylates in fish, however, it was investigated regarding the uncertainty related to the effects of branching to the susceptibility for enzymatic hydrolysis.

No correlation was found between the probability of biodegradability and predicted BCF values (both properties were calculated with EpiSuite v. 4.0). The endpoint biodegradation was studied with the aim to analyse the clustering of substances according to the branching, as it is implemented in the EpiSuite biodegradation models. Based on agreed cut-off of 0.5 between ready and not ready biodegradable substances on Figure 6, a clear cluster is formed with low probability (not readily biodegradable). A total of 16 substances were identified in this cluster and are listed in Table 5. It might be argued that to the extent the bioaccumulation itself can be related to ready biodegradability, these 16 substances should be considered separately from the group.

This finding could be relevant for an integrated assessment of persistency and bioaccumulation, for example for a PBT assessment of short chain alkyl methacrylates.

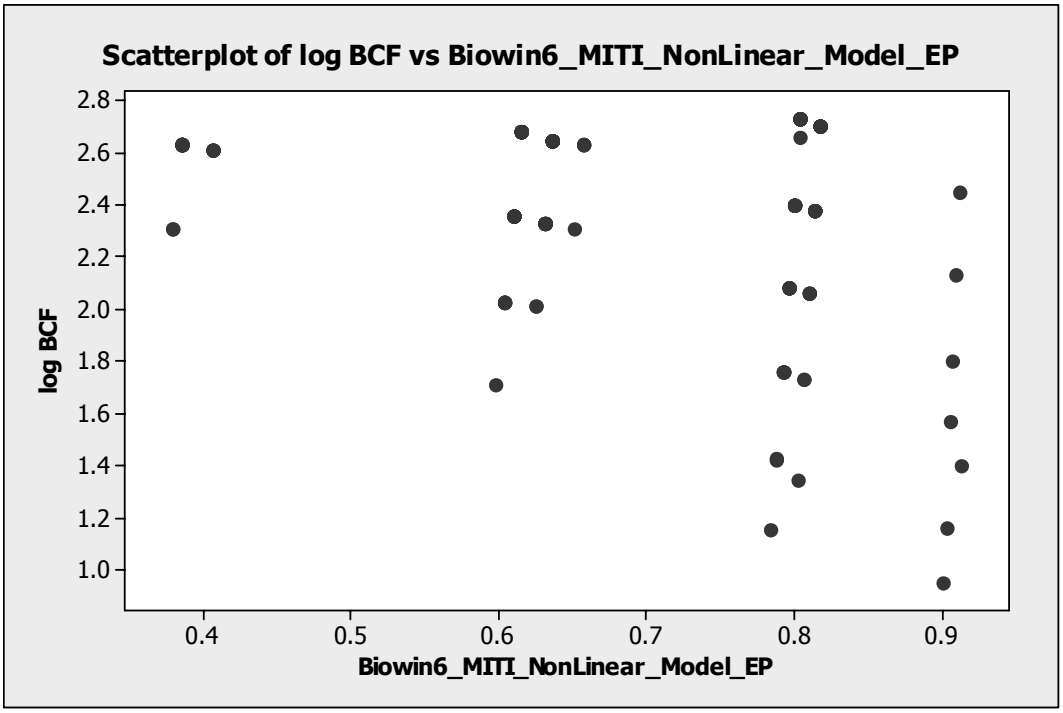
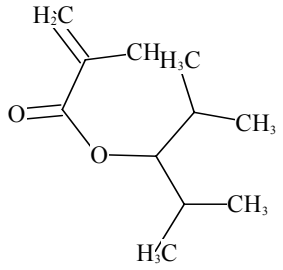
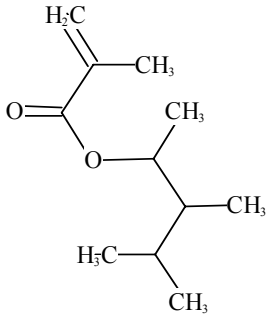
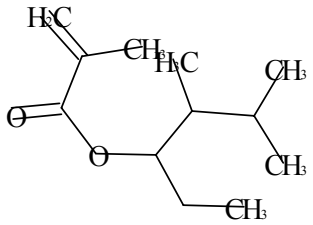
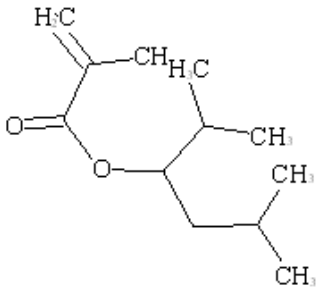
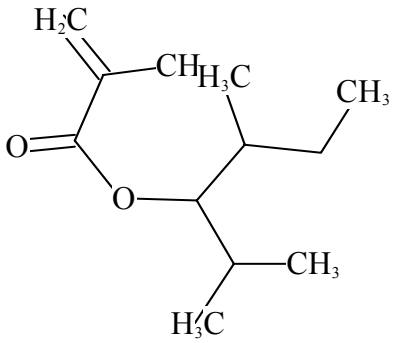
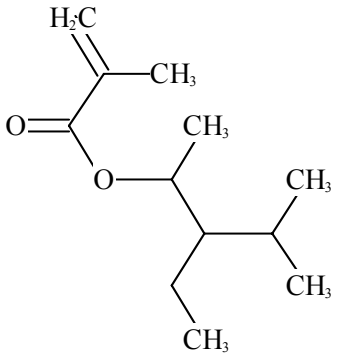
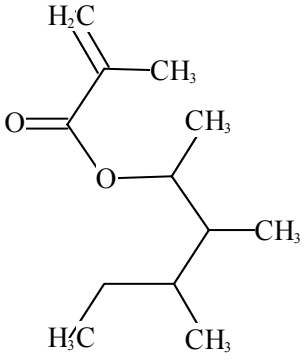
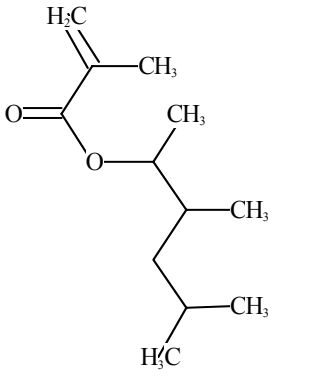
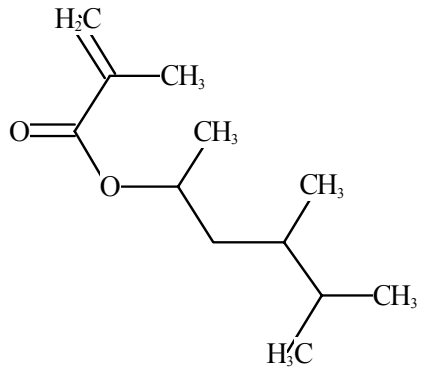
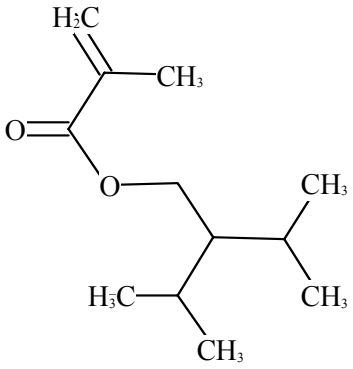
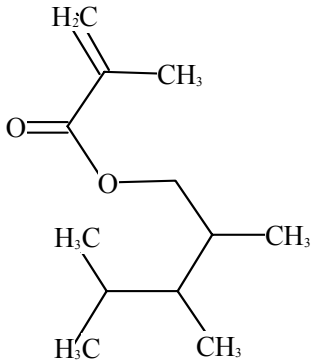
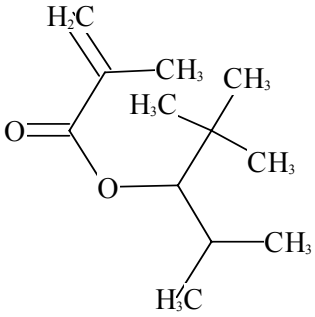
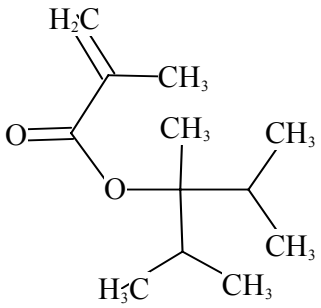
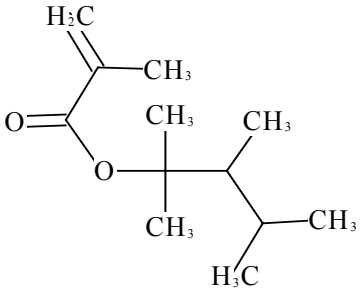
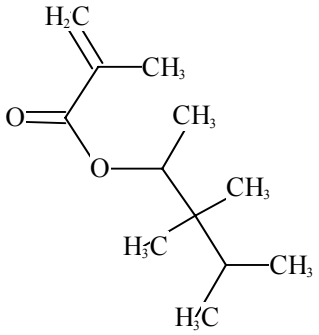
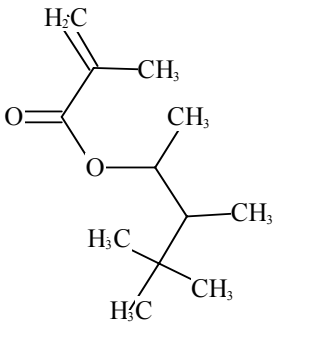


Figure 6. Bioaccumulation and ready biodegradability as estimated by EpiSuite.

Table 5. Sixteen analogues estimated as "not ready biodegradable" by Biowin_6.

Finally, we looked for a relationship between the estimated BCF and estimated metabolism rate constant in fish (again from EpiSuite, BCFBAF module). A good correlation was observed (Figure 7). However, it is acknowledged that our knowledge for modelling both events is based on the same principle, namely on the importance of hydrophobicity for estimation of both bioconcentration and the metabolism rate constant.

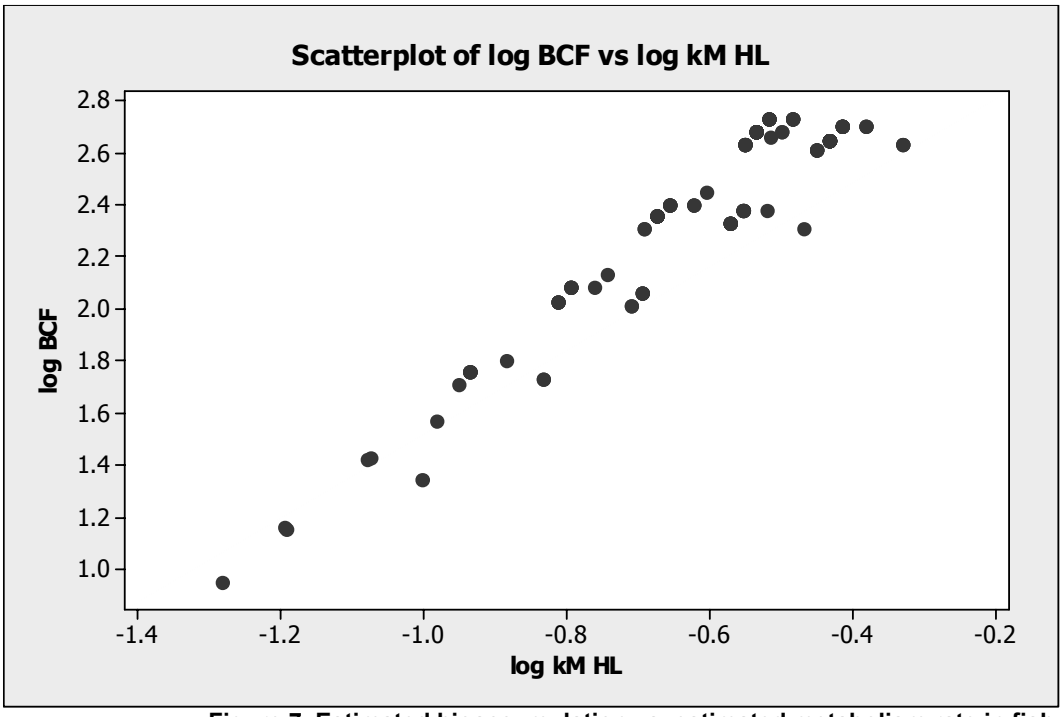


Figure 7. Estimated bioaccumulation vs. estimated metabolism rate in fish.

Despite the different hypotheses we tried to form, no justification for any of them was found and the illustrations made are based just on estimations, which is considered insufficient to provide any justification to any of the hypotheses outlined above.

2-EHMA was not among those substances predicted as not ready biodegradable. Assuming comparability between the enzymatic hydrolysis of the esters bond in humans, rats and microorganisms, there may be branched isomers of the extended category which are not as rapid biotransformed as the BCF data for 2-EHMA suggest.

v. Reliability of logKow predictions

Experimental and estimated logKow were compared. Only five experimental logKow values were retrieved from EpiSuite. From the limited evidence it could be concluded that in reality the experimental logKow values show higher variability with branching compared to the estimated values (see the cluster in the middle) on Figure 8.

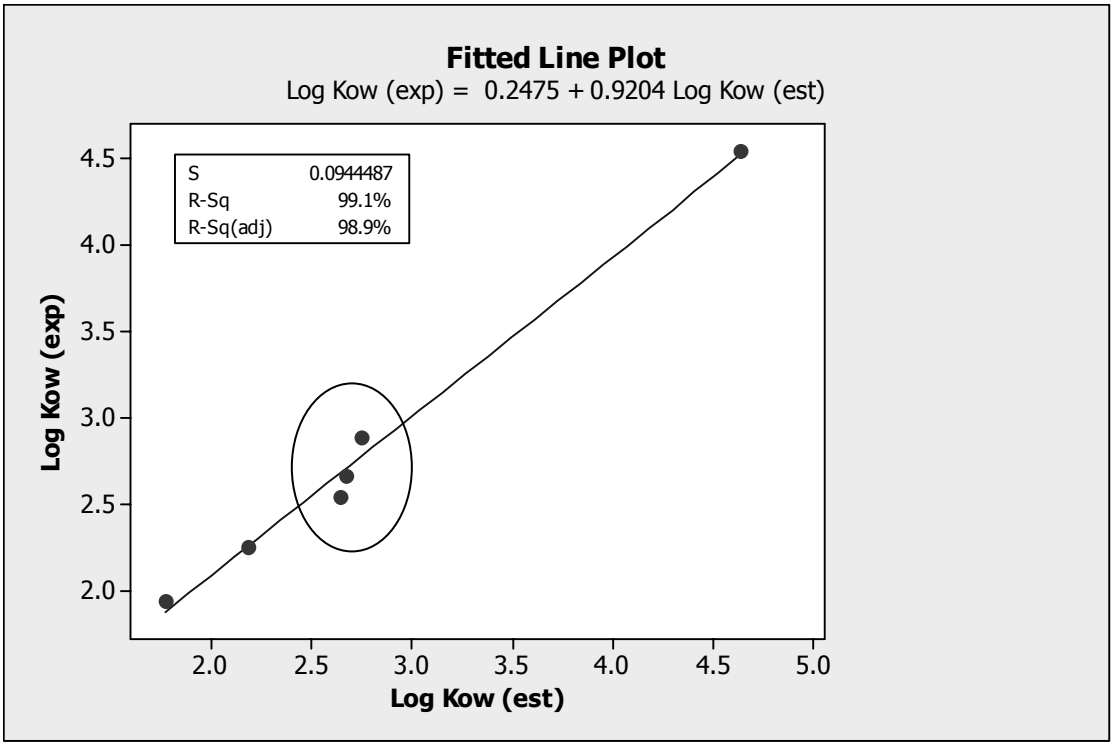
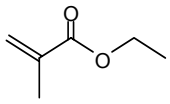
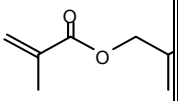
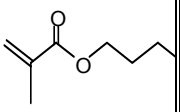
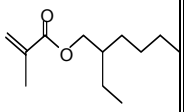


Figure 8. Correlation between experimental and estimated (Epi) logKow .

SUMMARY TABLE

CAS No.	97-63-2	97-86-9	97-88-1	688-84-6	160 members
Chemical	Ethyl Methacrylate	Iso-Butyl Methacrylate	n-Butyl Methacrylate	2-Ethylhexyl Methacrylate	methacrylates
Structural Formula					
Molecular weight	114	142	142	198	114-198
logKow (calculated; EpiSuite)	1.77	2.67	2.75	4.64	1.77-4.71
BCF experimental	-	-	-	1.57	-
Log BCF base line model (OASIS)[5]	1.48	2.03	2.08	3.46	1.48-3.42
Log BCF base line model (OASIS)[6]	0.97	0.99	1.02	1.51	0.97-1.58
Log BCF BCFBAF; (EpiSuite)[7]	0.71	1.27	1.44	2.22	0.71-2.38
Log BCF CEASAR	0.57	0.84	0.94	1.83	0.57-2.56

[5] Without mitigating factors

[6] With mitigating factors size and metabolisation (updated model)

[7] Arnot Gobas (including biotransformation rate estimates), mid trophic level,

Annex 1. Extract from the SIDs dossier concerning the OECD category

Bioaccumulation

$LogK_{ow}$ values of 1.87 for EMA, 2.95 for i-BMA and 2.99-3.03 for n-BMA indicate a low to moderate bioaccumulation potential for those esters while a $LogK_{ow}$ of 4.95-5.59 for 2-EHMA would indicate a high bioaccumulation potential (Table 8). Data from a fish bioconcentration test, (similar to OECD TG 305 but of shorter duration) however, indicates a low bioconcentration factor (BCF) of 37 for 2-EHMA (Schäfers, 2006).

Table 8 Calculated BCF values

Ester	Molecular weight	Octanol-water partition coefficient (log_{10} and 20/25ºC)	BCF*	Reference (LogPow)
EMA	114.12	1.87	8	Jones, 2002
i-BMA	142.2	2.95	64	Jones, 2002
n-BMA	142.2	2.99-3.03	69-75	CERI, 1998a; Jones, 2002
2-EHMA	198	4.95-5.59	3217-11259	CERI, 1998c; Jones, 2002
2-EHMA	198	---	37**	Schäfers, 2006

* Calculated according to Veith et al. (1979) with LogBCF = 0.85 x LogKow – 0.7

** Measured BCF in a fish bioconcentration test similar to OECD guideline 305, with duration of uptake equal to 56 hours and a depuration phase of 30 hours, which is shorter than suggested in Guideline 305.

Metabolism studies with the alkyl methacrylate ester series (Jones, 2002; see mammalian toxicology; metabolism) have demonstrated rapid metabolism for all members of the category indicating elimination by ubiquitous metabolic pathways (carboxyl esterases, tricarboxylic acid cycle, CoA aliphatic alcohol pathway). Further, the citric acid and fatty acid cycles are biotransformation pathways present in many species. Finally, as noted above, the measured BCF for 2-EHMA (the most lipophilic category member) is low, which suggests a low potential for bioaccumulation.

Annex 2: Experimental data for 2-EHMA (member of the original OECD category); extract from the SIDS dossier

3.7 BIOACCUMULATION

Species	:	other: Danio rerio
Exposure period	:	56 hour(s) at °C
Concentration	:	.3 mg/l
BCF	:	= 37
Elimination	:	
Method	:	OECD Guide-line 305
Year	:	2006
GLP	:	yes
Test substance	:	other TS: Ethylhexyl methacrylate

Method : Stock Solution
The stock solution was prepared two times a day under sterile conditions without solvent in a concentration close to the water solubility limit of ethylhexyl methacrylate. To prepare a stock solution of 2.5 mg/l, 25 mg of the test item were mixed with 10 L of water in sterilized vessels and agitated at room temperature for at least 21 h. Then, 9 L of the aqueous phase (the saturated solution) were drained from the port at the bottom of the mixing vessel into a sterilized stock solution bottle.

Fish exposure
The challenge of this study was to maintain the test concentration in water despite the rapid dissipation of the test item by diverse processes, i.e., degradation by bacteria, adsorption and volatilization and metabolism by fish. A non-GLP pre-study was performed to develop and optimize the approach resulting in maintaining a 5fold exchange of test solutions at a low fish density (< 0.1 g/L*day). Groups of 48 fish each were exposed to nominal concentrations which were based on the results of the non-GLP pre-study and aimed to be 0.3 mg/L and 0.06 mg/L 2-Ethylhexyl methacrylate in a flow-through system at a test temperature of 24 +/- 1°C. The concentrations of the test substance in the fish and in the water were determined throughout the different phases of the test. An effect control group was kept under comparable conditions without adding test substance; the fish were adequately reduced and used for the determination of fat content.
All-glass aquaria containing 20 L of test solution were used as test vessels. A continuous flow of approximately 4.2 L/h test solution (uptake phase) or water (depuration phase) was maintained throughout the test using metering pump systems, resulting in a water exchange rate of 5 times per day. Observations were made throughout the test period on fish behaviour and mortalities. Temperature, pH and the oxygen concentrations in the test vessels were measured daily. The light/dark cycle was adjusted to 12/12 hours.

Sampling schedule

Uptake hours	time	Fish sampled	Water sample
007:00		X	
108:00	4	X	
209:00	4	X	
411:00	4	X	
815:00	4	X	
1219:00	4	X	
3215:00	4	X	
5615:00	4	X	
Depuration			
116:00	4	X	
419:00	4		
1607:00	4		
4007:00	4		
sum	44		

During the uptake phase scheduled for 56 h, the fish were continuously exposed to the test substance. Thereafter the fish were transferred into new aquaria served with test substance-free dilution water further 30 h. The duration of both, uptake and depuration phase, were fixed based on the results of a non-GLP pilot study. On each sampling time, samples of 4 fish per concentration were removed from the test vessels according to the sampling schedule (Table 1) and immediately rinsed in dilution water, blotted dry and killed. To avoid degradation of the test substance by residual activity of esterases, the fish were either immediately frozen in liquid nitrogen or homogenized in organic solvent (see paragraph 7). The fish were individually analyzed for the test item by GC-MS/MS. At each fish sampling time during the uptake phase, and 1 h after start of the depuration phase, adequate amounts of water were analyzed per concentration. The water samples were analysed immediately after sampling.

Chemical analyses

Analysis of water samples
Extraction of the aqueous samples with cyclohexane, determination of EHMA by gas chromatography with quadrupole mass spectrometry detection (GC-MS/MS) in a non-polar capillary column.

Analysis of fish samples
For EHMA extraction after defrosting the fish were doused with 4 ml cyclohexane, 1 mL dichloromethane and with 100 µL of the IS working solution (500 ng n-Hexyl methacrylate [NHM] in cyclohexane). The fish were homogenized by using an Ultra-Turrax® . After it the mixture was treated with ultrasound and centrifuged . To eliminate fat and high molecular matrix components from the fish extracts, the whole organic phase was decanted into a Zymark® sample tube and concentrated to approx. 1 mL in the Zymark® concentration workstation. The concentrate was cleaned by adsorption chromatography using activated silica gel analysed by GC-MS/MS.
For the definition of the LOQ the method was validated

according to the EU guidance documents SANCO/3029/99 and SANCO/825/00 on the target LOQ of 0.05 mg/kg fresh weight and 10 times the target.

Calculation of the uptake and depuration rate constants and the BCF
The uptake rate constant (k1), the depuration rate constant (k2), the kinetic steady state bioconcentration factor (BCFk) were calculated by linear and nonlinear regression functions using data for concentrations of 2-Ethylhexyl methacrylate in whole fish measured in the extracts. Calculations of means and ranges were done with Excel spreadsheets (Microsoft Inc.) while linear and non-linear regressions were conducted with the program SigmaStat 2.03 (SPSS Inc. 1997).

Calculation of the steady state BCF
The test substance is known to be taken up quickly due to the high partition coefficient and to be rapidly metabolized, leading to a very fast elimination. A non-GLP pre-study showed that the steady state can be expected to be achieved within the first 8-12 hours. Two further sampling dates after 32 h and 56 h were included to provide certainty about the BCF. The BCFSS was calculated by dividing the mean of the values for the 2-Ethylhexyl methacrylate concentration in fish which represent the worst case steady state by the mean measured relevant concentrations in the water.

Calculation of the depuration rate constants
The depuration rate constant (k2) was calculated using the measured concentrations in fish during the depuration phase by applying a model regarding fish as one compartment. The model assumes that the concentration of the test substance in the fish (Cf) is decreasing exponentially:
Cf(t) = Cf(ti) * e(-k2*t)
Cf(t): concentration in fish at sampling time in days (µg/Kg)
Cf(ti): steady state concentration in fish corresponding to the concentration at start of the depuration phase (= 100%)
k2: depuration rate constant
k2 was calculated by linear regression applied to the ln-transformed concentrations in fish.

Calculation of the uptake rate constant
The uptake rate constant k1 was calculated by a non-linear regression of the ratios Cf/Cw against time during the uptake phase and using the depuration rate constant fitted before. The fitted model assumes an attenuation of uptake by simultaneous depuration, increasing with increasing Cf up to an steady state between uptake and depuration.
For the one compartment kinetics eq. 3 was fitted:
Cf/Cw = k1/k2 * (1-e(-k2*t))
k1: uptake rate constant
Cf: concentration in fish (µg/kg)
Cw: concentration in water (µg/L)

k1 was calculated by non-linear regression using the k2 values obtained in the depuration phase .

Calculation of kinetic BCFk
The kinetic BCF for the one compartment model is given by
BCFk = k1/k2

Result : No adverse effects or mortalities were observed during the study.
During the uptake phase, all concentrations in water and fish were above the LOQ.
After equilibration of the test system, the nominal test concentrations estimated from the non-GLP pre-study of 0.3 and 0.06 mg/L were nearly met. After addition of the fish, the concentration in water in the low treatment group, rapidly decreased to 50% of nominal during the first 2 h of exposure. It remained sufficiently constant at a mean measured concentration of 0.030 mg/L thereafter. In the high treatment, the concentration in water rapidly decreased to 30% of nominal during the first 4 h of exposure. It remained sufficiently constant at a mean measured concentration of 0.082 mg/L thereafter.

Test item concentrations in water and fish, and ratios between both for the low and high test concentrations

time		c_water [mg/L]		c_fish [mg/kg]		c_fish/
D	h	low	high	low	high	c_water*
0.0	0	0.066	0.378	0	0	0
0.04	1	0.049	0.181	1.146	4.607	20
0.08	2	0.035°	0.107	1.232°	5.482	29
0.17	4	0.028°	0.088	1.072°	3.452	34
0.33	8	0.032°	0.081°	1.064°	3.140°	33
0.5	12	0.038°	0.081°	1.310°	2.634°	34
1.33	32	0.027	0.084°	0.651	2.171°	24
2.33	56	0.025	0.082°	0.574	3.085°	23
Steady state**		0.033	0.082°	1.170	2.758	35
Deviation ***		15%	0%	11%	18%	

* For 1 - 4 hours the means of the concentrations of the actual and last sample were used
** Means were calculated from data marked with "°" considered to represent steady state conditions. BCFs were calculated from these steady state concentrations.
*** Deviation was calculated as half of the range of those data which were regarded as representing the steady state.

Uptake of 2-EHMA by fish and steady state BCF (BCFss)
The steady state is reached at 1.170 mg/kg fish at 0.033 mg/L and 2.758 mg/kg fish at 0.082 mg/L. When calculating the BCFss by dividing the conservative steady state concentrations in fish by the mean concentration measured in water for the same sampling dates, the results are
BCFss = 35 at the low treatment concentration
BCFss = 34 at the high treatment concentration

Depuration
In the depuration phase concentrations in the fish were below LOQ after 16 h. Consequently, only the data for 0, 1 and 4 h were used to calculate the depuration rates. The depuration rates were calculated to be 0.51 and 0.47/h and thus very similar for the low and the high treatment groups, respectively, indicating a depuration half-life of less than 1.5 h and 95 % depuration within approximately 6 hours.

Determination of k1 and BCFk
Uptake rates were calculated to be 19.2 and 17.4/h and thus very similar for the low and the high treatment level, respectively. From the kinetic rates the BCFk was calculated to be 37.5 and 37.4 for the low and high treatment group, respectively.

Test substance : Ethylhexyl methacrylate, purity: 99.02 %, produced by Röhm GmbH & Co. KG

Conclusion : Depuration
At the end of the study at the sampling points after 72 and 96 h and at both treatment levels the concentrations of 2-Ethylhexyl methacrylate in tissue were below the LOQ, corresponding to more than 99 % depuration at the high test concentration.

Water concentrations and uptake
The 2-Ethylhexyl methacrylate concentrations in the water during the uptake phase (with fish) were considerably lower than those achieved during the system equilibration phase (without fish). In both treatments the concentrations during the first hours decreased considerably, most probably due to rapid uptake by the high number of initially present fish and adsorption processes. For the period considered for steady state conditions, the 2-Ethylhexyl methacrylate concentrations were within 20 % of the mean measured concentration. Due to the very fast uptake and elimination processes, the concentrations can be regarded as sufficiently constant for determining a reliable BCF. At the low treatment level concentrations in fish decreased, which might indicate an increase of metabolic transformation, being less efficient at the higher treatment level.

Uptake rate constant and BCF
The fitted uptake rate constant k1 was also very similar at both concentrations. The deviation from the mean was approximately 5 % and the standard errors were < 20 % of the mean

Summary of kinetic study results +/- standard error

	Low treatment	High treatment	Deviation from mean
k1 [1/h]	19.2 +/- 1.1	17.4 +/- 1.6	4.9 %
k2 [1/h]	0.51 +/- 0.09	0.47 +/- 0.03	4.1 %
BCFk	37.5	37.4	0.1 %

As concluding result of the study, a BCF of 37 has been found for 2-Ethylhexyl methacrylate, calculated as mean BCFk derived from the two test concentrations.

This value is near the range of 34 - 35 calculated for the BCFss.

Reliability : (1) valid without restriction
Flag : Critical study for SIDS endpoint

Annex 3: List of chemicals in SMILES notation complying with the category definition "short chain (C2-C8) saturated linear and branched alkyl methacrylates".

SMILES
C(=O)(C(=C)C)OC(C(C)(C)C)C(C)C
C(=O)(C(=C)C)OC(C(C)(C)C)CC
C(=O)(C(=C)C)OC(C(C)(C)C)CCC
C(=O)(C(=C)C)OC(C(C)(C)CC)CC
C(=O)(C(=C)C)OC(C(C)C(C)C)CC
C(=O)(C(=C)C)OC(C(C)C)(CC)CC
C(=O)(C(=C)C)OC(C(C)C)C(C)C
C(=O)(C(=C)C)OC(C(C)C)CC
C(=O)(C(=C)C)OC(C(C)C)CC(C)C
C(=O)(C(=C)C)OC(C(C)C)CCC
C(=O)(C(=C)C)OC(C(C)C)CCCC
C(=O)(C(=C)C)OC(C(C)CC)C(C)C
C(=O)(C(=C)C)OC(C(C)CC)CC
C(=O)(C(=C)C)OC(C(C)CC)CCC
C(=O)(C(=C)C)OC(C(C)CCC)CC
C(=O)(C(=C)C)OC(C(CC)CC)CC
C(=O)(C(=C)C)OC(C)(C(C)C)C(C)C
C(=O)(C(=C)C)OC(C)(C(C)C)CC
C(=O)(C(=C)C)OC(C)(C(C)C)CCC
C(=O)(C(=C)C)OC(C)(C(C)CC)CC
C(=O)(C(=C)C)OC(C)(C)C
C(=O)(C(=C)C)OC(C)(C)C(C)(C)C
C(=O)(C(=C)C)OC(C)(C)C(C)C
C(=O)(C(=C)C)OC(C)(C)C(C)C(C)C
C(=O)(C(=C)C)OC(C)(C)C(C)CC
C(=O)(C(=C)C)OC(C)(C)C(C)CCC
C(=O)(C(=C)C)OC(C)(C)C(CC)CC
C(=O)(C(=C)C)OC(C)(C)CC
C(=O)(C(=C)C)OC(C)(C)CC(C)(C)C
C(=O)(C(=C)C)OC(C)(C)CC(C)C
C(=O)(C(=C)C)OC(C)(C)CC(C)CC
C(=O)(C(=C)C)OC(C)(C)CCC
C(=O)(C(=C)C)OC(C)(C)CCC(C)C
C(=O)(C(=C)C)OC(C)(C)CCCC
C(=O)(C(=C)C)OC(C)(C)CCCCC
C(=O)(C(=C)C)OC(C)(CC(C)C)CC
C(=O)(C(=C)C)OC(C)(CC)CC
C(=O)(C(=C)C)OC(C)(CCC)CC
C(=O)(C(=C)C)OC(C)(CCC)CCC
C(=O)(C(=C)C)OC(C)(CCCC)CC
C(=O)(C(=C)C)OC(C)C
C(=O)(C(=C)C)OC(C)C(C(C)C)CC
C(=O)(C(=C)C)OC(C)C(C)(C)C
C(=O)(C(=C)C)OC(C)C(C)(C)C(C)C
C(=O)(C(=C)C)OC(C)C(C)(C)CC
C(=O)(C(=C)C)OC(C)C(C)(C)CCC
C(=O)(C(=C)C)OC(C)C(C)(CC)CC
C(=O)(C(=C)C)OC(C)C(C)C

C(=O)(C(=C)C)OC(C)C(C)C(C)(C)C
C(=O)(C(=C)C)OC(C)C(C)C(C)C
C(=O)(C(=C)C)OC(C)C(C)C(C)CC
C(=O)(C(=C)C)OC(C)C(C)CC
C(=O)(C(=C)C)OC(C)C(C)CC(C)C
C(=O)(C(=C)C)OC(C)C(C)CCC
C(=O)(C(=C)C)OC(C)C(C)CCCC
C(=O)(C(=C)C)OC(C)C(CC)CC
C(=O)(C(=C)C)OC(C)C(CCC)CC
C(=O)(C(=C)C)OC(C)CC
C(=O)(C(=C)C)OC(C)CC(C)(C)C
C(=O)(C(=C)C)OC(C)CC(C)(C)CC
C(=O)(C(=C)C)OC(C)CC(C)C
C(=O)(C(=C)C)OC(C)CC(C)C(C)C
C(=O)(C(=C)C)OC(C)CC(C)CC
C(=O)(C(=C)C)OC(C)CC(C)CCC
C(=O)(C(=C)C)OC(C)CC(CC)CC
C(=O)(C(=C)C)OC(C)CCC
C(=O)(C(=C)C)OC(C)CCC(C)(C)C
C(=O)(C(=C)C)OC(C)CCC(C)C
C(=O)(C(=C)C)OC(C)CCC(C)CC
C(=O)(C(=C)C)OC(C)CCCC
C(=O)(C(=C)C)OC(C)CCCC(C)C
C(=O)(C(=C)C)OC(C)CCCCC
C(=O)(C(=C)C)OC(C)CCCCCC
C(=O)(C(=C)C)OC(CC(C)(C)C)CC
C(=O)(C(=C)C)OC(CC(C)C)CC
C(=O)(C(=C)C)OC(CC(C)C)CCC
C(=O)(C(=C)C)OC(CC(C)CC)CC
C(=O)(C(=C)C)OC(CC)(CC)CC
C(=O)(C(=C)C)OC(CC)CC
C(=O)(C(=C)C)OC(CCC(C)C)CC
C(=O)(C(=C)C)OC(CCC)(CC)CC
C(=O)(C(=C)C)OC(CCC)CC
C(=O)(C(=C)C)OC(CCC)CCC
C(=O)(C(=C)C)OC(CCCC)CC
C(=O)(C(=C)C)OC(CCCC)CCC
C(=O)(C(=C)C)OC(CCCCC)CC
C(=O)(C(=C)C)OCC
C(=O)(C(=C)C)OCC(C(C)(C)C)CC
C(=O)(C(=C)C)OCC(C(C)C)C(C)C
C(=O)(C(=C)C)OCC(C(C)C)CC
C(=O)(C(=C)C)OCC(C(C)C)CCC
C(=O)(C(=C)C)OCC(C(C)CC)CC
C(=O)(C(=C)C)OCC(C)(C(C)C)CC
C(=O)(C(=C)C)OCC(C)(C)C
C(=O)(C(=C)C)OCC(C)(C)C(C)C
C(=O)(C(=C)C)OCC(C)(C)C(C)CC
C(=O)(C(=C)C)OCC(C)(C)CC
C(=O)(C(=C)C)OCC(C)(C)CC(C)C
C(=O)(C(=C)C)OCC(C)(C)CCC
C(=O)(C(=C)C)OCC(C)(C)CCCC
C(=O)(C(=C)C)OCC(C)(CC)CC
C(=O)(C(=C)C)OCC(C)(CCC)CC

C(=O)(C(=C)C)OCC(C)C
C(=O)(C(=C)C)OCC(C)C(C)(C)C
C(=O)(C(=C)C)OCC(C)C(C)(C)CC
C(=O)(C(=C)C)OCC(C)C(C)C
C(=O)(C(=C)C)OCC(C)C(C)C(C)C
C(=O)(C(=C)C)OCC(C)C(C)CC
C(=O)(C(=C)C)OCC(C)C(C)CCC
C(=O)(C(=C)C)OCC(C)C(CC)CC
C(=O)(C(=C)C)OCC(C)CC
C(=O)(C(=C)C)OCC(C)CC(C)(C)C
C(=O)(C(=C)C)OCC(C)CC(C)C
C(=O)(C(=C)C)OCC(C)CC(C)CC
C(=O)(C(=C)C)OCC(C)CCC
C(=O)(C(=C)C)OCC(C)CCC(C)C
C(=O)(C(=C)C)OCC(C)CCCC
C(=O)(C(=C)C)OCC(C)CCCCC
C(=O)(C(=C)C)OCC(CC(C)C)CC
C(=O)(C(=C)C)OCC(CC)(CC)CC
C(=O)(C(=C)C)OCC(CC)CC
C(=O)(C(=C)C)OCC(CCC)CC
C(=O)(C(=C)C)OCC(CCC)CCC
C(=O)(C(=C)C)OCC(CCCC)CC
C(=O)(C(=C)C)OCCC
C(=O)(C(=C)C)OCCC(C(C)C)CC
C(=O)(C(=C)C)OCCC(C)(C)C
C(=O)(C(=C)C)OCCC(C)(C)C(C)C
C(=O)(C(=C)C)OCCC(C)(C)CC
C(=O)(C(=C)C)OCCC(C)(C)CCC
C(=O)(C(=C)C)OCCC(C)(CC)CC
C(=O)(C(=C)C)OCCC(C)C
C(=O)(C(=C)C)OCCC(C)C(C)(C)C
C(=O)(C(=C)C)OCCC(C)C(C)C
C(=O)(C(=C)C)OCCC(C)C(C)CC
C(=O)(C(=C)C)OCCC(C)CC
C(=O)(C(=C)C)OCCC(C)CC(C)C
C(=O)(C(=C)C)OCCC(C)CCC
C(=O)(C(=C)C)OCCC(C)CCCC
C(=O)(C(=C)C)OCCC(CC)CC
C(=O)(C(=C)C)OCCC(CCC)CC
C(=O)(C(=C)C)OCCCC
C(=O)(C(=C)C)OCCCC(C)(C)C
C(=O)(C(=C)C)OCCCC(C)C
C(=O)(C(=C)C)OCCCC(C)CC
C(=O)(C(=C)C)OCCCC(CC)CC
C(=O)(C(=C)C)OCCCC(CCC)CC
C(=O)(C(=C)C)OCCCCC
C(=O)(C(=C)C)OCCCCC(C)(C)C
C(=O)(C(=C)C)OCCCCC(C)C
C(=O)(C(=C)C)OCCCCC(C)CC
C(=O)(C(=C)C)OCCCCCC
C(=O)(C(=C)C)OCCCCCC(C)C
C(=O)(C(=C)C)OCCCCCCC

C(=O)(C(=C)C)OCCCCCCCC
C(C)(C)(C(C)(C)CC)OC(=O)C(=C)C
C(C)(C)(C)C(C)(C)COC(=O)C(=C)C
C(C)(C)(C)C(C)(CC)OC(=O)C(=C)C

Annex 4: BCF base-line model and bioconcentration of methacrylates (provided by OASIS)

1. Domain

The applicability domain of BCF base-line model has three components:

- Parameter domain – responds for the range of variation logKow, MW and Sw within the training set
- Structural domain – responds for the variation of surrounding of atoms in the target chemical and training set
- Mechanistic domain – responds for the driving forces of bioaccumulation (passive diffusion, protein binding, etc.)

All studied methacrylates are out of the structural domain. Without exception, for all methacrylates atoms constituting the methacrylic group are unknown and not presented in the training set. Figure 1 illustrates out of domain atoms constituting methacrylic group.

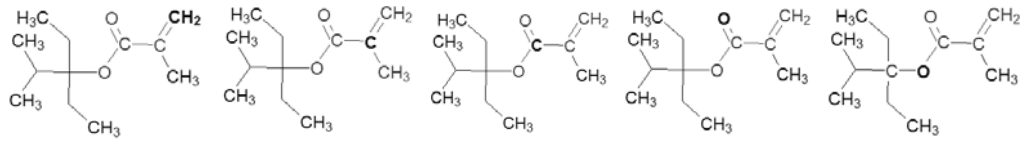


Figure 1. Out of domain atoms constituting methacrylate group.

Because of the lack of metacrylic group in the training chemicals metabolism of this group was not studied and not implemented in the simulator of metabolism. As a consequence the simulator doesn't recognize methacrylic group and metabolic attack is focused on the double bond (ignoring the presence of vicinal ester group) resulting in formation of epoxide. In this respect, the structural domain of the model is warning that metabolism of methacrylic group is not correct and prediction is really out of the domain. Indeed, literature search showed that the only first step of metabolism is hydrolysis of ester group [1-5], followed by addition of water molecule to the double bond and then entering the thricarboxylic acid cycle [6]. Hydrolysis is fast and starts 5 minutes after administration [2]. In a radiolabeled study it was found that up to 88% of administrated methacrylate is exhaled as CO2 within 10 days [7]. The metabolic logic of rapid hydrolysis resulted in low toxicity of administered methacrylates. Experimental data from the report (illustrated on Figure 2) showed reduction of BCF after 12 days.

Summary: The case study with methacrylates provides an excellent example for the importance and usefulness of the applicability domain and its interpretation. Comments in the report related to the BCF base-line model should be focused on the applicability domain instead on the generated numbers.

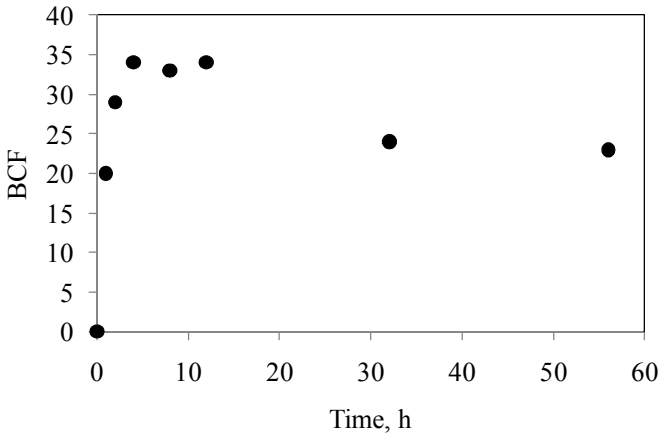


Figure 2. Uptake of 2-EHMA by fish.

2. Reactivity

Methacrylates are highly reactive and toxic compounds. Metylmethacrylate is unstable –light initiate its polymerization. In order to prevent this process metylmethacrylate is kept at low temperature and specific inhibitors of polymerization are used. As a result of reactivity bioavailability of methacrylates could be a problem during the testing. In addition reduction of concentration in fish could be expected because of covalent bonding to macromolecules in organisms.

3. Upgrade of the model

Based on reported experimental data for 2-EHMA and information collected from literature the model was upgraded to reproduce correctly metabolism of methacrylates. Inhibiting fragments with methacrylic structure were added to transformations responsible for incorrect epoxidation of double bond in methacrylic group. New transformation for hydrolysis of methacrylic group was added, Figure 3.

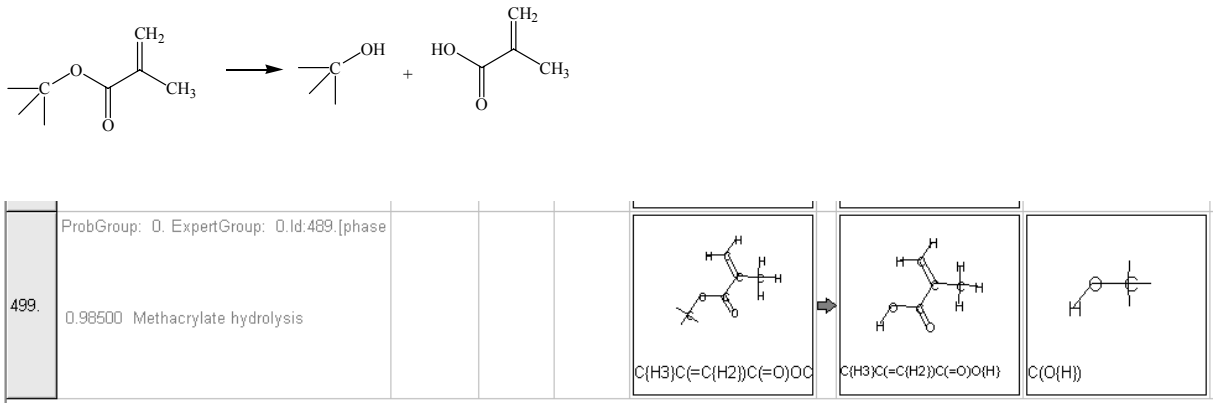


Figure 3. Hydrolysis of methacrylates and new defined transformation *Methacrylate hydrolysis*.

After these modifications the BCF base-line model correctly reproduces first metabolic fate of methacrylates and observed BCF value for 2-EHMA (logBCF predicted = 1.51). 24 out of 160 methacrylates are classified in the model domain. The remaining 136 methacrylates are out of the domain due to the replacement of C(sp3) atom with hydrogen. Option *Fragment with inert addition* from the *Domain set* window could be used to expand the applicability domain assuming that replacement of C(sp3) atom with hydrogen in the "tail" of methacrylates will not affect significantly hydrolysis of methacrylic group. It should be mentioned also that the probability of transformation *Methacrylate hydrolysis* is tentative because only one experimental value was available for bioconcentration of methacrylates.

4. References

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