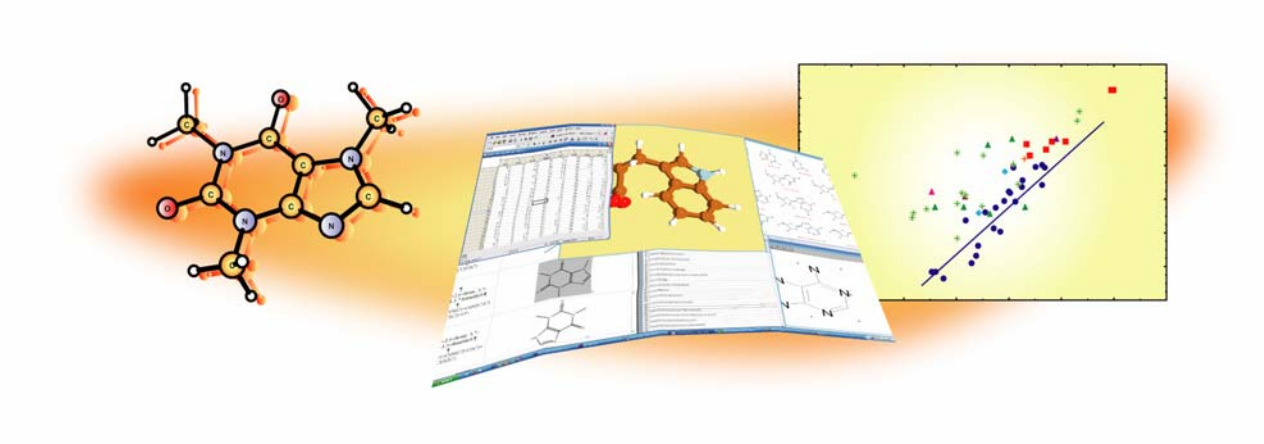

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The mission of the IHCP is to provide scientific support to the development and implementation of EU policies related to health and consumer protection.

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SUMMARY

This report presents the preliminary results from a (Q)SAR investigation of the acute toxicity to fish (fathead minnow) for a dataset of phthalate esters. A chemical set of 341 phthalates was compiled by using different searching engines. Their acute toxicity to fathead minnow was calculated with the ECOSAR and TOPKAT software. A good correlation between the predictions from the two programs was established ($r^2 = 0.81$). The chemicals were classified initially into four groups on a basis of their predicted by ECOSAR LC_{50} values: 1) no reasons for concern ($LC_{50} > 100$ mg/L), 2) harmful (10 mg/L $< LC_{50} < 100$ mg/L), 3) toxic (1 mg/L $< LC_{50} < 10$ mg/L), 4) very toxic $LC_{50} < 1$ mg/L). This prediction effort resulted in classification of the vast majority of the phthalates in the "very toxic" group. The reason for this result is that ECOSAR uses linear relationships with the octanol-water partition coefficient (log K_{ow}) for chemicals with log $K_{ow} < 5$ (warning is issued for chemicals with log $K_{ow} > 5$). The predictions from TOPKAT (only predictions within the optimum prediction space were considered) correlated relatively well with those from ECOSAR.

There were many high molecular weight phthalate esters in the chemical series, which appeared clearly outside the applicability domain of the ECOSAR models. This fact, as well as the understanding that beyond certain limits of hydrophobicity the toxicity of the organic chemicals decreases as a result of reduced bioconcentration, motivated the development of an algorithm for refinement of acute toxicity predictions of the phthalate esters using the bilinear relationship with log K_{ow}. In addition, water solubility limits were considered.

Long-term toxicity studies were not considered in this study. Transformation (e.g. biodegradability) of the parent compounds was not considered either. This could potentially be important as, theoretically, the transformation of very hydrophobic chemicals (log $K_{ow} > 7$) or extremely hydrophobic chemicals (log $K_{ow} > 8.0$) into more hydrophilic degradation/transformation products may increase the acute toxicity to fish.

This case study provides an illustration of how (Q)SAR methods can be used in the development of chemical categories and how (Q)SAR results can be used to perform an initial screening in support of classification and labelling. The results are discussed and interpreted with a view of what constitutes a category, how it can be defined and described, what are its boundaries, and the need to define subcategories that might be useful for deciding on the level of acute toxicological hazard associated with different structural modifications. Due to the preliminary nature of the (Q)SAR models, the results of this study should be regarded as an illustration of the applicability of (Q)SAR methods. The actual model results and rule-based classification scheme will need validation and refinement before they could be considered for regulatory use.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

CEFIC	European Chemical Industry Council
EPA	Environmental Protection Agency
ESR	Existing Substances Regulation
EU	European Union
HMWPE	High Molecular Weight Phthalate Esters
K_{ow}	Octanol-water partition coefficient
NGO	Non-Governmental Organisation
OECD	Organisation for Economic Co-operation and Development
PMG	Project Management Group
QSAR	Quantitative Structure-Activity Relationship
REACH	Registration, Evaluation, Authorisation of Chemicals (European Union)
RIP	REACH Implementation Project
SAR	Structure-Activity Relationship
SIAM	SIDS Initial Assessment Meeting
SIAR	SIDS Initial Assessment Report
SIDS	Screening Information Data Sets

1. Introduction

Currently under European legislation, Directive 67/548/EEC requires new substances to be tested and assessed for possible risks to human health and the environment before they are marketed in volumes of 10 kg or more. In contrast, existing substances are assessed under the provisions of Regulation (EEC) No 793/93, the Existing Substances Regulation (ESR) that requires the identification of priority substances, which are then subjected to comprehensive risk assessment carried out by Member States. Consequently, existing substances do not require testing unless identified as a priority substance. Concerns over lack of data, and thus lack of regulatory consideration on the vast majority of existing substances in commerce, led to the Commission's White Paper on a 'Strategy for a Future Chemicals Policy'.

Further discussions resulted into the Commission's proposal for REACH, published in 2003. REACH stands for the Registration, Evaluation, Authorisation and Restriction of chemicals. The REACH proposal was adopted by the Council on 18 December 2006 and will enter into force on 1 June 2007. REACH provides a legislative framework for industrial chemicals marketed in quantities of more than 1 tonne/enterprise/year. The 30,000 existing substances affected will be processed on a phased basis over a period of 11 years from implementation, starting with those marketed in the highest volumes, as well as those with very high hazard. An important part of this policy is the fostering of research on development and validation of alternative (to animal testing) methods, including (Q)SAR models.

This need to use (Q)SAR models has also been expressed by the European Parliament, who have requested 'the use of screening procedures based on simplified risk assessment using data modelling, e.g., quantitative structure activity relationships ((Q)SARs) and use patterns to prioritise substances of possible concern '…in order to speed up risk assessments…'.

Of particular importance for (Q)SAR applications is Annex XI in REACH which outlines the use of Structure-activity relationship (SAR) and Grouping of substances and read-across approaches for using non-testing information. The development and use of non-

testing methods are based on the expectation that structurally similar chemicals will have similar physical attributes and biological effects. This underlying premise of similarity can be used in hazard and risk assessment when there are inadequate test data to estimate missing values. Approaches developed for describing such relationships between similar chemicals include:

- Analogues/read-across. The use of read across/nearest analogue analysis is a possibility to obtain relevant data when there are no experimental studies on the compound of interest, and/or to evaluate the reliability of predicted estimates for a particular substance. Read across is the process by which one or more properties of a given chemical are inferred by comparison of that chemical with a chemical(s) of similar molecular structure(s) and physicochemical properties, for which the properties of interest are known. This approach can be used to assess physicochemical properties, toxicity, and environmental fate.

- SAR and (Q)SAR. A (Q)SAR consists of a relationship between the chemical structure, or physical-chemical representations thereof, and the outcome in a laboratory measurement for a test endpoint (biological or other physical-chemical. property). SARs are qualitative relationships in the form of structural alerts that incorporate molecular substructures or fragments related to the presence or absence of activity. (Q)SARs are quantitative models which estimates the relative chemical activity of chemicals presumed to behave according to the same mechanisms.

- Chemical Categories. A chemical category is a group of chemicals whose physicochemical and toxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity. These structural similarities may create a predictable pattern in any or all of the following parameters: physicochemical properties, environmental fate and environmental effects, and/or human health effects.

Within the context of the new EU Chemicals Policy, the European Commission has initiated a number of REACH Implementation Projects (RIPs) with the intention of developing tools and guidance for the new legislation. The RIPs are coordinated closely with the main stakeholders, namely Member States, Industry and Non-Governmental Organisations (NGOs). The overall aim of RIP 3 is to develop appropriate guidance documents and tools for industry in order to facilitate a smooth implementation of the legislation. RIP 3.3 is the sub project focused on developing guidance documentation on Information Requirements on Intrinsic Properties of substances.

One of the conclusions that arose from the first phase of RIP 3.3 (abbreviated as RIP 3.3-1) was with respect to read across and chemical categories, in terms of how to carry out a read-across or build a category, conduct them, how to justify the read-across / category proposal, and how to document the supporting argumentation. These issues have been taken up in the second phase of RIP 3.3 (abbreviated as RIP 3.3-2), which is being coordinated by CEFIC and steered by a multi-stakeholder Project Management Group (PMG). It was agreed that the European Chemicals Bureau (ECB) and the Organisation for Economic Cooperation and Development (OECD) should lead the Task on the development of guidance for categories (including read-across) and a limited number of case studies will be selected to derive and illustrate general principles and approaches.

The purpose of this report, therefore, was to assist in developing the concept of what constitutes a chemical category, to provide an example of how the chemical categories might be formed, to help identifying the borders, or the applicability domain, of different categories within given chemical class (e.g. phthalates) and for given toxicological endpoint (acute toxicity to fish), and to support the development of guidance for the Industry and Regulators on use of categories and read-across approaches for screening, prioritization, classification and labeling, and eventually risk assessment. It is believed that by analysing one particular example it would be possible to derive trends and rules with larger applicability (e.g. other chemical classes and other toxicological endpoints).

2. Materials and Methods

2.1. Compilation of the phthalate chemical set

The basis for collection of the chemical set was the category of High Molecular Weight Phthalate Esters (HMWPE), including 7 chemicals and chemical mixtures of "esters with an alkyl carbon backbone with 7 carbon atoms or more". This category was reviewed by the OECD Screening Information Data Sets (SIDS) Initial Assessment Meeting (SIAM) (SIAM 19, 19-22 October 2004, FR + JP/ICCA). The SIDS Initial Assessment Report (SIAR) contains the robust study summaries of the SIDS dossier.

To achieve the aims of this report, the scope of the SIAM example was extended to higher and lower molecular weight phthalate esters. In-house tools and public resources were used to identify potential analogues to supplement the existing category membership. The tools used for analogue identification included AMBIT (http://ambit.acad.bg/ambit/php/), Leadscope (www.leadscope.com), Danish (Q)SAR Database (http://ecbqsar.jrc.it/), Chemfinder (www.chemfinder.com), ChemID plus (http://chem.sis.nlm.nih.gov/chemidplus/) and the US EPA Analog Identification Method (AIM) (http://esc.syrres.com/analog/). The main searching methods included fingerprints/ Tanimoto distance, use of modified Tanimoto coefficient, and substructural search. It was accounted that different databases allow different level of detail to be specified when defining the query. As a result of these database/software specificities and the size of the databases, different number of chemicals was retrieved. Of the initial search, a total of 341 unique chemicals were recognised.

2.2. EU environmental hazard classification system

The environmental hazard criteria within Directive 67/548/EEC require information on acute aquatic toxicity, degradation and bioaccumulation. The categories for environmental hazard classification relevant to aquatic toxicity within the current EU system are given below:

Category R50 (Very toxic to aquatic organisms)

96h LC_{50} (fish)	$\leq$ 1 mg/L and /or
48h EC_{50} (for Daphnia or crustacean)	$\leq$ 1 mg/L and /or
72 or 96h EC_{50} (for algae or other aquatic plants)	$\leq$ 1 mg/L

Category R51 (Toxic to aquatic organisms)

96h LC_{50} (fish)	> 1 $\leq$ 10 mg/L and /or
48h EC_{50} (for Daphnia or crustacean)	> 1 $\leq$ 10 mg/L and /or
72 or 96h EC_{50} (for algae or other aquatic plants)	> 1 $\leq$ 10 mg/L

Category R52 (Harmful to aquatic organisms)

96h LC_{50} (fish)	> 10 $\leq$ 100 mg/L and /or
48h EC_{50} (for Daphnia or crustacean)	> 10 $\leq$ 100 mg/L and /or
72 or 96h EC_{50} (for algae or other aquatic plants)	> 10 $\leq$ 100 mg/L

In this study, only the limits for acute toxicity to fish were used but the approach might be applied to the most sensitive species as well. It was assumed that to be able to do read-across between members of a category, they should have toxicity in the same range according to the definitions above. It should be noted that when reliable experimental data exists, it should be considered. However, for filling data gaps, missing information might be collected by making (Q)SAR predictions.

2.3. Calculation of octanol-water partition coefficient (log K_{ow})

The Log Octanol-Water Partition Coefficient Program (KOWWIN) estimates the logarithmic octanol-water partition coefficient (log K_{ow}) of organic compounds.

KOWWIN requires only a chemical structure to estimate a log K_{ow}. Structures are entered into KOWWIN by SMILES (Simplified Molecular Input Line Entry System) notations. Users unfamiliar with SMILES notations can consult the document “A Brief Description of SMILES Notation” or the KOWWIN help file (accessed by pressing the F1 key or selecting "Help" from the program menu). Structures might be imported also as a Chemical Abstract Service (CAS) number and prediction will be done if the CAS is recognized in the KOWWIN database. The KOWWIN program and estimation methodology were developed at Syracuse Research Corporation. A journal article by Meylan and Howard (1995) describes the program methodology. The “fragment constant” methodology of KOWWIN is also briefly discussed in the help of the program.

In this chapter, measured log Kow were also considered (as provided by KOWWIN program). The measured log Kow values were preferred when available. As a result, the log Kow used in this section is a mixture of measured and estimated values.

2.4. Calculation of water solubility (WSol)

The WSKOWWIN program estimates the water solubility (WSol) of an organic compound using the compounds log octanol-water partition coefficient (log K_{ow}). A journal article by Meylan et al., 1996 describes the methodology. WSKOWWIN requires only a chemical structure to estimate WSol. Structures are entered into WSKOWWIN through SMILES. Users unfamiliar with SMILES notations can consult the document "A Brief Description of SMILES Notation". CAS numbers may be used to enter SMILES notations automatically through use of a supplemental database containing SMILES for 103,000+ compounds.
In this chapter, measured WSol were also considered (as provided by KOWWIN program). The measured WSol values were preferred when available. As a result, the WSol used in this section is a mixture of measured and estimated values. WSol was calculated by WSKOWWIN in mg/L.

2.5. Calculation of acute toxicity to fish using ECOSAR (v. 099g)

ECOSAR, which is freely available from the U.S. EPA, uses a number of (quantitative) structure-activity relationships [(Q)SARs] in order to predict the toxicity of chemicals to aquatic organisms. (Q)SARs are developed for chemical classes based on measured test data that have been submitted by industry to the U.S. Environmental Protection Agency (U.S. EPA) or they are developed by other sources for chemicals with similar structures, e.g., phenols. Using the measured aquatic toxicity values and estimated log K_{ow} values, regression equations can be developed for a class of chemicals. Toxicity values for new chemicals may then be calculated by inserting the estimated log Kow into the regression equation and correcting the resultant value for the molecular weight of the compound. Most SAR calculations in the ECOSAR

The main model used for prediction of the phthalates was the one for esters:

$$\text{Log } LC_{50} = -0.535 \log K_{ow} + 0.25 \qquad [1]$$

ECOSAR indicates with an asterisk if a chemical may not be soluble enough to measure the predicted effect and warns that the fish acute toxicity cutoff is at log $K_{ow} = 5.0$.

The ECOSAR Class Program has been developed primarily for the following scenario: (1) enter a SMILES notation, (2) computer determination of appropriate ECOSAR classes for the SMILES notation, and (3) calculate the ecotoxicity SARs using a log K_{ow} value. The program might be executed in batch mode and the result is available in text format. ECOSAR produces warnings in several occasions (e.g. when the water solubility is very low, or when the prediction is outside the range of log K_{ow}). The 96-hour acute toxicity to fish (LC_{50}) was calculated in mg/L.

2.6. Calculation of acute toxicity to fish using nonlinear relationship with log K_{ow}

Log K_{ow} represents the ratio between the concentration of a chemical into an octanol, used as a model of a lipid phase, and water, in equilibrium. Log K_{ow} might be used as a measure of chemical hydrophobicity. When the log K_{ow} is used to indicate the trend of penetrating of chemical through biological membranes, however, the relationship between log K_{ow} and the penetrated amount of chemical is not linear in a large log K_{ow} range. The nonlinearity can be described with different mathematical functions such as quadratic or bilinear function. Both functions assume that the relationship between log K_{ow} and the penetrated amount goes trough a maximum. The log K_{ow} associated with the maximum of the function depends on many factors such as the nature of the membrane, the pH of the water medium, the temperature, etc. Nevertheless, the nature of the factors that might influence the log K_{ow} range at the maximum are relatively constant when considering penetration through the fish gills or body surface, although, some interspecies variability can exist.

More often, quadratic function is used to describe the relationship between log K_{ow} and the toxicity, when the former vary in a large range (e.g. more than five log units):

$$\text{Log } (1/LC_{50}) = -a * (\log K_{ow})^2 + b *(\log K_{ow}) - c, \qquad [2]$$

where a, b and c are coefficient in the regression model, which are determined experimentally. Equation of this type was used by Hermens et al. (1984) to describe the relationship between the log K_{ow} and the 24-days toxicity of chemicals to guppy:

$$\text{Log } (1/LC_{50}) = -0.150 * (\log K_{ow})^2 + 1.67 *(\log K_{ow}) - 4.56, \qquad [3]$$

where the LC_{50} was expressed in μmol/L. Graphically, the relationship is presented on Figure 1. It is evident from Figure 1 that the toxicity increases till about log K_{ow} = 6 and decreases after that value.

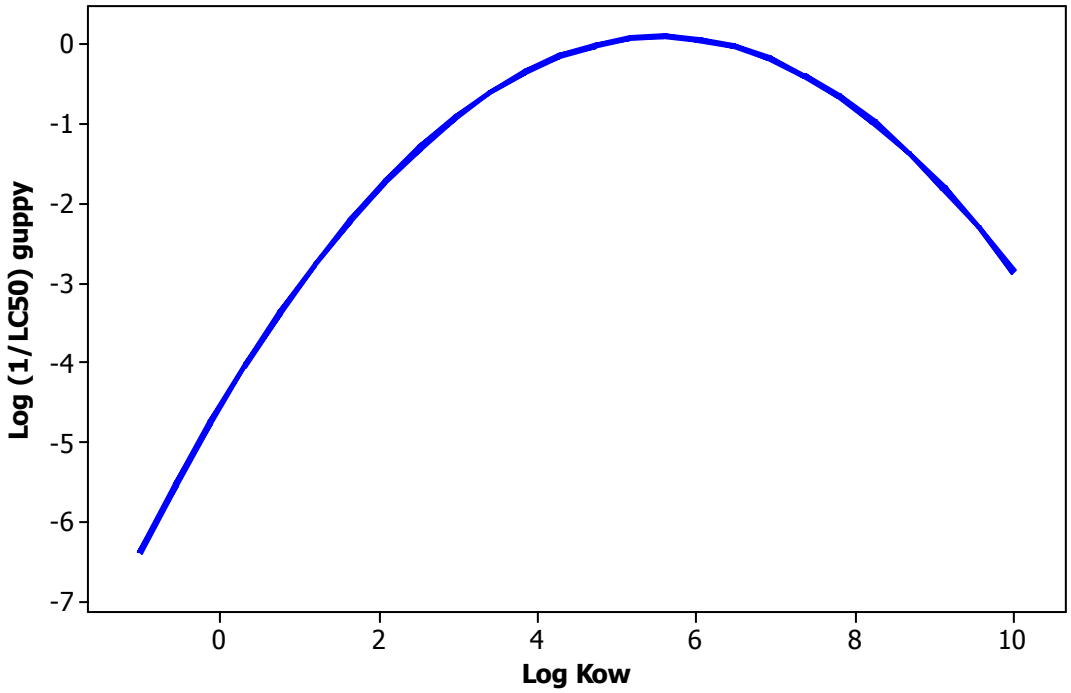


Figure 1. Plot of calculated according to Equation [2] acute toxicity to guppy and log Kow.

When a quadratic relationship for the exact species, test protocol and chemical class is missing, one can assume bilinear relationship between the hydrophobicity and toxicity. In the case of the phthalates, the toxicity of the chemicals with log K_{ow} > 6 (under the assumption that the linear ester model holds true in the range of log K_{ow} between 5 and 6) was predicted by the following linear model:

$$\text{Log } LC_{50} = 0.535 * \log K_{ow} - \text{Intercept} \qquad [4]$$

The slope of Equation [4] is the same as in Equation [1] but with opposite sign. Thus, for chemicals with log K_{ow} > 6 the toxicity decreases with increasing hydrophobicity. The slope in Equation 4 was adjusted in such a way that the line with the reversed slope begins at log K_{ow} = 6 and ends at log K_{ow} = 10 (due to the upper limit for hydrophobicity where no

bioavailability is expected). Note, that the ECOSAR equations predict the toxicity in mmol/L but when it is reported in the output window, it might be converted already to mg/L.

2.7. Calculation of acute toxicity to fish using TOPKAT (v. 6.2)

TOPKAT, which is a commercial product developed by Accelrys, assesses the toxicity of chemicals solely from their 2D molecular structure (SMILES notation but other input formats are also available). The program uses a range (Q)SAR models for assessing specific toxicological endpoints, including 96-hours acute toxicity to fathead minnow (LC_{50}). The (Q)SAR models in TOPKAT use electrotopological (E-state) fragments. (Q)SAR models (so called submodels) are available for different chemical classes and the program automatically selects the equation form the structural input. The program might be executed in batch mode and the result is available in format, directly readable by Excel for Windows.

TOPKAT produces information for the (Q)SAR applicability domain at several levels: 1) the prediction is within the "optimum prediction space (OPS) of the model; 2) the prediction is within the limits of OPS; 3) all fragments identified in a molecule are known to the model. In this chapter the predictions from TOPKAT are considered only if they fulfill all the three conditions placing the molecule of interest in the model applicability domain. The results for acute toxicity to fathead minnow are produced in different units of measurement (depending on the submodel) and there is a need for conversion. TOPKAT also makes visible experimental test data if such is available for the query chemicals (presumably used in the (Q)SAR training set).

3. Results and Discussion

The acute toxicity to fish (LC_{50}, in mg/L) was calculated by both ECOSAR and TOPKAT. The predictions by ECOSAR were further refined by use of limits for hydrophobicity and water solubility, as well as non-linear relationship with log K_{ow}. Only chemicals, which fit the applicability domain (the Optimum Predictions Space – OPS) of the acute fish toxicity module of TOPKAT were considered. The predicted LC_{50} values from both programs were compared. Further, the classification continued with the predictions from ECOSAR and an algorithm was suggested to allow classification from chemical structure in absence of measured toxicity data.

3.1. Characterisation of the chemical set

The total of 341 chemicals encoded as Simplified Molecular Input Line Entry Specification (SMILES) strings. These were processed in batch mode through KOWWIN and WSKOWWIN (both part of the EPIWIN, or shorter – EPI). A total of 324 chemicals were imported successfully. The input of 17 chemicals failed, which is not necessary due to the inability of EPIWIN and ECOSAR to read them. Their measured/calculated values for WSol and log K_{ow} are given in Appendix I. Figures 2-4 illustrate the distribution of the chemicals according to the number of carbon atoms in the molecule, WSol and log K_{ow}.

It can be seen from Figure 2 that the range of the number of carbon atoms vary from below 10 to more than 50, with a mean value of 22. This large range demonstrates that the studied chemical series covers low molecular weight chemicals as well as high molecular weight chemicals and thus provides a suitable diverse set of phthalate esters to allow meaningful investigations. The logarithm of the water solubility shows almost normal distribution with a mean value of 0.78. Interestingly, the log K_{ow} distribution shows two maximums at about 2.3 and 9, which was not specifically targeted. Otherwise, the mean value is 6.6, which show prevalence of the hydrophobic chemicals in the data set. As the complied set includes all unique chemicals retrieved from several chemical searching engines, it might be assumed that the set is representative for the group of the known phthalate esters.

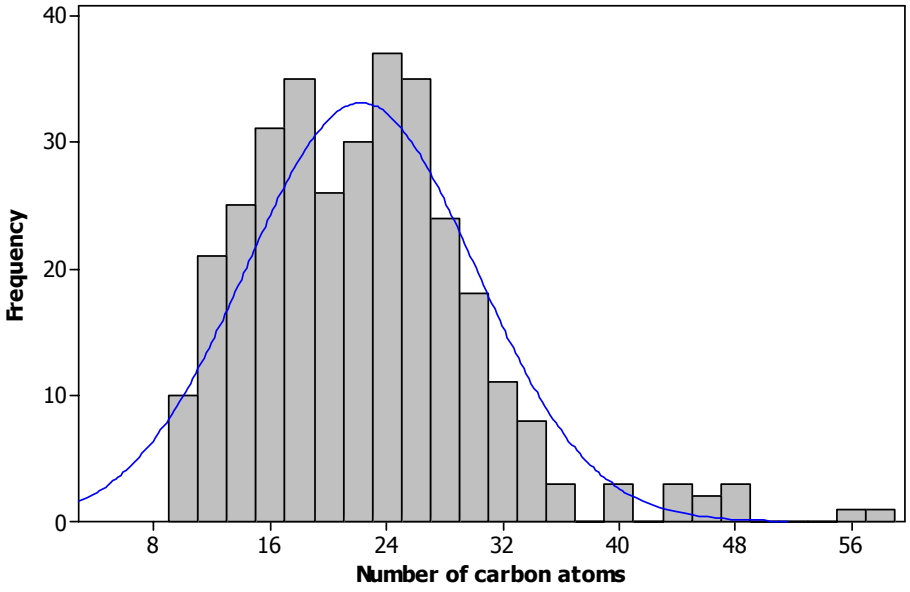


Figure 2. Distribution of the number of carbon atoms in the compiled set of phthalate esters.

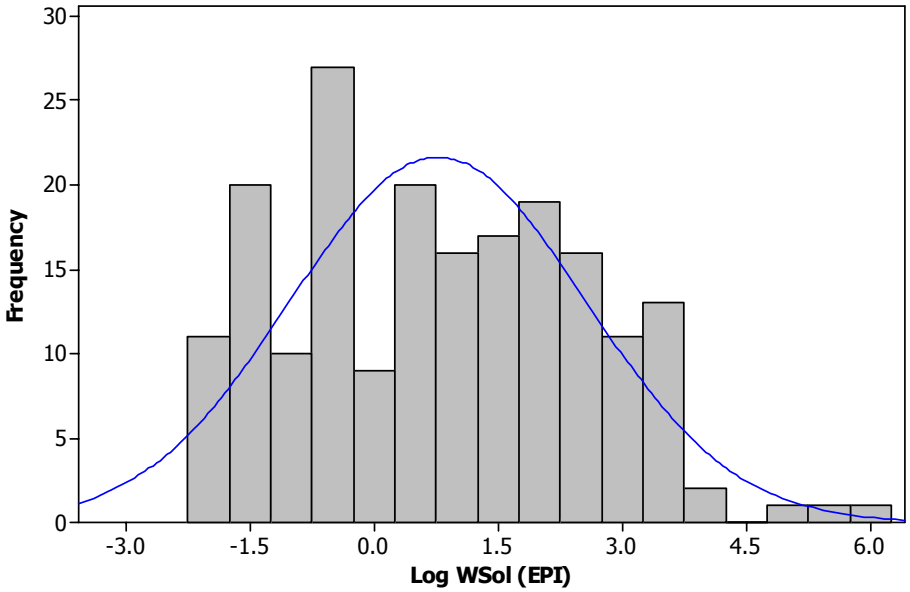


Figure 3. Distribution of the water solubility (presented as decimal logarithm of the concentration in mg/L) in the compiled set of phthalate esters.

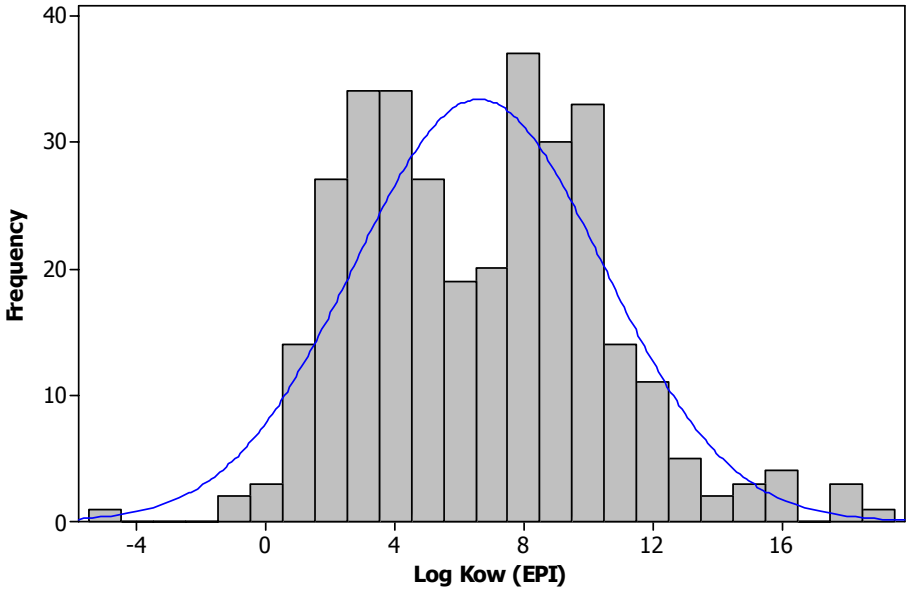


Figure 4. Distribution of the logarithm of the octanol-water partition coefficient in the compiled set of phthalate esters.

3.2. Prediction of toxicity using ECOSAR

A total of 324 chemicals (those with readable SMILES) were processed through ECOSAR and the LC_{50} values calculated in mg/L were recorded. For several chemicals, more that one predicted value was available. This results from the fact that sometimes one chemical trigger QSAR models for more than one class in presence of several functional groups. In such case, it might be argued whether a chemical belongs to the considered category (i.e. the phthalate esters) or should be treated as member of other category. Although the selection of a predicted value is not automated, the offer of a choice of a model to predict the LC_{50} value using models for different chemical classes is an advantage of ECOSAR. A list of models that were triggered by the selected set of phthalate esters (in addition to the ester model, Equation [1]), is shown in Table 1. When more than one predicted value was available, the more conservative one (i.e. the lower value) was selected.

Table 1. A list of models used at least once for prediction of the acute fish toxicity.

Model	**Equation***
Acrylate	Log LC_{50} = -1.46 - 0.18 log K_{ow}
Aldehyde	Log LC_{50} = -0.4487 log K_{ow} - 0.314
Aliphatic amine	Log LC_{50} = 0.72 - 0.64 log K_{ow}
Aromatic amine	Log LC_{50} = 0.956 - 0.739 log K_{ow}
Diepoxide	Log LC_{50} = -1.184 - 0.263 log K_{ow}
Epoxide	Log LC_{50} = -0.290 - 0.382 log K_{ow}
Ester-acid	Not found
Neutral organics	Log LC_{50} = -0.94 log K_{ow} + 1.75
Peroxy acid	Log LC_{50} = -3.037 + 0.122 log K_{ow}
Phenol	Log LC_{50} = 0.399 - 0.616 log K_{ow}
Quinone/hydroquinone	Not found

* For the specifics and limitations of the models, see ECOSAR help.

The predicted toxicity values were used for chemical classification in one of the four categories: no concern, harmful, toxic, and very toxic. The result of the initial classification is shown in Figure 5. Table 2 gives the exact numbers of the chemicals in each toxicity category, as well as the percentage of the total, which can be interpreted as a prior probability of the phthalate esters to demonstrate a certain level of the toxic effect (note that the prior probabilities change in the course of the study).

As the “no prediction” is not an eligible group for classification, and because the reason for its appearance is not specific to the chemical group, or the calculation method/ software, it was excluded to allow more precise calculation of the prior probabilities for a phthalate to belong to one of the four eligible toxicity classes. (see Figure 6 and Table 3).

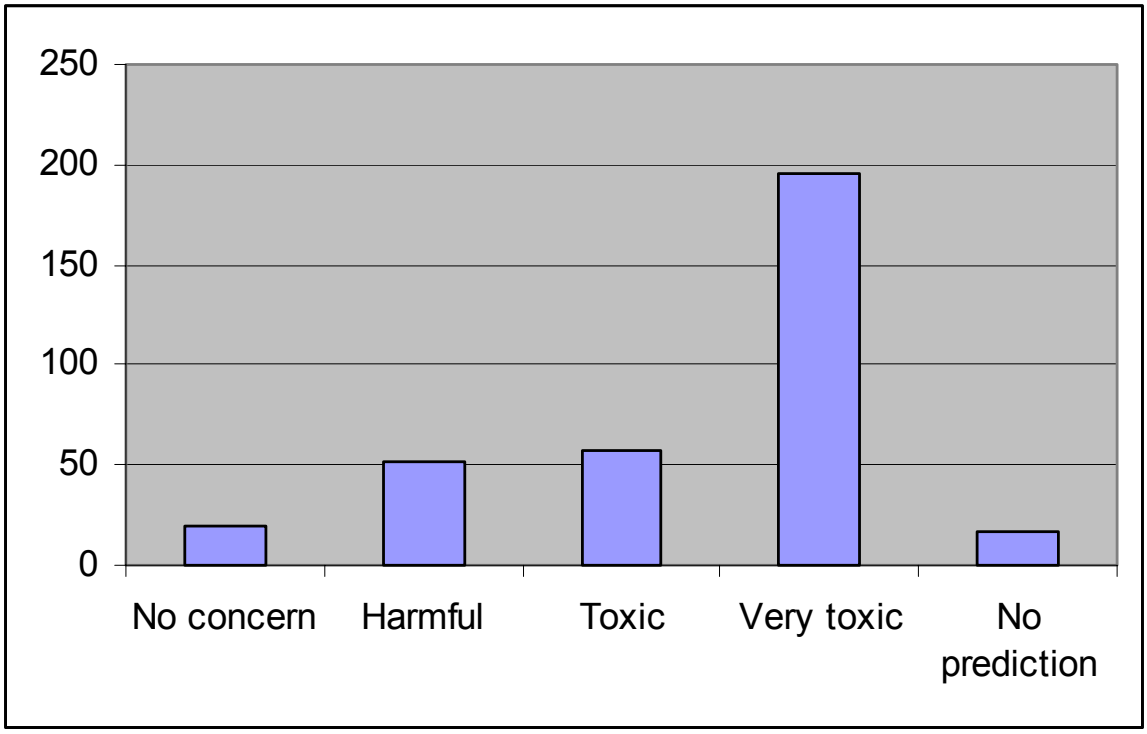


Figure 5. Initial classification of the phthalate esters in four toxicity groups. No prediction means the chemical input as a SMILES string failed for some reason.

Table 2. Number of chemicals classified into four toxicity groups and prior probabilities as calculated by the initial classification.

Category	**Number of Chemicals**	**Percentage of total (prior probability)**
No concern	19	5.57
Harmful	52	15.25
Toxic	57	16.72
Very toxic	196	57.48
No prediction	17	4.99
Total	**341**	

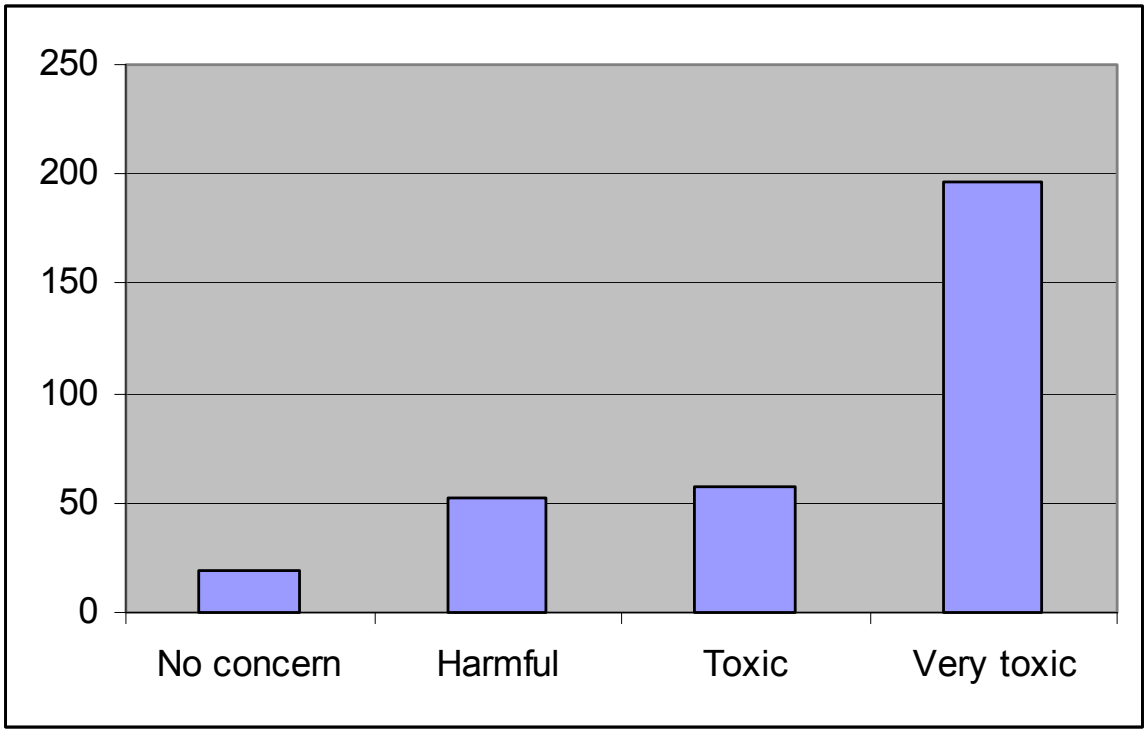


Figure 6. Initial classification of the phthalate esters in four toxicity groups.

3.3. Prediction of toxicity using TOPKAT

The compiled list of SMILES string were processed trough TOPKAT in batch mode. Only chemicals that were: 1) within the OPS; 2) within the limits of the OPS; and 3) all fragments recognized in the training set of the models were considered. This resulted in 264 predictions with expected reliability. These predictions were done by use of 3 from the 8 models for predicting of LC_{50} to fathead minnow. A list of models triggered by the phthalate series is given in Table 4.

The "Benzene (Subst.=2) Model" model contains the octanol-water partition coefficient as a descriptor (squared term), while the other two models are based only on electro-topological indices. The TOPKAT models are not presented explicitly and also due to the nature of descriptors they are difficult for reproduction outside the program. For this reason, as well as the fact that TOPKAT is commercial program and is not freely available to the

user, the predictions from TOPKAT were not used further in this study for more than comparison with the predictions done with ECOSAR.

Table 3. Number of chemicals classified into four toxicity groups and prior probabilities as calculated after exclusion of the "no prediction" group.

Category	**Number of Chemicals**	**Percentage of total (prior probability)**
No concern	19	5.86
Harmful	52	16.05
Toxic	57	17.59
Very toxic	196	60.49
Total	**324**	

Table 4. List of TOPKAT models used for prediction of fish toxicity and number of chemicals within OPS predicted by each model.

Model	**Number of chemicals predicted**
Fathead Minnow LC_{50} Benzene (Multiple & Fused) Model	42
Fathead Minnow LC_{50} Benzene (Subst.=2) Model	209
Fathead Minnow LC_{50} Benzene (Subst.=3) Model	13

To facilitate the comparison, 217 chemicals (47 further deleted due to predicted toxicity values of zero) were classified into four toxicity groups, similarly to the classification of predictions from ECOSAR. The distribution of the chemicals is shown in Figure 7 and Table 5.

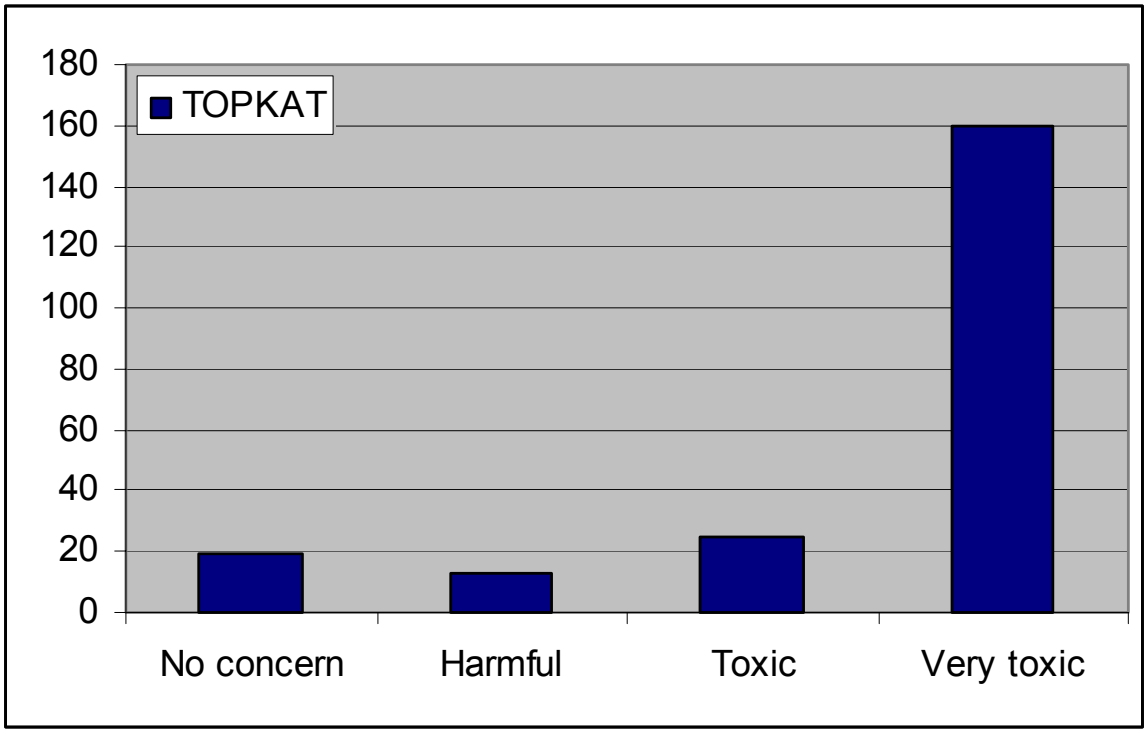


Figure 7. Classification of the phthalate esters in four toxicity groups according to their predicted by TOPKAT toxicity values.

3.4. Comparison between ECOSAR and TOPKAT

Based on the different algorithms and training sets that both programs use for calculation of acute toxicity to fathead minnow, it was expected that there will be substantial differences between the predicted toxicity values. Actually, it was found that there is a relatively good correlation between the predictions from ECOSAR and TOPKAT with a squared correlation coefficient of 0.80 (after exclusion of one significant outlier - 1,2-Benzenedicarboxylic acid, 1,2-ethanediyl dimethyl ester). The relationship is presented in Equation [5] and Figure 8.

$$\text{Log } (LC_{50})^{-1} \text{ ECOSAR} = 0.350 * \text{Log } (LC_{50})^{-1} \text{ TOPKAT} - 0.576 \qquad [5]$$

Table 5. Number of chemicals classified into four toxicity groups and prior probabilities after calculation of toxicity with TOPKAT.

Category	Number of Chemicals	Percentage of total (prior probability)
No concern	19	8.76
Harmful	13	5.99
Toxic	25	11.52
Very toxic	160	73.73
Total	**217**	

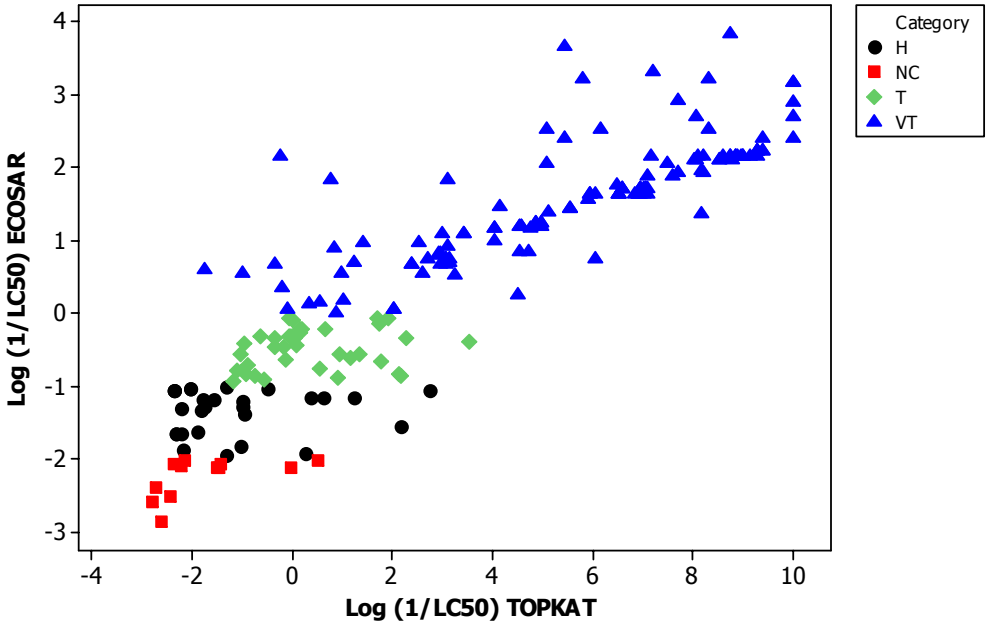


Figure 8. Correlation between the predicted by ECOSAR and TOPKAT log toxicity values to fathead minnow. The (toxicity) category grouping was done using ECOSAR predictions.

The relative correlation between the two columns of predictions resulted in a relatively similar classification of the phthalates into the four toxicity categories. As it can be seen from Figures 6 and 7 (and from Tables 3 and 5, respectively), the vast majority of the phthalates were classified in the category of very toxic chemicals with LC_{50} lower than 1

mg/L. This classification means that the prior probability of a phthalate to be very toxic, in absence of any measured toxicity data and/or suitable QSAR model, will be more than 60%, with TOPKAT being more conservative with approximately 74% prior probability for very high toxicity. In the same time, TOPKAT also gives higher percentage of chemicals with no concern compared to ECOSAR, so it is difficult to judge on average which program makes more conservative predictions.

3.4. Refinement of toxicity prediction using EPIWIN and ECOSAR

The analysis of the chemicals that fall into different toxicity groups revealed that most of the HMWPE are classified as very toxic. In the same time, the SIDS Initial Assessment Profile (October 2004, FR+JP/ICCA) says that there is a little concern regarding the acute and chronic aquatic toxicity of the HMWRE due to their low solubility (equal or less than 0.017 mg/L). Intuitively, and as a result of numerous studies available in the literature, it was felt that the toxicity does not increase to infinity with increase of hydrophobicity, as it tends to be predicted using Equation [1] and similar equations (listed in Table 1), and as it is shown in Figure 9. The chemicals on the main line are esters, predicted by the same model. The chemicals above the line were predicted using QSAR models for different chemical classes, if the prediction was more conservative than that for esters. Chemicals below the line were predicted by different models without option to use the ester model. Therefore, refinement of the prediction strategy was sought in order to improve the category classification using QSAR predictions.

On the presumption that the toxicity decreases above certain value of log K_{ow}, and this value is 6, a bilinear relationship was applied to predict the toxicity for the hydrophobic chemicals with log $K_{ow} > 6$. It is visualized in Figure 10. The log K_{ow} value of 6 and the type of the function (bilinear) were arbitrary chosen.

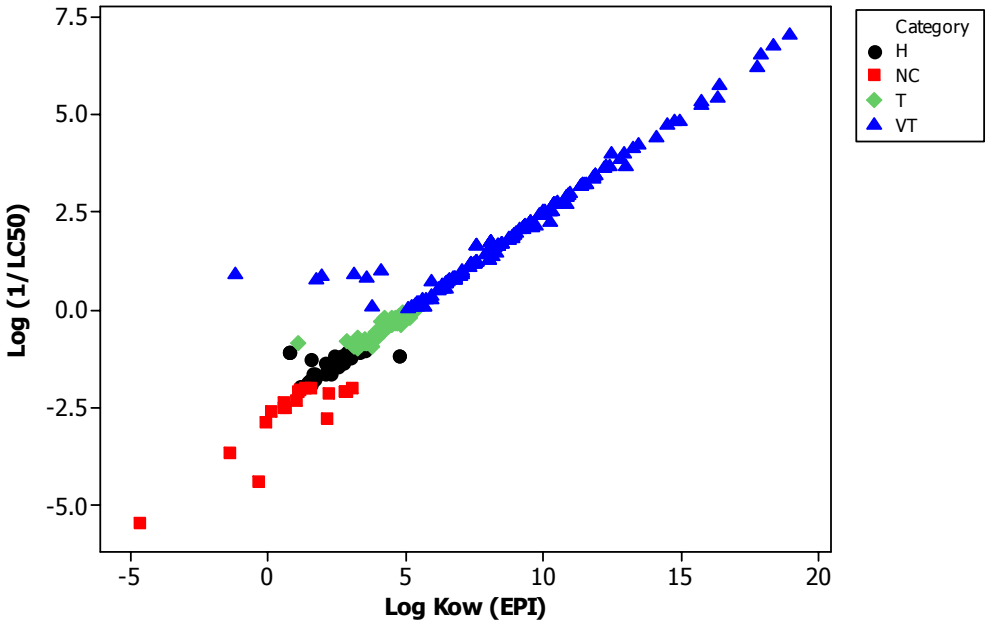


Figure 9a. Plot of predicted by ECOSAR toxicity to fathead minnow vs. hydrophobicity. The chemicals are coded by toxicity class.

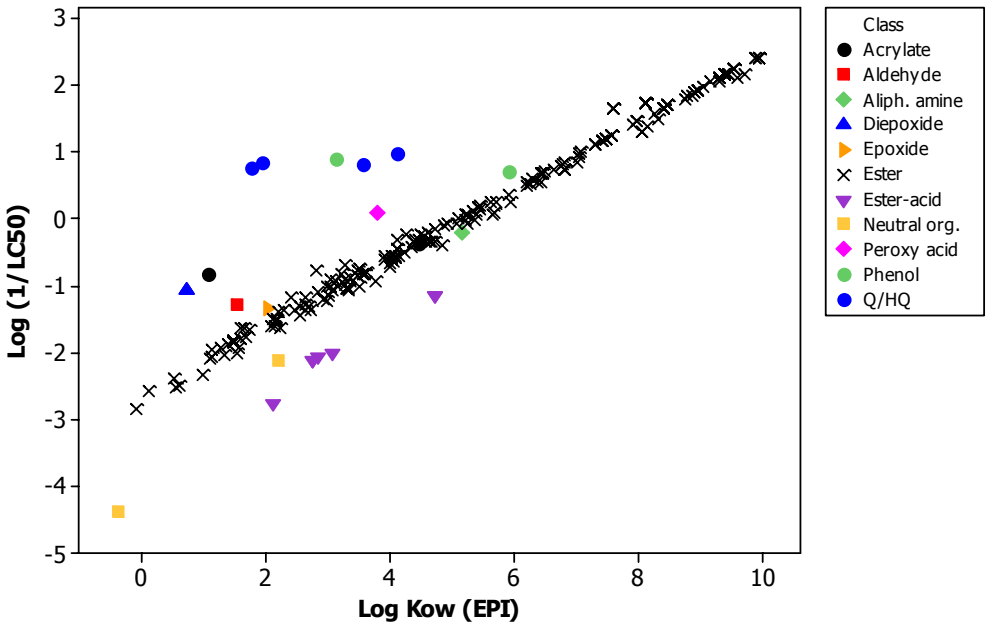


Figure 9b. Plot of predicted by ECOSAR toxicity to fathead minnow vs. hydrophobicity. The chemicals are coded by chemical class (from ECOSAR).

A better approximation of the real relationship between hydrophobicity and toxicity might be achieved by: (1) using experimental subchronic or chronic toxicity data with fathead minnow or, in the absence of these experimental toxicity data by (2) applying quadratic function with coefficients, derived from use of experimental data. However, a suitable function of this type was not found for fathead minnow.

From a theoretical point of view it should be noted that biodegradation or biotransformation of hydrophobic compounds may increase the acute toxicity. This could potentially be important as transformations of very hydrophobic chemicals (log K_{ow} > 7) or extremely hydrophobic chemicals (log K_{ow} > 8.0) into more hydrophilic degradation/ transformation products may lead to increased acute or long-term toxicity to fish. Transformations and biodegradation of the parent compounds were not considered in this study.

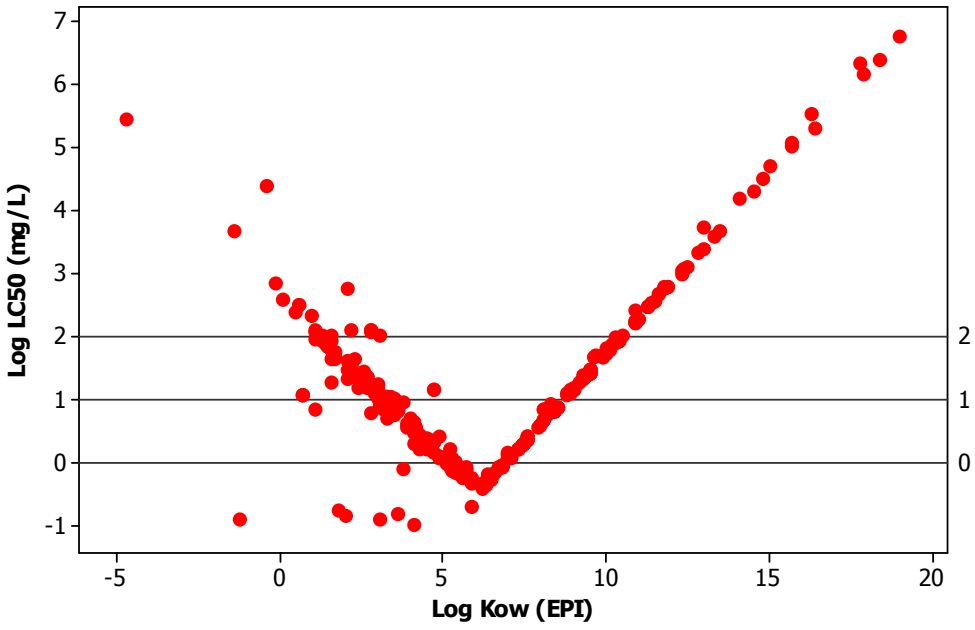


Figure 10. A plot of calculated acute toxicity using bilinear relationship with the octanol-water partition coefficient. The three reference lines indicate the regions of the four toxicity categories (very toxic below 0, toxic between 0 and 1, harmful between 1 and 2, and no concern above 2).

Figure 10 might be converted in rules of thumb for prediction of acute toxicity to fish of (phthalate) esters from log K_{ow}. Thus, it might be concluded that log K_{ow} >10.5 is associated with no concern. Log K_{ow} between approximately 8.8 and 10.5 indicates harmful effect. Chemicals with log K_{ow} between 7.0 and 8.8 are toxic. Finally, those with log K_{ow} between 5.0 and 7.0 are very toxic. Below 5.0 it is recommendable that toxicity is predicted by class-specific models and the more conservative value is taken, if several models can be applied. The classification of the esters according to the calculated acute toxicity using the bilinear relationship is shown in Figure 11 and in Table 6.

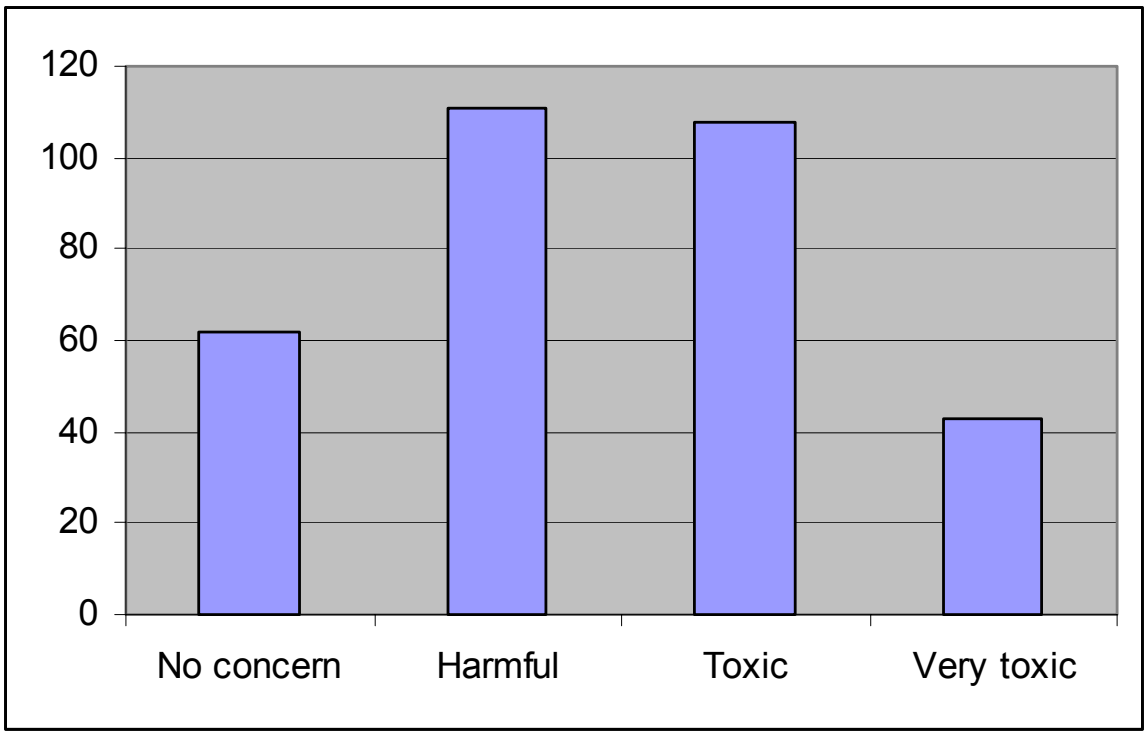


Figure 11. Classification of phthalate esters according to their calculated acute toxicity using the bilinear relationship with log Kow.

The last classification follows in some degree the log K_{ow} distribution, which is more intuitive, compared to the linear relationship with the hydrophobicity. As evident from Figure 11 and Table 6, now the prior probabilities for a phthalate ester to be harmful or

very toxic are almost equal and 33-34%. There is less probability to be very toxic and higher probability to be of no concern compared to the initial classification (Figure 12).

Table 6. Number of chemicals classified into four toxicity groups and prior probabilities after calculation of toxicity using the bilinear relationship with log Kow.

Category	**Number of Chemicals**	**Percentage of total (prior probability)**
No concern	62	19.14
Harmful	111	34.26
Toxic	108	33.33
Very toxic	43	13.27
Total	**324**	

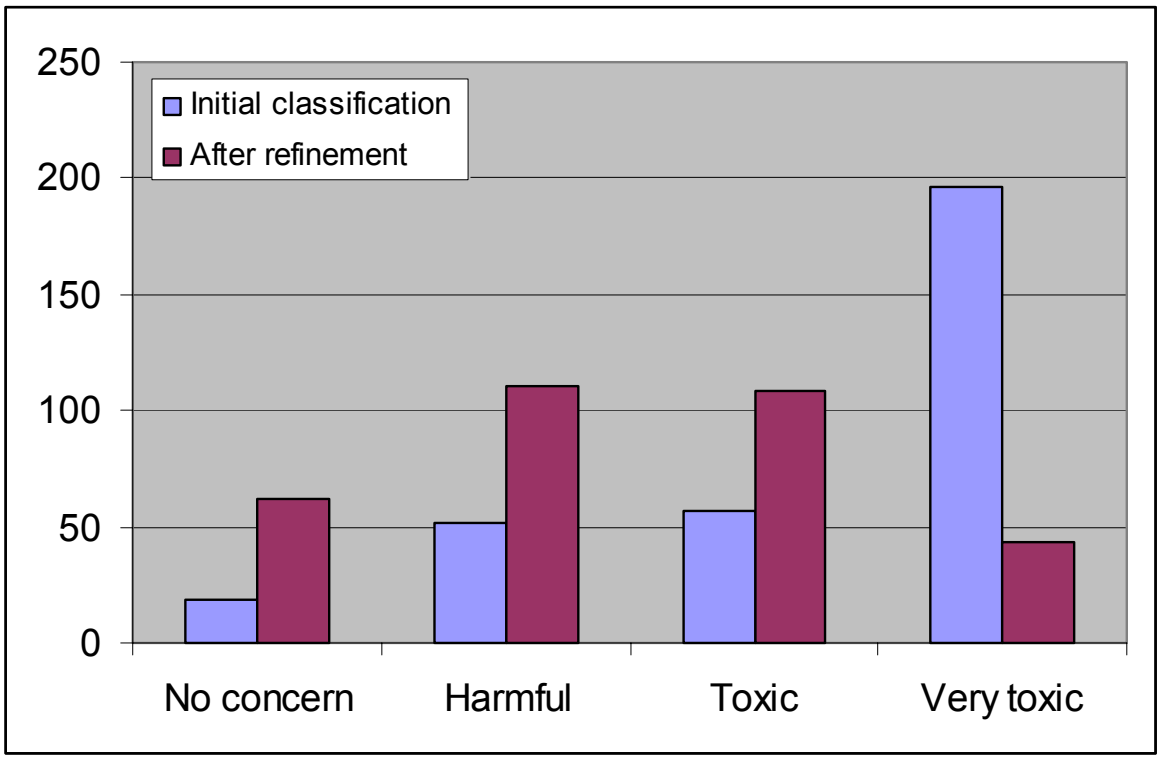


Figure 12. Distribution of phthalates into four toxicity groups after toxicity prediction using linear (initial classification) and bilinear (after refinement) relationships with log Kow.

The proposed approach for classification of chemicals on a basis of QSAR predictions has the advantage that it is built upon property, which is generally available trough the KOWWIN and other software. To be applied to other categories, in which there is a relationship between toxicity and hydrophobicity, only the coefficients in the models should be changed accordingly. The problem is, however, that it is based on prior knowledge for this relationship.

3.6. Analysis of predicted toxicity categories

On a basis of predicted toxicity values and accepted cut-offs for classification of chemicals into harmful, toxic, very toxic, and of no concern, the results of the previous analysis could be converted in structural rules for classification of phthalate esters with only hydrocarbon substituents at both ester groups, one benzene ring, and without substituent on the benzene ring. The results of the classification are summarized in Table 7.

It can be seen from Table 7 that the simplest phthalate ester (with two methyl groups) falls in the harmful category and the toxicity increase quickly with elongation of the hydrocarbon chain. When a new ester has to be classified, it has to be checked firstly for more reactive groups at the benzene ring and the side chains, and only in absence of such to be classified according to the rules in Table 7. The SIAR reports that there is no concern for acute toxicity associated with the C7 analogues and higher and the reason for that is looked for in the water solubility (see next section).

Table 7. Structural rules for classification of phthalate esters with two hydrocarbon substituents and one ring with no substituents on it.

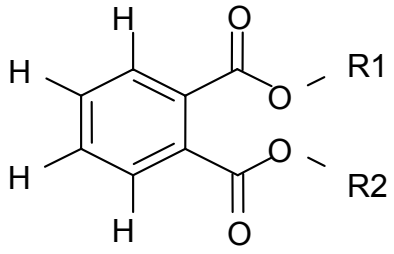


Table 7. Continued.

R_1, R_2	**Log K_{ow} range**	**Toxicity group**
-	log K_{ow} < 1.5	No concern
$R_1 = R_2 = CH_3$ $R_1 = R_2 = C_2H_5$	1.5 < log K_{ow} < 3.2	Harmful
$R_1 = R_2 = C_3H_7$ $R_1 = R_2 = C_4H_9$	3.2 < log K_{ow} < 5.0	Toxic
$R_1 = R_2 = C_5H_{11}$ $R_1 = R_2 = C_6H_{13}$	5.0 < log K_{ow} < 7.0	Very toxic
$R_1 = R_2 = C_7H_{15}$ $R_1 = R_2 = C_8H_{17}$	7.0 < log K_{ow} < 8.8	Toxic
$R_1 = R_2 = C_9H_{19}$ $R_1 = R_2 = C_{10}H_{21}$	8.8 < log K_{ow} < 10.5	Harmful
$R_1 = R_2 > C_{10}H_{21}$	log K_{ow} > 10.5	No concern

As a general trend, introduction of hydrophilic groups in the molecule of the phthalate esters reduces log Kow, and therefore – toxicity. For example, the 1,2-benzenedicarboxylic acid, bis(2-hydroxyethyl) ester have similar structure to the diethyl phthalate (classified as harmful) but the calculated log Kow = 0.12, and there are no reasons to assume different mechanism of action. As a result, this chemical is classified as “no concern” for acute toxicity. Conversely, introduction of hydrophobic groups such as halogen atoms at the side chains or on the benzene ring increases toxicity. An example is diethyl 3,4,5,6-tetrachlorophthalate, which, although containing the diethyl phthalate fragment, has estimated log Kow = 5.22 and is classified as very toxic. The structures are shown in Figure 13. Thus, each structural modification should be checked first against existing knowledge is it going to change the mechanism of toxic action, and then judged what its contribution to hydrophobicity is, subsequently, in which toxicity group the chemical will be classified.

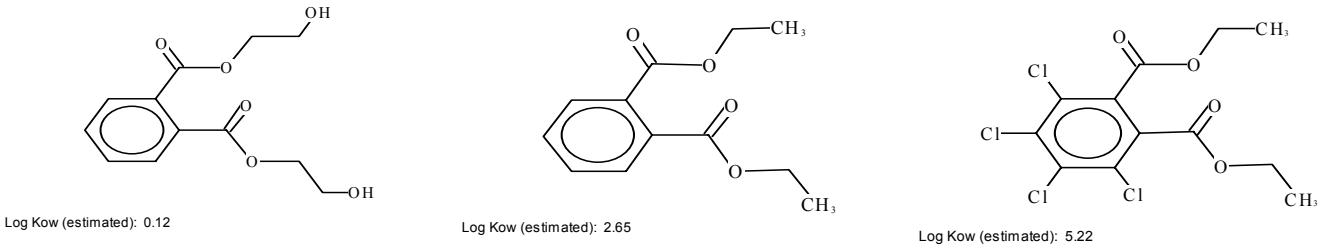


Figure 13. Examples for modifications of chemical structure that can change the toxicity group compared to similar compound.

3.7. Predicted toxicity and water solubility

There is a general understanding that chemical with water solubility less than 0.1 mg/L are insoluble in water. If a chemical is not soluble, it can not be absorbed by the organisms and therefore can not reach the system circulation and exhibit an acute toxic effect. This does not exclude the possibility of accumulation with the time at continuous exposure and appearance of chronic effects but this possibility is not considered in this study. The analysis of calculated water solubility for a series of symmetric homologue phthalate esters with hydrocarbon substituents showed that the analogue with C5 (di-n-amyl) has predicted water solubility of 0.17 mg/L. However, a reference with the WSKOWWIN database indicated a measured value of 100 mg/L at 20C. The next analogue in the series with C6 (dihexylphthalate) had a calculated value of 0.0115 mg/L and its reported measured toxicity was 0.24 mg/L at 20C. The discrepancy between the estimated and measured data provoked further analysis of this property.

A total of 40 measured values for water solubility were collected from the WSKOWWIN database for the set of 324 chemical and these were compared with the estimated ones. The result of the comparison is shown in Figure 14.

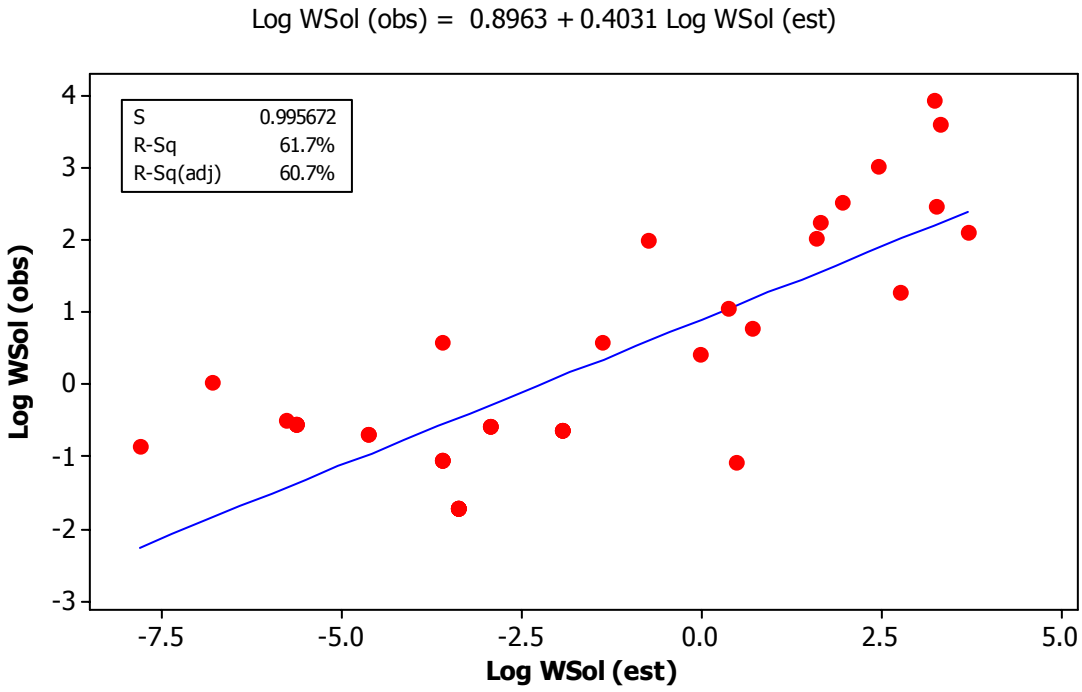


Figure 14. Relationship between measured and calculated by WSKOWWIN water solubility (n = 40, one outlier removed).

It has to be noted that the measured solubility values were measured at slightly different temperature (e.g. at 20 or 25C) and probably following different protocols. It is also properly described in the WSKOWWIN program that the algorithm for calculation of water solubility is based on log K_{ow} and molecular weight, and not on a correlation with measured values. This probably explains the relatively low correlation coefficient in the relationship between observed and estimated by WSKOWWIN water solubility ($r^2 = 0.61$). Nevertheless, the positive intercept in Figure 14 indicates that, on average, the WSKOWWIN program calculates water solubility lower with 0.9 log units compared to the experiment. Whilst this finding can not be extrapolated to all organic chemicals, certainly the trend applies to the group of phthalate esters and should be kept in mind as a need for correction of calculated values.

There was an expectation that for slightly soluble and insoluble chemicals the molecules can form micelles and thus to affect the measurement of solubility. Subsequently, another specific feature of the WSKOWWIN was noted for this group of chemicals. The WSKOWWIN models assume a linear relationship with log K_{ow} in all the range of its variability in the training set. The use of measured water solubility values, where available, showed that solubility below a limit of 0.02 mg/L can not be expected for this set of chemicals. This limit corresponds to measured log K_{ow} value of approximately 8. To account for the variability of measured data at very low concentrations and to take into account the fact that the WSKOWWIN predicts the water solubility of the phthalate esters lower than the experiment, a safety factor might be applied (at least a factor of 10 = 1 log unit for calculated water solubility). Thus, it would be possible to assume that at WSol < 0.002 mg/L the phthalate esters are not sufficiently soluble to be classified as toxic. This assumption can change the rules of thumb for the log K_{ow}-based classification, presented from Table 7, as shown in Table 8.

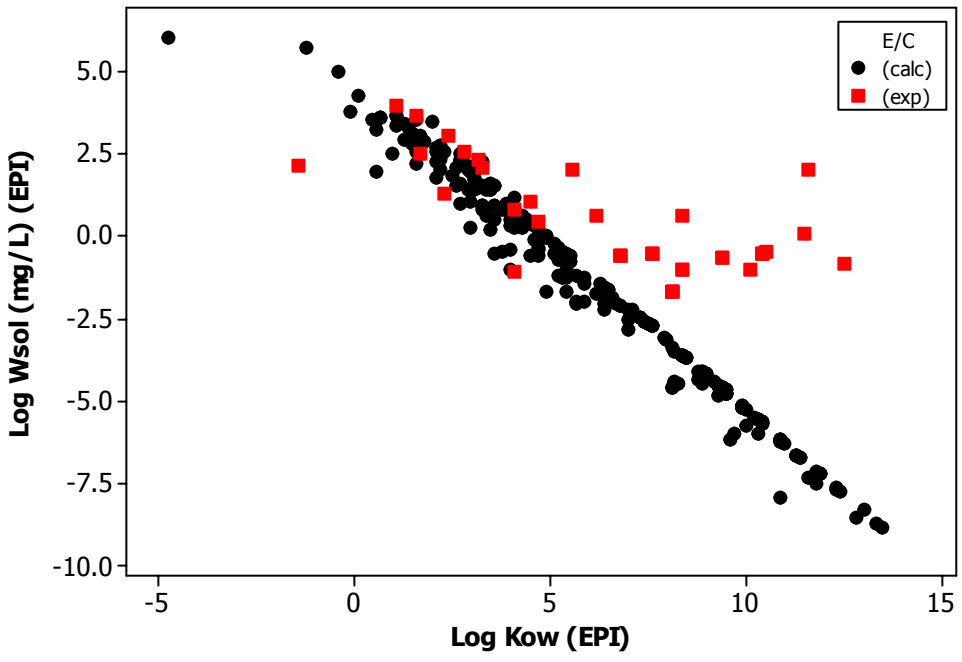
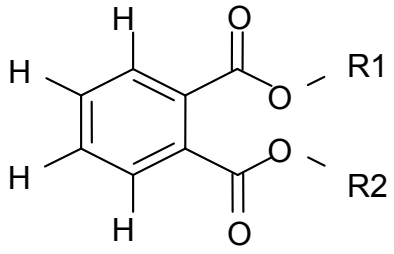


Figure 15. Plot of water solubility (calculated and experimental) against Log Kow.

Table 8. Structural rules for classification of phthalate esters with two hydrocarbon substituents and one ring with no substituents on it.



R_1, R_2	**Log K_{ow} range**	**Toxicity group**
-	log K_{ow} < 1.5	No concern
$R_1 = R_2 = CH_3$ $R_1 = R_2 = C_2H_5$	1.5 < log K_{ow} < 3.2	Harmful
$R_1 = R_2 = C_3H_7$ $R_1 = R_2 = C_4H_9$	3.2 < log K_{ow} < 5.0	Toxic
$R_1 = R_2 = C_5H_{11}$ $R_1 = R_2 = C_6H_{13}$	5.0 < log K_{ow} < 7.0	Very toxic
$R_1 = R_2 = C_7H_{15}$	7.0 < log K_{ow} < 8.0	Toxic
$R_1 = R_2 = C_8H_{17}$ $R_1 = R_2 => C_8H_{17}$	log K_{ow} > 8.0	No concern

The amended strategy considering the water solubility limits resulted in a new refined classification, shown in Table 9 and Figure 16. The perception for the acute fish toxicity hazard of chemicals of the series changed once again, with increase of the number of chemicals that could be expected to be of no concern.

Table 9. Number of chemicals classified into four toxicity groups and prior probabilities after calculation of toxicity using the bilinear relationship with log Kow and accounting for solubility limit of 0.02 mg/L.

Category	Number of Chemicals	Percentage of total (prior probability)
No concern	146	45.06
Harmful	52	16.05
Toxic	83	25.62
Very toxic	43	13.27
Total	**324**	

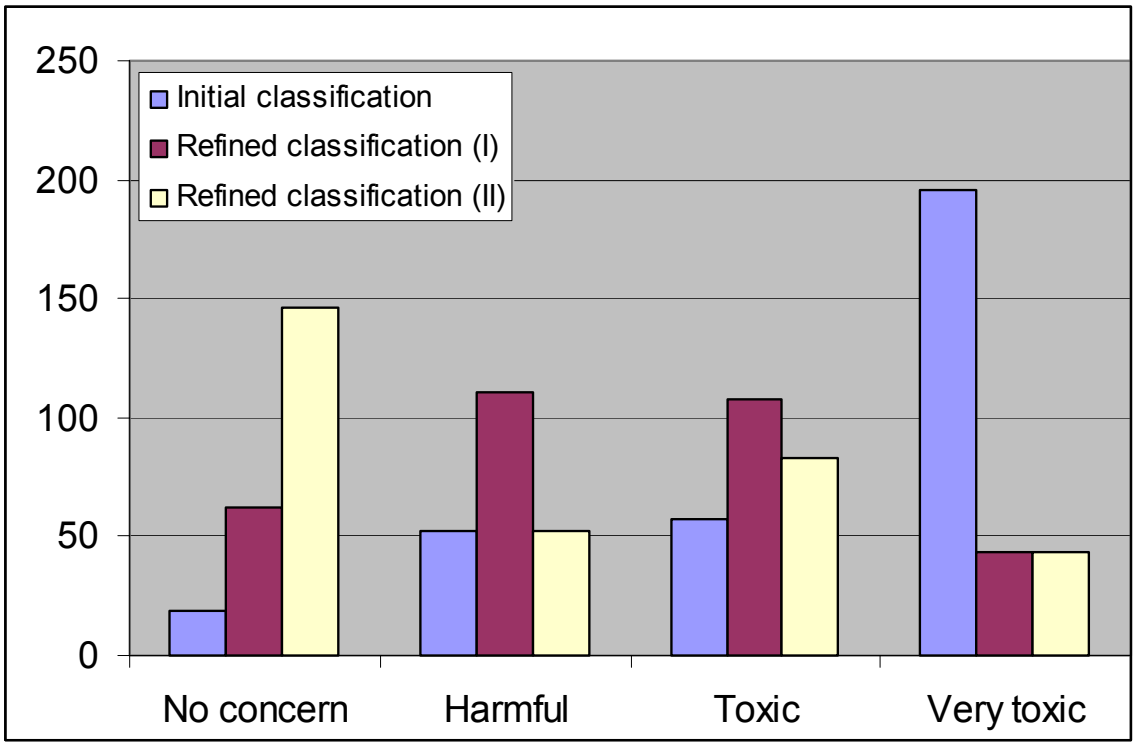


Figure 16. Distribution of phthalates in four toxicity groups after toxicity prediction using linear log Kow – based models (initial classification), bilinear relationships with log Kow (refined classification I), and considering water solubility limit (refined classification II).

3.8. Comparison of predicted toxicity with experimental data

Reported acute toxicity data to fish were collected for several phthalate esters. These are listed in Table 10. It is evident from Table 10 that the first to phthalate esters (dimethyl phthalate and diethyl phthalate) can be classified as harmful according to their measured acute toxicity to four species in five tests. Dibutyl phthalate is predicted as toxic. Based on experimental data it lays between the categories of toxic and very toxic chemical, with two measured values below 0.1 mg/L and two measured values above 2 mg/L. Dibutyl phthalate is a typical example for a chemical that is difficult to be classified, not because of inconsistency of data (actually, the measured values are quite similar) but because of using cut-offs to decide on the toxicity class (borderline case). Such chemicals will need additional information (e.g. acute toxicity to other species) to decide how to classifiy it more realistically. Butyl benzyl phthalate was classified as toxic according to its calculated log K_{ow} value (log K_{ow} = 4.35). Dihexyl phthalate was classified as very toxic according to its calculated log K_{ow} value (log K_{ow} = 6.57). This classification was confirmed by the experimental LC_{50} value available and shown in Table 10. Unfortunately, there are no measured LC_{50} values for the more hydrophobic chemicals because no acute toxicity at the limits of solubility was found.

Similar classification approach based on log K_{ow} and water solubility was applied by Parkerton and Konkel (2000). The authors suggested that phthalates with log $K_{ow} < 6$ show acute and chronic toxicity to aquatic organisms while those with log $K_{ow} > 6$ do not. This places the PE with $R_1 = R_2 = C_6H_{13}$ in the group with no concern, which, however, contradicts the measured LC_{50} value, found in this study for dihexyl phthalate (Table 10).

Thus, the initial classification presented in this study appears quite conservative for several HMWPE. It should be noted that the refined algorithm using bilinear relationship with log K_{ow} is less conservative than the algorithm based on linear relationship with log K_{ow}, and the additional consideration of solubility limits makes the classification even less conservative. However, for deciding on classification of borderline cases ($R_1 = R_2 = C_6H_{13}$ and $R_1 = R_2 = C_7H_{15}$), more experimental toxicological data might be needed.

Table 10. Collected experimental data for acute toxicity (in mg/L) of phthalate esters to fish.

Chemical	CAS	Sheepshead minnow, flow-through, 96h	Rainbow trout, flow-through, 96h	Bluegill, static, 96h	Fathed minnow, static, 96h	Fathead minnow, flow-through, 96h
Dimethyl phthalate	131-11-3	29	56	67	120	39
Diethyl phthalate	84-66-2	29	12	22	17	17
Dibutyl phthalate	84-74-2	NATBLS	1.6	0.85	3	0.92
Butyl benzyl phthalate	85-68-7	NATBLS			NATBLS	1.5
Butyl 2-ethylhexyl phthalate	85-69-8	NATBLS	NATBLS	NATBLS	NATBLS	NATBLS
Dihexyl phthalate	68515-50-4	NATBLS	0.82	NATBLS	NATBLS	NATBLS
Di-sec-octyl phthalate	117-81-7		NATBLS	NATBLS		NATBLS
1,2-Benzenedicarboxylic acid, di-C6-10-alkyl esters	68515-51-5	NATBLS	NATBLS	NATBLS		
1,2-Benzenedicarboxylic acid diisooctyl ester	27554-26-3	NATBLS	NATBLS	NATBLS	NATBLS	NATBLS
Di(C7-C9-C11)phthalate	68515-42-4			NATBLS		
Diisononylphthalate	68515-48-0	NATBLS	NATBLS	NATBLS	NATBLS	NATBLS
Diisodecylphthalate	68515-49-1	NATBLS	NATBLS	NATBLS	NATBLS	NATBLS
Diundecyl phthalate	3648-20-2	NATBLS	NATBLS	NATBLS	NATBLS	NATBLS
Ditridecyl phthalate	68515-47-9	NATBLS	NATBLS	NATBLS	NATBLS	NATBLS

NATBLS – No acute toxicity below the limit of aqueous solubility.

Collected from http://www.epa.gov/opptintr/chemtest/pubs/alkpht.pdf

4. Conclusions and Recommendations

This case study on phthalate esters with regard to their acute toxicity to fish was limited by the availability of high quality experimental fish toxicity data.

Our study showed that the development of a category on the basis of a common structural group might be necessary but is not sufficient to allow read-across between the members of the category because the phthalate esters cover a very large toxicity range depending on the substituents in the ester side chains and on the benzene ring.

A chemical category should be precisely defined not only by inclusion rules but also by exclusion rules if necessary. In the case of phthalate esters, the definition of the category "HMWPE" as "esters with an alkyl carbon backbone with 7 carbon atoms or greater" is not sufficient to embrace all possible variations of substituents on the benzene ring and on the carbon backbone.

The applicability domain of the category should be determined not only on a basis of physicochemical properties but also using structural rules, which are able to identify chemicals with different mechanism of action (e.g. acrylates, quinines/hydroquinones, etc.). One can argue that if there are chemicals acting by different mechanism of action, they should be considered outliers from the category and evaluated for membership in other category.

In a bottom-up approach to category development, the inclusion of new members in a category should be considered on a case-by-case basis. For example, ethyl phthalate is similar to methyl phthalate and therefore they have similar fish acute toxicity (both are classified as harmful). This statement does not hold anymore when butyl phthalate is included. This chemical is classified as toxic. Therefore, a more refined approach is necessary for the development of a category by analysing all potential members and the mechanisms of toxicity, including where necessary one or more subcategories.

An approach starting from assembly of all possible candidates and reducing them on a basis of available data, and/or existing knowledge (e.g. about the mechanism of action), and/or (Q)SAR predictions, has a granted advantage of increasing the diversity in the

development of the testing plan, if such is foreseen. It could also help to identify potentially safer alternative chemicals when a large chemical set is considered.

An introduction of subcategories within a category is recommended in order to facilitate the application of classification and labelling to category members in cases where the latter may cross different thresholds of formal classification criteria. For example, the members might be classified in two, three, four or more subcategories which reflect different levels of toxicological hazard associated with subtle changes in molecular structure. The definition of the subcategories also requires identification of their boundaries in terms of physicochemical and structural space.

Pragmatic approaches should be introduced, e.g. for very hydrophobic substances with very low water solubility. For example, a chemical might have different functional groups, and therefore may belong to a different mechanistic category. However, if it has a log K_{ow} above 10 and/or water solubility lower than 0.01 mg/L, the outcome regarding its acute toxicity to fish will probably be the same as a result of a lack of bioavailability.

A strong way to argue a category is to show that there is a clear trend in toxicity which may parallel a trend in some physicochemical property (e.g. for the phthalates, acute toxicity to fish correlates with hydrophobicity). However, the trend might not necessarily be linear. This might make it difficult to define the boundaries of the category or to determine whether analogues should be assigned to different subcategories to different categories.

The larger the number of the properties/endpoints associated with the category, the stronger the rationale for its existence. However, read across between category members should be carried out with caution, taking into account the presence of subcategories.

5. References

1. European Commission (2001). White Paper on a Strategy for a Future Chemicals Policy. Brussels: Commission of the European Communities (COM(2001) 88 final), 32pp. Belgium, Brussels: the Commission of the European Communities. [http://europa.eu.int/comm/environment/chemicals/whitepaper.htm].
2. Commission of the European Communities (2003). Proposal for a Regulation of the European Parliament and of the Council concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), establishing a European Chemicals Agency and amending Directive 1999/45/EC and Regulation (EC) {on Persistent Organic Pollutants} / Proposal for a Directive of the European Parliament and of the Council amending Council Directive 67/548/EEC in order to adapt it to Regulation (EC) of the European Parliament and of the Council concerning the registration, evaluation, authorisation and restriction of chemicals. Brussels, 29 October 2003, Brussels, Belgium.
3. Cronin MTD and Livingstone DJ, eds. (2004). Predicting Chemical Toxicity and Fate. CRC Press, Boca Raton, Florida.
4. Hermens J, Leeuwangh P and Musch A (1984). Quantitative structure-activity relationships and mixture toxicity studies of chloro- and alkylanilines at an acute lethal toxicity level to the guppy (*Poecilia reticulata*). *Ecotox. Environ. Saf.*, **8**: 338-394.
5. Meylan WM and Howard PH (1995). Atom/fragment contribution method for estimating octanol-water partition coefficients. *J. Pharm. Sci.* **84**: 83-92.
6. Meylan WM, Howard PH and Boethling RS (1996). Improved method for estimating water solubility from octanol/water partition coefficient. *Environ. Toxicol. Chem.* **15**: 100-106.
7. Parkerton TF and Konkel WJ (2000). Application of quantitative structure-activity relationships for assessing the aquatic toxicity of phthalate esters. *Ecotoxicol. Environ. Saf.* **45**: 61-78.

Appendix I. Chemical identification, physicochemical properties and calculated toxicity. CAS is Chemical Abstract Service number, MW is molecular weight, WSol is water solubility, Kow is the octanol-water partition coefficient, LC is lethal concentration. M/C stands for measured (experimental)/calculated (estimated) value. LC50 corrected incorporates the toxicity values calculated using the bilinear relationship with log K_{ow} (the values for log K_{ow} > 6 are coded in red). (NC) in the last column indicates chemicals that are considered of no concern due to water solubility below the observed limit.

ID	NAMES	CAS	MW	WSol (mg/L) (EPI)	M/C	Log K_{ow} (EPI)	M/C	LC_{50} (mg/L) ECOSAR	Mo-del	LC_{50} (mg/L) ECOSAR corrected	Toxicity Group
1	Dicyclohexyl phthalate	84617	330.43	4.00E+00	(exp)	6.20	(est)	2.83E-01		3.86E-01	VT
2	Diphenyl phthalate	84628	318.33	8.20E-02	(exp)	4.10	(est)	3.63E+00		3.63E+00	T
3	1-Butyl 2-cyclohexyl phthalate	84640	304.39	2.80E-01	(est)	5.41	(est)	6.90E-01		6.90E-01	VT
4	Diethyl phthalate	84662	222.24	1.08E+03	(exp)	2.42	(exp)	1.51E+01		1.51E+01	H
5	Diisobutyl phthalate	84695	278.35	6.20E+00	(exp)	4.11	(exp)	2.04E+00		2.04E+00	T
6	1,2-Benzenedicarboxylic acid, 2-ethoxy-2-oxoethyl ethyl ester	84720	280.28	2.17E+02	(est)	2.19	(est)	3.36E+01		3.36E+01	H
7	1,2-Benzenedicarboxylic acid, bis(2-hydroxyethyl) ester	84731	254.24	1.76E+04	(est)	0.12	(est)	3.90E+02		3.90E+02	NC
8	Dibutyl phthalate	84742	278.35	1.12E+01	(exp)	4.50	(exp)	1.69E+00		1.69E+00	T
9	1,2-Benzenedicarboxylic acid, dihexyl ester	84753	334.46	2.40E-01	(exp)	6.82	(exp)	1.82E-01		8.38E-01	VT
10	1,2-Benzenedicarboxylic acid, dinonyl ester	84764	418.62	1.74E-05	(est)	9.52	(est)	6.00E-03		2.92E+01	H (NC)
11	1,2-Benzenedicarboxylic acid, didecyl ester	84775	446.68	3.30E-01	(exp)	10.50	(est)	1.92E-03		1.04E+02	NC
12	1-Butyl 2-octyl phthalate	84786	334.46	2.40E-01	(exp)	6.82	(exp)	1.82E-01		8.38E-01	VT
13	1,2-Benzenedicarboxylic acid, butyl phenylmethyl ester	85687	312.37	2.69E+00	(exp)	4.73	(exp)	1.43E+00		1.43E+00	T
14	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	85698	334.46	2.16E-02	(est)	6.50	(est)	1.98E-01		5.65E-01	VT
15	1,2-Benzenedicarboxylic acid, 2-butoxy-2-oxoethyl butyl ester	85701	336.39	2.14E+00	(est)	4.15	(est)	3.60E+00		3.60E+00	T
16	1-(2-Ethoxy-2-oxoethyl) 2-methyl phthalate	85712	266.25	6.85E+02	(est)	1.70	(est)	5.83E+01		5.83E+01	H

17	1,2,4-Benzenetricarboxylic acid, trioctyl ester	89043	546.79	2.92E-08	(est)	11.81	(est)	4.67E-04		6.40E+02	NC
18	1,2-Benzenedicarboxylic acid, 2-ethylhexyl 8-methylnonyl ester	89134	418.62	2.32E-05	(est)	9.37	(est)	7.00E-03		2.43E+01	H (NC)
19	1,2-Benzenedicarboxylic acid, bis(8-methylnonyl) ester	89167	446.68	2.80E-01	(exp)	10.36	(est)	2.00E-03		8.76E+01	H (NC)
20	Not found	89189	362.51	2.12E-03	(est)	7.48	(est)	6.40E-02		2.05E+00	T
21	Phthalic acid, butyl decyl ester	89190	362.51	1.83E-03	(est)	7.56	(est)	5.80E-02		2.26E+00	T
22	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	117817	390.57	2.70E-01	(exp)	7.60	(exp)	2.30E-02		2.56E+00	T
23	1,2-Benzenedicarboxylic acid, bis(2-methoxyethyl) ester	117828	282.3	8.50E+03	(exp)	1.11	(est)	1.28E+02		1.28E+02	NC
24	1,2-Benzenedicarboxylic acid, bis(2-butoxyethyl) ester	117839	366.46	1.68E+00	(est)	4.06	(est)	4.39E+00		4.39E+00	T
25	1,2-Benzenedicarboxylic acid, dioctyl ester	117840	390.57	2.00E-02	(exp)	8.10	(exp)	1.90E-02		4.73E+00	T (NC)
26	1,2-Benzenedicarboxylic acid, bis[2-(2-ethoxyethoxy)ethyl] ester	117851	398.46	1.49E+02	(est)	1.55	(est)	1.05E+02		1.05E+02	NC
27	1,2-Benzenedicarboxylic acid, ditridecyl ester	119062	530.84	1.48E-09	(est)	13.45	(est)	6.01E-05		4.69E+03	NC
28	1,2-Benzenedicarboxylic acid, decyl octyl ester	119073	418.62	1.74E-05	(est)	9.52	(est)	6.00E-03		2.92E+01	H (NC)
29	1,4-Benzenedicarboxylic acid, dimethyl ester	120616	194.19	1.90E+01	(exp)	2.25	(exp)	4.47E+01		4.47E+01	H
30	1,2-Benzenedicarboxylic acid, dimethyl ester	131113	194.19	4.00E+03	(exp)	1.60	(exp)	4.47E+01		4.47E+01	H
31	1,2-Benzenedicarboxylic acid, bis(1-methylheptyl) ester	131157	390.57	2.39E-04	(est)	8.39	(est)	2.30E-02		6.77E+00	T (NC)
32	1,2-Benzenedicarboxylic acid, dipropyl ester	131168	250.3	1.08E+02	(exp)	3.27	(exp)	5.09E+00		5.09E+00	T
33	1,2-Benzenedicarboxylic acid, di-2-propenyl ester	131179	246.26	1.82E+02	(exp)	3.23	(exp)	6.98E+00		6.98E+00	T
34	1,2-Benzenedicarboxylic acid, dipentyl ester	131180	306.41	1.00E+02	(exp)	5.62	(exp)	5.57E-01		5.57E-01	VT
35	1,2-Benzenedicarboxylic acid, monobutyl ester (	131704	222.24	1.26E+02	(est)	2.84	(est)	1.20E+02	acid-esters	1.20E+02	NC
36	1,3-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	137893	390.57	2.39E-04	(est)	8.39	(est)	2.30E-02		6.77E+00	T (NC)
37	1,2-Benzenedicarboxylic acid, bis(4-methylpentyl) ester	146509	334.46	2.49E-02	(est)	6.43	(est)	2.16E-01		5.18E-01	VT

38	Phthalic acid, 4-fluoro-, diethyl ester	320967	240.23	9.94E+01	(est)	2.85	(est)	1.28E+01		1.28E+01	H
39	1,2-Benzenedicarboxylic acid, bis(phenylmethyl) ester	523319	346.39	3.00E-01	(est)	5.08	(est)	1.18E+00		1.18E+00	T
40	bis(2,2,3,3,4,4,5,5-Octafluoropentyl) phthalate	572941	594.25	2.30E-05	(est)	8.06	(est)	5.20E-02		6.86E+00	T (NC)
41	1,2-Benzenedicarboxylic acid, bis(1-methylethyl) ester	605458	250.3	3.32E+02	(exp)	2.83	(exp)	6.12E+00		6.12E+00	T
42	1,2-Benzenedicarboxylic acid, bis(3-methylbutyl) ester	605505	306.41	2.52E-01	(est)	5.45	(est)	6.62E-01		6.62E-01	VT
43	1,2-Benzenedicarboxylic acid, bis(2-ethoxyethyl) ester	605549	310.35	1.73E+02	(est)	2.10	(est)	4.15E+01		4.15E+01	H
44	Dimethyl 4-(hydroxy(oxido)amino)phthalate	610220	239.19	6.01E+02	(est)	1.48	(est)	6.87E+01		6.87E+01	H
45	Tetramethyl 1,2,4,5-benzenetetracarboxylate	635109	310.26	7.76E+02	(est)	1.33	(est)	1.07E+02		1.07E+02	NC
46	Cyclohexyl 2-ethylhexyl phthalate	1169988	360.5	3.14E-03	(est)	7.30	(est)	8.00E-02		1.63E+00	T
47	1,2-Benzenedicarboperoxoic acid	1203403	198.13	5.04E+05	(est)	-1.23	(est)	1.29E-01	peroxy acids	1.29E-01	VT
48	Not found	1225850	270.29	2.41E+01	(est)	3.37	(est)	7.57E+00		7.57E+00	T
49	1,2-Benzenedicarboxylic acid, octyl phenylmethyl ester	1248437	368.48	7.33E-03	(est)	6.81	(est)	1.49E-01		9.12E-01	VT
50	1,2-Benzenedicarboxylic acid, isodecyl octyl ester	1330967	418.62	2.01E-05	(est)	9.45	(est)	7.00E-03		2.68E+01	H (NC)
51	1,3-Benzenedicarboxylic acid, dimethyl ester	1459934	194.19	2.90E+02	(exp)	1.66	(est)	4.47E+01		4.47E+01	H
52	1,2,4-Benzenetricarboxylic acid, triheptyl ester	1528489	504.71	9.90E-07	(est)	10.34	(est)	3.00E-03		9.66E+01	H (NC)
53	Tri-n-hexyl trimellitate	1528490	462.63	3.33E-05	(est)	8.87	(est)	1.50E-02		1.45E+01	H (NC)
54	Benzoic acid, 4-formyl-, methyl ester	1571080	164.16	3.14E+03	(est)	1.55	(est)	1.90E+01	aldehydes	1.90E+01	H
55	Tributyl 1,2,4-benzenetricarboxylate	1726234	378.47	3.67E-02	(est)	5.92	(est)	4.58E-01		4.58E-01	VT
56	Tetraethyl pyromellitate	1729060	366.37	7.52E+00	(est)	3.30	(est)	1.12E+01		1.12E+01	H
57	Diethyl 4-(hydroxy(oxido)amino)phthalate	2050193	267.24	6.08E+01	(est)	2.46	(est)	2.30E+01		2.30E+01	H
58	1,2-Benzenedicarboxylic acid, 1,2-ethanediyl dimethyl ester	2055007	386.36	1.05E+01	(est)	2.99	(est)	1.73E+01		1.73E+01	H

59	1,2-Benzenedicarboperoxoic acid, bis(1,1-dimethylethyl) ester	2155717	310.35	6.22E+00	(est)	3.79	(est)	8.26E-01	peroxy acids	8.26E-01	VT
60	Phthalic acid, bis(2-ethyl-4-methylpentyl) ester	2229552	390.57	3.19E-04	(est)	8.24	(est)	2.70E-02		5.62E+00	T (NC)
61	1,2-Benzenedicarboxylic acid, didodecyl ester	2432908	502.78	1.40E-01	(exp)	12.47	(est)	1.02E-04		1.33E+03	NC
62	Trimethyl 1,2,4-benzenetricarboxylate	2451798	252.23	1.21E+03	(est)	1.50	(est)	7.07E+01		7.07E+01	H
63	1,2,4-Benzenetricarboxylic acid, trimethyl ester	2459101	252.23	1.21E+03	(est)	1.50	(est)	7.07E+01		7.07E+01	H
64	Not found	2545246	521.53	3.14E-01	(est)	3.77	(est)	8.92E+00		8.92E+00	T
65	Triallyl Trimellitate	2694544	330.34	2.91E+00	(est)	4.04	(est)	4.05E+00		4.05E+00	T
66	1,3-Benzenedicarboxylic acid, dibutyl ester	3126907	278.35	3.77E+00	(est)	4.26	(exp)	1.69E+00		1.69E+00	T
67	1,2,4-Benzenetricarboxylic acid, tris(2-ethylhexyl) ester	3319311	546.79	1.00E+02	(exp)	11.59	(est)	6.13E-04		4.88E+02	NC
68	1,2-Benzenedicarboxylic acid, bis (tetrahydro-2-furanyl)methyl ester	3388010	334.37	3.75E+01	(est)	2.71	(est)	2.11E+01		2.11E+01	H
69	1-Hexyl 2-methyl phthalate	3461232	264.32	5.98E+00	(est)	4.12	(est)	2.94E+00		2.94E+00	T
70	1-Butyl 2-pentyl phthalate	3461298	292.38	5.99E-01	(est)	5.10	(est)	9.72E-01		9.72E-01	VT
71	1,2-Benzenedicarboxylic acid, butyl nonyl ester	3461312	348.49	5.85E-03	(est)	7.07	(est)	1.02E-01		1.19E+00	T
72	1,2-Benzenedicarboxylic acid, diundecyl ester	3648202	474.73	1.11E+00	(exp)	11.49	(est)	6.02E-04		3.75E+02	NC
73	1,2-Benzenedicarboxylic acid, diheptyl ester	3648213	362.51	1.83E-03	(est)	7.56	(est)	5.80E-02		2.26E+00	T
74	Allyl 2,3-epoxypropyl phthalate	3814582	262.26	3.60E+02	(est)	2.05	(est)	2.22E+01	epoxides	2.22E+01	H
75	bis(2-(Hydroxy(oxido)amino)butyl) phthalate	4131844	368.35	9.21E+00	(est)	2.72	(est)	2.30E+01		2.30E+01	H
76	Ethylene phthalate	4196989	192.17	1.19E+02	(est)	3.05	(est)	7.98E+00		7.98E+00	T
77	Mellitic trianhydride	4253241	288.13	1.83E+02	(est)	2.22	(est)	1.33E+02	neutral organics	1.33E+02	NC
78	Mono(2-ethylhexyl)phthalate	4376209	278.35	1.49E+00	(est)	4.73	(est)	1.46E+01	ester-acids	1.46E+01	H
79	Di(sec-butyl) phthalate	4489616	278.35	2.53E+00	(est)	4.46	(est)	2.04E+00		2.04E+00	T

80	1-Cyclohexyl 2-ethyl phthalate	5333608	276.34	2.80E+00	(est)	4.42	(est)	2.12E+00		2.12E+00	T
81	1-Cyclohexyl 2-isobutyl phthalate	5334098	304.39	3.23E-01	(est)	5.33	(est)	7.62E-01		7.62E-01	VT
82	Bis(2-ethoxy-1-methyl-2-oxoethyl) phthalate	5396929	366.37	3.20E+01	(est)	2.56	(est)	2.78E+01		2.78E+01	H
83	1-(2-Butoxy-1-methyl-2-oxoethyl) 2-butyl phthalate	5420768	350.42	7.73E-01	(est)	4.57	(est)	2.24E+00		2.24E+00	T
84	Not found	5453247	390.48	3.59E-01	(est)	4.67	(est)	2.20E+00		2.20E+00	T
85	Bis(3-methoxybutyl) phthalate	5470019	338.4	2.28E+01	(est)	2.93	(est)	1.63E+01		1.63E+01	H
86	Not found	5950765	356.47	1.25E-02	(est)	6.62	(est)	1.82E-01		6.98E-01	VT
87	Phthalic acid, bis(2,2,2-trinitroethyl) ester	6093307	492.23	1.30E+02	(exp)	-1.38	(est)	4.79E+03		4.79E+03	NC
88	Cotarnine phthalate	6190369	604.62	6.07E-02	(est)	5.15	(est)	1.60E+00	aliph. Amine	1.60E+00	T
89	Hexamethyl 1,2,3,4,5,6-benzenehexacarboxylate	6237598	426.34	2.91E+02	(est)	1.00	(est)	2.21E+02		2.21E+02	NC
90	Bis(2-chloroethyl) phthalate	6279874	291.13	2.80E+01	(est)	3.15	(est)	1.07E+01		1.07E+01	H
91	1-Butyl 2-vinyl phthalate	6280042	248.28	2.52E+01	(est)	3.49	(est)	5.99E+00		5.99E+00	T
92	Diphenyl 4-iodophthalate	6301628	444.23	5.18E-02	(est)	5.26	(est)	1.21E+00		1.21E+00	T
93	Phthalic acid, diester with lactonitrile	6380638	272.26	3.32E+03	(est)	0.54	(est)	2.49E+02		2.49E+02	NC
94	1,4-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	6422862	390.57	4.00E+00	(exp)	8.39	(est)	2.30E-02		6.77E+00	T (NC)
95	Tetraethyl 1,2,4,5-benzenetetracarboxylate	6634011	366.37	7.52E+00	(est)	3.30	(est)	1.12E+01		1.12E+01	H
96	Dipropyl 3,4,5,6-tetrachlorophthalate	6928672	388.08	1.82E-02	(est)	6.21	(est)	3.29E-01		4.58E-01	VT
97	bis(4-Chlorophenyl) phthalate	7144107	387.22	9.24E-02	(est)	5.39	(est)	9.00E-01		9.00E-01	VT
98	1,2-Benzenedicarboxylic acid, bis(oxiranylmethyl) ester	7195451	278.26	3.81E+03	(est)	0.74	(est)	1.16E+01	diepoxides	1.16E+01	H
99	1,2-Benzenedicarboxylic acid, bis(2-ethylbutyl) ester	7299890	334.46	2.49E-02	(est)	6.43	(est)	2.16E-01		5.18E-01	VT
100	1,2-Benzenedicarboxylic acid, bis(2,3-dibromopropyl) ester	7415863	565.88	1.92E-02	(est)	4.85	(est)	2.56E+00		2.56E+00	T
101	Dimethyl 3,6-dihydroxyphthalate	7474922	226.19	2.58E+03	(est)	1.96	(est)	1.43E-01	Q/HQ	1.43E-01	VT

102	Diethylene glycol, diester with butylphthalate	7483252	514.58	8.51E-03	(est)	5.66	(est)	8.58E-01		8.58E-01	VT
103	Not found	7717295	470.52	9.49E-03	(est)	5.93	(est)	5.62E-01		5.62E-01	VT
104	1,2-Benzenedicarboxylic acid, di-9-octadecenyl ester, (Z,Z)-	10578333	667.08	2.83E-14	(est)	17.93	(est)	3.03E-07		1.47E+06	NC
105	Tetraallyl Pyromellitate	13360980	414.42	2.33E-01	(est)	4.72	(est)	2.20E+00		2.20E+00	T
106	Dimethyl 3-(hydroxy(oxido)amino)phthalate	13365269	239.19	6.01E+02	(est)	1.48	(est)	6.87E+01		6.87E+01	H
107	1,2-Benzenedicarboxylic acid, dihexadecyl ester	13372184	615	1.27E-12	(est)	16.40	(est)	1.84E-06		2.06E+05	NC
108	2,5,8-Benzotrioxacycloundecin-1,9-dione, 3,4,6,7-tetrahydro-	13988266	236.23	2.29E+03	(est)	1.28	(est)	8.68E+01		8.68E+01	H
109	1,2-Benzenedicarboxylic acid, bis(3,5,5-trimethylhexyl) ester	14103618	418.62	3.59E-05	(est)	9.15	(est)	9.00E-03		1.85E+01	H (NC)
110	1,2-Benzenedicarboxylic acid, dioctadecyl ester	14117965	671.11	1.14E-14	(est)	18.36	(est)	1.80E-07		2.51E+06	NC
111	1,2-Benzenedicarboxylic acid, bis-(1-ethylhexyl) ester	15495940	390.57	2.70E-01	(exp)	7.60	(exp)	2.30E-02		2.56E+00	T
112	Phthalic acid, bis 2-(2-butoxyethoxy)ethyl ester	16672392	454.57	1.40E+00	(est)	3.51	(est)	1.07E+01		1.07E+01	H
113	bis(2-(2-Methoxyethoxy)ethyl) phthalate	16672712	370.4	1.53E+03	(est)	0.57	(est)	3.26E+02		3.26E+02	NC
114	2,9-Benzodioxacyclododecin-1,10-dione, 3,4,5,6,7,8- hexahydro-	16709505	248.28	2.41E+01	(est)	3.52	(est)	5.78E+00		5.78E+00	T
115	Phthalic acid, benzyl 3-hydroxy-1-isopropyl-2,2-dimethylpropyl ester isobutyrate	16883833	454.57	1.47E-03	(est)	7.00	(est)	1.45E-01		1.42E+00	T
116	Benzoic acid, 4-(1,1-dimethylethyl)-, sodium salt	17264538	200.21	8.85E+04	(est)	-0.36	(est)	2.45E+04	neutral organics	2.45E+04	NC
117	1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	17851535	278.35	2.19E+00	(est)	4.54	(est)	1.84E+00		1.84E+00	T
118	Phthalic acid, dicyclopentyl ester	18699382	302.37	4.14E-01	(est)	5.22	(est)	8.67E-01		8.67E-01	VT
119	Phthalic acid, benzyl 2-ethylhexyl ester	18750055	368.48	8.47E-03	(est)	6.74	(est)	1.62E-01		8.36E-01	VT
120	1,2-Benzenedicarboxylic acid, decyl undecyl ester	19295820	460.7	5.20E-07	(est)	10.99	(est)	1.08E-03		1.96E+02	NC
121	Dimethyl 3,4,5,6-tetrachlorophthalate	20098413	331.97	1.90E+00	(est)	4.24	(est)	3.18E+00		3.18E+00	T
122	Dimethyl 4-methylphthalate	20116658	208.22	5.14E+02	(est)	2.21	(est)	2.43E+01		2.43E+01	H

123	Phthalic acid, bis(3-phenylpropyl) ester	20198645	402.49	2.86E-03	(est)	7.04	(est)	1.23E-01		1.32E+00	T
124	Diethyl 4-aminophthalate	22572845	237.26	4.27E+02	(est)	2.12	(est)	3.10E+01		3.10E+01	H
125	1,2-Benzenedicarboxylic acid, butyl dodecyl ester (9CI)	23761344	390.57	2.00E-02	(exp)	8.10	(exp)	1.90E-02		4.73E+00	T (NC)
126	1,2-Benzenedicarboxylic acid, decyl hexyl ester	25724587	390.57	2.00E-02	(exp)	8.10	(exp)	1.90E-02		4.73E+00	T (NC)
127	Diisodecyl phthalate	26761400	446.68	2.80E-01	(exp)	10.36	(est)	2.00E-03		8.76E+01	H (NC)
128	1,2-Benzenedicarboxylic acid, isooctyl phenylmethyl ester	27215221	368.48	7.58E-03	(est)	6.79	(est)	1.53E-01		8.89E-01	VT
129	1,2,4-Benzenetricarboxylic acid, triisooctyl ester	27251758	546.79	4.51E-08	(est)	11.59	(est)	6.13E-04		4.88E+02	NC
130	1,2-Benzenedicarboxylic acid, diisotridecyl ester (9CI)	27253265	530.84	1.98E-09	(est)	13.30	(est)	7.24E-05		3.89E+03	NC
131	Diisododecyl phthalate	27554069	502.78	2.07E-08	(est)	12.32	(est)	2.29E-04		1.10E+03	NC
132	Diisooctyl phthalate	27554263	390.57	9.00E-02	(exp)	8.39	(est)	2.30E-02		6.77E+00	T (NC)
133	bis(Methylcyclohexyl) phthalate	27987253	358.48	5.38E-03	(est)	7.04	(est)	1.09E-01		1.18E+00	T
134	1,2-Benzenedicarboxylic acid, diisononyl ester	28553120	418.62	2.00E-01	(exp)	9.37	(est)	7.00E-03		2.43E+01	H (NC)
135	1,2-Benzenedicarboxylic acid, bis(1,1-dimethylethyl) ester	30448432	278.35	2.93E+00	(est)	4.39	(est)	2.22E+00		2.22E+00	T
136	Not found	30833535	222.24	1.45E+02	(est)	2.77	(est)	1.30E+02	ester-acids	1.30E+02	NC
137	Dimethyl 3-methoxyphthalate	32136520	224.22	1.06E+03	(est)	1.74	(est)	4.68E+01		4.68E+01	H
138	1,2-Benzenedicarboxylic acid, 2-butoxyethyl butyl ester	33374286	322.4	1.80E+00	(est)	4.34	(est)	2.73E+00		2.73E+00	T
139	Phtalic acid, allyl ethyl ester	33672945	234.25	7.91E+01	(est)	3.00	(est)	1.03E+01		1.03E+01	H
140	1,2-Benzenedicarboxylic acid, butyl methyl ester	34006763	236.27	5.90E+01	(est)	3.14	(est)	8.78E+00		8.78E+00	T
141	1-Methyl 2-propyl phthalate	34006785	222.24	1.84E+02	(est)	2.65	(est)	1.51E+01		1.51E+01	H
142	1-(2-Methoxyethyl) 2-methyl phthalate	36339614	238.24	1.79E+03	(est)	1.39	(est)	7.65E+01		7.65E+01	H
143	Not found	36388360	715.12	1.02E-12	(est)	15.73	(est)	4.88E-06		1.05E+05	NC
144	Not found	36631308	630.96	3.87E-11	(est)	14.54	(est)	1.87E-05		2.13E+04	NC
145	Not found	37099120	278.26	3.81E+03	(est)	0.74	(est)	1.16E+01	diepoxides	1.16E+01	H

146	Dimethyl 1-benzyl-2-isopropyl-1H-benzimidazole-5,6-dicarboxylate	37391289	368.44	1.85E+00	(est)	4.00	(est)	4.75E+00		4.75E+00	T
147	1,2-Benzenedicarboxylic acid, bis(3,3,5-trimethylcyclohexyl) ester	37832658	414.59	7.89E-05	(est)	8.78	(est)	1.50E-02		1.16E+01	H (NC)
148	Tetramethyl 9H-carbazole-1,2,3,4-tetracarboxylate	37914344	399.36	9.47E-02	(est)	3.99	(est)	5.21E+00		5.21E+00	T
149	1,2-Benzenedicarboxylic acid, 2-hydroxyethyl 2-[(1-oxo-2-propenyl)oxy]ethyl ester	38056881	308.29	4.28E+03	(est)	1.08	(est)	6.83E+00	acrylates	6.83E+00	T
150	Di-(5-methylhexyl)phthalate	41451289	362.51	2.45E-03	(est)	7.41	(est)	7.00E-02		1.88E+00	T
151	1,2-Benzenedicarboxylic acid, isodecyl isooctyl ester	42343351	418.62	2.00E-01	(exp)	9.37	(est)	7.00E-03		2.43E+01	H (NC)
152	1,2-Benzenedicarboxylic acid, butyl isodecyl ester	42343362	362.51	2.12E-03	(est)	7.48	(est)	6.40E-02		2.05E+00	T
153	1-Cyclohexyl 2-methyl phthalate	43195900	262.31	8.84E+00	(est)	3.93	(est)	3.68E+00		3.68E+00	T
154	Dimethyl 1,2,3,6,7,8-hexahydro-as-indacene-4,5-dicarboxylate	51037208	274.32	2.00E+00	(est)	4.61	(est)	1.67E+00		1.67E+00	T
155	Dimethyl 4-aminophthalate	51832316	209.2	4.16E+03	(est)	1.14	(est)	9.10E+01		9.10E+01	H
156	1,2-Benzenedicarboxylic acid, 2-ethylhexyl isodecyl ester	53272223	418.62	2.32E-05	(est)	9.37	(est)	7.00E-03		2.43E+01	H (NC)
157	Phthalic acid, bis(3-methylhexyl) ester	53306539	278.26	3.81E+03	(est)	0.74	(est)	1.16E+01	diepoxides	1.16E+01	H
158	1,2-Benzenedicarboxylic acid, bis(2-propylheptyl) ester	53306540	446.68	2.24E-06	(est)	10.36	(est)	2.00E-03		8.76E+01	H (NC)
159	1,2-Benzenedicarboxylic acid, decyl isooctyl ester	53363965	418.62	2.01E-05	(est)	9.45	(est)	7.00E-03		2.68E+01	H (NC)
160	Not found	53623599	222.24	1.45E+02	(est)	2.77	(est)	1.30E+02	ester-acids	1.30E+02	NC
161	Not found	56961047	415.28	8.97E-03	(est)	6.37	(est)	2.89E-01		5.97E-01	VT
162	bis(Pentabromophenyl) terephthalate	57212632	1107.29	4.79E-13	(est)	13.00	(est)	2.18E-04		5.61E+03	NC
163	Not found	59348651	454.48	5.40E+01	(est)	2.12	(est)	5.93E+02	ester-acids	5.93E+02	NC
164	Diethyl 1,4-dihydroxy-2,3-naphthalenedicarboxylate	59883077	304.3	1.34E+01	(est)	4.12	(est)	1.05E-01	Q/HQ	1.05E-01	VT
165	Dimethyl 3-chlorophthalate	61539353	228.63	3.31E+02	(est)	2.31	(est)	2.36E+01		2.36E+01	H

166	1,2-Benzenedicarboxylic acid, hexyl isodecyl ester	61702816	390.57	2.07E-04	(est)	8.47	(est)	2.00E-02		7.47E+00	T (NC)
167	1,2-Benzenedicarboxylic acid, hexyl octyl ester	61827621	362.51	1.83E-03	(est)	7.56	(est)	5.80E-02		2.26E+00	T
168	1-(2-Ethylhexyl) 2-isobutyl phthalate	61827643	334.46	2.49E-02	(est)	6.43	(est)	2.16E-01		5.18E-01	VT
169	1,2-Benzenedicarboxylic acid, isodecyl tridecyl ester	61886600	488.76	5.78E-08	(est)	11.90	(est)	3.74E-04		6.39E+02	NC
170	Not found	62116705	582.74	1.06E-06	(est)	9.71	(est)	7.00E-03		5.13E+01	H (NC)
171	Di(D-glucitol) phthalate	62736009	494.45	1.00E+06	(est)	-4.69	(est)	2.84E+05		2.84E+05	NC
172	1,2-Benzenedicarboxylic acid, bis(1-methylheptyl) ester, (S-(R*,R*))-	64535973	390.57	2.39E-04	(est)	8.39	(est)	2.30E-02		6.77E+00	T (NC)
173	1,2-Benzenedicarboxylic acid, bis(1-methylheptyl) ester, (R-(R*,R*))-	64535984	390.57	2.39E-04	(est)	8.39	(est)	2.30E-02		6.77E+00	T (NC)
174	1,2-Benzenedicarboxylic acid, (methylstannylidyne)tris(thio-2,1-ethanediyl) triisooctyl ester	67907146	1146.12	3.73E-16	(est)	16.32	(est)	3.78E-06		3.47E+05	NC
175	1,2-Benzenedicarboxylic acid, isooctyl 2-mercaptoethyl ester	67907168	338.47	1.57E-01	(est)	5.46	(est)	7.22E-01		7.22E-01	VT
176	1,2-Benzenedicarboxylic acid, (butylstannylidyne)tris(thio-2,1-ethanediyl) triisooctyl ester	67939280	1188.2	1.05E-17	(est)	17.80	(est)	6.34E-07		2.23E+06	NC
177	1,2,4-Benzenetricarboxylic acid, decyl octyl ester	67989235	574.85	2.78E-09	(est)	12.79	(est)	1.47E-04		2.25E+03	NC
178	1,2-Benzenedicarboxylic acid, mixed cyclohexyl and 2- ethylhexyl esters	68130494	360.5	3.14E-03	(est)	7.30	(est)	8.00E-02		1.63E+00	T
179	1,2-Benzenedicarboxylic acid, mixed cetyl and stearyl esters	68442706	516.81	4.79E-09	(est)	12.96	(est)	1.07E-04		2.49E+03	NC
180	Not found	68443436	779.03	9.05E-12	(est)	14.12	(est)	3.87E-05		1.57E+04	NC
181	1,2-Benzenedicarboxylic acid, benzyl C7-9-branched and linear alkylesters	68515402	256.26	5.17E+01	(est)	3.07	(est)	1.04E+02	ester-acids	1.04E+02	NC
182	1,2-Benzenedicarboxylic acid, di-C7-9-branched and linear alkyl esters	68515413	390.57	2.07E-04	(est)	8.47	(est)	2.00E-02		7.47E+00	T (NC)
183	1,2-Benzenedicarboxylic acid, di-C7-11-bracnched and linear alkyl esters	68515424	390.57	2.07E-04	(est)	8.47	(est)	2.00E-02		7.47E+00	T (NC)
184	1,2-Benzenedicarboxylic acid, di-C9-11-branched and linear alkyl esters	68515435	446.68	2.09E-06	(est)	10.39	(est)	2.00E-03		9.09E+01	H (NC)

185	1,2-Benzenedicarboxylic acid, diheptyl ester, branched and linear	68515446	362.51	2.45E-03	(est)	7.41	(est)	7.00E-02	1.88E+00	T
186	1,2-Benzenedicarboxylic acid, dinonyl ester, branched and linear	68515457	418.62	1.74E-05	(est)	9.52	(est)	6.00E-03	2.92E+01	H (NC)
187	1,2-Benzenedicarboxylic acid, di-C11-14-branched alkyl esters, C13-rich	68515479	502.78	2.40E-08	(est)	12.25	(est)	2.50E-04	1.01E+03	NC
188	1,2-Benzenedicarboxylic acid, di-C8-10-branched alkyl esters, C9-rich	68515480	418.62	1.74E-05	(est)	9.52	(est)	6.00E-03	2.92E+01	H (NC)
189	1,2-Benzenedicarboxylic acid, di-C9-11-branched alkyl esters, C10-rich	68515491	446.68	2.60E-06	(est)	10.28	(est)	6.00E-03	7.94E+01	H (NC)
190	1,2-Benzenedicarboxylic acid, dihexyl ester, branched and linear	68515504	334.46	2.32E-02	(est)	6.46	(est)	2.08E-01	5.38E-01	VT
191	1,2-Benzenedicarboxylic acid, di-C6-10-alkyl esters	68515515	334.46	2.40E-01	(exp)	6.82	(exp)	1.82E-01	8.38E-01	VT
192	1,2-Benzenedicarboxylic acid, mixed 2-ethylhexyl and isodecyl esters	68515526	418.62	2.32E-05	(est)	9.37	(est)	7.00E-03	2.43E+01	H (NC)
193	1,2-Benzenedicarboxylic acid, mono-C9-11-branched alkyl esters, C10-rich	68515548	446.68	2.59E-06	(est)	10.28	(est)	3.00E-03	7.94E+01	H (NC)
194	1,2,4-Benzenetricarboxylic acid, tri-C7-9-branched and linear alkyl esters	68515606	504.71	1.77E-06	(est)	10.04	(est)	4.00E-03	6.67E+01	H (NC)
195	1,2-Benzenedicarboxylic acid, C6-12-alkyl esters	68610822	334.46	2.40E-01	(exp)	6.82	(exp)	1.82E-01	8.38E-01	VT
196	1,2-Benzenedicarboxylic acid, di-C7-11-alkyl esters	68648919	362.51	1.83E-03	(est)	7.56	(est)	5.80E-02	2.26E+00	T
197	1,2-Benzenedicarboxylic acid, di-C9-11-alkyl esters	68648920	418.62	1.74E-05	(est)	9.52	(est)	6.00E-03	2.92E+01	H (NC)
198	1,2-Benzenedicarboxylic acid, mixed decyl and hexyl and octyl diesters	68648931	390.57	2.00E-02	(exp)	8.10	(exp)	1.90E-02	4.73E+00	T (NC)
199	1,2-Benzenedicarboxylic acid, mixed 2-ethylhexyl and hexyl and isodecyl diesters	68648942	362.51	2.12E-03	(est)	7.48	(est)	6.40E-02	2.05E+00	T
200	1,2-Benzenedicarboxylic acid, mixed isodecyl and tridecyl diesters	68648953	488.76	5.78E-08	(est)	11.90	(est)	3.74E-04	6.39E+02	NC
201	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, chlorinated	68784076	459.46	4.35E-05	(est)	8.75	(est)	1.70E-02	1.24E+01	H (NC)
202	1,2-Benzenedicarboxylic acid, (dibutylstannylene)bis(thio-2,1-ethanediyl) diisooctyl ester	68928789	907.86	2.21E-13	(est)	14.99	(est)	1.54E-05	5.34E+04	NC

203	1,2-Benzenedicarboxylic acid, C4-13-branched alkyl esters	68951393	278.35	1.49E+00	(est)	4.73	(est)	1.46E+01	ester-acids	1.46E+01	H
204	1,2-Benzenedicarboxylic acid, di-C4-13-alkyl esters	68988181	390.57	4.24E-04	(est)	8.10	(exp)	1.90E-02		4.73E+00	T (NC)
205	1,2-Benzenedicarboxylic acid, mixed decyl and lauryl and octyl diesters	70693300	418.62	1.74E-05	(est)	9.52	(est)	6.00E-03		2.92E+01	H (NC)
206	Di-n-2-propylpentylphthalate	70910371	390.57	2.39E-04	(est)	8.39	(est)	2.30E-02		6.77E+00	T (NC)
207	1,2-Benzenedicarboxylic acid, mixed decyl and hexyl and isooctyl and octyl diesters	70955532	362.51	2.12E-03	(est)	7.48	(est)	6.40E-02		2.05E+00	T
208	1,2-Benzenedicarboxylic acid, mixed heptyl and isooctyl and nonyl and undecyl diesters	70955543	376.54	6.62E-04	(est)	7.97	(est)	3.60E-02		3.89E+00	T
209	1,2-Benzenedicarboxylic acid, mixed heptyl and isooctyl and nonyl diesters	70955554	376.54	6.62E-04	(est)	7.97	(est)	3.60E-02		3.89E+00	T
210	1,2-Benzenedicarboxylic acid, mixed heptyl and nonyl diesters	70955565	390.57	2.00E-02	(exp)	8.10	(exp)	1.90E-02		4.73E+00	T (NC)
211	1,2-Benzenedicarboxylic acid, 2,2-dimethyl-1,3-propanediyl diisooctyl ester	71097284	390.57	9.00E-02	(exp)	8.39	(est)	2.30E-02		6.77E+00	T (NC)
212	1,2-Benzenedicarboxylic acid, 2-methylbutyl phenylmethyl ester	71463826	326.4	2.75E-01	(est)	5.26	(est)	8.91E-01		8.91E-01	VT
213	1,2-Benzenedicarboxylic acid, cyclohexyl isooctyl ester	71486481	360.5	3.14E-03	(est)	7.30	(est)	8.00E-02		1.63E+00	T
214	1,2-Benzenedicarboxylic acid, dinonyl ester, branched	71549785	418.62	2.68E-05	(est)	9.30	(est)	8.00E-03		2.22E+01	H (NC)
215	1,2-Benzenedicarboxylic acid, di-C8-10-alkyl esters	71662469	404.6	5.57E-05	(est)	9.03	(est)	1.10E-02		1.54E+01	H (NC)
216	1,2-Benzenedicarboxylic acid, diisohexyl ester	71850094	334.46	2.49E-02	(est)	6.43	(est)	2.16E-01		5.18E-01	VT
217	1,2-Benzenedicarboxylic acid, hexyl isooctyl ester	71850129	362.51	2.12E-03	(est)	7.48	(est)	6.40E-02		2.05E+00	T
218	1,2-Benzenedicarboxylic acid, di-C6-8-branched alkyl esters, C7-rich	71888896	362.51	2.45E-03	(est)	7.41	(est)	7.00E-02		1.88E+00	T
219	Tri-isotridecyl Trimellitate	72361354	757.2	9.40E-16	(est)	18.96	(est)	9.67E-08		5.93E+06	NC
220	1,2-Benzenedicarboxylic acid, bis 3-hydroxy-2-(hydroxymethyl)-2-methylpropyl ester	72829153	370.4	5.48E+03	(est)	-0.08	(est)	7.27E+02		7.27E+02	NC
221	1,2-Benzenedicarboxylic acid, mixed esters with 3-(1,2-dihydroxyethoxy)-1,2-propanediol and stearic	73049895	552.76	3.57E-05	(est)	8.15	(est)	4.30E-02		7.13E+00	T (NC)

222	1,2-Benzenedicarboxylic acid, 2-ethylhexyl hexyl ester	75673164	362.51	2.12E-03	(est)	7.48	(est)	6.40E-02	2.05E+00	T
223	Not found	76644627	308.33	2.50E+01	(est)	3.09	(est)	1.22E+01	1.22E+01	H
224	Not found	76644638	322.36	7.85E+00	(est)	3.59	(est)	6.88E+00	6.88E+00	T
225	Not found	76644649	322.36	7.85E+00	(est)	3.59	(est)	6.88E+00	6.88E+00	T
226	Not found	76644650	336.39	2.47E+00	(est)	4.08	(est)	3.93E+00	3.93E+00	T
227	Not found	76644661	336.39	2.47E+00	(est)	4.08	(est)	3.93E+00	3.93E+00	T
228	Not found	76644683	308.38	3.22E+01	(est)	3.56	(est)	6.83E+00	6.83E+00	T
229	Not found	76644694	308.38	3.72E+01	(est)	3.49	(est)	7.45E+00	7.45E+00	T
230	Not found	76644707	308.38	3.22E+01	(est)	3.56	(est)	6.83E+00	6.83E+00	T
231	Not found	78246556	308.38	3.72E+01	(est)	3.49	(est)	7.45E+00	7.45E+00	T
232	1,2-Benzenedicarboxylic acid, 4-hydroxybutyl propyl ester	79038236	280.32	2.79E+02	(est)	2.65	(est)	1.91E+01	1.91E+01	H
233	Tricresyl ethyl phthalate	81705013	284.31	6.82E+00	(est)	3.92	(est)	4.04E+00	4.04E+00	T
234	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester, labeled with carbon-14	82208433	390.57	2.70E-01	(exp)	7.60	(exp)	2.30E-02	2.56E+00	T
235	1,2-Benzenedicarboxylic acid, diundecyl ester, branched and linear	85507795	488.76	6.68E-08	(est)	11.83	(est)	4.07E-04	5.86E+02	NC
236	1,2-Benzenedicarboxylic acid, 2,5-dimethylheptyl 4-methyloctyl ester	85851769	418.62	2.68E-05	(est)	9.30	(est)	8.00E-03	2.22E+01	H (NC)
237	1,2-Benzenedicarboxylic acid, 2,5-dimethylheptyl 6-methyloctyl ester	85851770	418.62	2.68E-05	(est)	9.30	(est)	8.00E-03	2.22E+01	H (NC)
238	1,2-Benzenedicarboxylic acid, 4-methyloctyl 6-methyloctyl ester	85851781	418.62	2.32E-05	(est)	9.37	(est)	7.00E-03	2.43E+01	H (NC)
239	1,2-Benzenedicarboxylic acid, 3-ethylheptyl 4-methyloctyl ester	85851792	418.62	2.32E-05	(est)	9.37	(est)	7.00E-03	2.43E+01	H (NC)
240	1,2-Benzenedicarboxylic acid, 3-ethylheptyl 6-methyloctyl ester	85851805	418.62	2.32E-05	(est)	9.37	(est)	7.00E-03	2.43E+01	H (NC)
241	1,2-Benzenedicarboxylic acid, bis(2-ethyloctyl) ester	85851816	446.68	2.24E-06	(est)	10.36	(est)	2.00E-03	8.76E+01	H (NC)
242	1,2-Benzenedicarboxylic acid, bis(2-ethylnonyl) ester	85851827	474.73	2.16E-07	(est)	11.34	(est)	7.24E-04	3.11E+02	NC

243	1,2-Benzenedicarboxylic acid, bis(2-methyldecyl) ester	85851838	474.73	2.16E-07	(est)	11.34	(est)	7.24E-04	3.11E+02	NC
244	1,2-Benzenedicarboxylic acid, bis(2-propylhexyl) ester	85851849	418.62	2.32E-05	(est)	9.37	(est)	7.00E-03	2.43E+01	H (NC)
245	1,2-Benzenedicarboxylic acid, bis(2-propyloctyl) ester	85851850	474.73	2.16E-07	(est)	11.34	(est)	7.24E-04	3.11E+02	NC
246	1,2-Benzenedicarboxylic acid, 2-ethylhexyl nonyl ester	85851861	404.6	6.44E-05	(est)	8.96	(est)	1.20E-02	1.41E+01	H (NC)
247	1,2-Benzenedicarboxylic acid, isononyl octyl ester	85851883	404.6	6.44E-05	(est)	8.96	(est)	1.20E-02	1.41E+01	H (NC)
248	1,2-Benzenedicarboxylic acid, hexyl isotridecyl ester	85851894	432.65	6.24E-06	(est)	9.94	(est)	4.00E-03	5.06E+01	H (NC)
249	1,2-Benzenedicarboxylic acid, isotridecyl nonyl ester	85851907	474.73	1.87E-07	(est)	11.41	(est)	6.64E-04	3.39E+02	NC
250	1,2-Benzenedicarboxylic acid, isodecyl nonyl ester	85851918	432.65	6.24E-06	(est)	9.94	(est)	4.00E-03	5.06E+01	H (NC)
251	1,2-Benzenedicarboxylic acid, 2-ethylhexyl isononyl ester	85851929	404.6	7.44E-05	(est)	8.88	(est)	1.30E-02	1.28E+01	H (NC)
252	Not found	90164435	626.79	6.97E-07	(est)	9.59	(est)	8.00E-03	4.76E+01	H (NC)
253	Di-(5-hexenyl)phthalate	92569443	330.43	3.37E-02	(est)	6.30	(est)	2.50E-01	4.36E-01	VT
254	Di-(9-decenyl)phthalate	92569454	442.64	3.04E-06	(est)	10.23	(est)	3.00E-03	7.40E+01	H (NC)
255	1,2-Benzenedicarboxylic acid, mixed esters with 1,4-butanediol and tridecanol	94214525	436.59	5.80E-03	(est)	6.43	(est)	2.82E-01	6.76E-01	VT
256	1,2-Benzenedicarboxylic acid, mixed isohexyl and 2-phenoxyethyl esters	94214536	384.48	1.69E-02	(est)	6.27	(est)	3.02E-01	4.89E-01	VT
257	1,2-Benzenedicarboxylic acid, mixed 2-phenoxyethyl and tridecyl esters	94214547	468.64	1.33E-05	(est)	9.29	(est)	9.00E-03	2.46E+01	H (NC)
258	1,2-Benzenedicarboxylic acid, isooctyl isotridecyl ester	94979212	460.7	6.95E-07	(est)	10.85	(est)	1.28E-03	1.65E+02	NC
259	1,2-Benzenedicarboxylic acid, isodecyl isoundecyl ester	94979223	460.7	6.95E-07	(est)	10.85	(est)	1.28E-03	1.65E+02	NC
260	1,2-Benzenedicarboxylic acid, decyl nonyl ester	96507765	432.65	5.40E-06	(est)	10.01	(est)	3.00E-03	5.51E+01	H (NC)
261	1,2-Benzenedicarboxylic acid, isoundecyl nonyl ester	96507787	446.68	1.94E-06	(est)	10.43	(est)	2.00E-03	9.55E+01	H (NC)
262	1,2-Benzenedicarboxylic acid, isoundecyl	96507798	474.73	1.87E-07	(est)	11.41	(est)	6.64E-04	3.39E+02	NC

	undecyl ester									
263	1,2-Benzenedicarboxylic acid, decyl isoundecyl ester	96507801	460.7	6.01E-07	(est)	10.92	(est)	1.18E-03	1.80E+02	NC
264	1,2-Benzenedicarboxylic acid, isodecyl undecyl ester	96507812	460.7	6.01E-07	(est)	10.92	(est)	1.18E-03	1.80E+02	NC
265	1,2-Benzenedicarboxylic acid, isononyl undecyl ester	96507823	446.68	1.94E-06	(est)	10.43	(est)	2.00E-03	9.55E+01	H (NC)
266	1,2-Benzenedicarboxylic acid, decyl isononyl ester	96507834	432.65	6.24E-06	(est)	9.94	(est)	4.00E-03	5.06E+01	H (NC)
267	1,2-Benzenedicarboxylic acid, isooctyl undecyl ester	96507845	432.65	6.24E-06	(est)	9.94	(est)	4.00E-03	5.06E+01	H (NC)
268	1,2-Benzenedicarboxylic acid, isooctyl nonyl ester	96507856	404.6	6.44E-05	(est)	8.96	(est)	1.20E-02	1.41E+01	H (NC)
269	1,2-Benzenedicarboxylic acid, diisoundecyl ester	96507867	474.73	2.16E-07	(est)	11.34	(est)	7.24E-04	3.11E+02	NC
270	1,2-Benzenedicarboxylic acid, isononyl isooctyl ester	96532795	404.6	7.44E-05	(est)	8.88	(est)	1.30E-02	1.28E+01	H (NC)
271	1,2-Benzenedicarboxylic acid, isooctyl isoundecyl ester	96532808	432.65	7.21E-06	(est)	9.87	(est)	4.00E-03	4.64E+01	H (NC)
272	1,2-Benzenedicarboxylic acid, 2,5-dimethylheptyl 3-ethylheptyl ester	97692552	418.62	2.68E-05	(est)	9.30	(est)	8.00E-03	2.22E+01	H (NC)
273	1,2-Benzenedicarboxylic acid, decyl isotridecyl ester	98072276	488.76	5.78E-08	(est)	11.90	(est)	3.74E-04	6.39E+02	NC
274	1,2-Benzenedicarboxylic acid, 2-ethylhexyl isotridecyl ester	98072287	460.7	6.95E-07	(est)	10.85	(est)	1.28E-03	1.65E+02	NC
275	1,2-Benzenedicarboxylic acid, isotridecyl undecyl ester	98072298	502.78	1.79E-08	(est)	12.39	(est)	2.10E-04	1.20E+03	NC
276	1,2-Benzenedicarboxylic acid, 2-ethylhexyl isoundecyl ester (	98088961	432.65	7.21E-06	(est)	9.87	(est)	4.00E-03	4.64E+01	H (NC)
277	1,2-Benzenedicarboxylic acid, isononyl nonyl ester	98088972	418.62	2.01E-05	(est)	9.45	(est)	7.00E-03	2.68E+01	H (NC)
278	1,2-Benzenedicarboxylic acid, bis(2-oxo-2-phenylethyl) ester	101012822	402.41	4.11E+00	(est)	3.35	(est)	1.16E+01	1.16E+01	H
279	Not found	102148878	264.32	5.98E+00	(est)	4.12	(est)	2.94E+00	2.94E+00	T
280	1,2-Benzenedicarboxylic acid, heptyl nonyl ester, branched and linear	111381896	390.57	2.39E-04	(est)	8.39	(est)	2.30E-02	6.77E+00	T (NC)

281	1,2-Benzenedicarboxylic acid, heptyl undecyl ester, branched and linear	111381909	418.62	2.32E-05	(est)	9.37	(est)	7.00E-03		2.43E+01	H (NC)
282	1,2-Benzenedicarboxylic acid, nonyl undecyl ester, branched and linear	111381910	446.68	2.59E-06	(est)	10.28	(est)	3.00E-03		7.94E+01	H (NC)
283	Not found	119394455	446.68	2.80E-01	(exp)	10.36	(est)	2.00E-03		8.76E+01	H (NC)
284	O=C(OCCCCCC)c1ccccc1(C(=O)OCC(CCC)CCCCC)	Not found	390.57	2.07E-04	(est)	8.46	(est)	2.00E-02		7.38E+00	T (NC)
285	bis(2,2,2-Trifluoroethyl) 2,3-naphthalenedicarboxylate	Not found	380.25	4.31E-01	(est)	4.65	(est)	2.20E+00		2.20E+00	T
286	O=C(OCCCCCCCC(C)C)c1ccccc1(C(=O)OCCCC(C)C)	Not found	390.57	2.39E-04	(est)	8.39	(est)	2.30E-02		6.77E+00	T (NC)
287	O=C(OCC(CCC(C)C)C(C)C)c1ccccc1(C(=O)OCC(CCC(C)C)C(C)C)	Not found	446.68	9.00E-02	(exp)	10.06	(est)	3.00E-03		6.05E+01	H (NC)
288	O=C(OCCCCCCCC(C)C)c1ccccc1(C(=O)OCCC(C)C)	Not found	376.54	7.65E-04	(est)	7.90	(est)	4.00E-02		3.57E+00	T
289	O=C(OCCCCCC)c1c(C(=O)OCCCCCC)c(C(=O)OCCCCCC)c(C(=O)OCCCCCC)c(C(=O)OCCCCCC)c1(C(=O)OCCCCCC)	Not found	847.15	1.31E-13	(est)	15.74	(est)	5.72E-06		1.26E+05	NC
290	O=C(OC(C(=O)OCC(CC)CCCC)C)c1ccccc1(C(=O)OC(C(=O)OCC(CC)CCCC)C)	Not found	534.7	3.44E-05	(est)	8.31	(est)	3.40E-02		8.39E+00	T (NC)
291	O=C(OCCCCCCCC(C)C)c1ccc(cc1)C(=O)OCC(CC)CCCC	Not found	418.62	2.32E-05	(est)	9.37	(est)	7.00E-03		2.43E+01	H (NC)
292	O=C(OCC5CC4C(C(OC(=O)c1ccccc1)CC3CC(OC(=O)c2ccccc2)CCC34(C))C6CCC(CCCC(=O)OC)C56(C))c7ccccc7	Not found	734.94	1.11E-08	(est)	10.85	(est)	2.00E-03		2.64E+02	NC
293	bis(2,2,2-Trichloroethyl) phthalate	Not found	428.91	5.39E-02	(est)	5.36	(est)	1.04E+00		1.04E+00	T
294	CCCCC(CC)COC(=O)c1cc(c(cc1C(=O)OCC(CC)CCCC)C(=O)OCC(CC)CCCC)C(=O)OCC(CC)CCCC	Not found	703.02	7.83E-12	(est)	14.79	(est)	1.53E-05		3.23E+04	NC
295	Butanoic 2-(ethoxycarbonyl)phenyl anhydride	Not found	264.28	2.67E+02	(est)	2.19	(est)	3.17E+01		3.17E+01	H
296	2-(2-(Diethylamino)ethyl) 1-ethyl 3-aminophthalate	Not found	308.38	1.80E+02	(est)	3.27	(est)	9.76E+00		9.76E+00	T
297	Dimethyl 1-anilino-4-hydroxy-2,3-naphthalenedicarboxylate	Not found	351.36	5.39E-02	(est)	5.92	(est)	2.00E-01	phenols	2.00E-01	VT
298	Dimethyl 1,4-dihydroxy-2,3-naphthalenedicarboxylate	Not found	276.25	1.35E+02	(est)	3.13	(est)	1.26E-01	phenols	1.26E-01	VT

299	bis(2-Bromoethyl) phthalate	Not found	380.03	5.80E+00	(est)	3.33	(est)	1.12E+01		1.12E+01	H
300	1-Butyl 2-methyl 3,4,5,6-tetrachlorophthalate	Not found	374.05	5.83E-02	(est)	5.71	(est)	5.86E-01		5.86E-01	VT
301	Diethyl 3-chlorophthalate	Not found	256.69	3.37E+01	(est)	3.29	(est)	7.93E+00		7.93E+00	T
302	1-Cyclohexyl 2-propyl phthalate	Not found	290.36	8.86E-01	(est)	4.92	(est)	1.20E+00		1.20E+00	T
303	1-Ethyl 2-(2-oxopropyl) phthalate	Not found	250.25	1.04E+03	(est)	1.59	(est)	6.28E+01		6.28E+01	H
304	1-Butyl 2-(2-oxopropyl) phthalate	Not found	278.31	1.05E+02	(est)	2.57	(est)	2.09E+01		2.09E+01	H
305	1-(2-(Methoxymethoxy)ethyl) 2-methyl phthalate	Not found	268.27	2.04E+03	(est)	1.13	(est)	1.19E+02		1.19E+02	NC
306	Dimethyl 11-methyl-1,4-dioxo-4,11-dihydro-1H-benzo[a]carbazole-5,6-dicarboxylate	Not found	377.36	2.92E-01	(est)	3.57	(est)	1.52E-01	Q/HQ	1.52E-01	VT
307	1-Ethyl 2-(1-isopropyl-2-methylpropyl) phthalate	Not found	292.38	9.23E-01	(est)	4.88	(est)	1.27E+00		1.27E+00	T
308	1-(2-(Hydroxy(oxido)amino)-2-methylpropyl) 2-methyl phthalate	Not found	281.27	9.25E+01	(est)	2.16	(est)	3.50E+01		3.50E+01	H
309	1-Butyl 2-(2-(hydroxy(oxido)amino)-2-methylpropyl) phthalate	Not found	323.35	2.90E+00	(est)	3.63	(est)	6.57E+00		6.57E+00	T
310	Diethyl 3,4,5,6-tetrachlorophthalate	Not found	360.02	1.86E-01	(est)	5.22	(est)	1.03E+00		1.03E+00	T
311	Tetramethyl 1-methyl-1H-indole-4,5,6,7-tetracarboxylate	Not found	365.34	7.60E+00	(est)	3.30	(est)	1.12E+01		1.12E+01	H
312	Trimethyl 5-methyl-6-oxo-5,6-dihydro-7,8,9-phenanthridinetricarboxylate	Not found	383.36	8.68E+01	(est)	0.63	(est)	3.14E+02		3.14E+02	NC
313	bis(2-Methoxy-1-methyl-2-oxoethyl) phthalate	Not found	338.32	3.26E+02	(est)	1.58	(est)	8.59E+01		8.59E+01	H
314	Dimethyl 3,6-dihydroxy-4-methoxyphthalate	Not found	256.21	6.55E+02	(est)	1.78	(est)	1.71E-01	Q/HQ	1.71E-01	VT
315	Dimethyl 9,10-dioxo-9,10-dihydro-2,3-anthracenedicarboxylate	Not found	324.29	1.83E+00	(est)	3.01	(est)	1.41E+01		1.41E+01	H
316	Dimethyl 2,3-anthracenedicarboxylate	Not found	294.31	3.83E-01	(est)	4.02	(est)	3.70E+00		3.70E+00	T
317	1-Ethyl 4,5-dimethyl 7-(3-methoxy-1-(methoxycarbonyl)-3-oxo-1-propenyl)-1H-indole-1,4,5-tricarboxylate	Not found	449.42	2.27E-01	(est)	4.47	(est)	2.44E+00	acrylates	2.44E+00	T
318	O=C(OCC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)F)c1ccccc1(C(=O)OCC(F)(F)C(F)(F)C(F)(F)C(F)(F)C(F)F)	Not found	594.25	2.30E-05	(est)	8.06	(est)	5.20E-02		6.86E+00	T (NC)
319	1-((2-Methyl-2-oxiranyl)methyl) 2-(2-methyl-2-propenyl) phthalate	Not found	290.32	3.48E+01	(est)	3.05	(est)	1.02E+01		1.02E+01	H

320	1-(2-Butenyl) 2-butyl phthalate	Not found	276.34	2.97E+00	(est)	4.39	(est)	2.20E+00	2.20E+00	T
321	Dimethyl 4,9-dimethyl-9H-carbazole-1,2-dicarboxylate	Not found	311.34	1.96E-02	(est)	5.41	(est)	7.06E-01	7.06E-01	VT
322	tris(2-Chloroethyl) 4,5,6-trichloro-1,2,3-benzenetricarboxylate	Not found	500.98	1.02E-02	(est)	5.67	(est)	8.25E-01	8.25E-01	VT
323	2-(2-(Diethylamino)ethyl) 1-ethyl 3-(hydroxy(oxido)amino)phthalate	Not found	338.36	1.64E+02	(est)	2.65	(est)	2.30E+01	2.30E+01	H
324	2-(2-(Diethylamino)ethyl) 1-methyl 3-(hydroxy(oxido)amino)phthalate	Not found	324.34	5.23E+02	(est)	2.16	(est)	4.03E+01	4.03E+01	H

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Summary

This report presents the preliminary results from a (Q)SAR investigation of the acute toxicity to fish (fathead minnow) for a dataset of phthalate esters. A chemical set of 341 phthalates was compiled by using different searching engines. Their acute toxicity to fathead minnow was calculated with the ECOSAR and TOPKAT software. A good correlation between the predictions from the two programs was established ($r^2 = 0.81$). The chemicals were classified initially into four groups on a basis of their predicted by ECOSAR LC_{50} values: 1) no reasons for concern ($LC_{50} > 100$ mg/L), 2) harmful (10 mg/L $< LC_{50} <$ 100 mg/L), 3) toxic (1 mg/L $< LC_{50} <$ 10 mg/L), 4) very toxic $LC_{50} <$ 1 mg/L). This prediction effort resulted in classification of the vast majority of the phthalates in the "very toxic" group. The reason for this result is that ECOSAR uses linear relationships with the octanol-water partition coefficient (log K_{ow}) for chemicals with log $K_{ow} < 5$ (warning is issued for chemicals with log $K_{ow} > 5$). The predictions from TOPKAT (only predictions within the optimum prediction space were considered) correlated relatively well with those from ECOSAR.

There were many high molecular weight phthalate esters in the chemical series, which appeared clearly outside the applicability domain of the ECOSAR models. This fact, as well as the understanding that beyond certain limits of hydrophobicity the toxicity of the organic chemicals decreases as a result of reduced bioconcentration, motivated the development of an algorithm for refinement of acute toxicity predictions of the phthalate esters using the bilinear relationship with log K_{ow}. In addition, water solubility limits were considered.

Long-term toxicity studies were not considered in this study. Transformation (e.g. biodegradability) of the parent compounds was not considered either. This could potentially be important as, theoretically, the transformation of very hydrophobic chemicals (log K_{ow} > 7) or extremely hydrophobic chemicals (log K_{ow} > 8.0) into more hydrophilic degradation/transformation products may increase the acute toxicity to fish.

This case study provides an illustration of how (Q)SAR methods can be used in the development of chemical categories and how (Q)SAR results can be used to perform an initial screening in support of classification and labelling. The results are discussed and interpreted with a view of what constitutes a category, how it can be defined and described, what are its boundaries, and the need to define subcategories that might be useful for deciding on the level of acute toxicological hazard associated with different structural modifications. Due to the preliminary nature of the (Q)SAR models, the results of this study should be regarded as an illustration of the applicability of (Q)SAR methods. The actual model results and rule-based classification scheme will need validation and refinement before they could be considered for regulatory use.

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Classification of Phthalates According to Their (Q)SAR Predicted Acute Toxicity to Fish: A Case Study
Authors: Netzeva, Tatiana - Worth, Andrew

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Abstract

This report presents the preliminary results from a (Q)SAR investigation of the acute toxicity to fish (fathead minnow) for a dataset of phthalate esters. A chemical set of 341 phthalates was compiled by using different searching engines.
Their acute toxicity to fathead minnow was calculated with the ECOSAR and TOPKAT software. A good correlation between the predictions from the two programs was established ($r^2 = 0.81$).
The chemicals were classified initially into four groups on a basis of their predicted by ECOSAR LC50 values:
1) no reasons for concern (LC50 > 100 mg/L), 2) harmful (10 mg/L < LC50 < 100 mg/L), 3) toxic (1 mg/L < LC50 < 10 mg/L), 4) very toxic LC50 < 1 mg/L).
This prediction effort resulted in classification of the vast majority of the phthalates in the very toxic group.
The reason for this result is that ECOSAR uses linear relationships with the octanol-water partition coefficient (log Kow) for chemicals with log Kow < 5 (warning is issued for chemicals with log Kow > 5). The predictions from TOPKAT (only predictions within the optimum prediction space were considered) correlated relatively well with those from ECOSAR.
There were many high molecular weight phthalate esters in the chemical series, which appeared clearly outside the applicability domain of the ECOSAR models. This fact, as well as the understanding that beyond certain limits of hydrophobicity the toxicity of the organic chemicals decreases as a result of reduced bioconcentration, motivated the development of an algorithm for refinement of acute toxicity predictions of the phthalate esters using the bilinear relationship with log Kow. In addition, water solubility limits were considered.
Long-term toxicity studies were not considered in this study. Transformation (e.g. biodegradability) of the parent compounds was not considered either. This could potentially be important as, theoretically, the transformation of very hydrophobic chemicals (log Kow > 7) or extremely hydrophobic chemicals (log Kow > 8.0) into more hydrophilic degradation/transformation products may increase the acute toxicity to fish.
This case study provides an illustration of how (Q)SAR methods can be used in the development of chemical categories and how (Q)SAR results can be used to perform an initial screening in support of classification and labelling. The results are discussed and interpreted with a view of what constitutes a category, how it can be defined and described, what are its boundaries, and the need to define subcategories that might be useful for deciding on the level of acute toxicological hazard associated with different structural modifications. Due to the preliminary nature of the (Q)SAR models, the results of this study should be regarded as an illustration of the applicability of (Q)SAR methods. The actual model results and rule-based classification scheme will need validation and refinement before they could be considered for regulatory use.

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