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A step change towards risk assessment in the 21st century

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TABLE OF CONTENTS

1. ABSTRACT

Chemical Regulation and the means by which data is generated for the purposes of risk assessment is undergoing a tremendous shift. There is a strong impetus in Europe, in particular, to move towards non-animal approaches to address data gaps for specific endpoints either in lieu of testing or as part of weight of evidence approaches within integrated testing strategies (ITS). An Exposure assessment considering workers and/or consumers is a critical component of a robust risk assessment. The EU chemicals legislation REACH, for example, provides considerable flexibility in the application of non-testing approaches such as (Q)SARs, chemical categories and read-across for data gap filling. There have been a number of efforts aimed at developing technical guidance, tools, and techniques for non-testing and tiered exposure approaches. Despite these efforts, there remains limited practical insight about how these approaches can be applied in the assessment of substances. Here, we first provide a background of the available approaches and how they can and should be practically utilised to address REACH requirements.

2. INTRODUCTION

2.1. Regulatory background

Chemical Regulation and the means by which data are generated and translated into information for the purposes of risk assessment is undergoing a massive shift. One of the major drivers for this change comes from the move towards non-animal alternatives. Animal welfare concerns within Europe, in particular, have provided significant momentum to investigate potential alternatives to animal testing which encompass the 3 "R"s (refine, reduce, and replace animal testing). For example; the 7th Amendment to the Cosmetics Directive has placed a ban on the *in vivo* testing of cosmetics ingredients. The ban for acute testing came into effect in March 2009, the ban for repeat dose testing will commence in 2013 (1). The new chemicals legislation within the EU, REACH (**R**egistration, **E**valuation, **A**uthorisation and Restriction of **Ch**emical substances) specifically calls for the use of non-animal alternatives (2). Vertebrate testing should only be used as a last resort. REACH advocates the use of integrated testing strategies (ITS) as an efficient means of addressing the

information requirements for a given endpoint. ITS approaches comprise multiple elements such as optimised *in vivo* tests, *in vitro* tests, (Q)SARs and chemical categories (3). The elements themselves are not new but the integration in a framework where the generated information is non-standard provides a challenge for interpretation and decision making.

Another significant driver is the general public who play a role in demanding that chemicals are evaluated and determined to be safe for their intended applications. This places a burden on both Regulators and Industry to demonstrate that relevant and sufficient information is available to enable robust risk assessments to be undertaken and reviewed in a timely manner.

Thus, the regulatory landscape is dramatically evolving, opportunities and challenges remain in determining what the critical aspects in the evaluation of adverse outcomes are and how to interpret them. We will discuss the REACH regulatory programme where the new framework exists but the practical implementation is still evolving.

2.2. REACH

REACH (Registration, Evaluation, Authorisation and restriction of CHemicals) is the new EU legislation that came into force in June 2007 (2) and which superseded Directive 79/831/EEC. Under Directive 79/831/EEC, Existing chemicals comprised substances introduced between January 1971 and September 1981 and were listed on EINECS (European INventory of Existing commercial Chemical Substances) whereas New Chemicals were those substances introduced subsequently and listed on ELINCS (European LIst of Notified Chemical Substances). Under Directive 79/831/EEC, information requirements under the Notification of New Substances (NONS) procedure were mandated only for new substances with tonnages in excess of 10 kg. Existing substances were not subjected to the same information requirements hence the complement of data supporting their use has been extremely variable. For the c.a. 100,000 existing chemicals, supporting information rarely exists. Under REACH, this inconsistency should eventually become harmonised as REACH calls for equivalent information requirements for all new and existing chemicals manufactured or imported at quantities of 1 tonne or greater per annum. The specific information requirements for REACH depend on tonnage bands which are described in Annexes VII-X of the REACH legal text. The original estimates were that some 30,000 chemicals would need to be re-evaluated leading to additional information requirements, but given the number of pre-registrations (c.a.143,000) (4), this estimate appears conservative. In any case, the number of chemicals that are likely to require re-evaluation is vast and this does represent a significant challenge in terms of cost, animal numbers and resources in order to address the necessary information requirements. The REACH legal text affords considerable scope to utilise and exploit alternative (non-animal) approaches. Annex XI provides the framework for fulfilling these requirements by other means (examples include *in vitro*, Weight of Evidence (WOE), (Q)SARs, chemical grouping etc) thus limiting vertebrate testing to the fullest extent possible. There is an obligation to carry out vertebrate testing only as a last resort and to consider all other options before performing or requiring testing as described by Articles 13(1) and 25(1) of the REACH legal text.

2.3. Integrated testing strategies

Integrated testing strategies (ITS) are structured workflows of the different elements required to conduct a risk assessment. They conceptually describe how exposure information and effects information obtained from (Q)SARs, read-across methods, and *in vitro* tests prior to *in vivo* testing can afford a more rapid, efficient, and cost-effective way to perform risk assessment of chemicals (3). The REACH technical guidance describes endpoint specific ITS, to illustrate the hazard characterisation requirements. The technical guidance identifies the available approaches e.g. *in vivo* test methods, *in vitro*, (Q)SARs etc in brief for that endpoint and how to evaluate their respective outputs. The workflow (the ITS) provides the framework for how the information available/generated should be integrated together to arrive at an overall conclusion for hazard characterisation purposes (e.g. Classification & Labelling (C&L) and/or Risk Assessment) (5). The ITS does not address the practical aspects of how to perform an integrated assessment, it merely provides a conceptual framework to encourage a step change in thinking – instead of a checkbox approach of gathering data, a hypothesis based approach is advocated that considers what critical elements are really pertinent for risk assessment/management decisions. This ideally should take into account a weight of evidence approach; exploiting known synergies between endpoints as well as knowledge of mechanisms/modes of action.

2.4. Non-testing approaches

Under REACH, a registrant may adapt standard testing requirements for its chemicals based on scientific, technical or exposure informed considerations. This assumes that the same level of information can be potentially obtained by means other than vertebrate testing. Non-testing approaches comprising (Q)SARs and chemical categories are outlined in Annex XI as strategies to adapt standard testing requirements (2). Annex XI provides specific wording for (Q)SAR use and the conditions that must be satisfied:

"Results obtained from valid qualitative or quantitative structure-activity relationship models (Q)SARs may indicate the presence or absence of a certain dangerous properties. Results of (Q)SARs may be used instead of testing when the following conditions are met:

- Results are derived from a (Q)SAR model whose scientific validity has been established
- The substance falls within the applicability domain of the QSAR model
- Results are adequate for the purpose of classification and labelling and/or risk assessment and

- Adequate and reliable documentation of the applied method is provided.

The Agency in collaboration with the Commission, Member States and interested parties shall develop and provide guidance in assessing which (Q)SARs will meet these conditions and provide examples."

The wording emphasises how information provided by (Q)SARs may be used in lieu of experimental data provided certain conditions are met. In practice, (Q)SAR information can be used either as replacements for experimental testing or more likely as part of a weight of evidence evaluation. Detailed technical guidance for the use of (Q)SARs is available from ECHA (5).

It is perhaps useful to briefly clarify the terminology of these conditions. Scientific validity refers to the internationally agreed OECD principles for the validation of (Q)SARs. These were adopted by the 37th Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology in November 2004. Guidance to describe each of the principles in turn is provided in the following references (6-7). Whilst the principles are helpful to characterise a given (Q)SAR model, the critical factor to consider in the application of a model is whether the substance of interest lies within the domain of applicability, i.e. is the (Q)SAR model relevant for the chemical under evaluation?

The concept of the domain of applicability was first defined as part of a series of background papers to the ICCA-LRI Setubal workshop (8). ECVAM hosted a workshop 2 years later (9) and proposed the following definition:

"The applicability domain of a (Q)SAR model is the response and chemical structure space in which the model makes predictions with a given reliability."

There is no single way to characterise a domain for a QSAR model. Approaches depend on the type of QSAR, the endpoint of interest and the underlying training set – i.e. domain characterisation has to be context dependent. One approach could be to characterise a domain on the basis of structural fragments if these are indeed the factors pertinent to the endpoint of interest, another may be to use the descriptors from the training set to compute ranges or probability densities etc Ultimately whatever domain approach is derived for a given model, this is merely a first step in the evaluation. Careful examination of the extent to which the model being used is able to provide reasonable predictions for other related substances (analogues) is still required to provide the confidence that a robust and reliable prediction is feasible for the substance of interest. Software programs have been developed to implement various approaches for domain evaluation – notable examples include AMBIT Discovery v0.04 (released May 2006 by Ideaconsult Ltd, Bulgaria) freely accessible from http://ambit.acad.bg/downloads/AmbitDiscovery/ and Domain Manager which is developed and commercialised by the Laboratory of Mathematical Chemistry (LMC) (University ''Prof. As. Zlatarov,'' Bourgas, Bulgaria).

The technical guidance on (Q)SARs addresses the principles, the need to rationalise the reliability and adequacy of a (Q)SAR result as well as some of the available software tools in some detail in Chapter R6 (5). Work under the former QSAR Working Group, a subgroup under the EU's Technical Committee for New and Existing Substances (TCNES) agreed on reporting formats (templates) to capture the key pieces of information needed for REACH for both a (Q)SAR model and its prediction. Two formats were proposed and these are known as the QSAR Model Reporting Format and QSAR Prediction Reporting Format, abbreviated as QMRF and QPRFs. Both of these formats are structured on the OECD Validation Principles. Illustrative examples of these formats are provided on the website of the former ECB (see http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/QRF).

Annex XI also contains the specific wording for the use of grouping methods (read-across and chemical categories). Specifically:

''Substances whose physicochemical, toxicological and ecotoxicological properties are likely to be similar or follow a regular pattern as a result of structural similarity may be considered as a group or 'category' of substances. Application of the group concept requires that physicochemical properties, human health effects and environmental effects or environmental fate may be predicted from data for a reference substance within the group by interpolation to other substances in the group (read-across approach). This avoids the need to test every substance for every endpoint. The similarities may be based on:

(1) a common functional group,

(2) the common precursors and/or the likelihood of common breakdown products via physical and biological processes, which result in structurally similar chemicals, or

(3) a constant pattern in the changing of the potency of the properties across the category…."

Whilst the development of chemical categories and (Q)SARs are underpinned by the same principles of chemical similarity, there is no specific requirement to validate a category. Most likely this is because adhoc categories have been routinely used under the High Production Volume (HPV) programmes within the US and under the OECD. Under REACH, the adequacy and reliability of the category approach must be substantiated and documented in a format known as the Category (Analogue) Reporting Format (CRF/ARF). An ARF is used when a read-across is carried out for one substance to

another. A CRF considers a group of three of more substances. The technical guidance for chemical grouping is described in more detail in Chapter R6 (5). Case studies (10) developed by the drafting group authors of Chapter R6 are available at the former ECB website (http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/doc/EUR_22481_EN.pdf).

Read-across is the data gap filling mechanism used to interpolate predictions as part of the category. The approach can be qualitative or quantitative depending on the type of data available. Quantitative approaches might make use of a trend analysis (derivation of a local QSAR) using the category members themselves or rely on external QSARs or expert systems to derive the necessary predictions. It is beyond the scope to discuss the expert systems and other (Q)SARs here. There have been a several reviews that describe the state of the art of (Q)SARs for a number of REACH endpoints. Examples include references 11-18. Moreover the technical guidance for the different endpoints under REACH describe the availability of different (Q)SARs for each of the endpoints in turn. These (Q)SARs are not accepted for regulatory use, they are simply provided as a examples of what is described in the peer reviewed literature or available as software tools (both commercial and public).

For categories, the OECD Toolbox (OECD TB) is probably the best known tool since its prototype release in early 2008. Phase 2, a 4 year project funded by the European Chemicals Agency, ECHA commenced late 2008. The Toolbox aids in the development, evaluation, justification and documentation of chemical categories. It can verify whether a substance is part of an existing established category e.g. US EPA, OECD HPV category. It also possesses the functionality to develop endpoint specific categories making use of mode/mechanistic/empirical/structural "profilers". It is envisaged that the OECD Toolbox will be extensively used in evaluating the categories and (Q)SARs submitted for REACH and potentially for other regulatory programmes. The current version of the OECD Toolbox is v2.3 and is freely available from the following websites: www.oecd.org/existingchemicals/qsar and www.qsartoolbox.org. At the time of writing, the version of the OECD Toolbox was v2.0.

Other tools that can play a role in evaluating categories include the Industry funded AMBIT, and the Toxmatch chemical similarity tool – both of which were developed by Ideaconsult Ltd, the latter as part of a JRC contract and are freely available from http://ambit.sourceforge.net/ and http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxmatch websites.

Whilst there is extensive technical guidance for the development of chemical categories and the use of (Q)SARs for regulatory purposes such as REACH, there remains little practical guidance or example case studies for how some of these tools can or should be applied in either the evaluation of existing (Q)SARs or the formation of robust categories. No doubt further guidance will be published by ECHA in due course for REACH, we aim in section 3 to provide some representative examples to illustrate how the principles and approaches can be potentially used in practice.

2.5. Exposure assessment

REACH requires evaluation throughout the entire life-cycle of a substance for exposure to workers, to consumers, and to all spheres of the environment. An exposure scenario is developed for each use of the substance. Risk characterisation is achieved by comparing the final hazard benchmark to the estimated exposure for a particular use. If the exposure is below the hazard benchmark, the use is determined to be safe. Since measured data for all uses is seldom available, models are used to estimate exposures. There are several models recommended by ECHA (19) for exposure estimations and each model has different inputs and assumptions. In the example discussed in section 4, the results from the different models will be compared for a professional worker dermal exposure scenario and compared to available measured data.

3. CASE STUDY ON SELECTED METHACRYLATES

Butyl methacrylate [97-88-1] (BMA) and methyl methacrylate [80-62-6] (MMA) were chosen as case study substances simply because they have been previously assessed under the OECD HPV programme (20) and hence were likely to be associated with a reasonable complement of (eco)-toxicity data. A full EU risk assessment report has also been completed for methyl methacrylate [80-62-6] and can be found at the former ECB website under http://esis.jrc.ec.europa.eu/index.php?PGM=ora (21). There is no intent to modify or change any of the conclusions/recommendations from these assessments, merely we wish to illustrate how non-testing approaches can play a role to either complement existing data in a weight of evidence approach by providing consistent and corroborating estimates.

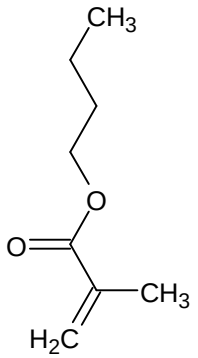
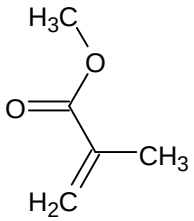
For a selection of endpoints, we will demonstrate how external (Q)SARs can be utilised to fulfil datagaps and the extent to which these estimates are concordant with known experimental values. A brief data matrix shown in Table 1 has been constructed for BMA and MMA using the summary information as reported in the respective SIDS documents (20). Selected endpoints from the four main domains: Physicochemical properties, mammalian toxicity, environmental fate and ecotoxicity will be illustrated.

3.1. Physicochemical properties

3.1.1. LogKow (octanol/water partition coefficient)

LogKow, the octanol/water partition coefficient is a parameter that is relied upon to give insight towards an array of properties cross cutting all these endpoint domains. LogKow serves as a good model for hydrophobicity. The hydrophobicity of a compound can provide an indication of how easily a substance might transverse across a cell membrane, whether it will be absorbed readily through the

Table 1. Summary information for butyl methacrylate [97-88-1] and methyl methacrylate [80-62-6]

Name	**Butyl methacrylate**		**Methyl methacrylate**	
CAS	97-88-1		80-62-6	
Structu				
	Experimental values taken from the SIDS Profile (SIAM 18)[1]	Estimated values	Experimental values taken from the SIDS dossier	Estimated values
Physicochemical roperties				
Melting Point	-50 deg C	nd	-48 deg C	nd
Boiling Point	163 deg C	nd	100-101 deg C	nd
Vapour Pressure	2.1 hPa @ 20 deg C	nd	36-47 hPa @ 20 deg C	
LogKow (partition coefficient)	2.99 @ 25 deg C	2.75 (KOWWIN v1.67) Substance within domain of KOWWIN	1.38 @ 20 deg C	1.27 (KOWWIN v1.67) Substance within domain of KOWWIN
Water solubility	0.36 g/L @ 25 deg C	nd	16 g/L @ 20 deg C	nd
Mammalian Toxicity				
Acute toxicity (oral)	Low	nd	LD50: 8420-10000 mg/kg (rat)	nd
Acute toxicity (dermal)	Low	nd	LD50: 5000-7500 mg/kg (rabbit)	nd
Acute toxicity (inhalation)	Low	nd	LC50: 7093 ppm (29.8 mg/l) (4hr) (rat)	nd
Irritation (eye)	Slight irritation (qualitative read-across)	nd	Slight irritant (rabbit)	nd
Irritation (skin)	Irritating in rabbits (qualitative read-across)	nd	Severe irritant (rabbit)	nd
Skin sensitisation	Likely weak sensitiser (qualitative read-across)	Potential sensitiser based on structural alerts, available experimental data within the OECD TB and TIMES refutes the predictions inferred	Sensitising (guinea pig) Reported EC3 of 60% in acetone and AOO 90% (Betts *et al*, 2006)	V Weak/Non sensitiser – TIMES could not discriminate between weak and non-sensitising. Part of the training set – categorised as non-v weak sensitiser experimentally
Mutagenicity (*in vitro*) Ames	No data (Methacrylate esters have been tested *in vitro* and *in vivo* for gene mutations, chromosome mutations and aneugenic effects over relevant dose ranges. There is no indication that methacrylate esters in the category cause gene mutagens	Predicted positive by TIMES, Flagged by OECD TB alerts. TIMES had a negative result for Ames for butyl methacrylate which was in its training set. The TB reported negative Ames and a category approach performed resulted in a negative call.	Negative w/wo activation)	Predicted positive by TIMES, Flagged by OECD TB alerts. TIMES had a negative result for Ames for methyl methacrylate which was in its training set. The TB reported negative Ames and a category approach performed resulted in a negative call.
Mutagenicity (*in vitro*) Chrom abs	No data	Predicted positive by TIMES. Qualitative read-across using the Toolbox and Leadscope suggest butyl methacrylate is positive for clastogenic effects *in vitro*	Positive in CHO cells and mouse lymphoma cells w/wo metabolic activation	No robust prediction possible. Substance 100 % outside of structural domain of model. Predicted CA on account of downstream metabolite
Subacute toxicity (inhalation)	Development of lesions in the olfactory region of the respiratory tract. LOEC 952 ppm (5626 mg/m3); NOEC 310 ppm (1832 mg/m3)	nd	No data	nd
Chronic toxicity	NOEL (male rats) 30 mg/bw day based upon reduced splenic weights and atrophy of the splenic red pulp. Noel (female rats) 300 mg/kg bw day based upon	nd	104 wk rat oral NOEL> 2000 ppm 104 wk rat inhalation NOAEC for local effects on the resp tract: 25 ppm (0.1 mg/L)	nd

	changes in blood and urine parameters indicative of effects on the kidneys, however these were not confirmed histopathologically (OECD 422)		104 wk rat inhalation NOAEC for systemic effects on the resp tract: 100 ppm (0.4 mg/L)	
Reproductive toxicity	Number of corpora luea & implantation sites was decreased at 1000 mg/kg bw day level giving a NOEL of 300 mg/kg/day (OECD 422)	nd	NOAEC = 9000 ppm inhalation (mouse) NOAEC> 2028 ppm inhalation (teratogenicity) (rat)	
Environmental Fate				
Biodegradation	Readily biodegradable	BOD of 0.8527 Catalogic	Readily biodegradable	nd
Adsorption	No significant presence in soil or sediment	nd	No data	nd
Bioaccumulation	Potential to bioaccumulate on the basis of LogKow	nd	No data	nd
Bioconcentration	No data	nd	No data	nd
Ecotoxicology				
Acute fish	Within the range of 100 mg/L (EMA) -2.78 mg/L (2-EHMA) based on category	5.6 mg/L reported in the MITI database for *O. latipes* 96 hr LC50 (OECD TB). ECOSAR predicts a 96hr LC50 value of 5.55 mg/L using the expt LogKow value	*L. macrochirus* LC50 (72hr): 264 mg/L; (96hr) 191 mg/L *O.mykiss* LC50 (96hr): >79 mg/L, NOEC (96hr): 40 mg/L	nd
Acute daphnia	Within the range of >66 mg/L (EMA) to 4.6 mg/L (2-EHMA) based on category	nd	EC50 (48hr): 69 mg/L; 84 mg/L (OECD TB v2.0)	
72 hr Algae acute	Within the range of >110 mg/L (EMA) to 7.68 mg/L (2-EHMA) based on category	nd	EC50 (96hr) = 170 mg/L (*S.capricornutum*)	nd
Chronic fish	No data	nd	No data	nd
Chronic daphnia (NOEC)	18 mg/L (EMA) – 0.105 mg/L (2-EHMA) based on category	nd	NOEC (21d): 37 mg/L	nd
Chronic algae (NOEC)	110 mg/L (EMA) – 5.8 mg/L (i-BMA) based on category	nd	NOEC (96hr): 100 mg/L (*S.capricornutum*)	nd

[1]Within SIAM 18, short chain linear and branched unsaturated alkyl methacrylates were grouped together as a chemical category. The members were respectively ethyl methacrylate (EMA), iso-butyl methacrylate (i-BMA), n-butyl methacrylate (n-BMA) and 2-ethylhexyl methacrylate (2-EHMA).

skin (22) or gut (23); whether it might be taken up in groundwater to pollute waterways (24). Many QSAR models for aquatic acute toxicity rely on LogKow as their driving factor due to a narcosis mechanism (25-26). Hydrophobicity is also useful in evaluating likely bioaccumulation potential (16).

There are numerous (Q)SAR models available both publically and commercially for the estimation of LogKow. Many of these are referenced in Dearden and Worth (27) as well as the Technical Guidance for REACH (5). One common model that is well known and commonly used is that of KOWWIN v1.68, developed by SRC Inc (formally Syracuse Research Corporation) and the US EPA (28). This model is routinely used as part of the US EPA's Premanufacture Notice (PMN) process (29) as well as the HPV programme under the OECD. Under REACH, a (Q)SAR model needs to be both characterised in accordance with its OECD principles and an assessment needs to be undertaken to substantiate that a substance of interest lies within the domain of applicability as defined for the (Q)SAR model (2). In an effort to illustrate this, we extracted the training set of compounds for KOWWIN v1.67 that is made available by the developers SRC, Inc. and the US EPA at http://esc.syrres.com/interkow/KowwinData.htm. At the time of writing, KOWWIN v1.67 was the most recent version. Since KOWWIN does not provide any explicit applicability domain aside from some guidance in terms of considering molecular weight and fragment descriptors as listed in the user manual; a domain was independently extracted using the commercial program, Domain Manager v1.02 software as cited previously. A structural domain on the basis of atom centred fragments was extracted owing to the fact that the (Q)SAR model itself as encoded in KOWWIN was based on structural fragments. The atom centred fragment approach is described in more detail in Dimitrov S *et al* (30). A set of rules are used to reflect the effect of different neighbours on a specified atom. The application of these rules allows the extraction of a set of atom centred fragments that can be used to characterise the structural domain of the atoms presented in a certain set of chemicals. The maximum and minimum values of molecular weight (MW) were also determined as a second criterion for assessing the KOWWIN domain. The MW range was as follows: Minimum MW 18.02, Maximum MW 719.92. In other words, for a substance to be considered within the domain of KOWWIN for our purposes, it had to be 100% within the structural domain as determined by atom centred fragments and have a MW value between 18 and 719. Butyl methacrylate and methyl methacrylate were introduced into the Domain Manager v1.02 software as a "test set" vs. the KOWWIN dataset as the "training set". The MW values of these substances are 142.18 and 100.12 which are well within the MW range of the KOWWIN training set. Both are also within 100% of

Table 2. Analogues similar to butyl methacrylate with respect to LogKow estimation

Chemical Name	CAS Number	SMILES	Exp LogKow	Kowwin Est	Similarity Index
Ethyl methacrylate	97-63-2	O=C(OCC)C(=C)C	1.94	1.77	0.727
Methacrylic acid, i-Butyl ester	97-86-9	C(=O)(OCC(C)C)C(C)=C	2.66	2.67	0.788
Methacrylic acid, n-Butyl ester	97-88-1	C(=O)(OCCCC)C(C)=C	2.88	2.75	1
Isobutyl acrylate	106-63-8	C=CC(=O)OCC(C)C	2.22	2.13	0.758
Butyl acrylate	141-32-2	O=C(OCCCC)C=C	2.36	2.2	0.939
t-Butyl methacrylate	585-07-9	CC(=C)C(=O)OC(C)(C)C	2.54	2.64	0.727

the structural domain as defined by the atom centred fragments. On the basis of our domain assessment of KOWWIN, butyl methacrylate and methyl would be considered "preliminary valid" since they satisfy these two conditions we have nominally set for being "in domain". Accordingly, we would expect LogKow estimates for both substances to be reasonable. Belonging to an applicability domain of a model increases the likelihood of a reasonable prediction but is not a guarantee. Some evaluation of related analogues and their predictions relative to their experimental values needs to be conducted to substantiate that any (Q)SAR estimate is indeed both reasonable and predictive. These analogues may be taken from the training set of the (Q)SAR model itself, i.e. are there any related substances within KOWWIN or from other sources and how do their predicted/experimental LogKow values compare. In this case, Toxmatch v1.06 (Ideaconsult Ltd) was used to search and retrieve analogues from the KOWWIN training set to enable a comparison of experimental and estimated LogKow values for butyl methacrylate. The training set of KOWWIN model was imported into the Toxmatch v1.06 software. The Tanimoto distance (fingerprints,kNN) was chosen as the similarity index approach. The "Tanimoto distance (fingerprints,kNN)" method calculates the average Tanimoto index between the fingerprints for each query chemical within the KOWWIN dataset and the fingerprints for the k most similar chemicals from the set (where k = 10 as default). The most similar chemicals are those with the highest Tanimoto index values. Butyl methacrylate, our substance of interest was then imported as the test set chemical and a pairwise similarity performed using the Tanimoto distance relative to the training set. The most similar analogues according to this Tanimoto distance index were then reviewed, where an index of 0.7 was arbitrarily taken as a quantitative measure of "most similar". Five other analogues were identified which are shown in Table 2 together with their respective similarity index values, experimental LogKow values and estimated LogKow values (by KOWWIN). All the methacrylates were reasonably predicted by KOWWIN v1.67 compared with their reported experimental LogKow values. Accordingly the estimated LogKow of butyl methacrylate, is considered to be a reasonable one. KOWWIN actually has butyl methacrylate as part of its training set of compounds. The estimated and experimental values are in good agreement with each other. The same procedure was conducted for methyl methacrylate, results not shown.

LogKow is well recognised as an important parameter for a range of different endpoints. KOWWIN v1.67 is an example of one (Q)SAR model that enables the prediction of LogKow. A evaluation of KOWWIN v1.67 with respect to the OECD principles is required to meet the conditions for (Q)SARs as laid out in Annex XI. Here we have described one of the principles – domain of applicability and illustrated how this can be extracted using the available training set of data from KOWWIN v1.67. We have applied this approach to butyl methacrylate and methyl methacrylate and found both to satisfy the two criteria specified for domain inclusion. To substantiate that the predicted value of butyl methacrylate was reasonable, an evaluation of related analogues within the training set using Toxmatch v1.06 identified 5 other analogues with predicted and experimental values in good agreement. This demonstrated that methacrylates are indeed well represented as a chemical class in the training set with robust predictions. Subject to the appropriate documentation being drafted (i.e. QMRF, QPRF) to substantiate this evaluation made, the conditions of use for REACH have been met.

3.2. Mammalian toxicity

3.2.1. Skin sensitisation

Much is understood about the mechanisms underpinning skin sensitisation. Further information can be found in the following references (31-32). A substance must negotiate a number of steps before sensitisation is induced. A sensitising chemical must penetrate through the *stratum corneum* and form a stable association with a carrier protein in order to deliver dermal trauma sufficient to induce and upregulate epidermal cytokines. These processes are necessary for the mobilisation, migration and maturation of LC, and for the chemical to be inherently immunogenic in such a way that a T lymphocyte response of sufficient magnitude is stimulated (32). All these steps are not considered to be equally important. The step which is dependent on the sensitising chemical itself, the rate determining step, provides insight on how to evaluate the skin sensitisation potential of a given chemical and has been discussed at some length in references (33-34). Efforts to predict skin sensitisers typically focus on identifying electrophilic features in chemicals (structural alerts) and relating these back to skin sensitisation potential.

There have been many efforts to develop models that relate the electrophilic features of chemicals to the skin sensitisation potential as shown in references (35-36). Most recent efforts have been described by Roberts and Aptula (37) who described a set of principles that characterised sensitisers by their reaction mechanisms which have since been implemented into Smiles ARbitrary Target Specification (SMARTS) codes for easy re-use (38) (available as a module within Toxtree, from the former ECB website, see http://ihcp.jrc.ec.europa.eu/our_labs/computational_toxicology/qsar_tools/toxtree). Other initiatives have focused on the development of expert systems. Derek for Windows

(DfW) is a well known knowledge based system that encodes chemistry and toxicity information in the form of toxicophores (39-40). The hybrid expert system TIssue MEtabolism Simulator for Skin Sensitisation (TIMES-SS) encodes structure toxicity and structure metabolism relationships through a number of transformations simulating skin metabolism and interaction of the generated reactive metabolites with skin proteins. The skin metabolism simulator mimics metabolism using 2D structural information. Metabolic pathways are generated based on a set of 236 hierarchically ordered principal transformations including spontaneous reactions and enzyme catalysed reactions (phase I and II). The covalent reactions with proteins are described by 47 alerting groups (structural alerts). Some of these alerts are additionally underpinned by mechanistically based 3D-QSARs to refine the predictions. These 3D-QSAR models depend on both the structural alert and factors that influence its reactivity - steric effects, molecular size, shape, solubility, lipophilicity and electronic properties (41-43). The rules implemented into TIMES-SS have also since been incorporated into the OECD Toolbox.

Butyl methacrylate and methyl methacrylate were processed within TIMES, the OECD Toolbox and the SMARTS implementation within Toxtree to gain some insights about likely skin sensitisation potential.

The SMARTS implementation within Toxtree identified both methacrylates as potential Michael acceptor. This is commensurate with the reaction mechanistic domains outlined in Roberts and Aptula (37). The same alerting group is flagged by the protein binding alerts as encoded in the OECD Toolbox v2.0. The rationale provided is as follows:

"Michael acceptors are double or triple bonds with neighbouring electron-withdrawing group. They can clearly be seen to have an electron-deficient double bond that is susceptible to nucleophilic attack. Michael-type addition provides a means of covalent adduct formation at an electrophilic centre, without any leaving group. Direct addition of a nucleophile can take place across a double or triple carbon-carbon bond if it is attached to a highly polarised substituent that permits the resultant negatively charged transition state to be stabilised, as for example in acrolein, acrylamide. Compounds with double or triple bounds adjacent to a C=O group are known as alpha,beta-unsaturated carbonyl compounds. Nucleophiles will undergo conjugate additions with them. C=O group profoundly affects the reactivity of the double or triple bond. Isolated C=C bonds are nucleophilic but conjugated C=C bonds are electrophilic. Proteins are good nucleophiles for conjugate addition reactions with these compounds. In the LLNA, most of acrylates and methacrylates are much less potent than expected from their reactivity. This is believed to be partly due to their volatility (for lower homologues such as ethyl acrylate, theoretical EC3 = 1.3 %, experimental EC3 = 28 %) and partly due to their rapid polymerisation (with loss of electrophilic reactivity) when exposed to air. Acrylates and methacrylates usually fail to show their true potential to sensitise because of polymerisation and in some cases evaporation under normal LLNA conditions and under many occupational and domestic exposure conditions".

Within TIMES, both methacrylates form part of the training set, in that there is a flag that experimental results exist. For butyl methacrylate, this value is an assignment made by the BfR (44) which categorises butyl methacrylate as a questionable or unlikely sensitiser and methyl methacrylate is cited as a very weak – non-sensitising. The respective predictions from TIMES were non-sensitiser for butyl methacrylate and "unable to predict" for methyl methacrylate owing to TIMES being unable to discriminate between a weak and non sensitising classification. An assessment of domain is automatically conducted for butyl methacrylate within the TIMES system. TIMES uses a modular approach to domain assessment starting with global requirements, structural domain, metabolic domain and interpolation domain. More explanation is provided in the following references that describe how TIMES processes its structures and assesses the relevance of the predictions made. Reference (42) in particular describes the overall characterisation of the TIMES sensitisation model with respect to all the OECD principles. Data from the OECD Toolbox for butyl methacrylate cites several data points, 3 from guinea pig assays, as Freund's complete adjuvant test and a final data point which is cited within the TIMES training set. In two out of the three guinea pig cases, the overall call was negative. Overall, the weight of evidence would support a negative call for butyl methacrylate. For methyl methacrylate, the calls are conflicting with a sensitising call in guinea pigs and a negative call in the LLNA owing to a reported EC3 of 60% and 90% in Acetone and AOO respectively where AOO is an acetone/olive oil mix (45). The OECD Toolbox was used to collect data on related methacrylates; on the basis of a qualitative read-across, based on 5 nearest neighbours (Figure 1), an overall call of positive was inferred for methyl methacrylate. In Figure 1, the nearest neighbours are marked in grey circles, the target substance MMA is a black triangle (note data exists for MMA hence there is more than one triangle reflected in the figure) and other related analogues as black squares.

Butyl and methyl methacrylate have the potential to be sensitisers due to their ability to react via a Michael addition route. Experimental evidence available within TIMES and the OECD Toolbox suggest that sensitisation is unlikely to be observed for butyl methacrylate. Equivocal data exists for methyl methacrylate and on the basis of read-across within the Toolbox, a conservative estimate of sensitising would be made.

3.2.2. Mutagenicity (*in vitro*): Ames

Covalent bond formation as a rate determining step is not unique to skin sensitisation. Schultz *et al.* (46) described a conceptual framework for predicting the toxicity of reactive chemicals where plausible molecular initiating events were based on covalent reactions with nucleophiles in proteins or DNA, and would ultimately lead to a variety of different adverse outcomes such as aquatic fish toxicity, mutagenicity, hepatocyte cytoxicity or

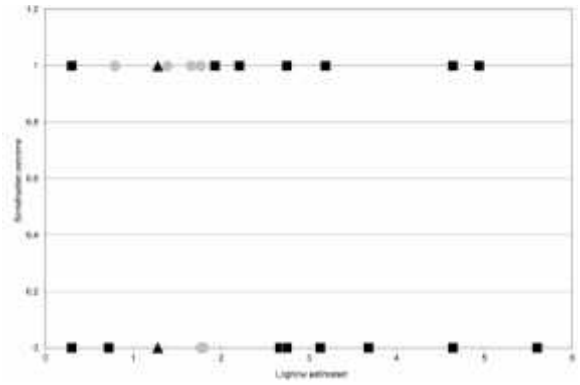


Figure 1. Read-across prediction for methyl methacrylate.

respiratory toxicity. Aptula and Roberts (37) illustrated this concept using aquatic toxicity and sensitisation as example endpoints. Electrophilicity is well known to be an important factor in driving mutagenicity and carcinogenicity (47). Seminal work by Ashby and Tennant (48-50) described structural alerts for carcinogenicity. They found that the electrophilicity of chemicals correlated very well with mutagenicity in the Ames test. Similar findings have been published by Benigni and co-workers (51). His extensive evaluations on structural alerts developed by Ashby and Tennant (48), Kazius *et al.* (52) and Woo, (53) have been additionally implemented into the software program Toxtree (54). Mekenyan *et al.* have developed the TIMES system for the prediction of Ames mutagenicity (55) and *in vitro* chromosomal aberrations (56). The structure and implementation is similar to that for skin sensitisation. Structure toxicity and structure metabolism relationships are encoded through a number of transformations simulating liver metabolism and interaction of the generated reactive metabolites with DNA.

Butyl methacrylate was processed through the OECD Toolbox and TIMES to determine what alerts if any were flagged and what the likely prediction in Ames was. The OECD Toolbox had experimental Ames data with/without metabolic activation which showed butyl methacrylate to be negative. A review of alerts identified no alerts from the DNA OASIS binding scheme but a polarised alkene/Michael addition flag from the DNA OECD binding profiler. The following rationale was provided: "An initial Michael addition mechanism has been suggested to be primarily responsible for the ability of these chemicals to alkylate DNA" (57). TIMES reported an overall negative call for butyl methacrylate since it formed part of the training set and was associated with a negative Ames result. It did flag an alerting group on account of the Michael addition mechanism and this actually drove a positive prediction. Butyl methacrylate was within 90% of the structural domain and 100% of the alert performance. It was categorised as being outside the domain on account of the 100% criterion for structural domain not being met.

Within the TIMES chromosomal aberration model, butyl methacrylate is predicted positive, the same alerting group for Michael addition being triggered which drives the potential response.

Thus the OECD Toolbox highlighted the same Michael addition route as potentially contributing to positive outcomes without activation in Ames and *in vitro* chromosomal aberration assays. The TIMES system predicted butyl methacrylate as being positive on account of this alerting group, however for Ames, it refuted the prediction in favour of the available experimental data.

A category approach was attempted within the OECD Toolbox to make complementary predictions of Ames and chromosomal aberration results. In this case, analogues with available data and similar with respect to this reaction mechanism were identified. The Oncologic profiler within the OECD Toolbox categorised butyl methacrylate as a substance containing a reactive acrylate functionality. This resulted in 1079 potential analogues including butyl methacrylate. Of these analogues, only 36 were associated with Ames experimental data (Ames without S9 since it was assumed that Butyl methacrylate was a direct acting electrophile). A subcategorisation was performed to ensure that the analogues used in the read-across were similar to butyl methacrylate in that they did not contain any other functional groups that would enable an alternative reaction pathway to occur. On the basis of the 5 nearest neighbours, butyl methacrylate is inferred to be negative in Ames. The category members substantiate the hypothesis that despite the potential for a reaction to occur with DNA, in practice, methacrylates and acrylates are not found to be experimentally mutagenic in Ames. Table 3 lists the category members together with their CAS numbers, names and SMILES codes.

A similar read-across exercise was conducted to elicit an estimated call for *in vitro* chromosomal aberration. In this case, from the starting set of analogues categorised by the Oncologic profiler, only a handful of analogues remained which inferred a likely positive outcome for butyl methacrylate. However in evaluating the analogues (Table 4) more closely, we see that the LogKow value of butyl methacrylate far exceeds that of the remaining 3 analogues, suggesting that this result is subject to considerable uncertainty since this would be an extrapolation for butyl methacrylate which lies outside of the domain of the read-across. The 3 analogues with available experimental data all are significantly less hydrophobic than butyl methacrylate.

A second search was conducted in Leadscope (http://www.leadscope.com), a data mining tool that contains structured data for a number of mammalian endpoints. A similarity search using butyl methacrylate as a key together with a flag to extract *in vitro* chromosomal aberration study results was undertaken. Table 5 identifies the substances and their associated data.

Again the set of data is limited but it reveals that short chain acrylates and methyl methacrylate have been shown to be positive. This provides an indication that butyl methacrylate may be a potential clastogen as part of a weight of evidence approach, even though a qualitative read-across for butyl methacrylate could not be reliably performed.

Table 3. Category members used in the read-across for Ames

CAS	Name	SMILES	Experimental Ames call
97-88-1	butyl methacrylate	C(=O)(C(=C)C)OCCCC	negative[1]
80-62-6	2-methyl-2-propenoic acid	C(=O)(C(=C)C)OC	negative
96-33-3	methyl acrylate	C(=O)(C=C)OC	negative
141-32-2	n-butyl acrylate	C(=O)(C=C)OCCCC	negative
103-11-7	2-ethylhexyl acrylate	C(=O)(C=C)OCC(CCCC)CC	negative
142-09-6	n-hexyl methacrylate	C(=O)(C(=C)C)OCCCCCC	negative
585-07-9	t-butyl methacrylate	C(=O)(C(=C)C)OC(C)(C)C	negative
623-91-6	diethyl (2e)-but-2-enedioate	C(=O)(C={t}CC(=O)OCC)OCC	negative
688-84-6	2-ethylhexyl methacrylate	C(=O)(C(=C)C)OCC(CCCC)CC	negative
923-26-2	2-hydroxypropyl methacrylate	C(=O)(C(=C)C)OCC(C)O	negative
999-55-3	allyl acrylate	C(=O)(C=C)OCC=C	negative
2157-01-9	n-octyl methacrylate	C(=O)(C(=C)C)OCCCCCCCC	negative
2210-28-8	propyl methacrylate	C(=O)(C(=C)C)OCCC	negative
3179-47-3	decyl methacrylate	C(=O)(C(=C)C)OCCCCCCCCCC	negative
4655-34-9	isopropyl methacrylate	C(=O)(C(=C)C)OC(C)C	negative
97-63-2	ethyl methacrylate	C(=O)(C(=C)C)OCC	negative
106-63-8	isobutyl acrylate	C(=O)(C=C)OCC(C)C	negative
868-77-9	2-hydroxyethyl methacrylate	C(=O)(C(=C)C)OCCO	negative
6983-79-5	9-cis-6,6'-diapo-psi,psi-carotenedioic acid	C(=O)(O)C=CC(C)=CC=CC(C)=CC=CC=C(C)C=CC=C(C)C=CC(=O)OC	positive
29964-84-9	isodecyl methacrylate	C(=O)(C(=C)C)OCCCCCCCC(C)C	negative

[1]Experimental data exists for butyl methacrylate which is added to this table for completeness.

Table 4. Analogues used in read-across of *in vitro* chromosomal aberration for BMA

CAS	Name	SMILES	LogKow estimated	ChromAbs
97-88-1	butyl methacrylate	C(=O)(C(=C)C)OCCCC	2.75	
140-88-5	ethyl acrylate	C(=O)(C=C)OCC	1.22	positive
80-62-6	methyl methacrylate	C(=O)(C(=C)C)OC	1.28	positive
96-33-3	methyl acrylate	C(=O)(C=C)OC	0.73	positive

Table 5. Output from Leadscope

Chemical Name	Study Type	Study Call	Species	Source
1,4-butanediol dimethacrylate	*in vitro* chromosome aberration	Negative	Chinese hamster (3)	cfsan-ofas
Ethyl methacrylate	*in vitro* chromosome aberration	Negative	Chinese hamster (2)	ntp
Ethyl Acrylate	*in vitro* chromosome aberration	Positive	Chinese hamster (3)	ntp
Methyl methacrylate	*in vitro* chromosome aberration	Positive	Chinese hamster (3)	ccris
Ethyl Acrylate	*in vitro* chromosome aberration	Positive	Chinese hamster (3)	ccris
n-Butyl acrylate	*in vitro* chromosome aberration	Positive	Chinese hamster (6)	ntp
Methyl methacrylate	*in vitro* chromosome aberration	Positive	Chinese hamster (3)	ntp
Ethyl Acrylate	*in vitro* chromosome aberration	Positive	Chinese hamster (3)	ntp

3.3. Environmental fate

3.3.1. Biodegradation

Catalogic (58), an expert system built on a same platform to that of TIMES, was used to make an estimate of ready biodegradation on the basis of the OECD MITI 301C study protocol. The Biochemical Oxygen Demand (BOD) value generated was 0.853, i.e. butyl methacrylate is likely to be readily biodegradable since the 60% threshold for 28 days is met on the basis of this 301C test guideline. In this case, within 28 days, 85% of BMA is expected to degrade, thus satisfying the criteria of ready biodegradability.

3.4. Ecotoxicity

3.4.1. Acute aquatic toxicity in fish

A reported value of 5.6 mg/L 96 hr LC50 for *O latipes* from the OECD Toolbox was actually available for butyl methacrylate. The profilers within the OECD TB assign BMA to a category of unspecific toxicity due to the methacrylate functionality. Verhaar *et* al (59) as encoded in the TB, places it in Class 3 (unspecific toxicity), OASIS MOA places it in unspecific reactivity and ECOSAR categorises it as a methacrylate vs. neutral organic (whereupon the mode of action would be simply narcosis and hence driven by hydrophobicity (25-26)).

ECOSAR, one of a suite of tools developed between SRC Inc and US EPA, was used to derive an acute fish LC50 prediction (29). A 96 hr LC50 of 5.55 mg/L was predicted based on the methacrylates QSAR and relying on the experimental LogKow value of 2.99 as reported in the SIDS document (20). The fish 96 hr LC50 values used to develop the SAR were measured and the octanol water partition coefficients (Kow) were calculated using KOWWIN v 1.67. The actual reported SAR equation is: Log 96 hr LC50 (mmol/L) = -0.6845 (LogKow) + 0.6381 The LC50 is in millimoles per litre (mM/L); N = 12 + 4; and the coefficient of determination (R2) = 0.7139. The dataset underpinning this QSAR is actually based on results from 9 different substances, one of these is subject to confidential business information (CBI), the remaining substances include methyl methacrylate amongst others. To evaluate the QSAR model further, the training set as reported in the ECOSAR Help manual was extracted as shown in Table 6.

This training set provided a basis for defining an applicability domain. The ranges of estimated LogKow and MW values together with the mode of action as being methacrylate as per ECOSAR's classes was proposed as appropriate criteria for a domain. The LogKow and MW

Table 6. ECOSAR training set for fish 96 hr LC50 methacrylate QSAR

Cas	Smiles	Chemical_name	LogKow_est	Expt_96hr_ LC50_ mg/l	Est_96hr_LC50 (mg/l)
868-77-9	CC(=C)C(=O)OCCO	2-hydroxyethyl methacrylate	0.3	227	269.65
2530-85-0	CC(=C)C(=O)OCCC[Si](OC)(OC)OC	2-Propenoic acid, 2-methyl-, 3-(trimethoxysilyl)propyl ester	0.75	175	1079.35
80-62-6	CC(=C)C(=O)OC	Methyl methacrylate	1.28	259	49.43
80-62-6	CC(=C)C(=O)OC	Methyl methacrylate	1.28	151	49.43
80-62-6	CC(=C)C(=O)OC	Methyl methacrylate	1.28	191	49.43
2370-63-0	CCOCCOC(=O)C(=C)C	2-propenoic acid, 2-methyl-, 2-ethoxyethyl ester	1.49	27.7	687.53
2455-24-5	CC(=C)C(=O)OCC1CCCO1	2-Propenoic acid, 2-methyl-, (tetrahydro-2-furanyl)methyl ester	1.8	34.7	739.73
96-05-9	CC(=C)C(=O)OCC=C	2-propenoic acid, 2-methyl-, 2-propenyl ester	2.12	0.99	548.27
4655-34-9	CC(C)OC(=O)C(=C)C	2-Propenoic acid, 2-methyl-, 1-methylethyl ester	2.18	38	16.06
2495-37-6	CC(=C)C(=O)OCC1=CC=CC=C1	2-Propenoic acid, 2-methyl-, phenylmethyl ester	2.98	4.67	14.20
		CBI	2.3	32	882.26
		CBI	2.3	34	882.26

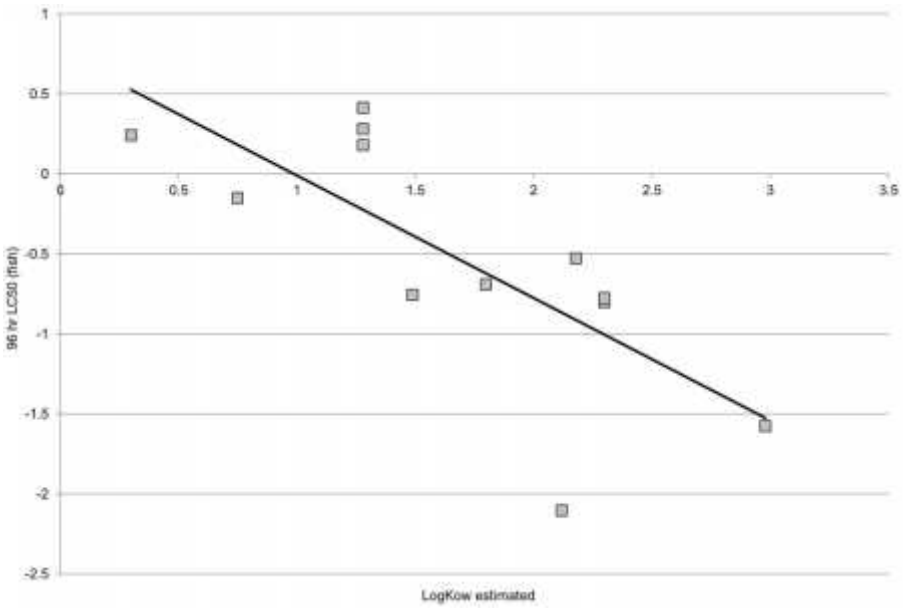


Figure 2. Re-derived regression equation for acute fish toxicity methacrylate QSAR in ECOSAR.

ranges were as follows: 0.3-2.98 and 100-248. Evaluating the ranges with respect to butyl methacrylate which has an estimated LogKow of 2.75 and a MW of 142.20 shows that the model should be appropriate for use. However as a second step, an attempt was made to re-derive the QSAR based on the training set to verify that the regression equation and corresponding reported statistics were reasonable. In fact, a simple linear regression performed within Minitab v15 statistical package resulted in a different regression equation with substantially poorer statistics than those reported by the ECOSAR user manual. The equation was as follows: Log 96 hr LC50 (mmol/L) = -0.7661 (LogKow) + 0.7578. The coefficient of determination in this case was only 0.554.

From the line plot depicted in Figure 2, it is evident that there is variability in the experimental values and a great deal of scatter with some substances notably allyl methacrylate [96-05-9] being poorly predicted. This raised some uncertainties in relying on the ECOSAR estimate alone and a trend analysis was attempted in the Toolbox to establish a more reliable estimate for the 96 hr LC50 in fish.

A category was defined based on the ECOSAR classes. This resulted in 116 substances. Of those, there were 13 substances with LC50 data in fish (a variety of species). Removing substances with very low water solubilities and subcategorising to remove additional functional groups resulted in a set of 9 analogues including butyl methacrylate from which an interpolated LC50 could be derived. A LC50 mol/L of 2.68E-04 mol/L was estimated for butyl methacrylate which equated to 38.1 mg/L. This appears to be a reasonable estimate of the potential toxicity of BMA and within an order of magnitude of that observed in *O. latipes* as referenced in the OECD Toolbox.

The ECOSAR estimate for methyl methacrylate gave rise to a conservative estimate of 49.43 mg/L. Methyl methacrylate lies within the domain of the model, on account of forming a part of the training set. However as evidenced from the evaluation already conducted, the ECOSAR model is not very robust and fails to provide reliable realistic predictions of methacrylates. Using the OECD TB, an estimated value was derived which provided a comparable yet conservative value of 82.8 mg/L. The

OECD SIDS dossier (20) quoted values ranging from >79 mg/L in *O. mykiss* to 191 mg/L in *L. macrochirus*.

3.4.2. Acute aquatic toxicity in *D. Magna*

Methyl methacrylate was assessed for its toxicity to *Daphnia magna*. An ECOSAR prediction was derived which gave rise to an estimated 48 hr LC50 of 33.112 mg/L. A closer evaluation of the SAR underpinning this prediction was performed and ascertained that the SAR within ECOSAR was based on a sole data point from a substance subject to CBI. The SAR quoted is given as 48 hr LogLC50 (mmol/L) = 0.377 - 0.6214 (LogKow). It is unclear how a regression equation was arrived from this datapoint. For REACH, it is unclear how the conditions of use could be fulfilled since the training set of 1 chemical is insufficient. Accordingly a trend analysis on the basis of deriving a category within the OECD TB was conducted. Initially a category was created on the basis of the OASIS Mode of Action to extract out a starting category of 29,309 substances. From that starting list, a trend analysis was undertaken to extract potential analogues that had associated *D. magna* 48 hr EC50 data. 135 analogues were identified with associated experimental data. These were filtered to remove substances with low predicted water solubility. The remaining analogues were subcategorised to remove inorganics, mixtures and dissociating substances. This resulted in 116 analogues, from which further subcategorisations were conducted to remove substances not acting by the same reaction mechanism. The remaining data points resulted in a fair correlation from which an estimated EC50 of 1.38E-03 mol/L (138 mg/L) was derived (results not shown). This is in reasonable concordance with the reported EC50 in the Toolbox (84 mg/L) as well as the SIDS experimental value of 69 mg/L (20).

3.5. Summary remarks

In the previous sections, we have outlined the REACH framework and the scope and flexibility of using non-testing approaches to address information requirements. For a selection of endpoints for 2 compounds, we have shown the practical possibilities of using different tools to evaluate existing (Q)SAR models, substantiate their estimates with analogues as well as derive new (Q)SAR models. The results were promising with estimates being derived that corroborated the experimental data available. In spite of the apparent stringent conditions of use required for non-testing approaches, we have demonstrated with practical examples how compliance for hazard characterisation may be adequately addressed for REACH.

4. EXPOSURE ASSESSMENT ON METHYL METHACRYLATE

Exposure assessment is a critical aspect of any risk assessment. Here we illustrate an exposure assessment for methyl methacrylate focusing in on dermal exposure given the hazard profile of this compound and its sensitising potential. Dermal exposures can be estimated under REACH using either measured data or recommended models. A toolbox of tiered models are described in the ECHA guidance to address both occupational and consumer exposures. The simplest models, Tier 1 models require few inputs and give rise to conservative outputs. Acceptable risk values obtained from Tier 1 models as quantified by risk characterisation ratios (RCR) less than 1 usually merit no further action. Tier 2 models require more detailed inputs. Occupational exposure estimation is outlined in Chapter R14 of the ECHA guidance (19). The ECETOC Targeted Risk Assessment Model (TRAM) is described as an acceptable Tier 1 model (60). The RCR is the estimated exposure divided by the derived no effects level (DNEL). The other dermal exposure model recommended for REACH is RISKOFDERM which is considered a Tier 2 model as it requires more detailed input (61).

4.1. Dermal sensitisation exposure assessment

A dermal sensitisation exposure assessment was undertaken for methyl methacrylate (MMA) using the ECHA guidance. Models available for estimating dermal exposure were compared to published dermal exposure data in order to determine whether there was control of risk for REACH. The exposure scenario considered was a dental technician working in a dental laboratory for 8 hrs a day. The scenario considered was a dental technician handling monomer MMA liquid whilst manufacturing or repairing orthodontic splints and dentures. Dermal exposure and inhalation exposure could therefore occur as the technician poured liquid MMA into moulds and added the polymerisation initiator.

4.2. Determination of the local effects dermal DNEL

MMA has been identified as a skin sensitiser. There is ample human evidence of skin sensitisation from workplace contact with MMA resulting in contact allergy (62). The former ECB published a risk assessment for MMA in 2002 (21). In this document, only systemic long term effects from dermal exposure based on the older Estimation and Assessment of Substance Exposure (EASE) model was addressed. The resulting daily dermal exposure estimate was 1000 micro g MMA/cm^2. Under REACH, local effects such as sensitisation must also be considered. Data from the local lymph node assay (LLNA) on the skin sensitisation potency of MMA (45) was available. The ECHA guidance from R.8 Appendix 8-10 Skin Sensitisation and the EC3 value from LLNA was used to derive a DNEL for local dermal effects. The EC3 is the effective concentration for a stimulation index (SI) of 3 in proliferation of lymph node cells. A SI of 3 in one or more test concentration categorises that substance as a sensitiser. The EC3 value was reported as 60% (categorised as a weak sensitiser; (63)) and ECHA guidance indicates this is considered as a LOAEL for induction (45). Following the R.8 Appendix 8-10 guidelines, the EC3 value can be converted from % to micro g/cm^2 by multiplying by 250 i.e. EC3 = 60% * 250 = 15,000 micro g/cm^2. The assessment factors are given in Table 7. The resulting local effects dermal DNEL is determined by dividing the EC3 by the overall AF. Thus, the Local effects dermal DNEL is 15,000 micro g/cm^2 / 250 = 60 micro g/cm^2.

Table 7. Assessment factors

Description		**Worker Factors**	**Remark**
Interspecies variation	AF1	10	As per ECHA R.8 Appendix 8-10
	AF2	2.5	Default as per ECHA R.8.4.3
	AF3	NA	Acute effect
	AF4	5	Default as per ECHA R.8.4.3
	AF5	2	LOAEL
	AF6	1	Robust
Overall AF[1]		**250**	

[1]Overall AF = AF1 x AF2 x AF3 x AF4

Table 8. Inputs for ECETOC TRAM model

Parameter	**Input**
Vapour Pressure	4.7E+03 Pa
PROCs	15, 13
Substance	Substance is a liquid, 100% pure
Time of Activity	8 hrs/day
Location and ventilation	indoors without any local exhaust ventilation
Skin Area exposed	Assume both hands are exposed (820cm^2) to be consistent with TRAM
Personnel Protective Equipment (PPE)	None

Table 9. ECETOC-TRA Model results for worker exposure

	Dermal exposure estimated (mg/kg/day)	**Dermal exposure converted to micro g/cm^2**	**Risk Characterisation Ratio (RCR)**
Dental technician scenario (PROC 15)	0.34	27	0.5
Dental technician scenario (PROC 13)	13.7	1060	18
ECB Risk Assessment Report from 2002	Not Reported	1000	17

Table 10. Inputs for RISKOFDERM model

Input parameters	Inputs
What is the quality of the ventilation?	Poor ventilation
What is the frequency of skin contact with the contamination?	More than rare contact
What kind of skin contact occurs?	Light contact
What type of product is handled?	Liquid
Do significant amounts of aerosols occur?	No
What is the level of automation of the task?	Manual task
Application rate of product (L/min or kg/min)	0.005
Cumulative duration of scenario per shift (min)	480

4.3. Methods for exposure assessment

Models can be used to estimate potential dermal exposure to the dental technicians based on defined exposure scenarios as follows. The dental technician (considered a professional worker under REACH, rather than an industrial worker) works 8 hrs a day. He/she mixes and pours small quantities of liquid MMA into moulds and after the cure process handles the finished product containing residual levels of MMA. The activities performed need to be matched with the ECHA R.12 Use descriptor system. Perfect matches do not always exist but in this case there were two possible matches. The most reasonable process categories (PROC) were PROC 15, use of substance at small laboratory scale (1 L or 1 kg) or PROC 13 treatment of articles by dipping and pouring. The first model considered was ECETOC-TRAM, the Tier 1 model. When a Tier 1 outcome indicates control of risk there is usually high confidence in the results. In practice, deciding on the appropriate PROC can be difficult which results in additional uncertainty as in this case. The inputs to the TRAM for worker exposure and are listed in Table 8 and the outputs from the TRAM are listed in Table 9.

The model reports potential systemic dermal exposure. This can be converted to a dose per surface area. The exposure occurs on the hands and for this scenario a typical male worker was used to match the defaults of the TRAM. The surface area exposed is 820 cm^2 and the body weight is 65 kg which enables conversion to micro g/cm^2 as follows:

(0.34 mg/kg/day x 65 kg)/ 820 cm^2 = 0.027 mg/cm^2 or 27 micro g/cm^2 for PROC 15
(13.7 mg/kg/day x 65 kg)/820 cm2 = 1.06 mg/cm^2 or 1060 micro g/cm^2 for PROC 13

These results are then divided by the DNEL to obtain the risk characterisation ratio (RCR). As can be seen from Table 9, PROC 15 shows control of risk but PROC 13 does not. In addition, the ECB report (21) as written would not have demonstrated control of risk under REACH. At this point it is difficult to feel confident that PROC 15 is sufficiently conservative for this scenario since both PROC 13 and the ECB report are more than 30 times higher (21). A higher tier model such as RISKOFDERM can be utilised which incorporates more information into the scenario. In RISKOFDERM, the type of activity is selected from six choices. For this scenario, filling, mixing and loading was deemed the best choice. The selected activity had specific questions to answer. The inputs selected are listed in Table 10. The 0.005 kg/min application rate results in the use of 2.4 kg/day which was selected to be higher than PROC 15 to ensure conservatism in the inputs. The text inputs are selected from a pull down menu where more information is given on the options.

Table 11. Refined dermal exposure estimates

Scenario	Potential dermal exposure (mg/day)	Time on skin (Eq. 1) (min)	Refined dermal exposure estimate (micro g/cm^2)	Risk Characterisation Ratio (RCR)
RISKOFDERM	296	53	39	0.7
Experimental Data	480	3	50	0.8

Table 12. Explanation of symbols

t:	Time on skin per day	answer	[s]
m:	mass, ECETOC estimate	From models	[mg]
R:	gas constant:	8.314	[J.K^{-1}.mol^{-1}]
T:	skin temperature	293	[K] (20°C – conservative estimate)
M:	molar mass for MMA	100	[g/mol]
beta:	coefficient of mass transfer in the vapour phase [m h^{-1}], for calculation: *beta* = 8.7 m/h, see below		
p:	vapour pressure of MMA		[Pa]
A:	area, EASE:		cm^2
K:	conversion factor:	3.60E+04	3.6.10^4

The 90th percentile result from RISKOFDERM was an exposure rate of 0.6 mg/min which when multiplied by 480 minutes results in an exposure to the hands of 0.36 mg/cm^2 or 360 micro g/cm^2. This results in an RCR of 6 which still does not show control of risk.

It must be remembered that these models evaluate potential dermal exposure. Neither of these models incorporate the fact that MMA is quite volatile and will evaporate off the skin while it is trying to absorb into the skin. ECHA R.14 Occupational Exposure Estimate details in Appendix 14.1 how to take the evaporation rate into account (19) with the following equation where the inputs are explained in Table 11:

$$t(s) = \frac{(mRT)\quad K}{(MbetapA)}$$

The total potential mass from the RISKOFDERM model is input into the equation. The only question is the dermal exposure area. In this scenario, there is no expectation for contamination to spread all over the 2 hands back and front since work is done on a small scale in a small area. The conservative approach is to put in a small area which will result in a longer time on the skin such as a finger tip or 2 cm^2. The resulting time on skin is 53 minutes rather than the entire workday of 480 minutes. When the 53 minutes rather than the 480 minutes are multiplied by the 0.6 mg/min exposure rate the refined result from RISKOFDERM is 39 micro g/cm^2.

As can be seen, the RISKOFDERM model with the inclusion of the evaporation effect is able to show control of risk and reinforces the earlier selection of PROC 15 which did not include the evaporation effect. To put into context, it is important to compare how these model results concur with actual measured dermal exposures. A recent publication which measured the potential dermal exposure to MMA for dental technicians was identified (64). Potential dermal exposures to the hands were evaluated using patches made of activated charcoal, one sandwiched between two layers of cotton fabric, which was attached to gloves donned by the technicians. The use of activated charcoal did not allow for MMA evaporation once adsorbed into the charcoal. Consequently, the results showed very high retention of MMA with a maximum of 25 mg/cm^2 and an average of 2.5 mg/cm^2; the estimated dermal exposure (95th percentile) was 8 mg/cm^2 based on the standard deviation of 3 mg/cm^2. The total surface area of the charcoal patches was 60 cm^2. The resulting potential dermal exposure was 8 mg/cm^2 x 60 cm^2 = 480 mg/day. Since the experimental data is based on 60 cm^2, this value is used to estimate the time on the skin. The resulting time on skin is only 3 minutes. RISKOFDERM can be used to estimate the actual dermal exposure in these experiments by forcing the model to output 480 mg/day (based on 60 cm^2) over 480 minutes and then using that resulting exposure rate with the actual time on skin. The estimated exposure rate was 1 mg/min, so the estimated actual dermal exposure was 3 mg/day over the measured area of 60 cm^2 or 50 micro g/cm^2. The final results are listed in Table 12 and indicate control of risk since the RCRs are less than 1.

4.4. Discussion

This exercise has shown how the tiered approach to risk assessment is performed when there is little to no experimental data available. Incorporating both details in the activity performed and physical properties of the substance can result in significant refinement and increases the confidence in the results. Based on this example, it probably would have been reasonable to use PROC 15 and stop there but the insights derived from the RISKOFDERM model and the evaporation rate increased overall confidence as part of a weight of evidence approach. Searching the literature and finding experimental data that can be compared to the models increases the level of confidence further. In addition, it demonstrates that experimental data is not always able to measure actual exposure and it can be necessary to refine both model and experimental data to arrive at meaningful dermal exposure estimates.

5. SUMMARY AND PERSPECTIVES

Regulation is changing and new approaches need to be exploited in an effort to identify hazards, evaluate exposure and perform robust risk assessments in a more timely and cost effective manner. REACH is one such example where a new framework has been laid out which advocates the use of alternative approaches for data gap filling and provides exposure assessment models to perform risk assessment requirements. We have aimed to show a practical perspective on this framework by providing illustrative examples of how non-testing

approaches can be undertaken and how exposure assessments can be performed. This is merely one step in the change towards a new risk assessment paradigm for the 21st Century.

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