CHAPTER 7

CHEMICAL CATEGORY FORMATION AND READ-ACROSS FOR THE PREDICTION OF TOXICITY

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Abstract: The aim of this chapter is to outline the principles of chemical category formation and the use of read-across methods to fill data gaps to aid regulatory toxicological decision making. The chapter outlines the Organisation for Economic Co-operation and Development (OECD) principles for the design of a chemical category. This section aims to give a flavour of the steps that need to be considered when forming a chemical category. This is followed by a description of the advantages that considering chemicals within categories bring in risk assessment. The importance of how to define chemical similarity and several commonly used methods is discussed. Finally a brief review of the limited literature available showing actual examples of read-across methods is presented

Keywords: Chemical categories, Read-across

7.1. INTRODUCTION

Chemical category formation and subsequent read-across analysis have been suggested as being essential if the objectives of REACH are going to be achieved without the excessive use of animals [1, 2]. The use of the chemical category approach is already common in a number of regulatory environments outside of the European Union namely in the United States and Canada. In terms of the Organisation for Economic Co-operation and Development (OECD) a chemical category has been defined as "a group of chemicals whose physiochemical 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 human health effects" [2]. On a practical level, this process involves treating a closely related (or similar) group of chemicals as a category. Within the category toxicological data will exist for some, but not all of the chemicals for the endpoints of interest. Thus data gaps are likely to exist for some of the properties or endpoints for each chemical, with it being likely that differing

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data gaps will exist for different chemicals within the category. It is for these data gaps that structure–activity relationship methods (such as read-across) will have to be utilised to make predictions for the missing toxicological data.

7.2. BENEFITS OF THE CATEGORY FORMATION

The recent OECD documentation detailing category formation highlighted a number of key benefits of the approach when applied to regulatory decision making about the safety of chemicals [2]. These can be summarised as follows:

1. Animal testing is reduced by the interpolation and/or extrapolation to other chemicals in the category. The use of existing data further reduces the need for additional testing.
2. Evaluation of chemicals using a category approach involves the use of a greater volume of data than assessing chemicals individually (as has been carried out in the past).
3. Development of a category aids the evaluation of chemicals which otherwise might not be assessed.
4. Chemicals which might not be able to be assessed in standard animal protocols can be investigated using the category approach [3, 4].
5. The category approach has the potential to aid in the risk assessment of chemicals for which animal tests do not reliably predict effects in humans [4].

As a practical benefit of the utilisation of such category approaches, the US EPA needed to conduct new animal tests for only 6% of 1257 chemicals assessed as part of the High Production Volume Challenge (HPVC) Program [5]. In this programme, existing data were available for 50% of the chemicals; a further 44% of the data required was estimated using methods such as read-across.

7.3. CHEMICAL SIMILARITY

The fundamental requirement for category formation is the ability to assess how similar a group of chemicals are that might form a category. Unfortunately no single measure of chemical similarity exists which can be universally applied across any endpoint. Instead one can consider a number of general approaches that have been suggested to be beneficial in the formation of a chemical category, with each one of them trying to ensure that for differing scenarios the resulting category contains chemicals acting via the same mechanism of action [2].

The first of these methods, and perhaps the simplest, is based upon forming a category around a common functional group such as an aliphatic aldehyde or aromatic ketone, the so-called “common functional group approach”. The second approach, generally suitable for categories dealing with physicochemical properties such as boiling point, aims to make use of simple counts of carbon chains lengths.

The third and fourth methods are more complex and aim to deal with category formation for complex mixtures and metabolically related chemicals. In terms of complex substances or biological material in which a single chemical substance

does not exist, it has been suggested that common constituents, similar carbon ranges or chemical class are likely to be useful in the formation of suitable categories. Such substances are referred to as "substances of unknown or variable composition, complex reaction products or biological material" (UVCB). Finally, chemicals can be grouped into a common category if they have a common precursor and/or common breakdown products; this can be thought of as the metabolic pathway category approach.

A related approach to chemical similarity that has been suggested to form useful chemical categories is the "mechanism-based approach", with it being suggested that a number of toxicological endpoints can be understood in terms of a common initialising event, usually a chemical reaction between an electrophilic chemical and a nucleophilic side chain in either amino or nucleic acids. A number of authors have documented such approaches [6–8].

Finally, it has been suggested that chemoinformatic approaches are able to form useful categories, especially in the identification of less obvious analogues from larger data sets [9]. Such methods rely upon the use of computational indices to encode structural information about chemicals; these indices can then be compared and chemicals within a certain distance located [10].

Given the numerous methods for developing chemical categories, it is unlikely a single method will always be the most appropriate, in contrast it being likely that more than a single method will be utilised in the formation of a single category. For example, a suitable category might be formed by the combination of assigning chemicals to a single electrophilic mechanism and then further restricting the chemicals within the category by the length of the carbon chains. Such decisions need to be made based on category by category basis with constant reference to the available experimental data. The aims of the remainder of this chapter are to highlight a general method by which chemical categories can be formed. In addition, the chapter will draw several examples from the literature to illustrate the differing ways of forming a chemical category, highlighting examples in which read-across has been used to fill data gaps.

7.4. GENERAL APPROACH TO CHEMICAL CATEGORY FORMATION

The recently published OECD guidelines for chemical category formation outlined nine steps required for the robust definition of a chemical category [2]. The first of these is to consider whether the chemical/chemicals of interest have already been assigned a category by other workers. A number of organisations provide resources for existing chemical categories for high volume chemicals, including the US EPA, OECD and the UN [11–13]. Assuming that the chemical of interest has not already been assigned to a category, eight further steps are suggested by the OECD; briefly these are

1. Development of a category hypothesis as the basis for the grouping of the chemicals. This definition should fully document the chemicals (names, structures)

and the endpoints that the category is applicable to. Care should be taken to fully document the structural domain that the category is applicable to. This definition covers molecular features such as chain lengths, molecular weight ranges, and the types of chemicals which should be included or excluded.
2. Gather data for each category member. This step involves the acquisition of all available toxicological and physicochemical data for each of the category members.
3. Evaluation of the quality and adequacy of the data available for each category member.
4. Construction of a data matrix showing the available data and crucially identifying gaps in the available data.
5. Evaluate the category hypothesis and if possible perform read-across to fill data gaps. This step aims to ensure that the hypothesis put forward in step 1 is fully valid and if so, and provided sufficient data exist, then the missing data in the data matrix be filled using appropriate read-across methods. Crucially if the data gathered in step 3 cannot or do not support the hypothesis proposed in step 1, then an alternate category might be required.
6. Should the data in step 5 support the category hypothesis but be insufficient for one or more of the endpoints covered by the category, further testing might need to be undertaken. Such testing should be designed in order to minimise animal usage whilst maximising information content.
7. If additional testing has been undertaken then a further assessment of the category should be undertaken. This is essentially a repeat of step 5.
8. If the category assessment is found to acceptable then the new category should be fully documented according to the OECD guidelines [2].

A common way to view the data matrix and how read-across methods might be used to fill any gaps in the data matrix is shown in Table 7-1.

Table 7-1. Schematic representation of data matrix required for a chemical category (X represents data points which are known and O represents missing data)

	Chemical 1	Chemical 2	Chemical 3	Chemical 4
Property 1	X	O	X	X
Property 2	O	X	X	O
Endpoint 1	X	O	X	X
Endpoint 2	X	X	O	X

7.5. EXAMPLES OF CATEGORY FORMATION AND READ-ACROSS

The above guidelines show the idealised methodology that should be employed in a regulatory environment for the formation of a chemical category. The remainder of the chapter will highlight studies in the literature into the development of categories of chemicals and then, in some cases, to perform read-across within these categories.

The focus of these sections is the illustration of three types of similarity method that can be used to aid category formation: these being chemical class, common mechanism of action and chemoinformatic approaches.

7.5.1. Chemical Class-Based Categories

A recent study utilised the category approach to assess the developmental toxicity of a group of phthalates esters with varying side carbon chain lengths [14]. The study used a further five phthalate esters of differing benzene substitution patterns and chain lengths to test the category hypothesis. The authors showed that differences in physicochemical properties, absorption rates or metabolism between the phthalate esters could not explain the differing reproductive toxicity profiles. The analysis of the chemicals in the study enabled a strict definition of the applicability domain of the category to be made, this being *ortho*-phthalate esters with carbon chain lengths between four and six carbons. The authors suggested that such chemicals acted via a common mechanism of action, most likely through binding to the anti-androgenic receptor. The study highlighted the use of both a chemical class and chain length restrictions in the formation of a suitable category. In addition, it showed that a clear mechanistic rationale could be offered for a complex endpoint within a well-defined chemical category.

7.5.2. Mechanism-Based Categories

A number of authors have demonstrated the use of mechanistic categories (rather than chemical class-based categories) for skin sensitisation and acute fish toxicity [6–8, 15–18]. Research has suggested that five principle organic chemistry mechanisms can be used as the basis for categorisation [15]. Briefly these mechanisms involve the attack by nucleophilic amino acid side chains (typical sulphur or nitrogen) on electrophilic fragments of potentially toxic chemicals; the mechanisms are summarised in Figure 2-1. Methods to enable chemicals to be assigned to these so-called reactive mechanisms have been published in the literature [19, 20] and included in the OECD (Q)SAR Application Toolbox which is freely available from the OECD website.

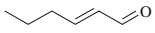
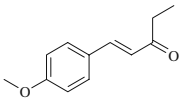
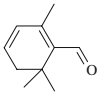
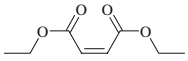
Additional studies have highlighted the ability of both QSAR and read-across methods to fill data gaps within these reactive mechanisms for both skin sensitisation and acute fish toxicity [18, 21–24]. One recent study demonstrated the utility of a computational measure of electrophilicity in making quantitative read-across predictions for a series of skin sensitising chemicals within the Michael mechanistic domain [21]. The study suggested the following methodology should be used to make a prediction for a "query chemical":

1. Calculate the electrophilicity for a database of chemicals in the Michael mechanistic domain with known EC3 values. The database was ranked based on electrophilicity (Table 7-1).

2. Select the two closest chemicals to the "query chemical" in terms of electrophilicity, one with a lower electrophilicity value, the other a higher electrophilicity value. Given that the database was ranked by electrophilicity the closest chemical with lower electrophilicity would be the chemical immediately preceding the "query chemical", whilst the closest chemical with greater electrophilicity would be the one immediately following the "query chemical". For example, to make the prediction for chemical 3 in Table 7-2, chemicals 2 and 4 would be chosen.
3. Linear extrapolation between electrophilicity and pEC3 using the two closest chemicals selected in step 1 allows a prediction to be made for the "query chemical". This step is equivalent to plotting electrophilicity against pEC3 for the two closest chemicals and using the electrophilicity value of the "query chemical" to predict its pEC3 value.
4. The predicted pEC3 value is then converted into an EC3 value.

Examples of the predictions possible from this methodology are shown in Table 7-2.

Table 7-2. Examples of read-across predictions made using the method described in the text. NP means a prediction has not been made as there is not a chemical more electrophilic (larger ω) or less electrophilic (smaller ω) in this small, four-chemical, example database

Name	Structure	Experimental EC3	Predicted EC3	ω
trans-2-hexenal		5.5	NP	1.608
1-(4-methoxyphenyl)-1-penten-3-one		9.3	9.87	1.734
Safranal		7.5	5.29	1.796
diethyl maleate		5.8	NP	1.804

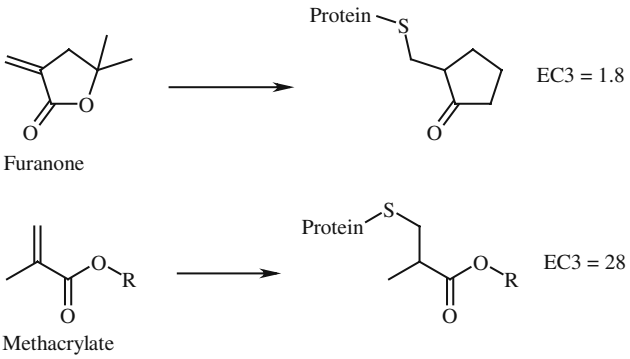


Figure 7-1. Ring strain release leading to increased skin sensitisation in 5,5-dimethyl-3-methylene-dihydro-2(3H)-furanone

Also highlighted was the need for sub-categories within the Michael domain, as 5,5-dimethyl-3-methylene-dihydro-2(3H)-furanone was found to be a significantly more potent skin sensitiser than would be suggested from its calculated electrophilicity. The authors suggested that upon reaction with a skin protein the furanone ring undergoes release of ring strain energy and thus is more reactive than the equivalent aliphatic molecules (Figure 7-1). It is therefore likely that for chemicals such as these, in which additional factors such as the release of ring strain energy are important, separate categories within the Michael domain will be required.

The use of calculated electrophilicity to make read-across predictions demonstrated that for good quality, interpretable predictions to be made requires subtle mechanistic understanding and appropriate categories and sub-categories to be formed. This suggested use of sub-categories within a mechanistic category is in keeping with the phthalates study in which sub-categories were used within a larger chemical class-based category [14].

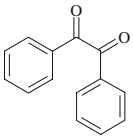
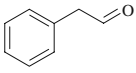
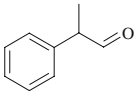
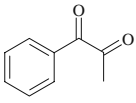
Another study [25] grouped compounds containing α,β-unsaturated carbonyl compounds together. Such compounds are believed to be able to interact covalent with proteins, enzymes and DNA through various mechanisms. As such, they are able to stimulate a range of environmental toxicities and adverse health effects. Koleva et al. [26] assume that compounds in this category (aldehydes and ketones) act by a common mechanism of action (Michael-type addition). The acute aquatic toxicities to *Tetrahymena pyriformis* of compounds within the category were obtained in an effort to develop approaches for (qualitative) read-across. In addition, *Salmonella typhimurium* (strain TA100) mutagenicity data were analysed to establish the structural differences between mutagenic and non-mutagenic compounds. These structural differences were compared with the structural characteristics of molecules associated with acute aquatic toxicity in excess of narcosis as well as other end points, for example, skin sensitisation. The results indicate that a category can be formed that allows structural information and boundaries to be elucidated.

7.5.3. Chemoinformatics-Based Categories

Chemoinformatics-based similarity measures have also been suggested for its use in the development of chemical categories [9, 26]. The primary example of this approach in the scientific literature makes use of a range so-called fingerprint methods. Such methods involve encoding the structural information within a molecule as a bit string in which each "bit" indicates the presence (if the bit is set as 1) or absence (if the bit is set as 0) of a particular molecular feature. These methods have been widely used in the drug discovery paradigm for locating similar chemicals from large chemical inventories [10, 27].

A recent study highlighted the usefulness of such approaches by using the freely available Toxmatch software (freely available from http://ecb.jrc.it/qsar/qsar-tools) to develop a small category of chemicals starting from a query chemical of interest [9]. The starting point for the study was a Schiff base chemical whose pEC3 was not known. By using the in-built fingerprint and similarity functions the software was able to locate three analogues from the 210 chemical local lymph node assay database [28] (Table 7-3).

Table 7-3. Schiff base category formation and subsequent read-across predictions using similarity indices

Chemical	EC3 (% wt)	Similarity
	1.07 (predicted)	"query chemical"
	3.00	0.60
	6.30	0.60
	1.30	0.87

The authors then used the similarity measures to perform linear extrapolation between the similarity measures and pEC3 values of the three most similar chemicals. It was then possible to use this relationship to obtain a predicted pEC3 value for the query chemical (Table 7-3). Additional category formation and subsequent read-across examples were also presented using the bioaccumulation and fathead minnow data sets. A further study [10] has illustrated the use of the Toxmatch to form groupings of compounds from which it is possible to make assessment of teratogenicity.

7.6. CONCLUSIONS

This chapter has demonstrated the general concepts that are required for the regulatory usage of chemical categories. It is clear from the material presented that the formation of a chemical category is a complex process requiring expert knowledge about both the physicochemical properties of the suggested group of chemicals and crucially their mechanisms of action across the endpoints of interest. In addition, the chapter has highlighted a number of read-across examples from the literature. Whilst examples of read-across predictions in the wider literature are currently limited, those presented in this chapter show that given a well-defined category (or indeed sub-category) good quality read-across predications can be made. These publications support the category hypothesis and help show that within these categories simple read-across methods enable mechanistically interpretable predictions to be made for complex toxicological endpoints.

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