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Application of in silico modelling to estimate toxicity of migrating substances from food packaging

Nicholas Price, Qasim Chaudhry *

The Food and Environment Research Agency, Sand Hutton, York YO41 1LZ, UK

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ABSTRACT

This study derived toxicity estimates for a set of 136 chemical migrants from food packaging materials using in silico (computational) modelling and read across approaches. Where available, the predicted results for mutagenicity and carcinogenicity were compared with published experimental data. As the packaging compounds are subject to safety assessment, the migrating substances were more likely to be negative for both the endpoints. A set of structural analogues with positive experimental data for carcinogenicity and/or mutagenicity was therefore used as a positive comparator. The results showed that a weight of evidence assembled from different in silico models and read-across from already-tested structurally similar compounds can provide a rapid and reliable means for rapid screening of new yet-untested intentional or unintentional chemical compounds that may migrate to packaged foodstuffs.

1. Introduction

1.1. The FACET project

The work reported here was designed to test the in silico predictive strategy used in the FACET project (Flavourings, Additives and food Contact materials Exposure Task – www.ucd.ie/facet), which was funded by the European Commission under the framework programme to produce a risk management tool consisting of a database of information on the levels of food additives, flavourings, food packaging migrants, and corresponding food consumption data (Hearty et al., 2011). The objectives within the food packaging migrant element of the project (Oldring et al., 2014a,b) also aimed at establishing the physicochemical properties that are related to migration of chemicals from food packaging, classification of foods in relation to their migration behaviour, mathematical modelling to estimate migration from packaging to various food types, and the possible use of in silico (computational) predictive models to evaluate specific toxicological endpoints of migrating compounds.

1.2. Predictive toxicology of food migrating substances

The migration of chemicals into food is a potentially serious issue relating not only to the quality of food but also to the safety of the consumer (Barnes et al., 2007). Some of the chemicals used to make food packaging have been shown to migrate from packaging into packaged foodstuffs. Generally, such compounds have been assessed by expert panels for safety, and have published toxicological data available. The amount and quality of the available data, however, differs widely among the compounds. In addition, for some compounds, the migrating substances have been found to be the breakdown products or impurities rather than the parent compound itself. Pragmatic approaches such as Threshold Level of Concern (TTC), are often based on rather crude indices of toxicity, such as the Cramer index, (Pinalli et al., 2011), and could be improved with a more data-intensive approach. The aim of this study was therefore to attempt to establish a set of "in silico" procedures that would add to the body of information on the existing migrating compounds, and serve as a starting point for assessment of new yet-untested compounds. The techniques used included a combination of expert systems and predictive computational models based on Structure Activity Relationships (SAR), and Quantitative Structure Activity Relationships, (QSAR).

Since the range of chemical types that may migrate from packaging into food can be very wide, no single model was deemed likely to suffice for all types of compounds or for all toxicological endpoints. A "weight of evidence" (WoE) approach was therefore

* Corresponding author. Tel./fax: +44 1904462584.
E-mail address: qasim.chaudhry@fera.gsi.gov.uk (Q. Chaudhry).

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taken as currently recommended for regulatory predictive toxicology (Balls et al., 2006). In addition, since the methodology was intended to be used widely in the industry and by regulatory bodies, all software platforms used were those that are publicly available.

By way of validating the approach taken in the FACET project, the current work assessed the quality of mutagenicity and carcinogenicity endpoints on the packaging migrant compounds that had measured toxicological data available for the two endpoints. Since all compounds used in plastics food packaging go through a rigorous process of assessment by expert panels, it was expected that the migrant substances would not be carcinogens or mutagens. Similarly, substances used in other packaging materials such as paper/board, inks and adhesives, will have undergone some degree of toxicity pre-selection by users. Although a predictive system that would have returned a result of "negative" for these compounds could be deemed to have performed well, it would have not allowed validation of the predictive strategy used. In view of this, a series of structural analogues of the migrant compounds that were tested positive for mutagenicity and carcinogenicity was also included in the study as a positive control. Structural and compositional similarity of this "test set" to the FACET set was considered a good test of the WoE approach in identifying those structural characteristics which confer carcinogenicity or mutagenicity.

2. Materials and methods

2.1. The OECD QSAR toolbox

The OECD Toolbox is a software application intended to be used to fill gaps in toxicity and ecotoxicity data needed for assessing the hazards of chemical substances. The Toolbox incorporates databases on chemical data (e.g. properties), experimental toxicological and ecotoxicological data, and estimated values from a large range of QSAR tools, together with incorporated QSAR modelling and Expert Systems, built within a regulatory application chassis. This package therefore allows the user to perform a number of functions (OECD, 2008), e.g. to:

- identify analogues for a chemical, retrieve experimental results available for those analogues and fill data gaps by read-across or trend analysis;
- categorise large inventories of chemicals according to mechanisms or modes of action;
- fill data gaps for any chemical by using the library of QSAR models;
- evaluate the robustness of a potential analogue for read-across;
- evaluate the appropriateness of a (Q)SAR model for filling a data gap for a particular target chemical; and
- build QSAR models.

For this study version 2.3 of the Toolbox was used that had been augmented with a number of extra publicly available databases with carcinogenicity and mutagenicity data.

2.2. The VEGA platform

The VEGA platform (www.vega-qsar.eu), has been developed by the Istituto di Ricerche Farmacologiche Mario Negri in Milan with a number of collaborating organisations and through a series of EU-funded projects. The models used in VEGA for carcinogenicity and mutagenicity originated in the EU project CAESAR, (www.caesar-project.eu/), with subsequent improvements and additions from contributing organisations, and incorporating some of the models in the US-EPA Toxicity Estimation Software Tool; T.E.S.T (www.epa.gov/nrmrl/std/qsar/qsar.html).

VEGA models generate transparent, reproducible, and verifiable results. The system comprises a series of tools that have been optimised so that the results obtained for a target compound can also be related to those for other structurally related compounds. VEGA also has a comprehensive 5-point validation system that allows the user to assess the reliability of predictions.

2.3. Study substances

Initially 78 "substances" were selected for the study that are used in inks and plastic food packaging. Using the summary information in the expert assessments of the EFSA Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids, published in the EFSA Journal, a set of discrete chemical compounds representing the actual, or most likely migrating moieties of each of the plastic substances was identified. In most cases but not all, the migrating moiety was the same as the parent compound. However, particularly in the case of oligomers, polymers, or mixtures, it was necessary to identify the specific migrant compound(s) and in some cases there were two or more. In some cases the same compound was found to be the most appropriate migrating substance for more than one parent. This resulted in a total of 136 discrete chemical compounds that were used in this study, being derived from the initial 78. The study set of 136 consisted of a wide variety of organic compounds including alcohols, aldehydes, alkanes, alkenes, amides, carboxylic acids, esters, ketones, organophosphates, phenols, phenolic acids, piperidines and sulphonamides. Names and SMILES notation for all of the compounds, (including where a the same compound was a migrant from more than one parent substance) can be found in Supplementary Table 1.

Where possible, experimentally measured values for the endpoints under study were used for comparison against those predicted by the software used in this study. Sources of measured endpoint data for the molecules in the study included the EFSA Journal www.efsa.europa.eu/en/publications/efsajournal.htm; Chembase: www.chembase.com/; ChemIDplus: http://chem.sis.nlm.nih.gov/chemidplus/; ChemSpider: www.chemspider.com/; Pubchem: http://pubchem.ncbi.nlm.nih.gov/; Carcinogenic Potency Database: http://toxnet.nlm.nih.gov/cpdb/cpdb.html; DSSTox: www.epa.gov/comptox/dsstox/; European chemical Substances Information System: http://esis.jrc.ec.europa.eu/; NTP Database: http://ntp.niehs.nih.gov/; IPCS: www.inchem.org/; ToxNet: http://toxnet.nlm.nih.gov/; and eChemPortal: www.echemportal.org/echemportal/.

Of the 136 study compounds, 70 had published results of Ames mutagenicity tests, whilst only 37 had data for carcinogenicity. For the purposes of this study, it was assumed that the remaining compounds were negative for these endpoints based on the expert scrutiny to which they had been subjected prior to approval as food packaging substances.

All 136 compounds were input into software using SMILES strings or .mol files. This structural information was obtained by searching the same databases as described above. Where structural data were not available, molfiles were constructed from known fragments using Accelrys Draw, http://accelrys.com.

2.4. Carcinogenic and mutagenic analogues

Further structural analogues of the migrant compounds were identified from toxicity databases for which measured mutagenicity or carcinogenicity data were available to regard them carcinogens or mutagens. This was done to assess the ability of the SAR and QSAR systems to predict the endpoints of structurally similar, but toxicologically dissimilar compounds. The selection criteria used were that the analogue had to be more than 70% similar to the parent compound, with positive published results for either mutagenicity and/or carcinogenicity, and be within the prediction domain for the QSARs in the OECD QSAR Toolbox. Structural similarity of analogues was assessed by using the similarity module in the QSAR Toolbox. Each parent compound was submitted to a search of the Toolbox databases for >70% similarity based on the Dice algorithm (Dice, 1945), using atom pairs, atom type and cycles as the basis for comparison. The resulting analogues were screened for those with positive carcinogenicity and/or mutagenicity data, and also for their domain suitability for the Toolbox QSARs for those endpoints. From these, compounds that were most similar to the target packaging migrant compounds were selected for further study. Not all of the migrant compounds yielded suitable analogues with >70% structural similarity, and only 49 compounds were found that met the criteria and are shown in Supplementary material.

2.5. Carcinogenicity and mutagenicity predictions

2.5.1. Predictive QSAR models

A number of validated QSARs are built into the Toolbox and the ones used for this study were all developed or adopted by the Danish Environmental Protection Agency for use in their Danish (Q)SAR Database (http://qsar.food.dtu.dk/). These were: male mouse carcinogenicity (MultiCASE commercial model AG3); female mouse carcinogenicity (MultiCASE commercial model AG4); male rat carcinogenicity (MultiCASE commercial model AG1); female rat carcinogenicity (MultiCASE commercial model AG2); and Ames mutagenicity (MultiCASE commercial model A2H).

Other models used included male/female rat carcinogenicity and in vitro mutagenicity based on the Ames test, using Salmonella typhimurium, (http://130.226.165.14/User_Manual_Danish_Database.pdf); and VEGA QSARs as described above. The results were used only for those compounds that had a good or moderate reliability based on the VEGA domain assessment scores.

2.5.2. Read across

Read across is a technique for predicting toxicity endpoints for a given compound from the measured toxicity values of other compounds that have a close structural or mechanistic similarity to the query compound (Gallegos Saliner et al., 2005). Thus, for each target compound requiring predictions of carcinogenicity and mutagenicity, a category of analogues was constructed using one criterion for structural similarity, and another for mechanistic similarity. As before,