CRACK IT

Maximise: maximising confidence whilst minimising data generation for acute hazard classification of mixtures

Background

Innovations in chemical products are characterised by novel formulations of existing and new ingredients. For agrochemicals, these are often mixtures of one or more active substances with any known toxicity concerns and co-formulants such as surfactants, buffers and solvents that may contribute to the overall toxicity. The toxicity profiles of these mixtures are complex and are often more than the simple sum of the ingredient endpoints with the potential for additive, synergistic and compensatory effects to be observed.

Even though a proportion of these ingredients may have already been tested for their hazard characterisation in previous uses, the novel formulation must be assessed for its classification and labelling according to international guidelines such as the UN Globally Harmonized System of Classification and Labelling of Chemicals (GHS) requirements. These guidelines require *in vivo* studies with toxicological endpoints addressing oral, dermal and inhalation lethality, skin and eye irritation, corrosivity and skin sensitisation. The studies can cause pain and discomfort to the animals and frequently use death (or signs of imminent death) as an endpoint.

There has been progress in the use of *in silico* and *in vitro* methods for specific acute endpoints on single chemicals and mixtures. For example, there are a number of *in vitro* methods which can be used to identify severe eye irritants and allow classification without the need for *in vivo* testing. However, these eye irritation tests do not incorporate the repair mechanisms seen in the *in vivo* model which is considered the 'gold standard'. There are also a number of validated *in vitro* methods available to determine effects on the skin including the Organisation for Economic Co-operation and Development (OECD) *in vitro* skin irritation test guideline (TG) 439 (OECD, 2015b) and the *in vitro*/*ex vivo* skin corrosion tests, OECD TGs 430, 431 and 435 (OECD, 2014a; OECD, 2014b; OECD, 2015a) but their predictivity for agrochemical mixtures is not proven.

In silico methods such as acute toxicity estimation calculations (ATEs) can be conducted on agrochemical mixtures to predict acute systemic end points (acute oral, dermal and inhalation toxicity). Each ingredient and its proportion in the formulation is considered in the calculation. ATE calculations have good accuracy for the systemic endpoints but limited predictivity for skin/eye irritation and skin sensitisation and regulatory acceptability of such calculations is at an early stage.

Other *in silico* methods such as QSARs are routinely used for the prediction of single ingredients but none of these are individually developed enough to be considered sufficiently reliable to be used for mixtures. A recent CRACK IT Challenge (QSARs Mix, 2014) was successful in the development of a more specific QSAR approach for assessing skin and eye irritation for petroleum chemical mixtures but an integrated solution that considers multiple chemical and toxicological data points that can be used to assess hazard characterisation for agrochemical mixtures is required.

This Challenge aims to develop innovative, integrated *in silico* approaches to better predict the GHS classification category for acute oral, skin and eye irritation in the development of agrochemical formulations without using animals or generating new *in vitro* data. The

Challenge also requires a disruptive business model whereby access and storage of industry data can be managed to provide predictive testing strategies for complex mixtures.

It is expected that this approach will have broader applicability to other industry sectors.

3Rs benefits

Acute toxicity studies are used to assess the safety of the agrochemical products and to gather information for regulatory purposes. While industry continues to refine these studies, they can involve the use of high concentrations of test items which can lead to severe suffering, and sometimes death as an endpoint.

Acute Oral

Acute oral toxicity studies are usually performed in rats. The determined endpoint is the LD_{50} (dose that is lethal to 50% of the animals). Within the agrochemical industry, it is estimated that between 500 and 1500 animals are used for acute oral toxicity assessments of products annually.

Eye and Skin irritation

Acute eye irritation studies are performed on rabbits measuring conjunctival, iris and corneal effects. Rabbits are also used for skin irritation studies where irritation and/or corrosion are assessed. It is estimated that over 850 rabbits are used annually across the industry for skin and eye irritation studies.

A successful CRACK IT Challenge has the potential to deliver significant 3Rs impact. In the short term, *in silico* screening of novel formulations to provide an indication of toxicity could prevent mixtures being taken into the animal studies needed for regulatory approval. In the longer term, confidence in the prediction of classification may lead to regulatory acceptance and the waiving of the *in vivo* studies for acute toxicity endpoints for skin, oral and eye irritation.

Need for collaboration

Successful delivery of this Challenge requires expertise that includes but is not limited to:

- Classification and labelling of chemicals
- Toxicology
- Innovative mathematical modelling and algorithms
- Database generation and curation
- Secure IT framework and data handling
- Confidential data sharing in the commercial sector.

Overall aim

To develop reliable predictions which confidently classify mixtures of chemicals for acute oral toxicity, skin and eye irritation with a focus on relevance for human safety. These should fulfil acute GHS Classification and Labelling requirements for non-encapsulated agrochemical mixtures using existing information on components and formulations without the need for additional *in vivo*, and ideally *in vitro,* studies.

Deliverables

- Acquisition of acute oral toxicity, eye and skin irritation information for the individual components of a specified training set of formulations. This will include toxicological data, classifications and formulation compositions provided by the Sponsors and other relevant database sources, for ingredients and formulations that have been tested directly *in vivo* and *in vitro*, where appropriate.

- There will be instances where there are no data for individual ingredients or formulated products that will need to be addressed in the Challenge. Expert analysis of the data to develop and implement read-across and/or default assumptions for these instances is required.

- Innovative algorithms that predict mixture toxicity that will permit a prediction of GHS classification with an associated measure of the confidence. The read-out should include:

    - A confidence range with known limits.

    - Any additional information required to enable a confident classification for identified data gaps.

    - Transparency in the generation of the classification.

    - User friendly readouts.

    - Evidence and case studies of the reliability of the predicted classification.

    - Assessment of the performance of the platform will be made against formulations that have known *in vivo* data.

- A business model that enables the sharing of data between companies whilst preserving data ownership rights confidential information. This should include:

    - A designed process for meeting the Challenge brief whilst retaining the confidentiality of formulation compositions.

    - A database for the different kinds of toxicity data for formulation components

    - Awareness of the regulatory environment and expectations.

    - An innovative and flexible commercial model to facilitate sharing of ingredient information between companies.

It is important to note that the CRACK IT Challenges competition is designed to support the development of new 3Rs technologies and approaches, which will improve business processes and/or lead to new marketable products. The application must include a plan to

commercialise the results into a product or service. This should be taken into consideration when completing your application.

Sponsor in-kind contributions

- Expertise on regulatory toxicology including acute toxicity data on mixtures and components.
- Toxicological data, classifications and formulation compositions for formulations that have been tested directly *in vivo* for acute oral toxicity, eye and skin irritation.
- Acute oral, eye and skin irritation toxicity information for formulation components, where available. This will include combinations of *in vivo*, *in vitro* data and classifications as and when available.

Duration

Up to one year

Budget

Up to £100k

Co-funding provided by the Engineering and Physical Sciences Research Council

Sponsors

Syngenta, Dow

References

GHS Guidance documents:
http://www.unece.org/fileadmin/DAM/trans/danger/publi/ghs/ghs_rev06/English/03e_part3.pdf

OECD, 2014a: http://www.oecd-ilibrary.org/environment/test-no-430-in-vitro-skin-corrosion-transcutaneous-electrical-resistance-test-ter_9789264071124-en

OECD, 2014b: http://www.oecd-ilibrary.org/environment/test-no-431-in-vitro-skin-corrosion-human-skin-model-test_9789264071148-en

OECD, 2015a: http://www.oecd-ilibrary.org/environment/test-no-435-in-vitro-membrane-barrier-test-method-for-skin-corrosion_9789264242791-en

OECD, 2015b: http://www.oecd-ilibrary.org/environment/test-no-439-in-vitro-skin-irritation-reconstructed-human-epidermis-test-method_9789264242845-en

QSARs Mix, 2015: http://crackit.org.uk/challenge-19-qsars-mix