Introduction

Skin sensitization is an immunological response following previous exposure to a substance which results in an inflammatory skin reaction which is usually presented as a red, itchy rash. This effect can range from mild to debilitating, presenting the patient concerned with concerns regarding their normal life. Such effects, or the reporting of them, have become more prevalent in recent years as people are exposed to more chemicals and/or mixtures of chemical substances on a daily basis.

The first phase of skin sensitization is induction and the second phase is elicitation. In the first phase, development of specialized memory cells in the immune system of an individual occurs following the initial exposure to a skin sensitizer. In the second phase, an allergic skin reaction is produced following subsequent exposure to the skin sensitizer. The specialized memory cells produced in the individual’s immune system following the initial exposure respond to the subsequent exposure, i.e., an allergic reaction takes place.

For many years the potential of non-animal test methods to cause skin sensitization was assessed by the performance of tests in animals, e.g., a Guinea Pig Maximization or Baseline Test (OECD Test Guideline 407) or a Local Lymph Node Assay (LLNA) (OECD Test Guideline 429), adopted in 2002(3). However, with the advent of Registration, Evaluation, Authorization of Chemicals (REACH) in the EU and the pressure to reduce the use of animals in testing and application of the 3Rs (Replacement, Reduction, and Refinement), non-animal testing is now the default requirement. In December 2016 the REACH annexes were amended such that information needed for the classification or risk assessment of a substance then had to be obtained through non-animal methods as a first step. In our view this should have been used if in vivo or in vitro test methods would not be applicable for assessment of the substance or would not provide information adequate for classification and risk assessment.

Envigo devised a general strategy (see diagram) to meet these non-animal testing requirements for chemicals. This has been rehearsed over the past 2 years additional test methods have become available and acceptance of the in vitro testing alternative results has been refined.

Initially availability of testing data for a weight-of-evidence approach to determine test strategy is required. In silico assessments of substances using QSAR models to see if probability and/or alerts for skin sensitization are raised is also employed.

In chemico in vitro testing is required as an alternative to animal testing, addressing both in vitro and in vivo data. For example, OECD Test Guideline 442, Skin Sensitization Direct Peptide Reactivity Assay (OECD, 2004) and OECD Test Guideline 443, Skin Sensitization Post-test Repeatability Assay (OECD, 2018), and OECD Test Guideline 444, Skin Sensitization In vitro Repeatability and Reactivity Test (OECD, 2016). Where these are not available, in vivo testing may be performed from the start for some substances if certain criteria apply. Moreover, testing in vitro, in chemico or in vivo may not need to be performed in all circumstances.

For agrochemicals currently the LLNA is the preferred option although if certain criteria apply. Moreover, testing in vitro, in chemico or in vivo may not need to be performed in all circumstances.

The Envigo process

A process has been developed to advise on the appropriate strategy for testing and assessment of the test item for skin sensitization, and for integration of the data from all sources in order to conclude on skin sensitization potential, and whether appropriate, whether classification as Category 1A is required.

Supporting in vitro studies with in silico predictions

Gathering information from QSARs was performed according to a requirement to use alternative methods whenever possible in Article 13 paragraphs 1 and 2 of REACH Regulation (EC) No 1907/2006, and as part of Endpoint Specific Guidance (ESG) objectives (ECHA R.7A, Ver. 6, July 2017).

Further to this the QSAR methodology followed was similar to that in Pudenz and Ruzgyte Frère (2017)(5) using a battery of software, listed below, to allow formation of the predictions made. If further testing was considered to be required the potential to cancel all future testing data by Regulatory authorities was often still required.

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Skin sensitization assessment for Agrochemicals:

Below is a sample of projects from the last year. Throughout these projects it has been possible, with the use of this Envigo process, to reduce the number of required animal tests significantly by misleading the number of in vitro studies conducted where these were deemed unnecessary. This has resulted in an overall reduction in animal testing and spared the use of over 450 animals.

Review of QSAR and test results from 2018-2019 (REACH)

In vivo testing data not accepted for registration in the EU but fully accepted for chemicals

In vivo testing data, only accepted for regulatory purposes in the US

Japan

New regulation 1st April 2019 in vivo data only accepted for active substances and co-formulants

Malaysia

In vivo data only accepted

Australia

New regulations in 2020 for chemicals mandating use of in vitro in vivo testing

Korea

In vitro data accepted for chemicals

Turkey

KDDI in vitro test methods to be used, active substances and co-formulants in PPS are considered registered

Russia

New regulations in 2021 mandating use of in vitro test methods

Conclusions

This is the approach developed for the assessment of industrial chemicals for registration under REACH in the EU but what is its applicability for agrochemicals? Globally the acceptance of in vitro testing data by regulatory authorities is becoming more widespread, but in the EU, thanks to new legislation, there is considerable pressure for it to be accepted. Many countries are in the process of replacing an increasing number of in vivo tests, and which may take time for in vitro testing as the regulatory requirement to be mandated, interim policies for acceptance of in vitro data are in place and considered good practice for many countries. However the acceptance of in vitro data only applied to active or react ingredients but not formulations, where in vivo data is often still required.

Envigo has established a process/system to support customers through all stages of decision making process based on sound scientific knowledge, and understanding of the individual studies. This has helped reduce unnecessary testing for our customers.

The predictive power of the Envigo TOPKAT skin sensitization model has been improved by extending the training set with compounds that are structurally similar to the already validated TOPKAT model. Where the training set substances were not structurally similar to the target, the predictive power may be improved by extending the training set with compounds which are structurally similar. This led to the introduction of training set extension as a possible approach for improving the predictive power of a QSAR.

In the continuation of the work, the skin sensitization module of TOPKAT has been extended in multiple circumstances where similar compounds have been identified in literature. This has been a continual process which has seen the addition of 66 substances to the TOPKAT skin sensitization model, a 17% increase in the number of substances in the training set.

The individual predictions were assessed for various parameters, accuracy, concordance and applicability, etc. to assess their reliability. All models were then assessed collectively and collectively in terms of overall assessment drawing conclusions, where possible, on classification for the compound with reference, if possible, to the identified mechanism(s) of action (the DEREK OECD Toolbox/Trentox) and the identification of the potential for the formation of pro-drugs (the ACD/Actavis database) in silico in those cases where the classification was then used to determine the in vivo testing, where appropriate, and conclude an assessment of potency (DEREK, OECD Toolbox). Finally, where a conclusion on classification could not be made with certainty, as per current legislation, all future skin sensitization studies was considered and determined based on the merits of the predictions made. If further testing was considered to be required the information gathered in in silico methods would help guide decisions on studies going forward, with a view to avoiding unnecessary studies.

Using the combined weight of evidence approach, we have seen a number of conclusions on classification or supporting arguments to accompany in vitro studies which have led to the reduction in further in vivo studies.