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Review

Respiratory sensitization: Advances in assessing the risk of respiratory inflammation and irritation ☆

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ABSTRACT

Respiratory sensitization provides a case study for a new approach to chemical safety evaluation, as the prevalence of respiratory sensitization has increased considerably over the last decades, but animal and/or human experimental/predictive models are not currently available. Therefore, the goal of a working group was to design a road map to develop an ASAT approach for respiratory sensitisers. This approach should aim at (i) creating a database on respiratory functional biology and toxicology, (ii) applying data analyses to understand the multi-dimensional sensitization response, and how this predisposes to respiratory inflammation and irritation, and (iii) building a systems model out of these analyses, adding pharmacokinetic–pharmacodynamic modeling to predict respiratory responses to low levels of sensitisers. To this end, the best way forward would be to follow an integrated testing approach. Experimental research should be targeted to (i) QSAR-type approaches to relate potential as a respiratory sensitizer to its chemical structure, (ii) *in vitro* models and (iii) *in vitro–in vivo* extrapolation/validation.

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Contents

1. Introduction

Testing for the potential sensitizing capacity of chemicals has been done traditionally with skin tests in experimental animals such as the Guinea Pig Maximization Test (GPMT) and the Buehler Test. Currently, testing for sensitizing capacity is done using the mouse Local Lymph Node Assay (LLNA), which measures proliferation of lymphocytes in draining lymph nodes. While this test as such does not discriminate between skin and respiratory sensitisers, the cytokine responses in conjunction with this test help to inform on skin vs. respiratory sensitization. However, a validated test to detect respiratory sensitisers is currently lacking.

The lacking availability to prospectively identify respiratory sensitizers cannot be discharged by the conclusion that based on existing information from humans all required information is available. For example, new applications such as spray applications may increase exposure to respiratory sensitizers that may not have been identified as such based on existing information from humans. The increased exposure may result in passing the threshold for respiratory sensitization. An adequate *in vitro* or *in vivo* model should have identified the compound as respiratory sensitizer, precluding its use in a spray application.

There are efforts to replace animal tests to prospectively identify sensitizers, by *in vitro* assays, but so far all of them are oriented at skin sensitization, whereas there are no or hardly any efforts oriented at respiratory sensitization (Vandebriel and Van Loveren, 2010). It is remarkable that no generally accepted *in vivo* or *in vitro* assay to identify respiratory sensitizers exists, as respiratory sensitization can be truly incapacitating and poses a considerable weight on health, especially of workers exposed to sensitisers. Hence, for predictive testing as well as for risk evaluation of chemicals, both skin and respiratory sensitization should be considered, and alternative approaches that do not use animals will only be adequate if they address both aspects of sensitization. Remarkably, the LLNA, which is a validated and generally accepted *in vivo* assay to identify skin allergens, can also be used to identify respiratory sensitizers when used in conjunction with cytokine profiling, with skin allergens inducing a Th1 response and respiratory sensitizers a Th2, or mixed Th1/Th2, response (Dearman et al., 1995, 1996; Vandebriel et al., 2000, 2003; Van Och et al., 2002). Although promising, the use of cytokines in the LLNA to distinguish contact from respiratory sensitizers has not yet entered pre-validation. Testing for respiratory sensitization is not required within REACH and thus an important reason for validation is lacking.

In contrast to tests for skin sensitization, the performance of tests for respiratory sensitization is currently not required under the EU legislation ''Registration, Evaluation, Authorization and Restriction of Chemicals'' (REACH). In REACH, respiratory sensitisers are indicated for harmonized classification and labeling and regulated in Annex I of Directive 67/548/EEC. Annex XV in REACH lays down general principles for preparing dossiers to propose and justify harmonized classification and labeling of respiratory sensitisers. Although no testing strategy is available, a substance could be classified as respiratory sensitizer based on existing evidence.

According to the Global Harmonization System (GHS), respiratory sensitisers are categorized as hazard category 1 (respiratory sensitizer) and, if required by a competent authority and if sufficient data are available, further classified as subcategory 1A ''stronger respiratory sensitizer'' or 1B ''other respiratory sensitizer''. In order to arrive at these (sub-) categories, human and/or animal data are required. However, as discussed above, recognized and validated animal models for the testing of respiratory hypersensitivity are currently not available. This implies that human evidence is required to identify respiratory sensitisers and to subcategorize them. Identification as respiratory sensitizer is based on the induction of specific respiratory hypersensitivity, while attribution to a subcategory depends on the frequency of reactions in a population and their severity. Human evidence can be either lung function tests, supported by skin prick tests, serological analysis, or other, or bronchial challenge tests. Lung function tests should be complemented with medical and occupational history, whereas the results of bronchial challenge tests are considered sufficient evidence on its own. To attribute a chemical to one of the subcategories, the number of cases has to be related to the size of the population exposed as well as the extent of exposure.

From these considerations, it may be concluded that human data, although not stemming from well-controlled studies, are essential to identify respiratory sensitisers and evaluate their potency. The ''Assuring Safety without the need for Animal Testing'' (ASAT) vision comprises a view on chemical safety that differs from the traditional one. Instead of relying wholly on animal studies, the