TOXICOLOGICAL HIGHLIGHT
Nonanimal Alternatives for Skin Sensitization: A Step Forward?

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For both animal welfare reasons as well as to meet the demands of existing and impending legislation in Europe, there is an increasing emphasis on the replacement of animals in toxicology testing for end points such as skin sensitization (Basketter and Maxwell, 2007). However, many of the end points (chronic toxicity, teratology) may prove intractable, so there is a heavy focus on end points such as irritation and sensitization. In this issue of Toxicological Sciences, there is a fascinating new paper by Natsch and Emter (2008) on how genes involved in the response to antioxidants are impacted by exposure to chemicals which are potential skin sensitizers. Their hypothesis was that all (organic) chemicals must react covalently with skin protein in order to behave as skin sensitizers and that this reactivity could be detected at the cellular level via a regulatory pathway activated by electrophiles (reactive chemicals). The pathway depends on a sensor protein, Kelch-like ECH-associated protein 1, which contains highly reactive cysteine residues (Dinkova-Kostova et al., 2005; Wakabayashi et al., 2004). Approximately 100 chemicals were examined in the present study, a much larger number than normally associated with a first publication of an alternative method, with the finding that most of the skin sensitizers did indeed activate this pathway, essentially part of the cellular systems for eliminating toxic reactive substances. Importantly, in the context of skin sensitization, irritant materials generally failed to activate this pathway. Overall, a prediction accuracy of about 82% was achieved, which is only a little lower than was obtained during the validation of the local lymph node assay (LLNA) (NIH, 1999), although some might be concerned by the failure of this in vitro system to detect almost 20% of the sensitizers. The authors address this issue by invoking the conduct of complementary assays, such as peptide binding or in silico methods. For example, an example of a chemical class that is not well predicted is aldehydes, where the possibility of employing an existing predictive Quantitative structure activity relationships is suggested. Of course, such devices are most easily implemented for chemicals where we already know what the answer should be, whereas the most critical assessment is for new substances where we do not already know the answers.

Let’s put this work into some perspective. Present in vivo methods, notably the LLNAs chemical hazards but are also used to characterize the potency of the sensitizer and thereby permit the risks to human health to be assessed and appropriately managed (Basketter and Kimber, 2006; Basketter et al., 2007, Felter et al., 2002, 2003). However, nonanimal approaches tend to dissect the various elements of the immunobiological response to skin-sensitizing chemicals in order to examine bioavailability (e.g., Basketter et al., 2007), chemical reactivity (e.g., Gerberick et al., 2007; Natsch et al., 2007), keratinocyte responses (e.g., Coquette et al., 2003), and dendritic cell responses (e.g., Aeby et al., 2007; Sakaguchi et al., 2006). The end point measured in any of these individual elements of sensitization induction mechanism may reflect an intrinsic ability of a substance to cause skin sensitization but is unlikely to offer a complete insight regarding the potency of an identified sensitizer. Hence, a method for the integration of the various data elements will be required, and an outline strategy for this has been published quite recently (Jowsey et al., 2006).

How do these considerations relate to the current paper by Natsch and Emter? In this present work, the authors attempt only to develop a prediction model which discriminates chemicals which do sensitize from those which do not. Indeed, even a cursory examination of their raw data in Table 1 shows that their response measure really does not offer an adequate correlate of sensitizing potency. This is entirely reasonable as multiple factors are likely to impact of the potency of a skin sensitizer. Even at the level of chemical reactivity, potency may be determined as much by the site of the reactions on skin protein as the extent to which derivatization occurs (Divkovic et al., 2005).

An incidental topic raised in this paper concerns the in vivo data sets against which an in vitro skin sensitization alternative is to be judged. For the validation of the LLNA, data on some 200 chemicals were provided, for which there was human and/ or guinea pig data (NIH, 1999). Subsequently, a more extensive data set from the LLNA has been made available (Gerberick et al., 2005). However, as with any toxicology test, predictions from the LLNA are not perfect. Natsch and Emter

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rightly mention the importance of having an official reference list of chemicals for validation. Currently, no efforts are underway to produce such a list, although there is effort to produce a short list of reference chemicals for the validation of nonradioactive end points for the LLNA (Basketter, Cockshott, Corsini, Gerberick, Idehara, Kimber, van Loveren, Takeyoshi, Matheson, Mehling et al. unpublished data; ICCVAM, 2007) which could provide a starting point. These considerations do raise an important point in relation to \textit{in vitro} methods, namely that whereas the \textit{in vivo} models give an integrated output, the \textit{in vitro} system informs only on what happens to the particular element of the mechanism for skin sensitization which is under investigation. This reinforces the need to have multiple \textit{in vitro} tests whose data are reintegrated (Jowsey et al., 2006), but ultimately, it should also focus us on the need to have an agreed list of significant skin sensitizers that any acceptable \textit{in vitro} alternative should identify as positive together with a matching list of substances that are either entirely nonsensitizing or are sufficiently weakly sensitizing, that, as with the current \textit{in vivo} methods, a reasonable \textit{in vitro} test would be expected to identify them as negatives. Then, given that any \textit{in vitro} test, just as with the current \textit{in vivo} methods, will not be perfect, we have to decide what level of accuracy is actually acceptable now and in the future. So, although the work by Natsch and Emter provides an important breakthrough insight, substantial impediments which still exist must be faced if we are to meet the sort of deadlines (March 2013) imposed by politicians in Europe for total replacement of \textit{in vivo} sensitization tests.

**REFERENCES**


