



Workshop Report

Classification of dermal sensitizers in pharmaceutical manufacturing



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ABSTRACT

Workers in development and manufacturing of pharmaceuticals are at risk for occupational contact dermatitis (OCD) of irritative (ICD) or allergic (ACD) origin, due to contacts with reactive intermediates (IM) and drug substances (DS). We examined, if alternative methods could replace presently used animal tests for identification of ACD in pharmaceutical development and manufacturing, without apparent loss of worker health, in line with regulations. The status of alternative methods for regulatory toxicology for consumer products has recently been reviewed by the Organisation for Economic Co-operation and Development (OECD) and the European Commission's Joint Research Center (JRC) for the European Chemicals Agency (ECHA). They concluded that prediction of skin sensitization potential, extent and quality by *in vitro* methods, for regulatory assessments, will depend on the regulatory purpose and level of confidence required. Some alternative methods are currently in validation. Current Globally Harmonized System (GHS) regulations on classification, labeling and packaging of substances and mixtures depend on human and animal data, whereas alternative methods may provide supportive evidence. Since the levels of workplace skin exposure to DS and IM in manufacturing of pharmaceuticals are usually not known, it is not possible to conduct quantitative risk assessments based on threshold calculations for contact sensitizers.

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1. Introduction

Occupational contact dermatitis (OCD) constitutes up to 30% of all notified occupational diseases. The registered incidence rate for OCD in some countries is 0.5–1.9 cases per 1000 full-time workers per year, with substantial underreporting (Diepgen and Coenraads, 2012). Eczema or contact dermatitis are estimated to amount to approximately 90–95% of all occupational skin diseases. There is a notion that irritative contact dermatitis (ICD) is more frequent than allergic contact dermatitis (ACD). The latter may outnumber the former in specialized occupational dermatology clinics (Antonov et al., 2012). Prevalence rates for ACD range from 19%

to 50% depending on profession and source (Antonov et al., 2012; Diepgen and Coenraads, 2012). Workers in pharmaceutical manufacturing are also at risk for OCD. This is attributed in part to both, contact with reactive intermediates and drugs (Goossens and Vander Hulst, 2012). OCD to intermediates occurs almost exclusively in employees working in drug development and manufacturing plants (Bircher, 2012). Thus, well-documented processes for hazard identification and quantitative risk assessment for contact sensitizers among pharmaceutically active ingredients and chemical intermediates would be desirable.

In context with recent developments in the cosmetic and food industries, implementation of GHS (globally harmonized system) regulations, an internal workshop was held followed by internal and external discussions to analyze the status quo of *in vitro* and *in vivo* testing for detecting potential contact sensitizers. We examined, if alternative methods could replace presently used methods for hazard identification of contact sensitization in pharmaceutical development and manufacturing, without apparent loss of worker health, in line with current regulations. An additional evaluation

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considered the possibility of quantitative risk assessments based on threshold calculations for contact sensitizers.

The objective of this publication is to determine currently available approaches which are used to assess potential opportunities for hazard identification and risk assessment in occupational health settings by reviewing current publications and regulations regarding classification of dermal sensitizers.

2. Material and methods

Database searches were initiated in Embase, Medline and Biosis (OvidSP provided by Wolters Kluwer, Alphen aan den Rijn NL), by combinations of the keywords “contact sensitization”, “dermal sensitizer”, “occupational health”, and “classification” covering the span 1996–2014. Resulting hits were reviewed and integrated into this publication.

3. Hazard identification in the field of consumer products

The status of alternative methods for regulatory toxicology has recently been reviewed by OECD (2012) and by the European Commission's Joint Research Center (JRC) for the European Chemicals Agency (ECHA) (EC JRC, 2014).

In the European Commission's JRC “Alternative methods for regulatory toxicology – a state-of-the-art review” publication, the progress of non-standard methods for assessing the toxicological properties of chemicals has been compiled (EC JRC, 2014). The term non-standard methods refers to alternatives to animal experiments, such as *in vitro* tests and computational models, as well as animal methods that are not covered by current regulatory guidelines, but could contribute to a reduction in the number of animals needed for each test. This report therefore assesses the current scientific status of non-standard methods for a range of human health and ecotoxicological endpoints, skin sensitization among others. Reference is made to the information needs of GHS (UNECE GHS, 2013) and the European regulation on classification, labeling and packaging of substances and mixtures (EC No 1272/2008), which aligns existing EU legislation to the United Nations GHS. The report (EC JRC, 2014) is also informative in relation to the possible use of alternative and non-standard methods in sectors other than industrial chemicals, such as cosmetics and plant protection products.

In order to replace animal testing in the hazard assessment of skin sensitization, a combination of different alternative methods addressing the key events in the skin sensitization pathway are needed (EC JRC, 2014).

Sixteen *in vitro* test methods are under formal validation (Table 1) by the European Union Reference Laboratory for alternatives to animal testing (EURL-ECVAM) (Reisinger et al., 2015) and are assessed for their reliability, with a view to their possible use within integrated assessment approaches (Rovida et al., 2015). Ten of the tests also predict sensitizer potency. These methods included: (1) the Direct Peptide Reactivity Assay (DPRA) which addresses protein binding by monitoring the depletion of a nucleophile-containing synthetic peptide; (2) the KeratinoSens assay which measures the activity of the antioxidant/electrophile response element dependent pathway in keratinocytes and (3) the human Cell Line Activation Test (h-CLAT) which measures the induction of CD54 and CD86 protein markers on the surface of THP-1 cell lines. These methods appear sufficiently reproducible to justify their inclusion in integrated approaches for hazard identification and classification. Some do provide endpoints for potency assessment, as well. Supportive evidence by alternative methods may be included into an integrated testing strategy providing a mechanistic rationale for the Adverse Outcome Pathway for skin

sensitization (OECD, 2012; Basketter et al., 2013; Rovida et al., 2015). To foster scientific and regulatory acceptance of alternative methods, a number of the validated methods have been tested recently against a data set of 213 substances (151 sensitizers, 62 non-sensitizers). Test results were compared with available human and animal data in integrated testing strategy assessments. The predictive accuracies of 90% versus human data and 79% versus animal data achieved even slightly exceeded predictivity of the LLNA (Urbisch et al., 2015).

The status of a quantitative risk assessment for contact sensitization potency has also been addressed (EC JRC, 2014). Quantitative structure activity relationship (QSAR) tools may predict key events in the sensitization pathway. In Table 2, three selected QSAR tools have been listed with respective references.

A number of computational *in silico* methods are presently in development for evaluation of chemicals without experimental data for sensitization. Alves et al. (2015) have discussed properties of their QSAR models and compared them with those of other publicly available models. They also pointed out the need to use a validated data set. In direct comparison with the OECD QSAR toolbox, the model of Alves et al. (2015) showed a higher specificity and correct classification rate, but lower sensitivity. The authors suggest to use the tool for identifying putative sensitizers as a first step in a tiered testing strategy.

4. Quantitative risk assessment in the field of consumer products

Since consumer exposure data are usually available for cosmetics, fragrances, personal care and food additives, a number of calculation models using the toxicological threshold principle have been proposed such as the dermal sensitization threshold (DST) (Safford et al., 2011), the dermal sensitization quantitative risk assessment (QRA) (Api et al., 2008) and the determination of the no-expected-sensitization-induction-level (NESIL) (Goebel et al., 2012). Depending on the studies, hazard identification was based on human data (patch test, human maximization test) (Api et al., 2008; Keller et al., 2009), animal data (mostly local lymphnode assay, LLNA) (Goebel et al., 2012; Griem et al., 2003; Anderson et al., 2011) or *in vitro* endpoints (Ryan, 2008).

The report (EC JRC, 2014) concludes that the current status regarding prediction of skin sensitization potential extent and quality by *in vitro* methods, as needed in a regulatory assessment, will depend on the regulatory purpose and the level of confidence required. In general, a greater burden of proof will be required to conclude on the absence of skin sensitization potential than to conclude on its presence.

5. Current regulations

Besides research, development, and evaluation of drug safety for patients, the pharmaceutical industry aims to set safe protection measures for employees in research labs and manufacturing operations for DS and IM. In recent assessments on the application of the threshold of toxicological concern (TTC) concept to pharmaceutical manufacturing operations, thresholds for genotoxic pharmaceutical impurities were reviewed (Hennes, 2012). TTC values for carcinogenic and non-carcinogenic effects of compounds with limited toxicological data were proposed (Dolan et al., 2005). In contrast, there is no generally applicable method to calculate threshold levels for sensitizers at the workplace (DFG 2014).

According to the current GHS (UNECE GHS, 2013) and the European regulation on classification, labeling and packaging of substances and mixtures (EC No 1272/2008), DS and IM have to be assessed for contact sensitizing properties as well, since the

Table 1

Sixteen *In vitro* test methods, under formal ECVAM step 1 validation (data from [Reisinger et al., 2015](#)). Abbreviations: S = sensitizer; NS = non-sensitizer; n. a. = not applicable; n. av. = not available.

Test method	Test principle	AOP (adverse outcome path-way) key event impacted	Concordance for 10 chemicals tested: 7 S and 3 NS	OECD test guideline status
AREc32	Activation of the Keap1/Nrf2/ARE pathway in MCF7 cells	Haptenation: covalent modification of epidermal proteins	8/10	n. av.
DPRA	Direct peptide reactivity assay	Haptenation: covalent modification of epidermal proteins	10/10	TG442CFeb-2015
GARD	GARD assay uses proliferating MUTZ-3 cells (a human myeloid leukemia-derived cell line) to measure gene expression induced by test substances	Activation of epidermal keratinocytes and dendritic cells	9/10	n. av.
h-CLAT	Uses THP-1 cells (human monocytic leukemia cell line) as a surrogate for dermal dendritic cells	Activation of epidermal keratinocytes and dendritic cells	9/10	Draft July 2014
KeratiSens™	Metabolic-competent human keratinocyte HaCaT cell line	Activation of epidermal keratinocytes and dendritic cells	9/10	TG442DFeb-2015
Lu-Sens	Keratinocyte-derived cell line	Activation of epidermal keratinocytes and dendritic cells	8/10	n. av.
mMUSST	U937 cell line (human histiocytic leukemia cell line) to evaluate capacity to induce dendritic cell activation	Activation of epidermal keratinocytes and dendritic cells	9/10	n. av.
MUSST	U937 cell line (human histiocytic leukemia cell line) to evaluate capacity to induce dendritic cell activation	Activation of epidermal keratinocytes and dendritic cells	10/10	n. av.
NCTC2544	Intracellular IL-18 expression by the keratinocyte cell line NCTC 2544	Activation of epidermal keratinocytes and dendritic cells	6/10	n. av.
PMDC	Human peripheral blood monocyte-derived dendritic cells isolated from the fresh buffy coats of five different donors used. If a test substance induces on average P20% increase in CD86-positive cells compared to nontreated cells it is considered as a skin sensitizer	Activation of epidermal keratinocytes and dendritic cells	8/10	n. av.
PPRA	Peroxidase peptide reactivity assay	Haptenation: covalent modification of epidermal proteins	8/9	n. av.
SenCeeTox	Protein reactivity and expression of four housekeeping and seven target genes are evaluated	Haptenation: covalent modification of epidermal proteins and activation of epidermal keratinocytes and dendritic cells	9/10	n. av.
SensiDerm™	SensiDerm assay uses proliferating MUTZ-3 cells (a human myeloid leukemia-derived cell line) to measure pathway-specific biomarker proteins induced by test substances	Activation of epidermal keratinocytes and dendritic cells	6/6	n. av.
Sens-IS	Sens-IS method classifies sensitizers according to potency categories based on the expression profiles of 65 genes	Haptenation: covalent modification of epidermal proteins	10/10	n. av.
VITO-SENS	VITASENS assay uses differentiated CD34 + progenitor cells (from human cord blood) as surrogate for dendritic cells. Measure gene expression of CCR2 and the transcription factor cAMP responsive element modulator	Activation of epidermal keratinocytes and dendritic cells	9/10	n. av.
EE Potency	Epidermal equivalent <i>potency assay</i> detects intracellular IL-18 expression by the keratinocyte cell line NCTC 2544	Activation of epidermal keratinocytes and dendritic cells	n. a.	n. av.

Table 2

Selected QSAR tools presently used for prediction of chemical sensitizers.

QSAR – quantitative structure–activity relationship model	Model applied	Concordance and endpoint	References
<i>In Silico</i> predictions of skin sensitization using OECD QSAR toolbox	Read-across, structure activity	Accuracy 77%	Strickland et al. (2015)
Predictive QSAR model of skin sensitization	Structure–activity relationship	Correct classification rate 71–88%	Alves et al. (2015)
DEREK for Windows Ver. 10.0.2	Structural alerts	15 of 28 strong sensitizers identified	Gould and Taylor (2011)

synthesis process may take many steps to build the drug molecule from chemical intermediates that are generally reactive by nature ([Gould and Taylor, 2011](#)). Both, GHS and EU regulations depend on hazard classification of skin sensitizers by human data and results from validated animal testing. Positive data from non-standard methods and close structural analogs may be considered on a case-by-case basis. In addition, GHS suggests allocation of skin sensitizers into potency sub-category 1A, strong sensitizers or 1B for other skin sensitizers, where human or animal data are sufficient ([UNECE GHS, 2013](#)). The objective is to detect any indication for

skin sensitizing properties from human experience of skin allergy following exposure to the agent and/or from animal testing ([Cockshott, 2008](#)). The current GHS and EU classifications are both hazard based systems. Until 2014, GHS regulations have been adopted and integrated into legislation by 67 countries and numerous organizations ([UNECE GHS implementation, 2014](#)).

Independent of the current legal classification and labeling systems (GHS, [EC No 1272/2008](#)), there are organizations and national regulations, which provide guidance for worker protection in research labs and manufacturing operations (e.g. [Murakami et al., 2007](#); [WHO/IPCS, 2008](#); [DFG, 2014](#)). A comparison between classifications of skin sensitizing substances by the German DFG-MAK commission (Deutsche Forschungsgemeinschaft, Maximale Arbeitsplatz Konzentrationen; Commission for the investigation of health hazards of chemical compounds in the work area) and by the EU legislation has been published by [Lessmann et al. \(2011\)](#). Although the DFG-MAK commission uses its own criteria regarding the validity of available human and animal studies, weight of scientific evidence and presumed occupational exposure, there are few differences between the two classification schemes ([Schnuch et al., 2002](#); [Lessmann et al., 2011](#)).

Main criteria as used by the DFG-MAK commission are sufficiently documented clinical data (case reports, epidemiological data, and patch test results). As far as possible, presumed

occupational exposure is taken into account (Schnuch et al., 2002). This is in line with the European Chemicals Agency (ECHA) guidance on information requirements and chemical safety assessment Part E: Risk characterization, which states that “Since sensitisation is essentially systemic in nature, it is important for the purposes of risk management to acknowledge that skin sensitisation may be acquired by other routes of exposure than dermal. There is therefore a need for cautious use of known contact allergens in products to which consumers or workers may be exposed by inhalation” (ECHA, 2012). Workers in pharmaceutical manufacturing are potentially exposed to airborne and dermal DS and IMs.

In context with the quantitative aspect of risk assessment for skin sensitizers, the same authors stated in 2005 that “until quantitative data on potency and exposure become a formal part of the classification and regulation process, which is clearly an important aim, the surrogate of clinical experience and expert judgment should not be disregarded” (Schnuch et al., 2005). Finally, the DFG-MAK commission states that “it is still not possible to determine (and document scientifically) generally applicable threshold concentrations either for the induction of an allergy (sensitization) or for triggering the allergic reaction (elicitation) in an already sensitized person” (DFG, 2014; page 182). This means that presently there is no generally applicable method to calculate workplace exposure threshold concentrations (MAK value, maximum concentration at the workplace). The reason is that workplace skin exposure to DS and IMs in manufacturing are mostly not known. On the other hand, results from specific workplace exposure measurements would be applicable only for a particular setting, where the measurements were conducted in alignment with the complex relationships between exposure, co-exposure, personal susceptibility, pre-existing skin disease, gene-environment interaction and onset of contact allergic reactions. In addition, the methods applied for consumer product exposure are not transferable to the workplace exposure in pharmaceutical manufacturing.

Furthermore, exposure–response relationships between occupational allergy of the immediate type (immune globulin E mediated) and exposures to well-known workplace allergens such as flours, rat allergens, and detergent enzymes have been reviewed by Jones (2008). He pointed out that exposure–response relationships are also very dependent on the route of exposure and genetic susceptibility of the individual, thus contributing to the problem of defining a generally applicable threshold concentration calculation for workplace exposure of sensitizers.

6. Conclusions

In the development of pharmaceuticals, alternative methods for hazard assessment for contact sensitizers have reached successfully the status of validation and test guideline development. Qualitative comparison of test results with available data sets (to human and animal data), indicate that a good predictivity can be reached. For the time being, alternative methods may form part of a tiered hazard assessment model with subsequent confirmation in the LLNA. In addition, the QSAR tools are used for DS and IM in very early drug development to get a first alert for possible sensitizers.

In context with GHS (UNECE GHS, 2013) and the European regulation on classification, labeling and packaging of substances and mixtures (EC No 1272/2008), classification is based on human data and results from validated animal testing. Once, regulatory bodies such as GHS accept replacement of the LLNA with alternative non-animal tests, the situation has to be reanalyzed.

Hazard characterization for consumer products can be obtained from animal tests (or from alternative methods) as a quantitative measure and transformed in a threshold concentration (e.g. NESIL) of the ingredient in the finished product for consumers.

The threshold concentration is then compared with the presumed consumer exposure, which is sometimes difficult to obtain. The concentration of the ingredient in the finished product is then adjusted to a concentration below the one for induction of sensitization. This threshold, however, is higher than the threshold for elicitation of ACD in people already sensitized.

In contrast, airborne workplace exposure and skin exposure to DS and IM in pharmaceutical manufacturing and in research labs are usually not studied and not known. Thus, worker protection is based on hazard assessment and qualitative risk assessment, only. Risk management is then conducted with protective measures. Very strong sensitizers (e.g. certain antibiotics) are produced in dedicated manufacturing facilities with protective containments.

Whereas human data are mostly available for DS, they are usually absent for IMs. These conditions require a pragmatic approach using clinical data (animal data, if human data are unavailable) and presumed occupational exposure for classification. Since the sensitization levels at the workplace with the corresponding airborne and skin exposure are not known, and the extrapolation from animal data cannot be verified with human data, a quantitative risk assessment cannot be performed. Whereas it should be scientifically possible to calculate individual threshold concentrations for each DS and IM based on potency of the sensitizer, it is not possible to determine (and document scientifically) generally applicable threshold concentrations either for the induction of an allergy (sensitization) or for triggering the allergic reaction (elicitation) in an already sensitized person (DFG, 2014).

This assessment of the status of available alternative methods for detecting potential contact sensitizers and of the progress in quantitative risk assessment for consumer products has pointed toward fundamental differences between consumer and worker risk assessment. Whereas skin exposure of consumers by the finished product can be readily assessed, this is not possible in most cases for workers in pharmaceutical manufacturing and research labs. Even if suitable measurements of workplace skin exposure would be possible, it would apply only to a specific setting.

Nevertheless, the publication also indicates possible opportunities for implementing QSAR tools (as well as read-across and grouping) in the preliminary hazard assessment of DS and IM at a very early stage i.e. when chemical structures are available, but there is no available test material for any laboratory tests. On the other hand, non-animal testing methods can be integrated into tiered hazard assessment models (and possibly waiving animal tests). For this purposes, alternative methods provide valuable information and good predictivity.

Conflict of Interest

The authors declare that they have no conflict of interest.

Transparency Document

The [Transparency document](#) associated with this article can be found in the online version.

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