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Categorisation of protein respiratory allergens: The case of Subtilisin

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ABSTRACT

Characterisation of the relative sensitizing potency of protein and chemical allergens remains challenging, particularly for materials causing allergic sensitization of the respiratory tract. There nevertheless remains an appetite, for priority setting and risk management, to develop paradigms that distinguish between individual respiratory allergens according to perceptions of the hazards and risks posed to human health. One manifestation thereof is recent listing of certain respiratory allergens as Substances of Very High Concern (SVHC) under the provisions of REACH (Registration, Evaluation, Authorisation and restriction of Chemicals). Although priority setting is a laudable ambition, it is important the process is predicated on evidence-based criteria that are transparent, understood and owned. The danger is that in the absence of rigorous criteria unwanted precedents can be created, and confidence in the process is compromised. A default categorisation of sensitisers as SVHC requiring assessment under the authorisation process is not desirable. We therefore consider here the value and limitations of selective assignment of certain respiratory allergens as being SVHC. The difficulties of sustaining such designations in a sound and equitable way is discussed in the context of the challenges that exist with respect to assessment of potency, and information available regarding the effectiveness of exposure-based risk management.

1. Introduction

For effective toxicological evaluations it is necessary to identify the intrinsic hazards associated with substances, and to assess likely risks to human health. If such evaluations indicate a potential for substantial or severe human health risks then it is of course legitimate to focus particular attention on those materials that are deemed to be of particular concern.

Under the provisions of Article 57(f) of REACH (Registration, Evaluation, Authorisation and restriction of Chemicals) such materials may be designated as Substances of Very High Concern (SVHC) if they possess the intrinsic potential to cause severe, even life-threatening, effects in humans at relatively low levels of exposure (European Chemicals Agency, 2007; Ter Burg and Jongeneel, 2011). Both skin and respiratory sensitising chemicals are being considered under REACH for categorisation as SVHC. Three chemical respiratory sensitisers have been given first priority in the evaluation process (diazene-1,2-dicarboxamide [ADCA], hexahydro-2-benzofuran-1,3-dione [HHPA], and hexahydromethylphthalic anhydride [MHHPA]) and have already been listed on the REACH SVHC candidate list (http://echa.europa.eu/candidate-list-table).

In 2011, the National Institute for Public Health and the Environment (RIVM) in the Netherlands stated that a proper risk management option for respiratory sensitizers could be to qualify them as SVHC in accordance with Article 57(f) of REACH European Chemicals Agency, 2007. The provisions under article 57(f) concern "*substances – such as those having endocrine disrupting properties, or those having persistent, bioaccumulative and toxic properties or very persistent and very bioaccumulative properties, which do not fulfil the criteria of points (d) or (e) – for which there is scientific evidence of probable serious effects to human health or the environment which give rise to an equivalent level of concern to those of other substances listed in points (a) to (e) and which are identified on a case-by-case basis in accordance with the procedure set out in Article 59*".

In the RIVM report (Ter Burg and Jongeneel, 2011), Subtilisin (a proteolytic enzyme of bacterial origin widely used in detergent products) was listed as a possible SVHC candidate. In this respect, it is of note that bacterial and fungal enzymes have for many years been widely used industrially and are well recognized as being associated with occupational respiratory allergy. The ability of proteins to cause allergic sensitization of the respiratory tract is, however, not limited exclusively to enzymes. Although detergent enzymes, and enzymes generally have been implicated as respiratory allergens, there are a variety of other proteins from diverse sources that are known to be associated with sensitisation of the respiratory tract and occupational asthma (including, for example,

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egg and other food proteins, bakery proteins, and protein dander from experimental animals) (Basketter et al., 2012a,b; Martel et al., 2010; Baur, 2013; Holsapple et al., 2006; Basketter and Kimber, 2011).

There are approximately 400 enzymes listed in the European Inventory of Existing Commercial Chemical Substances (EINECS) EINECS, 2012. Of those, 17 enzymes (13 enzymes on EINECS, 1 enzyme on ELINCS [European List of Notified Chemical Substances], and 3 enzyme groups) are listed in Annex VI of the EU's CLP (Classification, Labelling and Packaging) regulation (United Nations Nations, 2011). The common harmonized classification of these 17 enzymes is that of respiratory sensitizer. In general, enzymes, regardless of their catalytic activities, are potential respiratory sensitizers, whereas the weight of evidence from humans indicates that enzymes are not able to cause skin sensitization (Basketter et al., 2008, 2010, 2012, 2012a; Human and Environmental Risk Assessment on ingredients of household cleaning products: protease (Subtilisin), 2007; Human and Risk Assessment on ingredients of household cleaning products: α-AMYLASES, 2005). Except where there is compelling evidence to the contrary, all enzymes should be regarded as having the inherent potential to cause sensitization of the respiratory tract and should be classified "H334: Hazard Category 1: May cause allergy or asthma symptoms or breathing difficulties if inhaled" in accordance with the CLP Regulation (United Nations Nations, 2011). However there have been successful innovations, such as encapsulation and other formulation changes to craft non-inhalable forms of the products that reduce consumer risk. In addition, it has been possible to determine safe exposure levels of occupational and consumer exposures (Basketter et al., 2010; Sarlo et al., 2010).

The above listing of respiratory sensitisers on the REACH SVHC candidate list has potentially important implications. These are reflected by a letter issued by CEFIC (European Chemical Industry Council) dated March 12th 2013 and entitled "*Industry's viewpoint on the Roadmap on Substances of Very High Concern*" (CEFIC, 2013). In that letter it is stated that there may be significant repercussions in designation of a material as a SVHC, and that there has emerged a "black list" effect. As a consequence the designation should be used only sparingly and in circumstances where there is a clear need. In the present paper, the argument is made that an appropriate analysis of available risk management options should be explored in advance of any substance being proposed to be placed on the SVHC candidate list. The point is that the designation as SVHC may not be appropriate or necessary if there are available relevant approaches and procedures to mitigate the potential risks to human health. Thus, SVHC designation should be applied with due discretion.

In this commentary we will consider the case for the proposal that Subtilisin is a candidate for designation as a SVHC. In this context we will specifically address two issues: (a) whether there exists a legitimate evidence-based argument for the differential categorisation of protein respiratory allergens according to their intrinsic potency, and (b) whether, in the case of Subtilisin in particular, there is reason to believe that there exists a substantial risk to human health. These are important issues because they inform our appreciation of the extent to which it is possible currently to evaluate accurately the relative sensitizing properties of allergen, and the principle of risk-based decision making.

2. The relative sensitizing potency of respiratory allergens

At least in theory there are several ways in which the potency of allergens could be defined. However, for the purposes of hazard characterization and risk assessment the most important and most valuable metric is based on an understanding of the level of exposure that will trigger the acquisition of sensitization. There is available a recent detailed critique of the uncertainties associated with evaluation of the potency of respiratory allergens (Basketter and Kimber, 2011), and therefore a similarly detailed account is not required here.

Although the difficulties in determining the relative potency of protein respiratory allergens are not as great as those associated with chemical respiratory allergens, there are nevertheless important challenges (Basketter and Kimber, 2011).

Those challenges can be best illustrated by reference to the characterization of contact allergens. Here, the local lymph node assay (LLNA) can be used to compare and contrast the ability of contact allergens to cause the proliferation and clonal expansion of responsive T lymphocytes in lymph nodes draining the site of exposure. It is well established that this proliferative response by draining lymph node cells provoked by encounter with a contact allergen is causally and quantitatively correlated with the acquisition of sensitization. At a practical level, therefore, the relative skin sensitizing potency of a contact allergen is measured by consideration of dose response profiles in the LLNA, and identification of the concentration of allergen required to induce a certain level of response (specifically, a 3-fold increase in proliferation compared with concurrent vehicle-treated controls). This value is defined as EC3; the **E**ffective **C**oncentration of a contact allergen required to stimulate a **3**-fold increase in proliferation (Kimber et al., 2001).

On this basis it is clear that for adoption of a similar approach for assessment of the relative potency of respiratory allergens it will be necessary to identify biomarkers or responses that correlate quantitatively with sensitization of the respiratory tract. There is a continuing debate about the immunological processes through which chemicals cause sensitization of the respiratory tract. The picture is somewhat clearer in the case of protein allergens, and there is a general consensus that IgE antibodies plays a pivotal role.

In brief the development of allergy induced by exposure to proteins can be summarized as follows. Encounter with sufficient protein allergen, by an appropriate route, provokes an adaptive immune response that is associated with the elaboration of allergen-specific IgE antibodies. These antibodies distribute systemically and associate with mast cells that are found in virtually all vascularized tissues. At this point sensitization has been achieved. If the now sensitized subject encounters the same protein (and for the purpose of this article by inhalation into the respiratory tract), then that protein will bind with, and cross-link, IgE antibodies displayed on the mast cell surface. This in turn causes mast cell activation and the release by degranulation of both preformed and newly synthesized mediators (such as vasoactive amines and leukotrienes) that act in concert to drive the inflammatory response that is recognized clinically as respiratory allergy. Symptoms may range from mild itching, congestion and rhinitis, to severe asthma.

Against this background there are two main strategies that have been pursued for the identification and characterization of protein respiratory allergens, although it is important to acknowledge that neither approach has been formally validated for this purpose. The first is to attempt to measure directly the ability of test proteins to induce and elicit respiratory hypersensitivity reactions in experimental animals. The second is to consider instead the capacity of proteins to provoke IgE responses. In many instances the two approaches have been used in tandem (Karol et al., 1989; Kawabata et al., 1986; Ritz et al., 1993; Robinson et al., 1996, 1998; Sarlo et al., 1991; Sarlo and Karol, 1994). The former approach lacks the resolving power to provide a useful and reliable approach for the assessment of relative sensitising potency, and therefore measurement of IgE responses is the more attractive approach.

However, although IgE antibody is clearly identified as a critical effect molecule, and is the most obvious candidate as a correlate of sensitization, there are both technical challenges and mechanistic