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A review of critical factors in the conduct and interpretation of the human repeat insult patch test

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Abstract

This paper reviews key factors that are critical to the conduct and interpretation of Human Repeat Insult Patch Tests (HRIPTs). A methodology for HRIP testing is described and general guidelines for evaluation of responses indicative of induction and elicitation of skin sensitization and skin irritation are detailed. Understanding and applying these key factors is crucial to the design of such studies and reliability of the resulting data when used in the overall risk assessment process.

Keywords: Skin; Contact allergy; HRIPT; Critical factors; Design; Evaluation

1. Introduction

Integral to the development of consumer products and their ingredients is the evaluation of their potential to cause skin sensitization and allergic contact dermatitis (ACD). This is done by risk assessment, a multi-step iterative process which has been reviewed elsewhere (Basketter et al., 1999; Felter et al., 2003; Gerberick and Robinson, 2000; Gerberick et al., 1993, 2001; Nusair et al., 1988; Robinson et al., 1989, 2000).

The skin sensitization potential of a material is established pre-clinically through (1) its analytical and structural characterization; (2) literature review and where appropriate (3) animal testing [e.g., murine local lymph node assay (LLNA) or guinea pig tests (GPT)]. The availability of data that confirm humans will not respond adversely remains an important element of the overall risk assessment process. In the absence of existing human data, it may be advantageous to perform Human Repeat Insult Patch Testing (HRIPT) either to confirm a No-Effect Level (NOEL) used as one of the data sources in the establishment of a No Expected Sensitization Induction Level (NESIL) as part of a recently described Quantitative Risk Assessment (QRA) framework (Api et al., 2008) or to demonstrate that humans will not respond adversely to a particular formulation. Other complementary but less reliable on their own sources of such human data may be clinical in-use testing and later monitoring/follow-up of consumer comments.

Human patch testing methodology has evolved over more than 50 years, since first proposed in 1944 by Schwartz and Peck (Schwartz and Peck, 1944) and has since been extensively reviewed (Griffith, 1969; Hardy, 1973; Kligman, 1966a; Marzulli and Maibach, 1976a, 1996;

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Patrick and Maibach, 1995; Stotts, 1980). In every method one or more induction exposures is followed by a rest period and then a challenge exposure, but variations exist as to patch type, number of subjects, skin site, induction patch number, patch application time, duration and rest period prior to challenge. In all, enhancement of the skin response, after challenge over that seen during early induction exposures has been the criterion by which induction of skin sensitization is measured.

With cumulative experience from the consumer products industry has come an awareness of certain factors that are believed to be critical to a reliable test result. This paper discusses those key factors that can affect the design, conduct and interpretation of the results, and that are believed to provide greater assurance of a test result of high quality obtained within ethical guidelines for human volunteer testing.

2. Critical factors affecting the design and interpretation of HRIPTs

In 1966, Kligman followed his critique of standard methods (Kligman, 1966a) with a review of factors influencing the induction and measurement of ACD (Kligman, 1966b). Marzulli and Maibach stated in 1974 that it is important to take into account factors that could adversely affect the calculated margin of safety, such as frequency of application, contact area, permeability of skin site and occlusion when conducting threshold studies using the human Draize procedure (Marzulli and Maibach, 1974). More recently, Emmet et al. have suggested that factors such as heat, moisture, pressure, occlusion, duration of contact and irritation may affect sensitization dose–response relationships (Emmet et al., 1994). There are several factors that experience indicates are critical to consider before conduct of an HRIPT and for interpretation of the results (Fig. 1).

- Vehicle/Matrix Effects
- Test Material Concentration (Dose/Unit Area)
- Amount of Test Material Applied
- Occlusion
- Chemistry
- Target Population
- Allergen Potency

Fig. 1. Critical factors for HRIPT conduct and interpretation.

2.1. Vehicle/matrix effects

If it is appropriate and possible, the preferred method is to use a test material either undiluted or at the NOEL concentration (chosen based on other human and/or pre-clinical data). Where irritation or other considerations necessitate dilution or an undiluted test material represents highly unrealistic exposure (e.g., fragrance oil), then selection of a suitable vehicle (diluent) becomes necessary. Since the choice of vehicle can have a profound effect on the physicochemical properties of the test material and its bioavailability, it is essential to choose the appropriate vehicle with due regard to whether it is inherently irritating (Robinson et al, 1991), potentially sensitizing (Stotts and Ely, 1977), enhances penetration of materials across the skin (Robinson et al., 1991; Heylings et al., 1996), can interact with or alter the test material (Calvin, 1992) and is a suitable solvent (i.e., can solubilize or produce a stable suspension) for the test material (Marzulli and Maibach, 1976b).

A vehicle can be a simple single moiety (e.g., water), mixtures (acetone/water, ethanol/water) or a complex matrix presented in undiluted or diluted form. The effect of a complex matrix, as a vehicle, on the physicochemical parameters and bioavailability of a test material may be substantially different from that of a simple vehicle. The test material may preferentially partition into one phase of such a vehicle, resulting in a higher (possibility of inducing contact sensitization) or lower (possible false negative result) concentration than anticipated.

The skin sensitization potential of a test material can be affected by the vehicle (Stotts, 1980; Kligman, 1966b; Marzulli and Maibach, 1976b). For this reason, a vehicle relevant to a final formulation is the optimum choice. Additionally, in the event of follow-up (or re-challenge) work with a subject who may be sensitized, testing of an individual product ingredient may require a different vehicle than that relevant for a product formulation. As such, any alternative vehicle(s) may influence reactivity of a sensitized subject at re-challenge (Stotts, 1980). Ethanol (1:1 in water), for example, has been shown to be a skin sensitizer under certain exposure conditions (Stotts and Ely, 1977). The potential of ethanol to sensitize under occluded patches can be reduced by allowing evaporation for 10 to 20 min before the patch is applied or by using an alternative patching technique. Evaporation, however, has the disadvantage of altering the composition of the material placed on the patch (Stotts, 1980). Mineral oil (liquid petrolatum), as another example, has important advantages of being non-irritating, non-sensitizing and often permits the testing of a high sample concentration. However, it may not solubilize all test material components. One special precaution is that allergic reactions may be weaker in intensity and slower to develop with mineral oil as a vehicle rather than water or an organic solvent (Stotts, 1980). Acetone (1:1 in water), corn oil, and glycerin are other vehicles which may be useful under certain conditions (Stotts, 1980).