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Abstract

There is continued interest in, and imperatives for, the classification of contact allergens according to their relative skin sensitising potency. However, achieving that end can prove problematic, not least when there is an apparent lack of concordance between experimental assessments of potency and the prevalence allergic contact dermatitis as judged by clinical experience. For the purpose of exploring this issue, and illustrating the important considerations that are required to reach sound judgements about potency categorisation, the lower alkyl methacrylate esters (LAM) have been employed here as a case study.

Although the sensitising potential of methyl methacrylate (MMA) has been reviewed previously, there is available new information that is relevant for assessment of skin sensitising potency. Moreover, for the purposes of this article, analyses have been extended to include also other LAM for which relevant data are available: ethyl methacrylate (EMA), n-butyl methacrylate (nBMA), isobutyl methacrylate (iBMA), and 2-ethylhexyl methacrylate (EHMA).

In addressing the skin sensitising activity of these chemicals and in drawing conclusions regarding relative potency, a number of sources of information has been considered, including estimates of potency derived from local lymph node assay (LLNA) data, the results of guinea pig assays, and data derived from *in silico* methods and from recently developed *in vitro* approaches. Moreover, clinical experience of skin sensitisation of humans by LAM has also been evaluated.

The conclusion drawn is that MMA and other LAM are contact allergens, but that none of these chemicals has any more than weak skin sensitising potency. We have also explored here the possible bases for this modest sensitising activity.

Finally, the nature of exposure to LAM has been reviewed briefly and on the basis of that information, together with an understanding of skin sensitising potency, a risk assessment has been prepared.

Introduction

It is now well established that contact allergens vary considerably (and by up to 5 orders of magnitude) with respect to their relative skin sensitising potency. As a result there has been considerable interest in the categorisation of chemical allergens with respect to potency (1). However, although this is a legitimate aim, and a potentially very useful development, classification of chemical allergens in this way is not without problems. It is necessarily the case that relevant data should be available that provide a sound evidential basis for differential categorisation. However, it is appropriate, in addition, to distinguish carefully between potency and prevalence. That is, an important potential confounder is that there may be a lack of apparent concordance between estimates of potency derived from experimental assessment of skin sensitising activity and reports of prevalence of allergic contact dermatitis (ACD) that derive from clinical experience. To explore these issues, and to illustrate the important considerations that need to be addressed, the skin sensitising activity of methyl methacrylate (MMA) and other lower alkyl methacrylate esters (LAM) have been reviewed.

Methyl methacrylate is an α/β -unsaturated monomeric ester that is produced in relatively high volumes and used in a number of industrial applications and consumer products (2). This chemical forms part of a series of lower alkyl methacrylate esters (LAM) that includes as well as MMA, ethyl methacrylate (EMA), n-butyl methacrylate (nBMA), isobutyl methacrylate (iBMA) and 2-ethylhexyl methacrylate (EHMA). The chemical structures and key physico-chemical properties of the LAM considered in this article are provided in Table 1. The ability of MMA to cause allergic sensitisation has been considered previously. It is acknowledged that MMA has the potential to cause skin sensitisation resulting in ACD (3), but, although it is associated with respiratory irritation, the available evidence indicates that MMA fails to cause sensitisation of the respiratory tract and allergic asthma (4).

For purposes of this article the skin sensitising properties and potency of MMA will be reviewed using new and previously available data, and this analysis will be extended to embrace also a similar assessment of EMA, nBMA, iBMA and EHMA. For the purposes of exploring the assessment of potency for the purposes of classification, the LAM provide a relevant case study, not least – for example - because of the recent assessment of MMA by the American Conference of Government Industrial Hygienists (ACGIH). In 2013 the ACGIH published a draft notice of change that identified MMA as a potent skin sensitiser (5). Although, on the basis of further review, that assessment was rescinded, the original proposal does serve to illustrate the potential difficulties in deriving accurate assessments of skin sensitising potential when there are apparently inconsistent data available.

Skin sensitisation and allergic contact dermatitis: the importance of potency, hazard characterisation and risk assessment

Skin sensitisation resulting in allergic contact dermatitis is the most common manifestation of immunotoxicity in humans, and an important occupational health issue. In fact, there are many hundreds of chemicals that have been identified as having skin sensitisation potential sufficient to cause ACD (6). Considerable progress has been made in developing a more detailed, but as yet incomplete, understanding of the cellular and molecular events that result in the acquisition and orchestration of skin sensitisation, and in the elicitation of ACD (7-10). That increased understanding of the immunological and biochemical processes that cause skin sensitisation have, in turn, paved the way to improved methods for toxicological evaluation (11). Naturally, the development of accurate risk assessments requires, in addition to effective hazard characterisation, a clear appreciation of likely considerations of exposure. The importance of exposure considerations in the context of LAM is explored later in this review.

The initial approach to the identification of skin sensitisation hazards made use of guinea pig test methods in which activity was measured as a function of visual assessment of challenge-induced skin reactions in animals exposed previously to the test chemical. The guinea pig tests most thoroughly evaluated and most commonly used were the guinea pig maximisation test (GPMT) and the occluded patch test (12, 13). These methods served the toxicology community well, but were subject to a variety of limitations, not least of which was the lack of an objective measure of sensitising potential. In an attempt to address some of those limitations the mouse local lymph node assay (LLNA) was developed in which skin sensitising chemicals are identified by their ability to provoke lymphocyte proliferative responses in lymph nodes draining the site of topical exposure to the test material (14, 15). The LLNA was evaluated extensively and was the subject of formal validation exercises. It is currently the preferred method for the identification of contact allergens and has been used extensively for this purpose. A detailed consideration of the LLNA is beyond the scope of this article, but there are several reviews available that document the development, validation, acceptance and practical application of this method (16-19). Experience has shown that the LLNA provides a generally accurate and reliable way of identifying skin sensitisation hazards, and compared with the guinea pig tests that it largely superseded, offers a number of important advantages.

However, reliable hazard identification is only the first step in the toxicological evaluation of skin sensitisation potential. Although considerations of potency are germane for all classes of toxicants,

this is of particular relevance for contact allergens that are known to differ by up to 5 orders of magnitude with respect to their relative skin sensitisation potency (1).

Skin sensitisation potency is best defined in terms of the amount of chemical that is required to cause the acquisition of sensitisation; the more potent the chemical allergen the smaller will be the threshold exposure concentration required to induce skin sensitisation. Although, in some instances it is possible to derive estimates of sensitising potency from the results of guinea pig assays, this is not always the case since such methods are not normally configured to allow characterisation of dose-response relationships during the induction phase of skin sensitisation (1).

In contrast, the LLNA has proven useful for characterisation of the relative potency of contact allergens. This is due to the fact that the end point used in the LLNA (proliferative responses by draining lymph node cells [LNC] provoked by local topical exposure to a contact allergen) is both causally and quantitatively related to the effectiveness with which skin sensitisation will be acquired (20-22). The central event in the acquisition of skin sensitisation is the activation and clonal expansion of allergen responsive T lymphocytes within lymph nodes draining the site of exposure to the chemical allergen. Evaluation of proliferation by draining LNC induced by exposure to chemical provides a convenient measure of this, and thereby of the vigour and potency of the response (20-22).

Given that relationship, the LLNA has been adapted to provide a means of deriving an assessment of the relative sensitising potency of contact allergens (1, 23). The approach used is to derive from dose response relationships in the LLNA an EC3 value; defined as the amount of test chemical required to elicit a 3-fold increase in proliferative activity compared with concurrent vehicle control values (24). The EC3 value has been shown to provide a reliable measure of skin sensitising potency (25-27). Importantly, there is a close association between EC3 values derived from the LLNA and the relative potency of contact allergens as judged by clinical experience (28, 29). Moreover, it has been shown that there exists a correlation between human skin sensitisation thresholds and potency as determined through LLNA EC3 values (30, 31).

The availability of a robust and reliable method for assessment of relative skin sensitising potency is of considerable importance for the classification of contact allergens and for the development of accurate assessments of risk (1, 32, 33). Moreover, a quantitative measure of potency allows for discrimination between the prevalence of ACD to a particular contact allergen and its inherent sensitising potency. The two metrics are independent. Thus, in the US and Western Europe nickel is regarded as a common allergen, and among the most prevalent causes of ACD. However, nickel is

not considered to be a potent contact allergen, and is in fact a relatively weak sensitiser. The high prevalence of ACD caused by nickel is not a reflection of skin sensitising potency, but rather the widespread opportunities that exist for exposure generally, and for intimate exposure in particular (18).

Recently, for a variety of reasons, there has been a considerable investment in the development of alternative (non-animal) methods for the assessment of skin sensitising activity. Among the generic approaches that have attracted interest are the following: (a) peptide reactivity assays for the identification of sensitising chemicals as a function of their inherent or acquired electrophilicity and the ability to form stable associations with protein (34, 35), (b) models of (quantitative) structure-activity relationships ([Q]SAR) (36, 37), and (c) various cell-based assay systems using most commonly dendritic (DC)/DC-like cells or keratinocytes (38-40). Some of these assays are approaching validation or have recently been validated (41), whereas others are less well developed and should be regarded as work in progress. It must be emphasised that the above list is by no means exhaustive and that there are other methods, and other strategies, for predictive testing that are currently being explored. The important point is, however, that although some of the above approaches may – in time – yield methods suitable for the identification of contact allergens, their ability to provide information about relative potency is much less certain. Although some progress is reported, and interesting paradigms have been proposed (42, 43), experience to date indicates that it will prove challenging to develop a method, or suite of methods, that can provide an accurate assessment of relative skin sensitising potency comparable with that available currently from the LLNA (44).

Against that background it is the case, therefore, that for the purposes of exploring the sensitising potency of contact allergens the most useful information derives from LLNA EC3 values, and such data will provide an important element of the consideration here of the skin sensitising properties of LAM. However, although the most direct evidence of potency is provided by the LLNA, it has to be acknowledged that other methods, including guinea pig predictive tests, certain *in silico* and *in vitro* approaches, and clinical experience, can all provide information that may contribute to a holistic appraisal of skin sensitising activity. Accordingly, all relevant sources of information will be included here for the purpose of exploring as a case study the skin sensitisation properties of LAM.

The lower alkyl methacrylate esters: chemistry and applications

Acrylic resins are thermoplastic polymers produced by the polymerisation of methacrylic acid esters (methacrylates), or acrylic acid esters (acrylates). MMA is by far the most important methacrylic acid ester and in 2011 the global manufacture of polymethyl methacrylate resins was estimated to be approximately 3 million metric tons (45). The market for EMA, nBMA, iBMA and EHMA is substantially smaller than for MMA because these monomers are typically incorporated in smaller amounts to modify the properties of the resulting polymer.

The use of LAM esters in liquid form is considerably less common although there are a number of important applications, including use in the building industry (reactive resins and paints), medical (bone cements) and dental (manufacture and repair of dentures), and in arts and crafts (cements and embedding systems).

Skin sensitisation potential and potency of lower alkyl methacrylate esters

In examining the sensitising properties of LAM the approach taken is to consider in turn the information available, from relevant sources, for MMA, EMA, nBMA, iBMA and EHMA.

(a) MMA

MMA is the most extensively studied of these compounds. It is well established that MMA has the potential to cause skin sensitisation and allergic contact dermatitis (46-50).

LLNA

As indicated above, for the purposes of exploring the skin sensitising potency of MMA, a key consideration is the availability of data on EC₃ values derived from the LLNA. A relatively recent report by Betts et al (3) described the activity of MMA in the LLNA when formulated either in acetone, or in a 4:1 mixture of acetone: olive oil (AOO). In the same investigations comparisons were made with 2,4-dinitrochlorobenzene (DNCB), a very potent contact allergen. It is possible to record EC₃ values in a number of ways: (a) as the percentage of chemical (in vehicle) required to elicit a 3-fold increase in LNC proliferative activity, (b) as the molar concentration of chemical required for a 3-fold response, or (c) as a function of the concentration of chemical required per unit area of skin. While it is the last of these that most accurately reflects the important metric in the acquisition of skin sensitisation, all approaches are legitimate in terms of evaluating relative potency. In the

investigations reported by Betts et al (3) EC3 values were recorded in terms of the percentage of chemical in vehicle. When delivered in acetone as vehicle MMA was found to have an EC3 value of 60%, and when formulated in AOO to have an EC3 of 90%. These are high EC3 values indicative of only weak skin sensitising activity. This is illustrated by comparisons with the results obtained using DNCB in the same series of experiments (3). As expected, DNCB elicited a very strong response in the LLNA, with a derived EC3 value of 0.036%. Collectively these data serve to reinforce the point made previously that contact allergens vary by several (up to 5) order of magnitude with respect to their relative skin sensitising potency. In the experiments reported by Betts et al the estimated potency of DNCB in AOO as judged by EC3 value was found to be greater than that of MMA in the same vehicle by a factor of approximately 2,500 (3).

As described above, the availability of EC3 values has prompted proposals for the categorisation of contact allergens as a function of their relative skin sensitising potency (1, 32, 33). One approach that has been suggested is to adopt 4 categories as follows: *Extreme Sensitiser* (EC3 value of less than 0.1%), *Strong Sensitiser* (EC3 value of less than 1%, but greater than 0.1%), *Moderate Sensitiser* (EC3 value of less than 10%, but greater than 1%), and *Weak Sensitiser* (EC3 value equal to, or less than, 100%, but greater than 10%). A non-sensitising chemical would naturally not achieve an EC3 value (32). Employing those criteria to the data reported by Betts et al (3) then DNCB would be considered to be an extreme skin sensitiser. In contrast, MMA – whether formulated in either acetone or AOO – would be categorised as a weak sensitiser.

Recently, in comparative studies of the standard LLNA with a version of the assay designed to avoid the use of radiolabelled thymidine for the measurement of LNC proliferation – the Lymph Node Cell Counts (LNCC) approach – MMA was one of the chemicals examined. In neither the standard LLNA, nor in the LNCC, did MMA elicit a positive response for classification as a sensitiser (51).

There is little other information regarding the sensitizing activity of MMA in mice. It is relevant to note, however, that in a report published by Rustemeyer et al in 1998 (52) the authors commented that to their knowledge no successful attempts to induce skin sensitisation to MMA in mice had by that date been reported. They commented also that they had attempted to induce skin sensitisation to MMA in mice using various procedures without success (52).

Guinea pig assays

Attempts to induce skin sensitisation in guinea pigs have commonly been problematic. Although some success was reported by Chung and Giles (53), the results could not be reproduced by other investigators using similar or different protocols (54). Others have met with variable, and often very

limited, success (55-57). In an investigation designed to compare various endpoints for assessment of skin sensitisation in guinea pigs, MMA was one of the chemicals evaluated. In that series of experiments MMA failed to induce sensitisation (measured as a function of challenge-induced skin reactions), and also failed to cause lymph node activation (58).

In studies using a standard GPMT, Boman et al (59) found that sensitisation could be induced with MMA but only with comparatively high test concentrations. An induction regime employing 10% of MMA for both intradermal injection and epicutaneous exposure resulted in each of 5 guinea pigs displaying reactions when animals were challenged subsequently with 100% of MMA (59).

A detailed summary in tabular form of guinea pig skin sensitisation assays conducted with MMA is available elsewhere (4). The authors of the article in which that table appears drew the general conclusion that results indicate weak sensitisation potential, with a significant proportion of tests giving negative results. The same conclusion is drawn here.

In vitro and in silico approaches

A wide variety of approaches for the development of alternative (non-animal) methods for the identification and characterisation of skin sensitising chemicals are currently being explored. Some of these methods are more fully developed and evaluated than others, and it is upon these that attention will focus primarily here. For the purposes of this article the methods that will be considered are as follows:

- (a) *Direct Peptide Reactivity Assay (DPRA)* (41, 60) in which the inherent electrophilicity of test chemicals is measured as a function of their ability to associate with model peptides. There has, more recently, been described a modification of the standard DPRA that seeks to facilitate the detection of pro-haptens that require metabolic activation to form an electrophilic species. This modified assay incorporates horseradish peroxidase and hydrogen peroxide to effect pro-hapten conversion; the so-called *Peroxidase Peptide Reactivity Assay (PPRA)*(35). However, in the context of LAM, the more relevant configuration of the test is the DPRA since the parent esters are inherently electrophilic, and therefore complete haptens in their own right.
- (b) Assays based on the activation of DC or DC-like cells. One such test that has received considerable attention is the *human Cell Line Activation Test (h-CLAT)* in which the ability of test chemicals to induce increases in the expression of specific plasma membrane determinants (CD54 and/or CD86 that are markers of activation on DC) on a human monocytic leukaemia cell line (THP-1) is measured (39, 61, 62). Another assay system, that

employs a very similar approach and uses the human myelomonocytic cell line U-937, is the *Myeloid U-937 Skin Sensitization Test (MUSST)* (63, 64). The readout most commonly used is an elevated expression of CD86. Finally, brief mention will be made of a series of experiments in which the impact of chemicals on a variety of activation markers were examined using human blood monocyte-derived DC (65). All such assays are intended to reflect *in vitro* the ability of contact allergens to provoke the activation and maturation of DC within the skin.

- (c) Approaches based on signalling pathway activation. One such is the *KeratinSensTM* assay (38, 66, 67) which is predicated on an appreciation of the nuclear factor-erythroid 2-related factor 2 (Nrf2)-Kelch-like ECH-associated protein 1 (Keap1)-antioxidant response element (ARE) pathway. Keap1 is a protein sensor that possesses cysteine (Cys) residues that are highly reactive with many electrophilic sensitising chemicals. Covalent modification of these Cys residues by contact allergens causes Keap1 to dissociate from Nrf2 which then accumulates in the nucleus causing the transcriptional activation of genes with an ARE in their promoter regions. The *KeratinSensTM* assay uses a keratinocyte-based luciferase reporter cell line to measure activation of the Keap1/Nrf2 pathway as a function of induced gene transcription (67). A very similar method similarly based on the activation of Nrf2, the *LuSens assay*, has been reported also (68).
- (d) *In silico* approaches that are based on an understanding of the relationship between physicochemical properties and skin sensitisation potential. There is a very substantial literature on (Q)SAR/SAR approaches (36, 37), and although it is hoped that these might provide a platform for potency assessment as well as hazard identification, there is probably still further development required to achieve that (69). One of many approaches that has been used to identify structural alerts for skin sensitisation is the OECD (Organisation for Economic Cooperation and Development) QSAR tool box (Version 2.0, 2010)(70).

Although there are other alternative methods that are being explored, it is those approaches summarised above that have information available regarding the skin sensitising activity of MMA.

There is limited experience with MMA in *in vitro* and *in silico* test systems. The evidence available can be summarised as follows. In the DPRA MMA has been shown to yield a positive response, displaying binding to both Cys-peptides and Lys(lysine)-peptides (68). A positive response with MMA in the DPRA is also cited in a separate report from the same group of authors (71-73). In a separate

investigation MMA gave comparable results in a DPRA, with a classification of moderate reactivity (CA Ryan; personal communication).

Data are available for the activity of MMA in the MUSST and h-CLAT assays. In both a positive response was recorded, with only weak reactivity being observed in the h-CLAT assay (68, 71-73). The only other information regarding the activity of MMA in a DC-based cellular assay comes from investigations using human peripheral blood monocyte-derived DC (65). In those studies the impact of chemicals on a variety of DC activation markers were examined: CD80, CD83 and CD86, CXCR4 and CCR5 (both chemokine receptors) and P-glycoprotein (Pgp) and lung resistance protein (LRP) (two multidrug resistance-related molecules). MMA (which was characterised by the authors as being a weak sensitiser based on clinical data and the results of guinea pig assays) was found, in common with other contact allergens tested, to elicit some increased expression of all markers examined, although in the case of CD83 and LRP responses to MMA were weaker than recorded with other allergens (65).

In the KeratinoSens assay, and in the very similar LuSens method, MMA was initially reported to be negative (68, 71). However, in subsequent studies reported elsewhere MMA consistently tested positive in the LuSens assay (72, 73), this being the result of continued developmental work with the method achieving increased sensitivity (R Landsiedel; personal communication).

The picture that emerges for MMA in *in vitro* assay systems is of positive responses (albeit sometimes relatively modest responses) in the DPRA, h-CLAT and MUSST, and LuSens methods.

Finally, it is known that MMA is a direct acting Michael acceptor electrophile, and on that basis is identified as a contact allergen in the OECD QSAR tool box (70). In attempts to develop quantitative models for Michael acceptors it has been acknowledged that MMA (and other short chain acrylates and methacrylates) are, in reality, less potent sensitisers than predicted by the model (74, 75).

Taken together, the evidence available from predictive test methods and other experimental systems, including animal models and *in vitro* and *in silico* assays, indicates that although MMA clearly has some potential to cause skin sensitisation, it is not a potent contact allergen. On the basis of the LLNA, MMA would be classified as a weak allergen (possessing only modest skin sensitising potency). Although, for the reasons discussed above, estimates of skin sensitisation potency are currently best furnished by EC3 values derived from the LLNA, there are no data available from guinea pig predictive tests, *in vitro* assay systems or *in silico* approaches that would contradict the view that MMA is a weak skin sensitiser.

It is relevant, therefore, to consider briefly whether the evidence available from human studies and clinical experience serves to support, or to contest, the view that MMA is not a potent contact allergen.

Human studies and clinical experience

Case study reports of skin sensitisation have been summarised and commented upon previously by Betts et al in 2006 (3), in which publication a list of reports was provided in tabular form. The data summarised in that article revealed extensive variation between the reports cited with respect to the number of subjects in whom a positive response was observed as a function of those tested. In fact, the prevalence was found to vary between studies from 0% to 16% (3). The higher prevalence rates were reported in those studies where relatively small numbers of patients with dermatitis had been referred to clinic. The corollary was that much lower prevalence rates were observed among larger cohorts, but even in those cases it could be argued that the figures obtained are an over-estimation of the frequency with which skin sensitisation develops among populations exposed to MMA. The data of Pflug (76, 77) were considered to perhaps provide the most accurate reflection of the prevalence of sensitisation to MMA in the dental industry (values of between 0.25% and 0.378%), although even in these reports there is possibly some over-estimation of true rates of sensitisation to MMA (3, 76, 77). The conclusion drawn at that time was that, although there was no doubt that MMA is a contact allergen, the available evidence from predictive test methods indicated relatively weak skin sensitising potential. The data available from reports of skin sensitisation to MMA among exposed human population was considered to be consistent with that conclusion (3).

It is, of course, important to consider whether there are any other data that have become available regarding human skin sensitisation to MMA since publication of the Betts et al article (3). Little new information has emerged that is relevant to the prevalence of sensitisation to MMA, or to its sensitising potency. Goon et al (78) described an investigation of 1632 subjects, comprising 1322 dental patients and 310 dental health professionals. It was found that 2.3% of patients and 5.8% of health professionals displayed positive patch test reactions to (meth)acrylate allergens. The frequency of patch test responses to MMA was low. Of the 2.3% of dental patients that showed positive patch test reactions to any (meth)acrylate allergen, only 13.3% of that number responded to MMA (equivalent to approximately 0.3% of the total patient population). Similarly, of the 5.8% of dental health professionals that reacted to any (meth)acrylate, 38.8% were sensitised to MMA, equivalent to approximately 2.2% (78).

In the absence of any other data that informs understanding of human sensitisation to MMA there is no reason to revise the conclusions published previously (3). Based on all available data it is concluded that MMA is a contact allergen, but displays weak skin sensitising potential. The relatively low frequency of sensitisation among those exposed to MMA is consistent with that conclusion.

Conclusion

MMA is a contact allergen but has weak skin sensitisation potential.

(b) EMA

EMA has not been investigated in the same depth as MMA, but there are relevant data available.

LLNA

The activity of EMA in the LLNA has been reported recently. In a standard assay, groups of mice were exposed to 25%, 50% or 100% EMA in vehicle (AOO). At those doses, respectively, the recorded stimulation indices were 0.93, 1.41 and 3.85. The EC3 value derived from these data is 82.6%; a result that would categorise EMA as a weak sensitiser (79).

Guinea pig assays

Other than the report by Chung and Giles in 1997 (53) who apparently enjoyed considerable success in sensitising guinea pigs to several LAME (EMA, MMA and nBMA), experience suggests that EMA at best elicits weak responses in guinea pigs. Studies employing the GPMT used increasing intradermal induction concentrations of EMA (0.17M, 0.50M and 1.50M), together with epicutaneous exposure to 1M EMA. Animals were subsequently challenged with 3M EMA. Only 1 in 10 animals responded to challenge following exposure to either 0.50M or 1.50M intradermal EMA; no animals responded following induction with 0.17M EMA (55). In another report of a commissioned study using the GPMT a negative result was obtained with EMA (80). On the basis of those data EMA would not be classified as a skin sensitiser in the GPMT; the threshold response for classification being 30%.

In vitro and in silico approaches

EMA has been evaluated in several methods. In the DPRA EMA tested positive with somewhat weaker activity than that observed with MMA (72, 73). EMA also elicited positive responses in the LuSens assay and in the MUSSTT (72, 73). In common with MMA, EMA is a direct acting Michael

acceptor electrophile, and on that basis is also identified as a contact allergen in relevant SAR models and the OECD (Q)SAR tool box (70).

Human studies and clinical experience

Skin sensitisation to EMA is most commonly associated with artificial fingernails and there are available in the literature a number of case studies that describe this. An illustrative example is provided by Conde-Salazar et al (1986) (81). In a review of patch testing with (meth)acrylates cited previously by Betts et al (3), it was found that among patients with a history of exposure to methacrylates and acrylates 7.4% of those tested reacted to EMA; exactly the same percentage that was shown to be responsive to MMA (82).

Conclusion

Like MMA, EMA is a contact allergen. However, the available evidence is that it has only modest (weak) skin sensitisation potency.

(c) nBMA

It is also the case with nBMA that there are only limited data available relevant for consideration of skin sensitising potency.

LLNA

There is available a single report on the activity of nBMA in the LLNA (83). In a standard LLNA, groups of mice were exposed to 25%, 50%, or 100% nBMA in vehicle (AOO). The stimulation indices recorded were, respectively, 2.19, 3.28 and 5.41. Derived from these data the EC3 value was calculated to be 43.6%. On this basis nBMA would be considered a weak skin sensitiser (83).

Guinea pig assays

In common with their studies of MMA and EMA, Chung and Giles (53) reported the successful sensitisation of guinea pigs to nBMA. Other investigators have been less successful. nBMA was among a series of acrylates and methacrylates studied by van der Walle and colleagues. In the GPMT no positive responses were recorded when animals were challenged at 21 days, and only 2 of 10 animals displayed reactions following challenge at 35 days (55). In a slightly different guinea pig assay, the Freunds Complete Adjuvant Test, nBMA was uniformly negative (55). The GPMT data would not result in classification of nBMA as a skin sensitiser.

In vitro and in silico approaches

In the DPRA, nBMA was shown to elicit a positive response, albeit somewhat weaker than recorded for MMA (72, 73). Positive responses with nBMA were also recorded in the LuSens assay and in the MUSST (72, 73). In common with other LAM nBMA is a direct acting Michael acceptor electrophile and is identified as a contact allergen in relevant SAR models and the OECD (Q)SAR tool box (70).

Human studies and clinical experience

Although nBMA is acknowledged to be a contact allergen, there is no evidence that it is a strong skin sensitiser among humans. Kanerva et al (1997) considered patch test results over a 10 year period with a large number of (meth)acrylates. In a total of 275 patients with a history of exposure to acrylates and methacrylates only 2.5% of those tested reacted to nBMA (82). In the same series the percentage of patients that responded to MMA or EMA was in each case 7.4% (82). Those data are consistent with a review of 6 cases of patients with a history of occupational exposure to methacrylates that were seen in a contact dermatitis clinic. Only 1 of the 6 patients reacted to challenge with nBMA, whereas 3 were found to be sensitised to MMA (84). Moreover, in a series of patients known to be exposed to artificial nails none of 12 subjects patch tested with nBMA were positive. In the same investigations 4 of 21 tested with MMA were positive, and 2 of 13 reacted to EMA (85).

Conclusion

The data indicate that although nBMA is a contact allergen, it has only weak skin sensitisation potency.

(d) iBMA

There is little information available on the skin sensitising potential of iBMA

LLNA

A single LLNA has been conducted with iBMA. Using a standard assay, groups of mice were exposed to 25%, 50% or 100% of iBMA in AOO. The stimulation indices recorded were, respectively, 1.78, 3.64 and 5.13. Those data translate into an EC3 value of 41.4%. On that basis iBMA would be categorised as being a weak skin sensitiser (86).

Guinea pig assays

No published data on the activity of iBMA in guinea pig assays are available, other than in the form of two toxicology reports. The first found iBMA to be negative in the Stevens test (a less commonly used guinea pig assay) (87). The second recorded a uniformly negative response to iBMA in the GPMT (88).

In vitro and in silico approaches

To our knowledge iBMA has not been tested in the *in vitro* assays systems identified previously in this article. However, like other LAME, iBMA is a direct acting Michael acceptor electrophile, and on that basis is identified as a contact allergen in relevant SAR models and the OECD (Q)SAR tool box (70).

Human studies and clinical experience

Very little information is available about the skin sensitising activity of iBMA in humans. However, there is a report by Taylor (89) in which cases of adverse reactions to acrylics were investigated. Of 12 subjects patch tested with iBMA all were negative.

Conclusion

Taken together the available evidence suggests that iBMA may be able to induce skin sensitization, but that it has only weak potency.

(e) EHMA

Similarly to some other LAM there is little information available on the skin sensitising potential of EHMA.

LLNA

In a standard LLNA, animals were exposed to 25%, 50% or 100% EHMA in AOO as the vehicle. The stimulation indices recorded were, respectively, 1.53, 2.66 and 2.85. At no test concentration was a stimulation index of 3 or more induced and on that basis EHMA is classified as a non-sensitiser in the LLNA (90).

Guinea pig assays

The activity of EHMA in guinea pig assays has been somewhat variable. Both negative and positive results have been published in investigations using the GPMT, but even the positive responses when observed have been of low grade (91-94).

In vitro and in silico approaches

There are no data available on the activity of EHMA in the *in vitro* test methods summarised above. In common with other LAME, and as a direct acting Michael acceptor electrophile, EHMA is identified as a contact allergen in SAR models and in the OECD (Q)SAR tool box (70).

Human studies and clinical experience

A small series of 4 patients that had developed occupational allergic contact dermatitis from working with dental prostheses were examined. All responded to challenge with MMA, but none displayed reactions following patch testing with EHMA (95).

Conclusion

The data on EHMA are equivocal. This ester failed to elicit a positive response in the LLNA, and there is an absence of evidence revealing that EHMA is a human contact allergen. The conservative conclusion is that EHMA may have some potential to cause skin sensitisation, but that sensitising potency is very weak.

A summary of the skin sensitisation data available for LAM is contained within Table 2.

(f) Skin sensitisation potency of LAM: general conclusion

The general conclusion drawn is that MMA, EMA, nBMA, iBMA and EHMA each possess no more than weak skin sensitising potency. The most persuasive evidence in reaching this conclusion derives from studies using the LLNA; a method that (as discussed above) is currently the most informative with regard to assessment of skin sensitising potency. In one case (EHMA) the LLNA was negative, and in the other 4 instances, where a positive result was obtained (MMA, EMA, nBMA and iBMA), EC3 values were never less than 40%, indicating weak skin sensitising activity. Although guinea pig assays are usually less suitable for the assessment of sensitising potency, the data available from these methods is, in most instances, entirely consistent with LAM having only modest potency. The utility of *in vitro* and *in silico* methods for potency assessment is still unproven and uncertain, but

what data are available for LAM from such methods does not contradict conclusions drawn from LLNA data. In the context of exploring the skin sensitising activity of these (or other) chemicals the potential value of the currently available *in vitro* methods is not that they provide readouts that are necessarily of direct relevance for potency assessment, but rather that those which employ cells or cell lines of human origin may arguably provide another perspective of sensitising potential in humans. Finally, although the evidence available from clinical experience indicates that the LAM can cause skin sensitisation they cannot be regarded as potent contact allergens. There is no information deriving from clinical reports that is inconsistent with the categorisation of LAM as having no greater than weak sensitising potency.

Factors that may limit the skin sensitising potency of LAM

If the conclusion drawn is that, as a class, LAM has only weak skin sensitising activity, then it is instructive to consider briefly the possible mechanistic basis, or bases, for this.

One general factor that is believed to influence reactivity and skin sensitising potential is chemical structure. It has been suggested that the modest sensitising activity of methacrylates is attributable to the α -methyl substitution of the α - β double bond that in turn destabilises the Michael transition state reducing capacity to react with glutathione and protein-SH groups (96, 97). This is in contrast to the situation that pertains to the structurally related acrylates that show rates of glutathione reactivity that are at least two orders of magnitude higher than seen with the methacrylates (98), and stronger reactivity in guinea pig assays (96), and the LLNA (99).

The effective acquisition of skin sensitisation requires highly orchestrated, and very complex, cellular and molecular interactions that combine to elicit a T lymphocyte response of the quality and vigour required to cause immunological priming to the inducing allergen (7-10). Despite that complexity, it is possible to identify some critical events or processes on the pathway to skin sensitisation. It has been proposed previously that, in effect, there are a number of 'hurdles' that must be negotiated successfully if topical exposure to a chemical is going to result in skin sensitisation. The corollary is that if such hurdles are not negotiated successfully then sensitisation will not be acquired, or at least will be acquired less effectively (11, 42, 100).

Those events and processes that have been identified as being on the critical path to skin sensitisation are: (a) the chemical gaining access to the viable epidermis, (b) the formation of stable hapten-protein conjugates, (c) the elicitation of 'danger' signals in the skin that support the activation of cutaneous DC which in turn are responsible for the recognition, processing and

transport of antigen to regional lymph nodes, and its subsequent presentation to responsive T lymphocytes, and (d) the proliferation and clonal expansion of allergen-responsive T lymphocytes (100).

There are *in vitro* and, in some instances, *in silico*, models that seek to identify contact allergens as a function of the ability of chemicals to accomplish one of these tasks along the critical pathway to skin sensitisation (11, 42). Although, in most part, such tests have been designed for the purposes of hazard identification, it has been proposed that the activity of chemicals in some of these assays could, in principle at least, contribute to an assessment of sensitising potency (42). The argument is that the level of activity *in vitro* tests, and/or quantitative aspects of Q(SAR), might inform assessment of sensitisation potency. Although, in theory, the argument is rational, it must be acknowledged that with many endpoints used in such assays it is difficult to identify what specific metrics might be most relevant in terms of potency assessment, or indeed whether or not certain endpoints have any quantitative relationship with the effectiveness of skin sensitisation (42). Moreover, there is currently no clear view about which events on the pathway to acquisition of skin sensitisation play the most influential roles in determining potency. Although there is no doubt that the vigour and quality of T lymphocyte responses provoked by exposure to chemical allergen correlate closely with the extent to which sensitisation will develop, it is not known which of the many events that lead ultimately to T lymphocyte activation and proliferation determine how strong that response will be (22).

In this context, in a recent examination of 3 *in vitro* test methods (DPRA, MUSST and KeratinoSens) the investigators reviewed the value of these approaches not only for hazard identification, but also for potency assessment (100). Although they acknowledged that currently the LLNA provides the most accurate evaluation of skin sensitising potency, they found that each of the 3 *in vitro* methods considered had, to a varying degree, some association with potency as judged by the LLNA. However, they pointed out also that such associations were far from perfect, and that the data showed a significant degree of spread between potency classes (101).

Nevertheless, despite those limitations, it may be legitimate to draw the conclusion that a chemical which fails to negotiate successfully any one of these requirements or hurdles would lack the ability to induce skin sensitisation, or would do so only ineffectively (42).

Against that background, and acknowledging the caveats summarised above, it is relevant to consider briefly the activity of LAM in test methods that have been designed to reflect *in vitro* or *in silico* certain key events in the development of skin sensitisation. The tests that will be considered

here are: (a) prediction *in silico* of dermal absorption, (b) peptide binding assays and other methods designed to evaluate electrophilicity, (c) activation of DC or DC-like cells or cell lines, and (d) the activation *in vitro* of primary T lymphocyte responses.

(a) Prediction in silico of dermal absorption

In a series of comparative analyses the dermal permeability of LAM was determined using a (Q)SAR approach based primarily on the octanol:water partition coefficient (Log P), together with consideration of molecular weight and saturated aqueous solubility. The approach used was based on an established model described by Potts and Guy (102). Chemicals were then ranked according to predicted flux through the skin measured in units of $\mu\text{g}/\text{cm}^2/\text{hr}$. On the basis of predicted flux MMA, EMA, nBMA, iBMA were each categorised as having moderate dermal absorption properties (in the rank order of MMA>EMA>iBMA>nBMA). Ethylhexylmethacrylate (EHMA) was classified as having low dermal absorption (103). These results would be consistent, possibly, with EHMA having lesser skin sensitising potency than other LAM.

(b) Peptide binding assays

As indicated above, the LAM are intrinsically electrophilic and would be expected, therefore, to elicit positive responses in the DPRA. This is the case for those LAM that have been tested in this assay: MMA, EMA and nBMA. In one series of experiments the levels of reactivity (measured as a function of mean depletion of Cys and Lys peptides) was comparable for MMA, EMA and nBMA being, respectively, 29.5%, 10.7% and 18.6% (72). The results, as would be expected, reflect the fact that LAM are direct Michael acceptor electrophiles and have the inherent potential to form stable associations with peptides and proteins, primarily through interaction with Cys residues, but also – to a lesser extent – with Lys. As indicated above, it is for this reason that LAM are positive in SAR models and the OECD (Q)SAR tool box (70). This property is also the basis for the positive responses seen with MMA, EMA and nBMA in the LuSens assay (72, 73).

(c) Activation of DC or DC-like cells or cell lines

In the better characterised DC-based assays (MUSST and h-CLAT) those LAM that have been tested have elicited positive responses, but these data do not provide any indication of relative potency (68, 71-73).

(d) Activation in vitro of primary T lymphocyte responses.

Such assays seek to identify contact allergens as a function of their ability to provoke the activation and clonal expansion of naive allergen-responsive T lymphocytes *in vitro* (104 -). To our knowledge LAME have not yet been tested using such methods.

As discussed previously, the data available from *in vitro* test methods and *in silico* models are consistent with LAM being contact allergens. However, these data do not identify any one specific characteristic that is necessarily the basis for their relatively weak skin sensitising potency. These chemicals are natural electrophiles, are able to gain access to the viable epidermis, and activate DC-type cells *in vitro*.

In the cause of exploring what factors might serve to influence the sensitising activity of LAM there are two other issues worthy of consideration.

The first of these is that it is possible that in certain circumstances the inherent skin sensitising activity of LAM may be augmented by local inflammation at sites of exposure. The 'danger hypothesis' proposes that the initiation of adaptive immune response is facilitated by, and sometimes dependent upon the receipt of endogenous or exogenous signals that indicate microbial invasion and/or tissue disruption. It is believed that such danger signals are relevant also for the elicitation of cutaneous immune responses and the acquisition of sensitisation (105). There is an increasing appreciation of the nature of the signals relevant for skin sensitisation (7, 10). It is now well established that irritant dermatitis associated with (meth)acrylates and other agents is not uncommon among healthcare workers, including dental technicians (106-108). It is possible that in some of these individuals the acquisition of skin sensitisation to LAM in the workplace may be facilitated by local irritation and inflammation that augments immunological priming.

The second issue is the possibility that cross-reactivity plays a role in contact allergic reactions to LAM. More than 130 potential chemical allergens have been identified among materials used in dental practice (109). From among these the methacrylates most commonly implicated as contact allergens are 2-hydroxyethyl methacrylate (2-HEMA) and ethyleneglycol dimethacrylate (EGDMA)(110, 111). As cross-reactivity between methacrylate esters has been observed in studies using experimental animals (53, 112), it is possible that some at least of patch test reactions to LAM result from sensitisation to other methacrylate esters.

The conclusion drawn is that the modest sensitising potency of LAM is likely due to a combination of a variety of factors, including possibly only moderate skin permeability and low overall immunogenicity. Whatever the root causes, the information available from the LLNA is that LAM are weak skin sensitisers, and the data available from other test methods, and from other sources, is

consistent with that view. It is possible that, despite this inherent weak sensitising potential, the development of sensitisation to LAM may be facilitated in some occupational settings by pre-existing eczema. Finally, it must be borne in mind that at least some cases of reactivity to LAM could result from initial sensitisation to other methacrylates and immunological cross-reactivity.

Exposure to lower alkyl methacrylate esters

Acrylic resins are thermoplastic materials produced by the polymerisation of methacrylic acid esters (methacrylates) or acrylic acid esters (acrylates). Methyl methacrylate monomer (MMA) is by far the most important methacrylic acid ester. As summarised above, in 2011 global manufacture of polymethyl methacrylate (PMMA) resins was estimated to be almost 3 million metric tons, production being in China, USA, Western Europe, Japan, Korea and Singapore with the principal consumption occurring in North America, China, Western Europe and Japan (45). There are no definitive data available for the global production and consumption of other LAM, but reasonable estimates are as follows: EMA 10,000 tons, nBMA 140,000 tons, iBMA 140,000 tons and EHMA 10,000 tons. Much of LAM production is destined for manufacture of acrylic homo-polymers, although there is some copolymerisation with other non-acrylate monomers and compounding with additives to produce a wide range of acrylic-based polymers. In 2011, production was divided between the manufacture of acrylic sheet (32%) (including cast and extruded), moulding compounds (32%), surface coatings (20%), impact modifiers/processing aids (5%), with a further 13% used for the production of acrylic latexes (emulsions), lacquers, enamels, powder coating resins and modified alkyd resins (45). Some LAM production finds application in the manufacture of monomer/polymer based systems aimed at relatively small-volume niche markets, such as the building industry (reactive resins and paints), medical (bone cements), dental (denture manufacture and repair), and arts and crafts (cements and embedment systems). Methacrylate monomer/polymer based systems are also used in artificial nails (acrylic nails and fillers etc.). Historically, this application used MMA exclusively, but since the US Food and Drug Administration (FDA) introduced a ban on nail products containing 100% MMA monomer in 1974 (113), and in the absence of regulations that specifically prohibit the use of MMA monomer at lower concentrations in cosmetic products, it continues to be found in nail products in the USA and many other countries (114, 115). Increasingly, EMA is the LAM preferred by the cosmetology industry. This is despite this application, and indeed any application with unavoidable direct skin or nail contact, not being supported by the monomer manufacturers (116).

In terms of potential for worker exposure, LAM are exclusively manufactured by industry on large scale manufacturing sites. Since the processes involve the use of highly toxic raw materials, such as hydrogen cyanide, they are subject to hazard regulations so there is an assumed high level of safety awareness and compliance with recommended control measures. Methacrylate based polymers are also almost exclusively manufactured either in large scale semi-closed systems, such those used for the production of sheet products, or in medium to large scale batch processes, such as those used for the production of moulding compounds or latexes. The numbers of workers involved in these activities are relatively small when compared with those in downstream industries handling polymers, and mechanical control measures combined with the use of personal protective equipment can avoid skin exposure to liquid monomers (117). Such assumptions cannot be necessarily made for all skilled trade and professional workers handling monomer/polymer based products since compliance with recommended handling practices may be less consistent.

In the dental industry there are some 200,000 registered dental technicians in North America and Europe [USA: 40,900 (118); Canada, 850 (119) and EU/EEA [27 countries of the EU, Iceland, Liechtenstein, Norway, Switzerland and Croatia], 150,189 (120)], and a further (approximately) 700,000 dental assistants [USA > 300,000 (121); EU/EEA, 400,000 (120)]. In terms of awareness and compliance with safe handling practices, there is in the USA no formal requirement for a vocational qualification to become a dental technician, although there is an entry requirement for a high school diploma, or equivalent, and moderate-term on-the-job training. There is only a post-secondary non-degree award entry requirement to become a dental assistant. In Canada, dental technologists and technicians must have a college diploma in dental prostheses technology (formerly dental technology) and dental laboratory bench workers require a high-school diploma. Within the EU/EEA there is a requirement for registration with a Competent Authority following formal vocational training and qualification in 23 countries, with overseas training required by 2 (Cyprus and Liechtenstein). Training is generally of two to five years duration and takes place in special schools, sometimes but not always, associated with dental schools; and often follows training as a dental chair-side assistant or dental technician. In the remaining 9 countries (Croatia, Czech Republic, Denmark, France, Ireland, Luxembourg, Netherlands, Poland and Spain (although technician registration is mandatory in some regions) formal training is available, but there is no requirement to register with a Competent Authority (120).

In the case of cosmetology, there were more than 62,000 manicurists and pedicurists in the USA in 2012 (122,) associated with an estimated annual revenue from nail services in 2013/13 of over \$7 billion (123). There is an entry level requirement for a post-secondary non-degree to become a

manicurist and pedicurist, followed by a state-approved cosmetology program (122). After the program is completed, all states except Connecticut require that a state examination is passed before a license is awarded. Statistics are not available for the EU/EEA. There are no formal national registers of nail technicians and training is either through National Vocational Qualifications (NVQ), short training courses, or on-the-job training.

There are apparently no organisations collecting statistical data for the other uses such as the building industry, and as a consequence analyses for these sectors cannot be conducted.

In terms of standards and practices in skilled trade and professional workers handling monomer/polymer based products it may be concluded that there are established standards of occupational hygiene practice, including the use of personal protective equipment such as gloves, within the dental industry; although these standards may not necessarily be uniformly applied in all countries. In the cosmetology industry there may well be established standards and practices within the USA, but this may not extend to Europe and other countries. Furthermore, since this use involves direct application of liquid LAM to the nails of customers, with the opportunity for coincidental skin contact, these standards and practices apply only to the cosmetology worker. For other skilled trade and professional uses, other than recommended handling practices, warnings and directions on products, no conclusion can be drawn regarding adoption of skin protection to avoid contact allergy due to LAM.

With respect to polymerised LAM, once polymerised, the levels of residual acrylic monomers in the polymer are extremely low (124). Therefore, although there are likely to be far greater numbers of individuals (both workers and consumers) exposed to these articles and polymer-based products, there is no concern for contact allergy during normal handling.

Risk assessment

It is clear that the available toxicological and clinical data support the conclusion that LAM are contact allergens, but importantly have only weak skin sensitisation potential. There is evidence of occupational ACD occurring in the dental industry but the reported number of cases compared with the extremely large numbers of individuals working in this industry would point to the actual prevalence being very low indeed (for example between 0.24% and 0.38% for MMA), and consistent with LAM being weak skin sensitisers. The reports of ACD and nail reactions in consumers of the cosmetology industry are not surprising since this use can involve direct application of the liquid LAM to the nail, and avoiding contact with surrounding skin cannot be guaranteed. Although

statistics for this industry are not available, and there is an impression that the prevalence of adverse reactions may again be low compared with the scale of the industry, such uses are on principal advised against. Although there are no data available on the standards and practices in other industries handling liquid LAM there is no evidence in the literature that this is a problem that warrants special consideration. On this basis the conclusion drawn is that cases of occupational ACD reported in the literature are commonly the result of extended and intimate exposure to the inducing methacrylate ester. It is the case that even weak contact allergens will induce skin sensitisation if the extent and duration of exposure is sufficient.

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Conclusion

MMA and other LAM are weak skin sensitising chemicals and should be categorised as such. Detailed evaluation of the sensitising properties of, and exposure patterns to, this series of chemicals illustrates the importance of considering carefully apparently inconsistent data in reaching decisions about categorisation on the basis of potency. In particular it is necessary to distinguish carefully between true skin sensitising potency and estimates of the prevalence of ACD deriving from clinical experience.

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Table 1. Chemical structures and key physico chemical properties

CAS No.	80-62-6	97-63-2	97-86-9	97-88-1	688-84-6
Chemical Name	Methyl Methacrylate (MMA)	Ethyl Methacrylate (EMA)	Iso-Butyl Methacrylate (i-BMA)	n-Butyl Methacrylate (n-BMA)	2-Ethylhexyl Methacrylate (2-EHMA)
Structural Formula					
Boiling point [°C]	100.36	118.2	163	155	227.6
Vapour pressure [hPa at 20 °C]	37	20	2.1	2.1	0.065
Water solubility [g/l @ 20°C]	15.3	4.69	0.36 (25°C)	0.47	0.003
Partition coefficient n-octanol/water (log value)	1.38	1.87	3.0	2.95	4.95

Table 1. Contact allergy data for lower alkyl methacrylate esters (LAM)

CAS No.	80-62-6	97-63-2	97-88-1	97-86-9	688-84-6
Chemical Name	Methyl Methacrylate (MMA)	Ethyl Methacrylate (EMA)	n-Butyl Methacrylate (n-BMA)	Iso-Butyl Methacrylate (i-BMA)	2-Ethylhexyl Methacrylate (2-EHMA)
QSAR tool box (Version 2.0)	positive	positive	positive	positive	positive
LLNA	60-90% (weak positive)	82.6% (weak positive)	43.6% (weak positive)	41.1% (weak positive)	negative
Guinea pig assays	positive / negative (weak)	positive (weak)	negative	negative	positive / negative (very weak)
Direct Peptide Reactivity Assay (DPRA)	positive (moderate)	positive (weak)	positive (weak)	No Data	No Data
Myeloid U-937 Skin Sensitization Test (MUSST)	positive	positive	positive	No Data	No Data
LuSens assay	positive	positive	positive	No Data	No Data
KeratinoSens™ assay	positive	No data	No Data	No Data	No Data
Human studies and clinical experience	positive (particularly in cosmetology (nails), health care and dental professions)	positive (comparable to MMA)	positive (very few cases)	negative	negative

Highlights

- We assessed the skin sensitising potency of lower alkyl methacrylates (MMA, EMA, nBMA, iBMA, EHMA).
- We reviewed LLNA, guinea pig, in silico in vitro and clinical (human) data.
- We found that MMA and other LAM are contact allergens but had weak skin sensitising potency.
- We found that the clinical prevalence of ACD to LAM was low compared with the numbers exposed.
- The greater incidence of ACD in certain industries was likely the result of extended and intimate exposure.