

Skin sensitization potency of methyl methacrylate in the local lymph node assay: comparisons with guinea-pig data and human experience

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There is compelling evidence that contact allergens differ substantially (by 4 or 5 orders of magnitude) with respect to their inherent skin-sensitizing potency. Relative potency can now be measured effectively using the mouse local lymph node assay (LLNA) and such data form the basis of risk assessment and risk management strategies. Such determinations also facilitate distinctions being drawn between the prevalence of skin sensitization to a particular contact allergen and inherent potency. The distinction is important because chemicals that are implicated as common causes of contact allergy are not necessarily potent sensitizers. One example is provided by nickel that is undoubtedly a common cause of allergic contact dermatitis, but is a comparatively weak sensitizer in predictive tests. In an attempt to explore other examples of contact allergens where there may exist a discrepancy between prevalence and potency, we describe here analyses conducted with methyl methacrylate (MMA). Results of LLNA studies have been interpreted in the context of historical clinical data on occupational allergic contact dermatitis associated with exposure to MMA.

Key words: dinitrochlorobenzene; local lymph node assay; methyl methacrylate; risk assessment; skin sensitization potency. © Blackwell Munksgaard, 2006.

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In recent years, there has been an increasing interest in assessment of the relative potency of skin-sensitizing chemicals, with the ultimate aim of providing more accurate assessments of human health risks (1, 2). This interest has been fuelled, in part, by a growing recognition that contact allergens differ by several (4 or 5) orders of magnitude with respect to their relative ability to cause the acquisition of skin sensitization (2). In tandem, there have been technical developments that facilitate the direct experimental assessment of sensitizing potency. For this purpose, the mouse local lymph node assay (LLNA) is most commonly used (1–3). This method was developed initially, and subsequently validated, for the identification of skin sensitization hazard, and in this context, activity is measured as a function of the vigour of draining lymph node cell (LNC) proliferative responses induced by topical exposure of mice to test chemicals (3). It is now appreciated that LNC turnover, in addition to providing a useful marker of contact allergic potential, is causally and quantitatively associated with the development of skin sensitization (3, 4). For this reason, it is legitimate,

therefore, to employ the LLNA for assessment of relative potency, and there is now available considerable experience with this approach.

One advantage of accurate measurement of relative potency is that it permits the development of effective risk assessments and risk management strategies that are based on objective data. Of particular benefit is the ability, with any particular contact allergen, to distinguish between *prevalence* (the overall frequency with which cases of allergic contact dermatitis are seen) and *potency* (which is best defined as the relative allergenic potential of a chemical as determined by the amount of material needed to cause the acquisition of skin sensitization). This distinction is important because a relatively high prevalence is not necessarily attributable to a high potency (2). An illustrative example is provided by nickel, one of the most common causes of skin sensitization in Europe and the USA. The prevalence of nickel allergy is, however, associated with ubiquitous opportunities for exposure, including intimate exposure through body piercing, rather than inherent sensitizing

activity. By all criteria, nickel is in fact a relatively weak allergen (2).

Against this background, the purpose of these investigations was to determine whether there exist other examples of contact allergens where a distinction can be drawn between prevalence and potency, and for this purpose, we have examined methyl methacrylate (MMA), a chemical used in the production of resins and plastics.

Allergic contact dermatitis to plastic materials is increasing (5), and methacrylates, including MMA, have been implicated as skin sensitizers. In the case of MMA, sensitization is commonly associated with dental surgeons and technicians, orthopaedic surgeons and nurses, and those using adhesives and artificial nail preparations (6, 7). Here we have sought to evaluate the skin sensitization potency of MMA using the LLNA and these data have been compared with results available from guinea-pig tests for skin sensitization, and with information deriving from clinical experience.

Materials and Methods

Mice

Young adult CBA/Ca (Harlan, Bicester, Oxfordshire, UK) 8–12 weeks of age were used throughout these studies. Mice were housed in metal cages, and food (SDS PCD pelleted diet; Special Diets Services Ltd, Witham, UK) and water were available *ad libitum*. The ambient temperature was maintained at 22 ± 3 celsius and relative humidity was $50 \pm 20\%$ with a 12 hr light/dark cycle. All experiments were conducted under the provisions of the UK Animals (Scientific Procedures) Act, 1986.

Chemicals

MMA (99.96% pure) was supplied by Lucite International UK Ltd via Cassel Works, Cleveland, UK. 2,4-Dinitrochlorobenzene (DNCB; 98.9% pure) was obtained from Sigma Chemical Co. (Poole, Dorset, UK). Dosing solutions (0.3%–75% w/v for MMA, 0.01%–0.25% w/v for DNCB) were prepared gravimetrically in acetone:olive oil vehicle (AOO; 4 : 1 ratio) or acetone alone. Animals were also dosed with 100% MMA (neat). Dosing solutions were prepared freshly for each application.

Local lymph node assay

The LLNA was performed according to the standard protocol described previously (8). Groups of CBA/Ca strain mice ($n = 4$) were exposed

topically on the dorsum of both ears to 25 μ l of various concentrations of chemical, or to the same volume of vehicle alone, daily for 3 consecutive days. Five days after the initiation of exposure, all mice were injected intravenously via the tail vein with 20 μ Ci of [^{3} H] methyl thymidine ([^{3} H]TdR; specific activity 2 Ci/mmol; Amersham International, Amersham, UK) in 250 μ l of phosphate-buffered saline (PBS). Five hr later, mice were killed and the draining auricular lymph nodes were excised and pooled for each experimental group. A single-cell suspension of LNC was prepared by gentle mechanical disaggregation through 200-mesh stainless steel gauze. Cells were washed twice with an excess of PBS and precipitated in 5% trichloroacetic acid (TCA) at 4 celsius. Approximately 12 hr later, pellets were resuspended in 1 ml of 5% TCA and transferred to 10 ml of scintillation fluid (Optiphase "Hisafe3"; Wallac, Turku, Finland). Incorporation of [^{3} H]TdR was measured by β -scintillation counting as disintegrations per minute per node for each experimental group. In each case, a stimulation index (SI) relative to the concurrent vehicle-treated control value was derived.

Mathematical analysis

The estimated concentration of chemical required to induce an SI of 3 relative to concurrent vehicle-treated controls, or EC3 value, was derived by linear interpolation of dose–response data as described previously (9). The EC3 value was calculated by interpolating between 2 points on the SI axis, one immediately above, and the other immediately below, the SI value of 3. The vehicle-treated control (SI = 1) cannot be used for the latter. Where the data points lying immediately above and below the SI value of 3 have the coordinates (a , b) and (c , d), respectively, the EC3 value may be calculated using the following equation:

$$EC3 = [(3 - d)/(b - d)](a - c).$$

Absorption of MMA through human epidermis

Absorption of MMA through human epidermis was measured *in vitro* using static glass diffusion cells according to a method described previously (10). Human abdominal whole skin (dermis plus epidermis) was obtained post-mortem from subjects of various ages. Following immersion in water at 60 celsius for 45 seconds, sheets of epidermis were gently separated from the dermis and stored at -70 celsius on aluminium foil. Samples

of epidermis (from 3 different subjects) were positioned in glass diffusion cells (AstraZeneca Engineering, Alderley Park, Macclesfield, UK) forming a sealed horizontal membrane (exposed surface area of 2.54 cm^2) between the donor (outer) and receptor (inner) chambers. Chambers were clamped and semi-immersed in a thermostatically controlled water to maintain skin temperature at 30 celsius. The integrity of all membrane preparations was assessed by measurement of permeability to tritiated water (10). Those membrane samples that had permeability coefficients to tritiated water of $>15 \times 10^{-4} \text{ cm/hr}$ were regarded as being abnormally permeable and were excluded from the study. Both receptor and donor chambers were emptied, rinsed with saline, 0.5 ml of saline applied to the receptor chamber, and samples incubated overnight at 30 celsius to hydrate naturally. The receptor chamber was filled with a measured volume of 50% (v/v) ethanol in water in order to allow free diffusion of MMA into this phase. Absorption of MMA was measured following application of neat MMA ($9430 \mu\text{g}/\text{cm}^2$ to the surface of the membranes). 6 separate cells were either occluded for the duration of the exposure (10 hr), or remained uncovered to allow the MMA to evaporate from the surface of the membrane. At various times (0–10 hr) samples of receptor fluid were removed and the concentration of MMA in the receptor fluid measured by gas liquid chromatography. MMA was measured using a 10% OV17 chromosorb WHP column (100–120 mesh) in helium carrier gas (flow rate 30 ml/min) using a flame ionization detector (detector gases: hydrogen at 40 ml/min and air at 250 ml/min). The column temperature was 80 celsius, the injection port temperature was 150 celsius, and the detector temperature was 300 celsius. The injection volume was 2 μl , and the limit of detection for MMA was 10 $\mu\text{g}/\text{ml}$. The amount of MMA absorbed was expressed as $\mu\text{g}/\text{cm}^2$ or as a percentage of the applied dose. Data are recorded as mean values $\pm$ SE.

Results

The absorption of neat MMA through human epidermis *in vitro* was examined under occluded and unoccluded conditions. These studies generate kinetic data reflecting the rate of delivery of the chemical from the external application site to the systemic circulation, expressed per unit surface area of skin per unit time. Maximum absorption rate or steady state flux under infinite dose and occluded conditions is usually used to determine a worst case body burden, though in practice absorption slows down as the chemical or its vehicle evaporates, or

the applied dose is depleted or washed off. Experiments were conducted over a 10-hr period and the rate of absorption and the percentage of the total applied dose absorbed were measured (Table 1). Significant levels of MMA were detected in the receptor fluid within 10 min of treatment in the presence or absence of occlusion. Under both conditions, maximal rates of absorption were recorded within the first hour of exposure, with higher values recorded following application under occlusion compared with open exposure (274 compared with 107 $\mu\text{g}/\text{cm}^2/\text{hr}$). The absorption rate for open application of MMA decreased very rapidly and profoundly thereafter, with an average rate of 3.5 $\mu\text{g}/\text{cm}^2/\text{hr}$ recorded over the 10-hr period. A less marked decrease in absorption rate was recorded over the same time period for occluded epidermis (152 $\mu\text{g}/\text{cm}^2/\text{hr}$). The differences in absorption rate were paralleled by differences in the mean percentage dose of MMA absorbed. During the first hour of exposure, very similar total amounts were absorbed irrespective of occlusion (0.036% and 0.047% from unoccluded and occluded epidermis, respectively). Within 1 hr of treatment, considerably more MMA had been absorbed from occluded, compared with unoccluded, tissue (2.41% and 0.48%, respectively). Given that the rate of absorption from unoccluded epidermis decreased very markedly 1 hr after application, it is not surprising that the total percentage of the dose absorbed after 10 hr had risen to only 0.56%. In contrast, after 10 hr of occlusion, 15% of the total applied dose of MMA was detected in the receptor fluid. Given the volatility of this material, the marked reduction in absorption of MMA under unoccluded conditions is likely to be a result of the loss, by evaporation, of MMA from the epidermal surface.

The skin sensitization potential of MMA was determined using the LLNA, a method in which

Table 1. Percutaneous absorption of methyl methacrylate through human epidermis^a

	Unoccluded exposure	Occluded exposure
Mean absorption rate ($\mu\text{g}/\text{cm}^2/\text{h}$) $\pm$ SE		
0.17–1 hr	107 $\pm$ 15	274 $\pm$ 21
0.17–10 hr	3.5 $\pm$ 0.6	152 $\pm$ 13
Mean percentage dose absorbed (%)		
0.17 hr	0.036	0.047
1 hr	0.48	2.41
10 hr	0.56	15.0

^aHuman epidermis was mounted in glass diffusion chambers. Absorption of neat methyl methacrylate ($9430 \mu\text{g}/\text{cm}^2$) was measured over 10 hr following application in the presence or absence of occlusion. Results are expressed as mean absorption rate ($\mu\text{g}/\text{cm}^2/\text{h}$) $\pm$ SE for 6 individual cells or as the percentage of applied dose.

the activity is measured as a function of induced proliferative responses in the lymph node draining the site of unoccluded topical exposure to the chemical. In this assay, chemicals are defined as positive if at one or more test concentrations a threefold or greater increase in proliferation compared with concurrent vehicle-treated controls is recorded (3). Mice received various doses of MMA (10%–75%) formulated in acetone vehicle, 100% (neat) MMA or acetone alone, on the dorsum of both ears daily for 3 consecutive days and on the fifth day after exposure LNC proliferation assessed by radiolabelled thymidine incorporation (Table 2; Fig. 1). A dose-dependent increase in proliferation in response to chemical was recorded, with concentrations of 75% and 100% (neat) MMA provoking stimulation indices of 4.4 and 7.3, respectively.

As described above (cf. Table 1), after the first 1 hr of open application, there is little increase in absorption of MMA through human epidermis, probably as a result of the volatility of the compound and loss from the surface of the skin. The use of a volatile vehicle such as acetone in the LLNA will also ensure rapid evaporation of MMA from the skin. In order to determine whether a less volatile vehicle would enhance sensitization by increasing the extent of skin contact with MMA, a second LLNA was conducted in which the chemical was formulated in AOO vehicle. This is a recommended vehicle for the LLNA that has excellent wetting qualities and has been used successfully for a variety of test materials of different classes (8). Five days after the initiation of exposure of mice to various concentrations of MMA (10%–75%) in AOO, 100% (neat) MMA, or to AOO vehicle alone, the extent of LNC proliferation was measured following intravenous injection of radiola-

belled thymidine. In parallel experiments, the activity of DNCB, a known human contact allergen for which the ED₅₀ (50% effective dose) for sensitization of normal adult volunteers has been determined previously (11), was measured. DNCB (0.01%–0.25%) was formulated in the same vehicle (AOO) in order to allow direct comparison between chemicals. Topical application of MMA dissolved in AOO stimulated increased levels of proliferation with a similar dose response to that provoked by the same material formulated in acetone (Fig. 1; Table 2). In both cases, a maximal SI of approximately 7 was recorded at doses of 100% (neat) MMA. As expected, DNCB dissolved in AOO vehicle also elicited a positive LLNA response, with vigorous proliferation recorded at doses of 0.05% and above, rising to a maximal SI of approximately 16 at 0.25%.

The LLNA dose–response data were used to derive mathematically by linear interpolation the EC₃ value, the estimated concentration necessary to provoke an SI of 3, the threshold for a positive response in the LLNA (Table 3). EC₃ values of 60% and 90% were observed for MMA formulated in acetone and AOO, respectively. For DNCB formulated in AOO, an EC₃ value of 0.036% was recorded; this value is very similar to EC₃ values derived in previous studies in this laboratory in which DNCB was formulated in the same vehicle (0.031% and 0.048%) (12, 13). These data demonstrate that there are differences that span several orders of magnitude between the threshold concentrations necessary for the induction of sensitization to DNCB and MMA (regardless of application vehicle), with approximately 2000-fold more MMA than DNCB required to stimulate a threefold increase in proliferation in the LLNA.

Table 2. Local lymph node assay dose responses to MMA and DNCB^a

Concentration (%w/v)	MMA (acetone vehicle) d.p.m. per node (SI)	MMA (AOO vehicle) d.p.m. per node (SI)	DNCB (AOO vehicle) d.p.m. per node (SI)
0	137 (1)	243 (1)	609 (1)
0.01	ND	ND	839 (1.4)
0.025	ND	ND	1352 (2.2)
0.05	ND	ND	2455 (4.0)
0.1	ND	ND	5971 (9.8)
0.25	ND	ND	9843 (16.2)
10	205 (1.5)	341 (1.4)	ND
30	312 (2.3)	363 (1.5)	ND
50	274 (2.0)	369 (1.5)	ND
75	605 (4.4)	513 (2.1)	ND
100 (neat)	999 (7.3)	874 (3.6)	ND

^aGroups of mice received 25 µl of various concentrations of MMA formulated in AOO or acetone or DNCB formulated in AOO, or an equal volume of vehicle alone, on the dorsum of both ears daily for 3 consecutive days. 5 days after the initiation of exposure, all mice were injected intravenously with phosphate-buffered saline containing [³H]TdR. Draining auricular lymph nodes were excised 5 hr later and pooled on an experimental group basis, a single-cell suspension prepared, and [³H]TdR incorporation measured by β-scintillation counting. Incorporation of [³H]TdR (d.p.m. per node and SI) is displayed for 2 independent experiments for MMA and for 1 experiment for DNCB. MMA, methyl methacrylate; DNCB, 2,4-dinitrochlorobenzene; AOO, acetone:olive oil; ND, not determined; d.p.m., disintegrations per minute; [³H]TdR, [³H] methyl thymidine; SI, stimulation index.

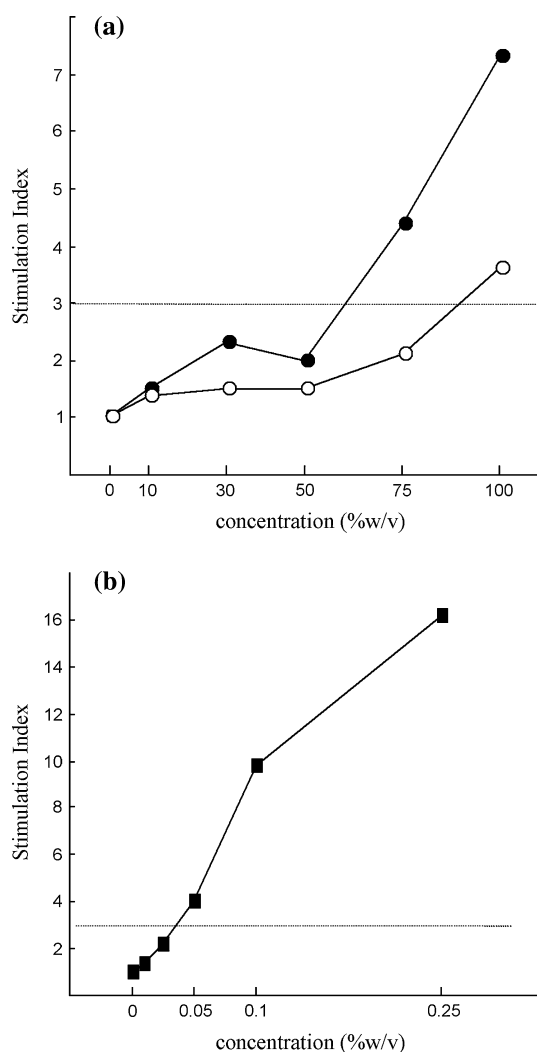


Fig. 1. Local lymph node assay dose responses to MMA and DNCB. Groups of mice received 25 μ l of various concentrations of MMA formulated in AOO (○) or acetone (●), DNCB formulated in AOO (■), or an equal volume of vehicle alone, on the dorsum of both ears daily for 3 consecutive days. 5 days after the initiation of exposure, all mice were injected intravenously with phosphate-buffered saline containing [^{3} H]TdR. Draining auricular lymph nodes were excised 5 hr later and pooled on an experimental group basis, a single-cell suspension prepared, and [^{3} H]TdR incorporation measured by β -scintillation counting. Incorporation of [^{3} H]TdR (SI) is displayed for 2 independent experiments for MMA **a** and for 1 experiment for DNCB **b**. The horizontal dotted line represents the threshold for a positive response in the local lymph node assay, an SI of 3. MMA, methyl methacrylate; DNCB, 2,4-dinitrochlorobenzene; AOO, acetone:olive oil; [^{3} H]TdR, [^{3} H] methyl thymidine; SI, stimulation index.

Discussion

The data contained herein show that MMA has the potential to induce skin sensitization, as judged by the results of 2 independent LLNAs performed with different vehicles. In the context of this report, however, the important observation was that MMA displays only very limited sensitization potency; with EC3 values of 60% and 90%

Table 3. EC3 values for MMA and DNCB^a

Chemical	EC3 value (%w/v)
MMA (acetone)	60
MMA (AOO)	90
DNCB (AOO)	0.036

^aLocal lymph node assay dose responses were performed as described in the footnote to Table 1. The concentration of chemical required in each experiment to induce an stimulation index of 3 (EC3 value) was calculated using linear regression. MMA, methyl methacrylate; DNCB, 2,4-dinitrochlorobenzene; AOO, acetone:olive oil.

recorded in the two studies performed. Such EC3 values are clearly markedly different from those associated with chemicals such as DNCB that are acknowledged to be very potent sensitizers. In previous investigations (in this and other laboratories), DNCB has been shown to have EC3 values ranging between approximately 0.03% and 0.08% (12, 13), and in the analysis reported here, an EC3 value of 0.036% was derived. To a first approximation, it can be calculated that on a %w/v basis, DNCB is inherently at least 3 orders of magnitude more potent than is MMA with regard to the effective acquisition of skin sensitization. Translating this more directly into consideration of risk assessment, the estimate would be that, other factors remaining equal, compared with DNCB, exposure to a 1000-fold or greater concentration of MMA would be required to induce an equivalent degree of skin sensitization.

EC3 values derived from LLNA dose responses have been used to classify contact allergens according to their relative potency. In one such approach, it is suggested that 4 categories may be used: *Extreme* = EC3 value less than 0.1%; *Strong* = EC3 value less than 1%, but greater than 0.1%; *Moderate* = EC3 value less than 10%, but greater than 1%; and *Weak* = EC3 value equal to, or less than, 100%, but greater than 10% (2). Using these criteria, DNCB would be categorized as an *Extreme* contact allergen (alongside potent skin sensitizers such as methyl/chloromethylisothiazolinone, diphenylcyclopropenone, and oxazolone (2)). In contrast, MMA would be categorized as a *Weak* contact allergen, based on EC3 values of either 60% using acetone as a vehicle or 90% using AOO as a vehicle. In attempts to evaluate the worth of EC3 values, and their utility in risk assessment, exercises have been conducted in which measurements of relative potency derived from calculation of EC3 values have been compared with estimates of skin sensitization potency based upon extensive clinical experience. Such comparisons have shown that there exists a very close correlation between clinical observations and estimates of potency based on EC3

values (14, 15). Given the growing confidence that EC3 values derived from LLNA dose responses provide an accurate indication of likely skin sensitization potency in human, and in light of the fact that there is no reason to believe that the data derived for MMA are not similarly correlative in a clinical context, the conclusion drawn from the mouse data is that MMA is indeed a weak skin sensitizer and possesses only a weak potency compared with many other contact allergens.

The results of guinea-pig tests are less informative regarding relative potency partly because comparable induction concentrations are rarely used and dose–response analyses are usually unavailable. Nevertheless, it is relevant to question whether the data available from the maximization test and/or other guinea-pig methods are informative regarding the sensitizing activity of MMA. Although the available data are variable, with respect to induction and elicitation concentrations used, in many instances the response rate recorded in test animals has been 30% or less (16–19). A notable exception to this pattern was provided by the report of Rustemeyer et al. (20). These investigators acknowledged that in previous studies, it had proven difficult to induce sensitization reliably to MMA and other methacrylates in guinea-pigs

using a range of either standard or modified test methods (and, incidentally, they noted also that in their hands attempts to induce skin sensitization to methacrylates in mice had failed) (20). However, in order to generate sufficiently robust models to allow investigation of patterns of cross-reactivity among methacrylates, Rustemeyer et al. used a modified Freunds complete adjuvant test method in guinea-pigs employing high sensitization and challenge doses. Using this method, that requires during the induction phase multiple intradermal injections of the chemical emulsified in adjuvant, more reproducible sensitization activity was found for MMA. However, given the doses used, and the nature of the induction regimen employed, we question whether the view expressed by the authors that MMA and other methacrylates are strong contact allergens in the guinea-pig is justified (20).

In the light of this interpretation, it is necessary to consider whether there exist any human data that are informative. There have been many reports of contact allergy allegedly resulting from exposure to MMA, usually among dental practitioners (see Table 4 for a summary). The prevalence of subjects reacting to MMA varies considerably between these investigations. The highest rates were commonly observed in those

Table 4. Case study reports of methyl methacrylate (MMA) sensitization: a summary

Test group (selection criteria)	Total no. tested	No. tested positive	Incidence (%)	Reference
Dental technicians (with dermatitis, details regarding exposure to MMA not reported)	93	17	16 ^a	24
Dental technicians with dermatitis (details regarding exposure to MMA not reported)	72	9	13 ^a	25
1619 patients with contact dermatitis [23 (meth)acrylate-exposed persons were tested, no further details]	23	3	13 ^a	26
Patients with dermatitis with previous contact with (meth)acrylate (no further details)	271	20	7.4 ^a	27
Patients with dermatitis with previous contact with (meth)acrylate (no further details)	352	17	4.8 ^a	28
Patients with dermatitis [suspected of (meth)acrylate allergy; no further details]	4900		1.4 ^a	29–32
	3080		1.2 ^a	
	4099		1.6 ^a	
	5812		1.4 ^a	
Patients with dermatitis [suspected of (meth)acrylate allergy; no further details]	4221	51	1.2 ^a	33
Patients with dermatitis with previous contact with (meth)acrylates (dental products, adhesives) [(meth)acrylate allergy suspected; no further details]	82	1	0.8 ^a	34
Patients with dermatitis [suspected of (meth)acrylate allergy; no further details]	1161	9	0.8 ^a	35
Dental technicians insured by the BGFE worker compensation fund	65 419	247	0.378 ^c	22
Dental technicians insured by the BGFE worker compensation fund	77 729	194	0.25 ^c	21
Dental technicians and students (with and without exposure to MMA; no details reported)	175	0	0 ^b	36

^aPositive bias as patients were referred to dermatologists with contact allergy.

^bNegative bias due to inclusion of individuals with no relevant exposure.

^cPositive bias due to inclusion of patients with dermatitis caused by other allergens such as latex.

studies with comparatively small numbers of subjects with dermatitis that had been referred to a clinical dermatologist. Lower prevalence rates were found in larger studies. However, even these latter do not necessarily provide an accurate indication of the size of the exposed populations from which selected cohorts were derived. The data of Pflug (21, 22) probably provide the most accurate indication of true prevalence rates in the dental industry (incidence of 0.25%–0.378%) as these reports capture information on the total number of dental technicians exposed, together with the number of insurance claims citing dermatitis. However, even in these examples, there is perhaps some over-estimation as the impact of other recognized skin allergens such as latex had not been excluded. These studies suggest that there is a relatively low frequency of sensitization to MMA among exposed populations.

The conclusion drawn from these studies is that, in line with clinical experience, MMA is a contact allergen. However, based on LLNA dose responses and available EC3 values, and the majority of the data derived from guinea-pig studies, the interpretation is that compared with other contact allergens, MMA has only a relatively weak potential to cause the acquisition of skin sensitization. This is largely consistent with epidemiological studies, provided that exposure is taken into account. This is an important observation and one that facilitates more informed decisions to be made in the future about appropriate risk management strategies.

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