

Comparative analysis of skin sensitization potency of acrylates (methyl acrylate, ethyl acrylate, butyl acrylate, and ethylhexyl acrylate) using the local lymph node assay

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There are currently available no systematic experimental data on the skin sensitizing properties of acrylates that are of relevance in occupational settings. Limited information from previous guinea-pig tests or from the local lymph node assay (LLNA) is available; however, these data are incomplete and somewhat contradictory. For those reasons, we have examined in the LLNA 4 acrylates: butyl acrylate (BA), ethyl acrylate (EA), methyl acrylate (MA), and ethylhexyl acrylate (EHA). The LLNA data indicated that all 4 compounds have some potential to cause skin sensitization. In addition, the relative potencies of these acrylates were measured by derivation from LLNA dose-response analyses of EC₃ values (the effective concentration of chemical required to induce a threefold increase in proliferation of draining lymph node cells compared with control values). On the basis of 1 scheme for the categorization of skin sensitization potency, BA, EA, and MA were each classified as weak sensitizers. Using the same scheme, EHA was considered a moderate sensitizer. However, it must be emphasized that the EC₃ value for this chemical of 9.7% is on the borderline between moderate (<10%) and weak (>10%) categories. Thus, the judicious view is that all 4 chemicals possess relatively weak skin sensitizing potential.

Key words: acrylates; local lymph node assay; skin sensitization potency. © Blackwell Munksgaard, 2007.

Accepted for publication 27 May 2007

The investigations described here were undertaken to assess the sensitizing potential of a panel of acrylates for which previously available data are incomplete, and in some instances, inconsistent. Both butyl acrylate (BA) and ethyl acrylate (EA) are included in commercial (meth)acrylate series used for diagnostic patch testing, and there are reports of patch-test-positive individuals, particularly among dental laboratory technicians, to these materials, although such are considerably less frequent than those reported for the methacrylates (1, 2). In 1 study in which 275 patients were patch tested and in which 17.5% of subjects tested positive to 1 or more methacrylates, no allergic reactions was observed in response to

ethylhexyl acrylate (EHA) (3). Furthermore, case studies collated from 13 833 patients detailing incidences of contact allergy to methacrylates indicated that 2-EHA is not reported to be a strong human sensitizer (4). Predictive tests in animals for the identification of skin sensitization potential have been performed only rarely with acrylates and then with somewhat conflicting results. For example, several guinea-pig assays have been conducted using BA, in which this material has elicited variable responses in the guinea-pig maximization test (GPMT), the Freund's complete adjuvant test, and the split adjuvant test (unpublished observations; 5, 6). Even less information is available from guinea-pig tests for other acrylates,

including those of interest here [EA, methyl acrylate (MA), and EHA]. There are limited local lymph node assay (LLNA) data available for these materials. In a standard LLNA conducted using CBA/Ca strain mice, EA was identified as a weak contact allergen (7), and in a non-standard assay conducted using B6C3F1 hybrid mice, BA was identified as a weak contact sensitizer, whereas EA failed to induce positive responses at the maximum concentration tested (30%) (8). It was the purpose of this study, therefore, to provide a more robust and structured evaluation of the skin sensitizing potencies of a series of acrylates using the systematic application of the LLNA. To this end, relative skin sensitization potency was determined by derivation of EC3 values from LLNA dose-response data. The EC3 value (the effective concentration of chemical required to induce a threefold increase in proliferation of draining lymph node cells compared with control values) has been used extensively for this purpose (9–11) and has been shown to correlate closely with estimates of relative skin sensitizing potency based on clinical experience (12, 13).

Materials and Methods

Mice

Young adult CBA/Ca strain mice (Harlan, Bicester, Oxfordshire, UK), 8–12 weeks of age, were used throughout these studies. Mice were housed in metal cages and food (RM1 pelleted diet; Special Diets Services Ltd, Witham, UK) and water were available *ad libitum*. The ambient temperature was maintained at $22 \pm 3^\circ\text{C}$ 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

BA (99.7% pure), EA (99.95% pure), MA (99.94% pure), and EHA (99.84% pure) were supplied by BASF Aktiengesellschaft, Ludwigshafen, Germany. Dosing solutions (1–25% w/v for BA and MA, 2.5–50% w/v for EA, and 0.5–10% for EHA) were prepared gravimetrically in acetone:olive oil (AOO) vehicle (4:1 ratio). Dosing solutions were prepared freshly for each application.

Sighting studies

Female CBA/Ca strain mice ($n = 1$) were exposed topically on the dorsum of both ears to 25 μl of various concentrations of chemical daily for 3 consecutive days. Control animals were untreated

(naïve). 5 days after the initiation of exposure, mice were terminated and auricular lymph nodes were excised and pooled per animal. A single cell suspension of draining lymph node cells (LNC) was prepared under aseptic conditions by mechanical disaggregation through sterile 200-mesh stainless steel gauze and resuspended in phosphate-buffered saline (PBS). Viable cell counts were performed by exclusion of trypan blue dye, and the cellularity per lymph node was recorded.

Local lymph node assay

The LLNA was performed according to the standard protocol described previously (14, 15). Groups of female CBA/Ca strain mice ($n = 4$) were exposed topically on the dorsum of both ears to 25 μl of various concentrations of chemical, or to the same volume of vehicle alone, daily for 3 consecutive days. The area around the exposure site was monitored for any signs of irritancy, which might prevent continued dose administration. 5 days after the initiation of exposure, all mice were injected intravenously via the tail vein with 20 μCi of [^3H] methyl thymidine (^3H -TdR; specific activity 2 Ci/mmol; Amersham International, Amersham, UK) in 250 μl of PBS. 5 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°C . 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 ^3H -TdR was measured by β -scintillation counting as disintegrations per minute (d.p.m.) 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 (16). The EC3 value was calculated by interpolating between 2 points on the SI axis, 1 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 co-ordinates

(a,b) and (c,d), respectively, then the EC3 value may be calculated using the following equation:

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

Results

The skin sensitization potential of a panel of 4 acrylates was determined using the LLNA, a method in which activity is measured as a function of proliferative responses induced in lymph nodes draining the site of topical exposure to chemical (14, 15). In this assay, chemicals are defined as positive if, at 1 or more test concentrations, a threefold or greater increase in proliferation compared with concurrent vehicle-treated controls is recorded. All materials were formulated in the same vehicle, the preferred vehicle for the LLNA, AOO in a 4:1 ratio (12). Preliminary experiments were conducted with BA, EHA, and MA (at concentrations of 50%, 10%, and 2.5%) to ensure that no detectable local or systemic toxicity was induced by topical application of these compounds. In addition, the level of immune activation provoked by these chemicals was assessed as a function of induced changes in cellularity in the lymph node draining the site of exposure in order to identify a dose range of chemical likely to incorporate a threshold-positive response in the LLNA. This was not necessary for EA, as data appropriate for dose setting were available from a previously published report (7). Topical exposure to EHA (50% and 10%), 50% BA, and 50% MA all resulted in marked increases in cellularity (approximately ninefold to fourfold changes compared with naïve controls). At lower doses, changes in cellularity were more modest, or were absent. On this basis, dose ranges for the LLNA were set as follows: BA and MA at

1–25%, and EHA at 0.5–10% (incorporating in each instance concentrations of chemical predicted to be above and below the threshold of a threefold SI).

In the LLNA, mice therefore received the following dose ranges: 1–25% w/v for BA and MA, 2.5–50% w/v for EA, and 0.5–10% for EHA. The site of exposure was monitored for signs of irritation, but no such observations were noted. Values for thymidine incorporation are given in Table 1. SIs deriving from these values are represented graphically in Fig. 1. Basal levels of proliferation recorded for the concurrent vehicle (AOO)-treated control groups were either 728 or 746 d.p.m./node. These levels are well within the normal range of thymidine incorporation of between 217 and 882 d.p.m./node (encompassing 5–95 percentiles) reported recently for AOO-treated mice from this laboratory (17). All 4 chemicals provoked positive responses in the LLNA, with dose-dependent increases in proliferation recorded in each instance. Maximal SIs recorded were as follows: 8.7 at 25% BA, 4.0 at 50% EA, 3.8 at 25% MA, and 3.1 at 10% EHA.

The LLNA dose-response data summarized above were used to derive mathematically by linear interpolation respective EC3 values. These are listed in Table 2. The lowest EC3 value recorded was for EHA (9.7%) and the highest for EA (36.8%).

Discussion

Taken together, the data summarized here demonstrate that each of the acrylates studied elicited positive responses in the LLNA, and on this basis, each is therefore defined as having some potential to cause skin sensitization. However, in no instance were responses very vigorous, and this is reflected in the derived EC3 values. There is

Table 1. Groups of mice received 25 μ l of various concentrations of BA, EA, MA, or EHA 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 PBS 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 (d.p.m./node) is displayed for each individual experiment

Concentration (% w/v)	BA (AOO vehicle), d.p.m./node	EA (AOO vehicle), d.p.m./node	MA (AOO vehicle), d.p.m./node	EHA (AOO vehicle), d.p.m./node
0	746	746	728	728
0.5	ND	ND	ND	772
1	590	ND	574	878
2.5	995	933	588	688
5	1027	1150	907	877
10	1895	1061	1152	2249
25	6487	1547	2755	ND
50	ND	2967	ND	ND

AOO, acetone:olive oil; BA, butyl acrylate; EA, ethyl acrylate; EHA, ethylhexyl acrylate; ND, not determined.

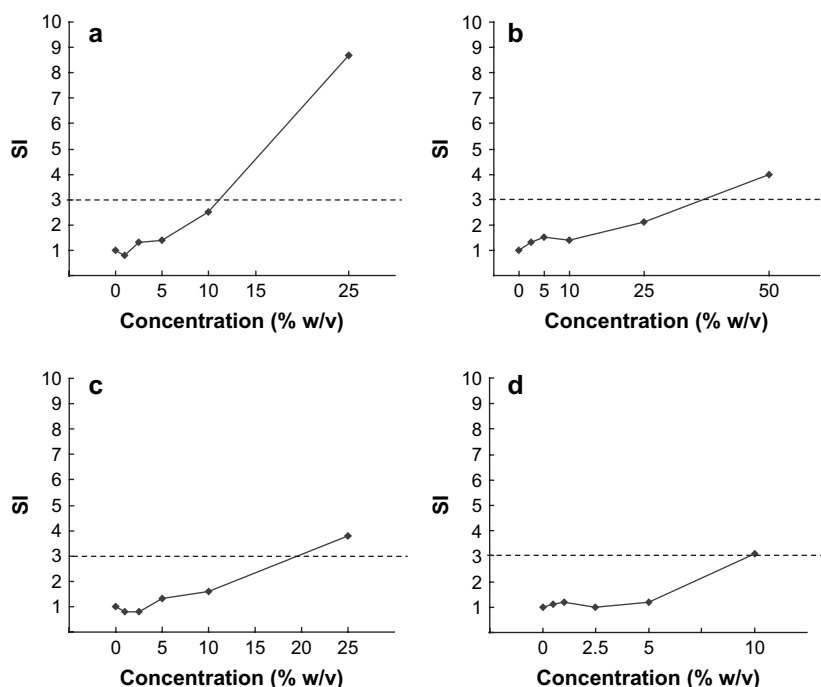


Fig. 1. Local lymph node assay (LLNA) dose responses to butyl acrylate, ethyl acrylate, methyl acrylate, and ethylhexyl acrylate. Groups of mice received 25 μ l of various concentrations of BA (a), EA (b), MA (c), or EHA (d) formulated in acetone:olive oil, or an equal volume of vehicle alone, on the dorsum of both ears daily for 3 consecutive days. 5 days after the initiation of exposure, all mice were injected intravenously with PBS 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 [stimulation index (SI)] is displayed for each individual experiment. The horizontal dotted line represents the threshold for a positive response in the LLNA, an SI of 3.

currently some interest in the possibility of categorizing relative potency on the basis of EC3 values derived from LLNA dose-response data (11, 18). Such an approach appears both reasonable and robust, particularly given that derived EC3 values have been shown to be reproducible between laboratories, and with time in longitudinal studies (19–21). There are a number of possible classification schemes that could be used for the purpose of categorization based on EC3 values, but 1 approach that has found some favour has been described in detail elsewhere (11). In this scheme, the proposal is to categorize chemicals as being extreme, strong, moderate, or weak skin sensitizers, or as lacking skin sensitizing potential. Extreme skin sensitizers are defined as displaying an EC3 value of less than 0.1%, strong skin sensitizers as having an EC3 value of equal to or

greater than 0.1% but less than 1%, moderate skin sensitizers as having an EC3 value of equal to or greater than 1% and less than 10%, and weak sensitizers as having EC3 values equal to or greater than 10% and below 100% (11). Using this scheme, and based on the investigations reported here, then EHA would be categorized as being a moderate skin sensitizer and the other 3 compounds (BA, EA, and MA) as being weak sensitizers. However, it must be emphasized that the EC3 value for this chemical of 9.7% is on the borderline between moderate (<10%) and weak (>10%) categories. Indeed, according to another scheme for categorizing skin sensitizers on the basis of applied dose per unit area (μ g/cm²), all 4 chemicals would be designated as weak or extremely weak (13). Thus, the judicious view is that all 4 chemicals possess relatively weak skin sensitizing potential. Consistent with the reproducibility of EC3 values, it should be noted also that the figure derived here for EA of 36.8% is comparable with the value of 28.7% obtained in a separate series of experiments reported in 2001 (7). In both cases, based on these EC3 values, EA would, according to the scheme described above, be categorized as having weak skin sensitizing activity (11).

There is little other experience of acrylates in the LLNA. The 1 additional source of information derives from investigations reported by Hayes and Meade (8) who used a non-standard LLNA using B6C3F1 (rather than CBA/Ca) strain mice to examine the skin sensitizing potential of BA and

Table 2. EC3 values for BA, EA, MA, and EHA. LLNA dose responses were performed as described Table 1. The concentration of chemical required in each experiment to induce an SI of 3 (EC3 value) was calculated using linear interpolation

Chemical	EC3 value (% w/v)
BA	11.2
EA	36.8
MA	19.6
EHA	9.7

BA, butyl acrylate; EA, ethyl acrylate; EC3 value, the effective concentration of chemical required to induce a threefold increase in proliferation of draining lymph node cells compared with control values; EHA, ethylhexyl acrylate; LLNA, local lymph node assay; MA, methyl acrylate.

EA. In those studies, BA was positive at 20%. These data are consistent with the investigations described here where BA was found to elicit positive LLNA responses at a concentration of 25%. That previous report described EA as being negative at all concentrations tested (of up to 30%). This is again compatible with the data presented here insofar as EA tested negative at 25% but positive at 50%. These comparisons provide further reassurance that the results generated here reflect accurately the behaviour of acrylates in the LLNA.

The LLNA results summarized above are consistent also with what is known of the ability of acrylates to cause sensitization among exposed human populations. Although methyl methacrylate is the compound used most commonly by dental assistants, here is evidence for relatively infrequent and sporadic skin sensitization to the acrylates tested. With regards to the acrylates, the more persuasive cases being for the sensitizing properties of BA and EA. There is little doubt that in humans, these chemicals are weak contact allergens (1, 2, 22–24).

Finally, it is worth considering what is known of the activity of these acrylates in guinea-pig tests. There is little published information available, and what reports have been published suggest show that, under the conditions tested, BA, but not EA, elicited a positive response in the GPMT (5, 6). The only other guinea-pig data are unpublished.

In conclusion, therefore, there is a lack of sound information about the skin sensitizing properties of common acrylates that is available for the purposes of risk assessment and risk management. There is little information deriving from LLNA studies and that derived from guinea-pig data are incomplete or contradictory. We provide here a systematic comparative analysis of 4 acrylates of occupational significance conducted using the LLNA. These studies show that all 4 compounds have some potential to cause skin sensitization but that, according to 1 scheme used currently for categorization according to potency, 3 of the acrylates (BA, EA, and MA) should be regarded as having only weak skin sensitizing potency and 1 (EHA) as being a borderline moderate sensitizer. These data will be of value in developing accurate risk assessments.

Acknowledgements

Publication was sponsored by Basic Acrylic Monomer Manufacturers, Inc (BAMM).

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