

High frequency of false-positive reactions in attempted patch testing with acrylate/methacrylate mixes

Anthony T.-J. Goon¹, Magnus Bruze², Erik Zimerson², An Goossens³, Chee-Leok Goh¹ and Marlène Isaksson²

¹National Skin Centre, 308205 Singapore, Singapore, ²Department of Occupational and Environmental Dermatology, Skåne University Hospital, Lund University, SE-205 02 Malmö, Sweden, and ³Department of Dermatology, University Hospitals Leuven, Katholieke Universiteit Leuven, 3000 Leuven, Belgium

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Summary

Background. Although acrylate/methacrylate allergy has been frequently reported, until now patch testing with this group of allergens has been unwieldy, requiring the application of large supplementary series in most centres.

Objectives. To formulate and evaluate two mixes of acrylate/methacrylate allergens in three centres (Malmö, Singapore, and Leuven).

Patients/materials/methods. All patients tested with the baseline series during the study period were also patch tested with the mixes. Mix 1 consisted of: triethyleneglycol diacrylate (TREGDA) 0.1% wt/wt, 2-hydroxyethyl methacrylate (2-HEMA) 1.0% wt/wt and ethyleneglycol dimethacrylate 1.0% wt/wt in petrolatum. Mix 2 consisted of: TREGDA 0.1% wt/wt and 2-HEMA 2.0% wt/wt in pet. The separate components of the two mixes were also tested simultaneously.

Results. There were 25 (5 males; 20 females) positive reactions to mix 1 with 16 in Malmö, 8 in Singapore, and 1 in Leuven. Positive reactions to mix 2 were seen only in Malmö, in 8 female patients. Thus, the positive reaction rate for mix 1 was 8.3% overall (Malmö 7.7%, Singapore 18.6%, and Leuven 2.1%), and that for mix 2 was 2.7% overall (Malmö 3.8%, Singapore 0%, and Leuven 0%). Of the 16 positive reactions to mix 1 in Malmö, only 4 were considered to be true allergic reactions, as the component allergen testing gave totally negative results in 12/16. For mix 2, only 3/8 positive reactions were considered to be true allergic reactions, as the component testing was negative in 5/8. Many doubtful (10–20%) and positive but non-allergic reactions were recorded, leading to early termination of the study.

Conclusions. Although this was an unsuccessful attempt to formulate an acrylate/methacrylate mix, our experience will be useful for those embarking on future attempts to do this.

Key words: acrylates; mix; patch test; plastics.

Correspondence: Anthony T.-J. Goon, National Skin Centre, 1 Mandalay Road, Singapore 308205, Singapore. Tel: +65 62532244; Fax: +65 62533225. E-mail: anthonygoon@nsc.gov.sg

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Acrylate contact allergy has been frequently reported since the 1970s, with a large number of articles having been published in dermatology journals so far. The current practice for the investigation of patients with suspected contact allergy is to apply a supplementary series containing acrylate/methacrylate allergens in addition to the baseline series; they are currently not present in baseline series in the majority of patch test centres worldwide. Hence, only suspected cases would

be patch tested with a supplementary series containing acrylate/methacrylate allergens.

Most patch test centres use a commercially available acrylate/methacrylate series that typically contains approximately 20–30 components. The benefit of developing a screening preparation to detect acrylate/methacrylate contact allergy in the general patch tested population would have clear practical benefits. It could detect contact allergy in patients where suspicion had not been raised during the clinical history taking, and a screening allergen or mix of high sensitivity would also obviate the need for an extensive supplementary acrylate/methacrylate series in most suspected cases.

We attempted to determine whether acrylate/methacrylate allergy can be detected by using a mix. The allergens were selected on the basis of the results of a previous study on screening for acrylate/methacrylate allergy in the baseline series in Malmö and Singapore (1). They were: triethyleneglycol diacrylate (TREGDA), 2-hydroxyethyl methacrylate (2-HEMA), and ethyleneglycol dimethacrylate (EGDMA).

Patients and Methods

We planned to test the ability of two screening mixes, mix 1 and mix 2, to detect acrylate/methacrylate contact allergy among the entire patch test populations in three centres. Simultaneous patches with the separate components of the two mixes were also applied at the time of testing. Mix 1 consisted of: TREGDA 0.1% (wt/wt) pet., 2-HEMA 1.0% (wt/wt) pet., and EGDMA 1.0% (wt/wt) pet. Mix 2 consisted of: TREGDA 0.1% (wt/wt) pet. and 2-HEMA 2.0% (wt/wt) pet. The separate components of the two mixes that were also tested simultaneously were: TREGDA 0.1% (wt/wt) pet., 2-HEMA 1.0% (wt/wt) pet., 2-HEMA 2.0% (wt/wt) pet., and EGDMA 1.0% (wt/wt) pet.

The allergens were purchased from Chemotechnique Diagnostics (Vellinge, Sweden), and the mixes were compounded in the laboratory of the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Lund University, Malmö, Sweden. After compounding, samples of all of the preparations (mixes and constituents) were also sent to the other two centres for patch testing on their patients. The participating clinics were in Malmö, Singapore, and Leuven, Belgium. All patch test preparations from the three participating centres were from the same batches from the supplier. We standardized the amount of allergen applied to the small Finn Chambers® to 20 mg each, or approximately 7 mm squeezed out from the provided syringes. This amount has been shown to be the optimal dose in a scientific study from Malmö (2).

We recorded data for all positive reactors, including occupation, location of dermatitis, and relevance of positive reactions. In some centres, we also used a patient-answered questionnaire prior to patch testing, asking all patients about details on exposure to artificial nails, dental work with plastic fillings, printing inks, paint production or use, furniture production, and car window repair work.

All patients tested with the baseline series during the study period were patch tested with the six aforementioned acrylate/methacrylate test preparations.

Patch testing was performed with Ø8 mm Finn Chambers® (Epitest Ltd Oy, Tuusula, Finland) on Scanpor® tape (Norgesplaster A/S, Vennesla, Norway). The test chambers were attached to the back for 48 hr. The patch test readings were carried out on day 3 or 4 and on day 7. The separate morphological features according to a protocol (Table 1) were filled in whenever there was a patch test reaction to register. This registration was performed without consideration of the individual history and clinical findings. The protocol was designed at our department in Malmö in 1995, and has been refined over the years (3, 4).

At analysis, the reactions seen were classified as follows:

Positive reaction. Erythema and infiltration covering the whole patch tested surface.

Doubtful reaction. Only slight erythema without infiltration, or infiltration not covering the whole area, or infiltration and erythema not covering the whole area of patch testing, papules without infiltration, or vesicles without infiltration.

Irritant reaction. Shiny surface, silk paper-like wrinkles, always without erythema and infiltration on the whole patch tested surface.

Table 1. List of morphological reactions sought for in the protocol

Erythema covering the whole test area
Erythema not covering the whole test area
Infiltration/oedema covering the whole test area
Infiltration/oedema not covering the whole test area
Papule(s)
Vesicle(s)
Bulla(e)
Dry skin
Scaling
Pustule(s)
Erosion
Necrosis
Petechiae
Shiny skin
Silk paper appearance
Other morphology

Table 2. Results of patch testing with acrylate/methacrylate mixes in the three centres (Malmö, Sweden; Leuven, Belgium; and Singapore)

Substance	Tested	PT+, n (%)	Doubtful, n (%)	Irritant, n (%)
Malmö				
Acrylate mix 1, 2.1% pet.	209	16 (7.7)	38 (18.2)	3 (1.4)
Acrylate mix 2, 2.1% pet.	209	8 (3.8)	19 (9.1)	1 (0.5)
Leuven				
Acrylate mix 1, 2.1% pet.	48	1 (2.1)	10 (20.8)	0
Acrylate mix 2, 2.1% pet.	48	0	3 (6.3)	0
Singapore				
Acrylate mix 1, 2.1% pet.	43	8 (18.6)	8 (18.6)	0
Acrylate mix 2, 2.1% pet.	43	0	13 (30.2)	0
Malmö				
Acrylate mix 1, 1.41% pet.	214	6 (2.8)	0	0
Acrylate mix 2, 1.37% pet.	214	5 (2.3)	2 (0.9)	0
Acrylate mix 1 (controls), 2.1% pet.	55	2 (3.6)	4 (7.3)	1 (1.8)
Acrylate mix 2 (controls), 2.1% pet.	55	2 (3.6)	0	1 (1.8)

PT+, at least 1 + reaction on patch testing with this acrylate mix.

Preliminary testing in Malmö was carried out from 1 February to 9 March 2008. All patients tested with the baseline series were tested with the two acrylate mixes and their individual components. After we had noticed frequent irritant reactions in the tested population, we suspended testing from 10 March to 1 June 2008. From 2 June to 5 October 2008, we started testing again, but with lower concentrations – acrylate mix 1 at 1.41% pet. and acrylate mix 2 at 1.37% pet. At the same time, we also analysed the concentrations of the initial mixes, and found that the concentrations of the constituents were accurate.

In Malmö, we also tested 55 controls from 27 June to 15 August 2008 with both mixes at 2.1% (wt/wt) pet. These controls were consecutive eczema patients (but not suspected to have acrylate/methacrylate allergy) who had been sent for patch testing at our clinic. Only 1 control patient had a toxic reaction to either mix, and so we decided that it was feasible to resume testing with this concentration in the baseline series. Furthermore, as there had been no reactions to the mixes at the lower concentrations, we resumed testing with acrylate mixes 1 and 2 at the original concentrations, that is, 2.1% (wt/wt) pet. This testing was performed in Malmö from 6 October to 31 December 2008. In November 2008, we also tested patients in the two other participating centres, Singapore and Leuven, Belgium. Again, testing was suspended after frequent false-positive reactions had been found in the patients tested. After much deliberation, we decided to suspend testing indefinitely.

The methodology of the high-performance liquid chromatography (HPLC) investigations used to test for the composition of our mixes has been published previously (5).

Results

The patch test results are shown in Table 2. Overall, 300 consecutive patients from each centre (excluding controls) were tested with both mixes at 2.1% (wt/wt) pet. in the three centres; 105 (35%) were male and 195 (65%) were female. There were 25 (5 male; 20 female) positive reactions to mix 1:16 in Malmö, 8 in Singapore, and 1 in Leuven. Positive reactions to mix 2 were only seen in Malmö, in 8 female patients. Thus, the positive reaction rate for mix 1 was 8.3% overall (Malmö 7.7%, Singapore 18.6%, and Leuven 2.1%), and that for mix 2 was 2.7% overall (Malmö 3.8%, Singapore 0%, and Leuven 0%).

Of the 16 positive reactions to mix 1 in Malmö, 4 were considered to be true allergic reactions, as 1 or more of the component allergens also showed at least a + reaction, or the doctor reading the test concluded that the reaction was a true allergic reaction even though there was no + reaction to any of the components. Similarly, of the 8 positive reactions to mix 2 in Malmö, 3 were considered to be true allergic reactions. Among the positive reactions to mix 1 in Leuven and Singapore, none was classified as a true allergic reaction.

Of the 4 Malmö patients concluded to have true allergic reactions, 3 had positive patch test reactions to both mixes, 1 had positive patch test reactions to all four simultaneously tested components, and 2 were positive for 2-HEMA 1% pet. and 2% pet. as well as EGDMA 1% pet. The final patient had a positive reaction only to mix 1, but did not have positive reactions to mix 2 or any of the components. The doctor who read this patient's patch test had considered the reaction to mix 1 to be an allergic reaction but of uncertain relevance. The patient was a 24-year-old male gardener who had been patch tested

because he had an area of dermatitis over a tattoo on his thigh.

There were doubtful reactions to mix 1 in 18.1% of Malmö patients, 20.8% of Leuven patients, and 18.6% of Singapore patients (overall 18.7%), as well as doubtful reactions to mix 2 in 9.1% of Malmö patients, 6.3% of Leuven patients, and 30.2% of Singapore patients (overall 11.7%).

Discussion

It has been suggested that any sensitizer should be considered a candidate for inclusion in the baseline series when routine testing of patients with suspected contact dermatitis results in a contact allergy rate exceeding 0.5–1% (6). Therefore, whether acrylates/methacrylates should be added to the European and other baseline series will depend on whether the contact allergy rate in patients with suspected contact allergy to these allergens reach this level.

During our earlier study (1) on screening for acrylate/methacrylate allergy in the baseline series in Malmö and Singapore, we had found that 1.6% of the Malmö patients and 1.0% of the Singapore patients presented with positive patch test reactions. Thereupon, we suggested that a mix of 2-HEMA, TREGDA and EGDMA could be formulated into a screening acrylate/methacrylate mix, taking care that such a mix would not actively sensitize patients. With this in mind, we were also aware that methacrylates and acrylates such as 2-HEMA, TREGDA or EGDMA could not be used to screen for contact allergy to cyanoacrylates or epoxy acrylates.

In practice, acrylates at 0.1% pet. and methacrylates at 2.0% pet. are used in commercial patch test preparations. In formulating our mixes, we chose to add an acrylate at 0.1% to one or two methacrylates up to a total concentration of 2.0%, giving a final concentration of 2.1%. This choice of formulation was rather arbitrary, but we felt that this concentration would be safe enough not to actively sensitize our patients. We had no prior information about whether the addition of these acrylates and methacrylates in such concentrations would have any additive or synergistic effect in causing irritation, or other false-positive reactions, as such studies had never been published before.

We compounded our mixes in the traditional way, whereby the correct proportions of allergen and pet. was combined weight for weight in a beaker. The mixture was then continuously manually stirred for at least 20 min. It is unlikely that interactions between the individual constituents had resulted in new irritating components: we repeatedly analysed the mixes used after the first batch

of testing in Malmö from 10 March to 1 June 2008, which had yielded many false-positive reactions, with HPLC, and the concentrations of the constituents in the mixes were found to be accurate. The methodology has been published in a previous article (5).

Positive reactions to a mix would be considered true allergic reactions if any one of its components also gave at least a + reaction during the same patch test session. In many of the documented positive reactions to the mixes in our tested population, the morphology of the reactions suggested true-positive reactions, that is, erythema and infiltration covering the whole patch tested surface. However, these readings were not consistent with the results of the testing with individual components of the mixes on the same patients at the same time. This led us to strongly suspect that these were false-positive reactions.

In view of the high frequency of doubtful reactions and positive but non-allergic reactions in patients tested in the three centres, the study was terminated early. There had been too many false-positive and irritant reactions. The penetration of the allergens may have been enhanced when they were formulated into a mix, which should be considered a possible explanation; however, we currently have no way of confirming or disproving this hypothesis.

Interestingly, we found a much higher rate of false-positive reactions in Singapore than in the other two centres. This may be because of Singapore's humid tropical climate, where patients would be sweating more under the test patches; as compared with the two European centres, where the drier, cooler climate would not have led to any significant sweating under the test patches. Another possible reason for the higher false-positive patch test reaction rate in Singapore could be an increased genetic susceptibility to irritant reactions in this different racial group.

Whether it is worthwhile to repeat the attempt in a different way has been a matter of debate. A suggestion for future attempts would include testing with acrylate-only and methacrylate-only mixes. Perhaps the combination of acrylate and methacrylate in the same preparation increased the irritant effect, either additively or synergistically. Another way would be to attempt to formulate mixes with lower concentrations [e.g. acrylate(s) 0.05% + methacrylate(s) 1.0% pet.], but the detection rate would also probably be correspondingly lower.

We would like to reiterate that the current practice of testing with a large number of commercially available acrylate/methacrylate allergens in a screening series leaves much to be desired. In a previous article, we have shown that a shorter screening series comprising six

allergens specifically targeted to our population may be sufficient to detect all retrospective cases of suspected acrylate/methacrylate allergies in our centre (7, 8). Besides the unwieldy number of allergens used, testing with a large number of allergens also increases the probability of a false rejection of the null hypothesis when attempting to make pairwise comparisons of the frequencies of the patch test results between two groups of tested patients (9). A further level of complexity is added when the individual patch tests that make up the series are not completely independent of each other; as when there is a high degree of collinearity between the component allergens within such a series (e.g. between chromium and nickel, or between various acrylates and methacrylates) (9).

This is the first publication of an attempt, albeit unsuccessful, to formulate screening acrylate/methacrylate mixes as patch test preparations that would be useful additions to the baseline series. It is hoped that future workers may learn from our failed attempt and achieve greater success in subsequent studies.

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