

Correlation between Stated and Measured Concentrations of Acrylate and Methacrylate Allergens in Patch-Test Preparations

Anthony Teik-Jin Goon, Magnus Bruze, Erik Zimerson, Östen Sörensen, Chee-Leok Goh, David Soo-Quee Koh, and Marlène Isaksson

Background: Contact allergy to acrylates and methacrylates is not uncommon. The allergy is confirmed by patch-testing patients with commercial patch-test preparations.

Objective: To investigate acrylate and methacrylate allergens used for patch testing in nine different dermatology departments from Europe, America, Asia, and Australia.

Methods: The acrylate and methacrylate (methyl methacrylate, 2-hydroxypropyl acrylate, 2-hydroxyethyl methacrylate, ethylene glycol dimethacrylate, and triethylene glycol diacrylate) allergen samples were analyzed with high-performance liquid chromatography to measure the allergen content.

Results: Variation in measured versus stated concentrations was seen in these samples. The ratio of measured to stated concentrations ranged from 0.11 to 1.1. Only 22 (63%) of 35 samples were within the arbitrary acceptable limits of 80 to 120% of the stated concentrations.

Conclusion: The results may have implications for individual diagnosis and prevention and when test results from various centers are compared.

ACRYLATES and methacrylates are used in many ways, including use in clear plastics (eg, Perspex, Lucite International, Lancashire, UK); water-absorbent material (eg, diapers); orthopedic, dental, and industrial adhesives; paints, tattoo ink, and ultraviolet-cured inks; artificial nails; laser discs and digital video discs; musical instruments such as guitars and drums; and optical fibers. Contact allergy to acrylates and methacrylates leading to allergic contact dermatitis has been frequently reported in medical literature. Confirmation of this diagnosis is made by patch-testing patients with preparations containing various acrylate and methacrylate allergens.

Many acrylates and methacrylates known to cause contact allergy are included in commercially available acrylate and methacrylate patch test series. Suppliers of

such patch-test preparations include Chemotechnique Diagnostics (Vellinge, Sweden), Hermal (Reinbek, Germany), FIRMA (Florence, Italy), SmartPractice Canada (Calgary, Alberta, Canada), and Brial (Greven, Germany). Patch-test departments from different dermatology centers have different preferred suppliers of patch-test preparations. Acrylates and methacrylates are volatile substances, and it has been demonstrated that volatile allergens may disappear from petrolatum patch-test preparations.¹

The aim of the present study was to measure the concentrations of five acrylate and methacrylate allergens in petrolatum patch-test preparations used in various dermatology departments around the world and to compare these with the concentrations stated by their suppliers. The allergens we requested from the departments were methyl methacrylate (MMA), 2-hydroxyethyl methacrylate (2-HEMA), 2-hydroxypropyl acrylate (2-HPA), ethylene glycol dimethacrylate (EGDMA), and triethylene glycol diacrylate (TREGDA). These are the same five allergens we used for acrylate and methacrylate allergy in the baseline series.² In that study, a significant number of patients with contact allergy would have been missed if acrylate and methacrylate allergens had not been included in the baseline series.

From the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Lund University, Malmö, Sweden.

Supported by Avtal om Läkarutbildning och Forskning (ALF), Sweden, and Alfred Österlunds Stiftelse, Malmö, Sweden.

Address reprint requests to Anthony Teik-Jin Goon, National Skin Centre, 1 Mandalay Road, Singapore 308205. E-mail: anthonygoon@nsc.gov.sg

DOI 10.2310/6620.2011.10083

© 2011 American Contact Dermatitis Society. All Rights Reserved.

DECKER



All chemical analyses for this study were done at the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö, Sweden.

Materials and Methods

Chemicals

High-performance liquid chromatography (HPLC)-grade heptane was purchased from Fisher Scientific (Loughborough, UK), and ethanol (99.5%) was purchased from Kemetyl (Haninge, Sweden). n-Butanol was purchased from EGA-Chemie (Steinheim, Germany); 2-HEMA (97%) was purchased from Fluka (Buchs, Switzerland); and MMA, 2-HPA, EGDMA (90%), and TREGDA were purchased from Chemotechnique Diagnostics (Vellinge, Sweden). Petrolatum (snow white quality E) was purchased from Apoteket (Gothenburg, Sweden).

Patch-Test Preparations

We contacted 18 dermatology departments around the world and received patch-test preparations (in the original syringes) that were being used for routine patch testing by nine of those departments: four in Europe, three in North America, one in Asia, and one in Australia. The patch-test preparations were supplied by four companies designated as suppliers 1, 2, 3, and 4. Eighty percent of the preparations came from supplier 1. The preparations, the suppliers used by the various departments, and the concentrations labeled on each syringe are shown in Table 1. A total of 35 test preparations from the nine departments were analyzed from August 2009 to November 2009. The syringes were stored in a freezer at -32°C from arrival until analysis.

The preparations that were past their expiration dates before arrival at our laboratory are also shown in Table 1. Thirteen of 32 (41%) of the preparations whose expiration dates were available had passed their expiration dates when analyzed: 2 of 7 (29%) EGDMA, 3 of 7 (43%) 2-HEMA, 2 of 5 (40%) 2-HPA, 4 of 8 (50%) MMA, and 2 of 5 (40%) TREGDA preparations.

Sample Preparation

Before taking the samples to be measured, 100 to 200 mg of each sample were squeezed out of the syringe and discarded. Approximately 15 to 25 mg of each sample were

accurately weighed into a test tube or a vial and diluted. The details are shown in Table 2.

High-Performance Liquid Chromatography

The ThermoFinnigan mass spectrometry system that was used (ThermoFinnigan, San José, CA) consisted of a P4000 quaternary pump, an ultraviolet 6000 diode array detector, an AS3000 autoinjector, an SN4000F control module, and *ChromQuest* 4.1 software (Thermo Fisher Scientific Inc., Waltham, MA) for spectral analysis. The injection volume was 20 μL for samples containing the methacrylates and 100 μL for those containing the acrylates. The column (4.6 mm internal diameter $\times$ 250 mm), from Phenomenex (Torrance, CA), was packed with 100-angstrom Luna 5 μm silica (Phenomenex). The detector scanned the eluate in the range of 200 to 360 nm. The chromatogram at 215 nm was chosen for detection and used for measurements. For 2-HEMA, 2-HPA, and TREGDA, the elution was isocratic with 90% heptane plus 10% ethanol, and the flow rate was 2.00 mL/min. For MMA and EGDMA, the elution was isocratic with 99% heptane plus 1% butanol, and the flow rate was 2.00 mL/min. Three samples from each syringe were analyzed, and the mean of the values of the three readings was used in the tables.

Standards were prepared freshly from the acrylate and methacrylate chemicals mentioned above. Standard curves were prepared in the concentration range of the analyzed samples.

We determined the repeatability of the HPLC method by repeating the analysis 10 times with a sample of each allergen in petrolatum. The percent coefficient of variation for these 10 values was 0.2.

Results

The acrylate and methacrylate patch-test preparations used by the various departments, the suppliers of the preparations, the preparations' stated and measured concentrations, and the test preparations whose expiration dates had passed before arrival at our laboratory are shown in Table 1. Sample A was from Australasia; samples B, E, G, and I were from Europe; samples C, D, and F were from North America; and sample H was from Asia. Expiration dates were available for 32 of 35 of the samples; of these samples, 19 had not yet expired. None of these non-expired samples passed their expiration dates between their receipt and the start of the analysis in our laboratory. The ratio of measured to stated allergen concentration varied from 0.11 to 1.10 (see Table 1); 22 of 35 samples were within the

Table 1. Detected vs Stated Concentrations of Allergens Obtained from Nine Dermatology Departments

<i>Dept.</i>	<i>Supplier</i>	<i>Stated Conc</i> (% w/w)	<i>Detected Conc*</i> (% w/w)	<i>CV (%)</i>	<i>Ratio of Detected to</i> <i>Stated Conc</i>	<i>Expiry Status</i> (mo-yr)
<i>Methyl Methacrylate</i>						
A	1	2.0	1.2	4.5	0.60	E (12-2008)
B	1	2.0	1.1	1.9	0.55	NA
C	1	2.0	0.21	1.7	0.11	E (09-2005)
D	1	2.0	1.1	0.6	0.55	E (11-2007)
E	2	2.0	1.7	1.1	0.85	V (04-2011)
F	1	2.0	1.4	1.3	0.70	V (03-2011)
G	3	5.0	1.4	2.0	0.28	E (04-2009)
H	1	2.0	1.2	3.5	0.60	V (11-2009)
I	1	2.0	0.78	2.6	0.39	V (11-2009)
<i>2-Hydroxyethyl Methacrylate</i>						
A	1	2.0	1.7	1.1	0.85	E (05-2009)
B	1	2.0	1.7	8.3	0.85	NA
C	1	2.0	1.6	0.5	0.80	E (01-2007)
D	4	2.0	1.5	0.8	0.75	V (12-2010)
E	2	1.0	0.82	2.3	0.82	V (02-2011)
G	3	5.0	4.5	2.2	0.90	E (02-2009)
H	1	2.0	1.7	2.5	0.85	V (01-2011)
I	1	2.0	1.7	1.3	0.85	V (02-2011)
<i>2-Hydroxypropyl Acrylate</i>						
C	1	0.1	0.050	3.3	0.50	E (03-2005)
D	1	0.1	0.041	5.0	0.41	E (07-2007)
F	1	0.1	0.086	1.1	0.86	V (02-2010)
H	1	0.1	0.079	1.1	0.79	V (02-2010)
I	1	0.1	0.078	1.3	0.78	V (02-2010)
<i>Ethylene Glycol Dimethacrylate</i>						
A	1	2.0	2.0	1.1	1.0	V (01-2010)
B	1	2.0	1.9	4.5	0.95	NA
C	1	2.0	1.9	2.0	0.95	E (03-2008)
D	1	2.0	1.8	0.2	0.90	E (08-2007)
E	2	2.0	2.1	0.2	1.1	V (06-2010)
F	1	2.0	2.0	0.5	1.0	V (01-2010)
H	1	2.0	2.0	0.8	1.0	V (01-2010)
I	1	2.0	2.0	2.8	1.0	V (04-2011)
<i>Triethylene Glycol Diacrylate</i>						
C	1	0.1	0.079	1.3	0.88	E (01-2005)
D	1	0.1	0.086	1.2	0.95	E (10-2007)
E	1	0.1	0.072	2.5	0.80	V (01-2011)
H	1	0.1	0.074	0.7	0.82	V (11-2009)
I	1	0.1	0.074	1.4	0.81	V (11-2009)

Conc = concentration; CV = coefficient of variation; E = expired; NA = not available; V = valid or not expired; w/w = weight per weight.

*Mean of three analyzed samples.

allowable range of 0.80 to 1.20, 13 samples were below the allowable range, and none of the samples exceeded it.

There were wide differences in the ratios of measured to stated allergen concentrations among the five allergens,

especially between MMA and the other allergens. We calculated these ratios for each allergen and found them to be 1.2 for 2-HEMA, EGDMA, and TREGDA; 2.1 for 2-HPA; and 7.7 for MMA.

Table 2. Sample Preparation Details

<i>Allergen Sample</i>	<i>Approx. Weight (mg)</i>	<i>Diluent</i>	<i>Volume (mL)</i>
MMA	25	99% heptane + 1% butanol	20.0
2-HEMA	25	99% heptane + 1% ethanol	20.0
2-HPA	25	99% heptane + 1% ethanol	1.5
EGDMA	25	99% heptane + 1% butanol	20.0
TREGDA	15	99% heptane + 1% ethanol	1.5

EGDMA = ethylene glycol dimethacrylate; 2-HEMA = 2-hydroxyethyl methacrylate; 2-HPA = 2-hydroxypropyl acrylate; MMA = methyl methacrylate; TREGDA = triethylene glycol diacrylate.

Discussion

Studies on other patch-test preparations (eg, isocyanates³ and disperse dyes⁴) have shown that actual allergen concentrations may differ from the concentrations stated by the suppliers. According to Isaksson and colleagues, producers of patch-test preparations allow for a variation of $\pm 20\%$ of the concentration stated on the label before expiry.⁵ Allergens prepared in petrolatum and stored in syringes in a refrigerator at 4°C have been thought to be stable for years.⁶

In this study, 22 of 35 (63%) of the analyzed allergens had ratios of measured to stated concentrations that were within the acceptable range of 0.80 to 1.20. Thirteen of 32 (41%) of the preparations whose expiration dates were available had passed the expiration date when analyzed. Of the allergens that were analyzed before their dates of expiry, 13 of 19 (68%) had ratios within the acceptable range, 6 (32%) had ratios below that range, and none had ratios that exceeded it. Among the 13 samples analyzed after their expiration dates, 7 (54%) had ratios that were within the acceptable range, 6 (46%) had ratios that were below that range, and none had ratios that exceeded it.

Seven of 8 2-HEMA samples (88%), 8 of 8 EGDMA samples (100%), and 5 of 5 TREGDA samples (100%) were within the acceptable range. However, 4 of 5 2-HPA samples (80%) were below the acceptable range; the lowest values were those of the two expired samples. Only 1 of 9 MMA samples (11%) had ratios within the acceptable range; of the 8 MMA samples with ratios below the acceptable range, 4 had expired.

In general, the variation in measured versus stated concentrations of acrylate and methacrylate allergens from different departments around the world may be due to many factors, including the volatility of allergens, the vehicles used, the source of the preparations, changes in temperature and pressure during transport, and the conditions under which the syringes were stored.

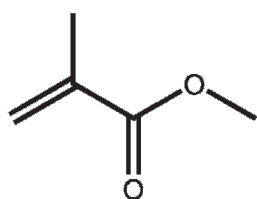
Allergens that are more volatile may evaporate more rapidly and be lost from the preparations more quickly than less volatile allergens. Evaporation from the different vehicles, and chemical interactions with them, may result in different rates of loss of allergens from the preparations. Preparations from different suppliers may contain different additives (eg, stabilizers and emulsifiers), which may lead to varying stabilities of the allergens in these preparations. Higher temperatures may lead to more rapid evaporation and chemical interactions within preparations. Lower ambient pressures may result in more rapid evaporation of volatile allergens. Storage conditions (eg, room, refrigerator, and freezer temperatures as well as other variables) may also affect the rate of loss of allergens from preparations.

Possible explanations for the lower concentrations are evaporation, chemical degradation (oxidation or hydrolysis), polymerization, diffusion into the plastic of the syringe, and suppliers' compounding errors. We found no differences among the companies in regard to the wide variation in measured versus stated concentrations. However, MMA, which has the highest vapor pressure of the investigated substances, also had the greatest variation in concentration. Patch-test preparations of MMA and 2-HPA (which also had a relatively high vapor pressure) had concentrations that most often were lower than the acceptable arbitrary concentration range. The chemical structures and physical characteristics of all five allergens are shown in Figure 1.

All nine departments that sent samples informed us that the syringes had been stored in refrigerators, as recommended by the suppliers; five departments had stored the syringes with caps on, three had stored them without caps, and one had stored three of their five samples without caps and had stored two samples with caps on. There is a possibility that allergen loss was more rapid in the uncapped syringes than in the capped syringes.

Our findings have implications for the clinical practice of patch testing. Lower-than-stated concentrations may lead to increased rates of false-negative patch-test results whereas higher-than-stated concentrations may increase the risk of active sensitization and irritant reactions.

We found that the MMA syringe obtained from the Malmö dermatology department had a measured concentration of only 0.78% despite not having passed its expiration date. This might explain why we had had such a low pick-up rate of MMA contact allergy in one of our previous studies.² MMA has the highest vapor pressure of the five acrylate and methacrylate allergens studied and is thus at a higher risk of loss through evaporation. This may explain why two of the

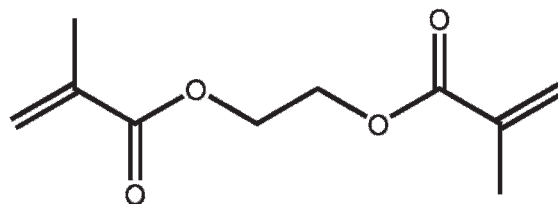
**Methyl methacrylate (MMA)**

CAS no: 80-62-6

bp: 102°C

vp: 36.9 torr = 4.9 kPa

MW: 100

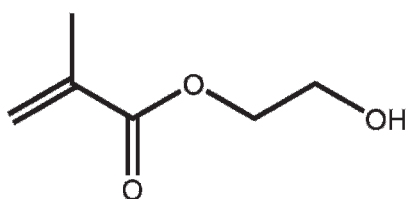
**Ethylene glycol dimethacrylate (EGDMA)**

CAS no: 97-90-5

bp: 260°C

vp: 0.0121 torr = 0.0016 kPa

MW: 198

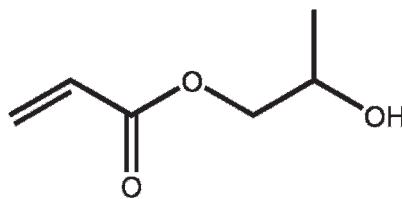
**2-Hydroxyethyl methacrylate (2-HEMA)**

CAS no: 868-77-9

bp: 189°C

vp: 0.159 torr = 0.021 kPa

MW: 130

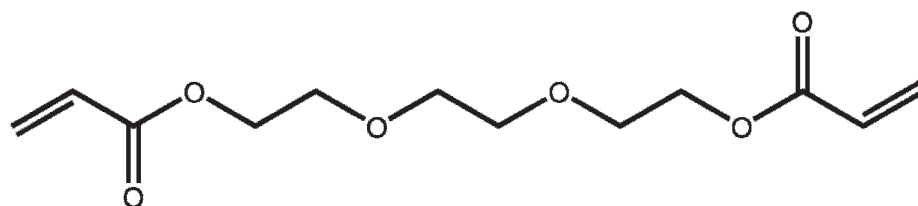
**2-Hydroxypropyl acrylate (2-HPA)**

CAS no: 999-61-1

bp: 225°C

vp: 0.115 torr = 0.015 kPa

MW: 130

**Triethylene glycol diacrylate (TREGDA)**

CAS no: 1680-21-3

bp: 350°C

vp: 9.5×10^{-5} torr = 0.000013 kPa

MW: 258

Figure 1. Chemical structures and physical characteristics of the five acrylate and methacrylate allergens used in the study. (bp = boiling point; CAS = Chemical Abstracts Service; kPa = kilopascals; MW = molecular weight; vp = vapor pressure.)

MMA samples had what were among the lowest ratios of detected concentration to stated concentration among the samples in this study.

Besides the concentration, the amount of applied patch-test preparation (dose per square centimeter) may influence the potential for active sensitization. We have recommended that a preparation of 20 mg in petrolatum is the optimal dose for 8 mm Finn Chambers (Epitest Ltd Oy, Tuusula, Finland)⁷ whereas 30 mg pet has been recommended by Chemotechnique Diagnostics, the maker of IQ Chambers.

Among our study samples, those from one of the centers stated the concentrations of 2-HEMA and MMA to

be 5% pet. DeGroot mentioned 2% and 5% as concentrations that have been used for patch testing with 2-HEMA, and 2% as the patch-test concentration for MMA.⁸ Methacrylates have been reported to cause sensitization at a 2% concentration⁹; that report, however, makes no comment on the dose of allergen applied to the chambers per square centimeter. Furthermore, the MMA concentration in the syringe with the test preparation might have been higher than stated. Currently, two of the three patch-test suppliers mentioned in DeGroot's article supply 2-HEMA in a 1.0% concentration in petrolatum, whereas the third still supplies it in a 2.0% concentration. In our experience of more than 20 years of patch testing with

commercial methacrylate patch-test preparations at a labeled concentration of 2.0% in petrolatum, we have not observed any cases of active sensitization.

The allergen concentrations in more than half (54%) of the syringes analyzed after expiry were within the acceptable range, whereas 68% of syringes analyzed before expiry had concentrations within the acceptable range. Thus, a greater proportion of syringes analyzed after expiry had concentrations below the acceptable range when compared to those analyzed before expiry (46% vs 32%).

From our data, it is clear that MMA samples must be carefully prepared and stored; expired samples should not be used. Samples of 2-HPA that have expired should also not be used.

Conclusion

We recommend that manufacturers assign earlier expiration dates to the more volatile allergens (eg, methyl methacrylate [MMA]) and instruct users to store these preparations in a freezer until they are needed. More-stringent checks on whether actual allergen concentrations correspond with stated concentrations should be performed. In all future studies using patch-test preparations of MMA and 2-hydroxypropyl acrylate (2-HPA), it would be prudent for investigators—before embarking on their investigations—to double-check the initial allergen content of the samples they will be using for their experiments.

Acknowledgments

The authors thank Donald V. Belsito (Kansas City, KS), Francisco M. Brandão (Almada, Portugal), Francesca Giusti (Modena, Italy), Jeanne Duus Johansen (Gentofte, Hellerup, Denmark), Rosemary Nixon (Melbourne,

Australia), Frances Storrs (Portland, OR), and James Taylor (Cleveland, OH) for providing patch-test preparations. We thank Lotta Thorsson for her valuable technical assistance.

Financial disclosures of authors and reviewer(s): None reported.

References

1. Mowitz M, Zimerson E, Svedman C, Bruze M. Stability of fragrance test preparations applied in test chambers. *Contact Dermatitis* 2008; 58 Suppl 1:34.
2. Goon AT, Bruze M, Zimerson E, et al. Screening for acrylate/methacrylate allergy in the baseline series: our experience in Sweden and Singapore. *Contact Dermatitis* 2008;59:307–13, doi:[10.1111/j.1600-0536.2008.01440.x](https://doi.org/10.1111/j.1600-0536.2008.01440.x).
3. Frick M, Zimerson E, Karlsson D, et al. Poor correlation between stated and found concentrations of diphenylmethane-4,4'-diisocyanate (4,4'-MDI) in petrolatum patch-test preparations. *Contact Dermatitis* 2004;51:73–8, doi:[10.1111/j.0105-1873.2004.00404.x](https://doi.org/10.1111/j.0105-1873.2004.00404.x).
4. Ryberg K, Gruvberger B, Zimerson E, et al. Chemical investigations of disperse dyes in patch test preparations. *Contact Dermatitis* 2008; 58:199–209, doi:[10.1111/j.1600-0536.2007.01298.x](https://doi.org/10.1111/j.1600-0536.2007.01298.x).
5. Isaksson M, Gruvberger B, Persson L, Bruze M. Stability of corticosteroid patch test preparations. *Contact Dermatitis* 2000;42: 144–8, doi:[10.1034/j.1600-0536.2000.042003144.x](https://doi.org/10.1034/j.1600-0536.2000.042003144.x).
6. Andersen KE, Rastogi SC, Carlsen L. The allergen bank: a source of extra contact allergens for the dermatologist in practice. *Acta Derm Venereol* 1996;76:136–40.
7. Bruze M, Isaksson M, Gruvberger B, Frick-Engfeldt M. Recommendation of appropriate amounts of petrolatum preparation to be applied at patch testing. *Contact Dermatitis* 2007;56:281–5, doi:[10.1111/j.1600-0536.2007.01098.x](https://doi.org/10.1111/j.1600-0536.2007.01098.x).
8. De Groot AC. Patch testing: test concentrations and vehicles for 4350 chemicals. 3rd ed. Wapserveen, the Netherlands: acdegroot publishing; 2008.
9. Vozmediano J, Arancha E. Active sensitization to (meth)acrylates. *Contact Dermatitis* 1998;39:314, doi:[10.1111/j.1600-0536.1998.tb05949.x](https://doi.org/10.1111/j.1600-0536.1998.tb05949.x).