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1) Title: **Variation in allergen content over time of acrylates / methacrylates in patch test preparations.**

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What's already known about this topic?

- It has already been shown that allergen content in patch test preparations such as textile dyes, isocyanates may vary with time.

What does this study add?

- This study confirms that acrylate / methacrylate patch test allergen content decreases with time.
- These allergens are lost rapidly from IQ<sup>TM</sup> chambers especially if stored under room temperatures.
- Allergens in syringes remain above 80% of their initial concentrations for longer periods compared to IQ<sup>TM</sup> chambers.
- In syringes and IQ<sup>TM</sup> chambers, there is a slower rate of decrease in concentration when the storage temperature is lower.

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*Background:* Acrylates / methacrylates are volatile substances. There might be a gradual decrease in acrylate / methacrylate allergen content over time in patch test preparations but this has not yet been documented.

*Objectives:* To determine the allergen content of acrylate / methacrylates in patch test preparations over time under different storage conditions.

*Methods:* Five acrylate / methacrylate allergens (2-hydroxyethyl methacrylate (2-HEMA), methyl methacrylate (MMA), ethylene glycol dimethacrylate (EGDMA), triethylene glycol diacrylate (TREGDA), and 2-hydroxypropyl acrylate (2-HPA)) in syringes and IQ<sup>TM</sup> chambers (Chemotechnique Diagnostics, Vellinge, Sweden) were analysed using gel permeation chromatography (GPC) and high-performance liquid chromatography (HPLC) to measure the allergen content over time in samples stored in the freezer, refrigerator and under room temperature.

*Results:* The concentration of allergens in syringes decreased with time. Those stored at room temperature had the fastest rate of decrease, followed by those in the refrigerator and freezer. In most cases, in syringes or IQ<sup>TM</sup> chambers under all storage conditions, the MMA decreased most rapidly, followed by 2-HPA, 2-HEMA, EGDMA and TREGDA. The allergens in the IQ<sup>TM</sup> chambers rapidly disappeared with almost all samples reaching non-detectable levels by Day 8. In fact, MMA was the first to reach a non-detectable level at Day 2.

*Conclusions:* Acrylate / methacrylate allergens are lost rapidly from IQ<sup>TM</sup> chambers especially if stored under room temperatures. Allergens in syringes remain above 80% of their initial concentrations for longer periods compared to IQ<sup>TM</sup> chambers. In syringes and IQ<sup>TM</sup> chambers, there is a slower rate of decrease in concentration when the storage temperature is lower. Allergens should be stored refrigerated, replaced regularly, and freshly applied onto test patches on the day of use.

*Key words:* acrylates; allergic contact dermatitis; contact allergy; gel permeation chromatography; high-performance liquid chromatography; patch test; stability; volatile.

Contact allergy to acrylates / methacrylates may lead to occupational and non-occupational allergic contact dermatitis. The diagnosis is often missed as there are no acrylate / methacrylate allergens in most baseline patch test series or when no acrylate / methacrylate allergens were tested due to lack of clinical suspicion in affected patients. In a study where we had screened for acrylate / methacrylate allergy in the baseline series, we reported that a significant number of patients with contact allergy would have been missed if acrylate / methacrylate allergens had not been included in the baseline series <sup>1</sup>. Acrylates / methacrylates have a myriad of uses, including clear plastics e.g. perspex; water absorbent material e.g. in diapers; adhesives – orthopaedic, dental, industrial; paints, tattoo ink, UV-cured inks; artificial nails; laser discs & DVDs; musical instruments – guitars, drums; and optical fibers. Many acrylates / methacrylates known to cause contact allergy are included in commercially available acrylate / methacrylate patch test screening series. Suppliers of such patch test preparations include Chemotechnique Diagnostics (Vellinge, Sweden), Hermal (Trolab) (Reinbek, Germany), FIRMA (Florence, Italy), AllergEAZE (Calgary, Canada) and Brial (Greven, Germany).

Not many studies on the stability of patch test preparations under different storage conditions have been done so far <sup>2-4</sup>. Whether the measured concentration of allergens in a patch test preparation would change over time and whether there are differences in stability when stored in different environments would have an impact on the practice of clinical patch testing if we find that certain allergens are not stable at higher or lower temperatures, as this would affect the patch test results and their interpretation. Furthermore, it has already been shown that allergen content in patch test preparations (e.g. textile dyes <sup>5</sup>, isocyanates <sup>6</sup>) may vary. We chose to study acrylates / methacrylates as opposed to other allergens, e.g. metals, as some of the acrylate / methacrylate allergens have high vapour pressures, i.e. are more easily lost through evaporation.

To determine the allergen content of acrylates / methacrylates in patch test preparations over time under different storage conditions, we chose to study patch test preparations of 5 allergens: 2-hydroxyethyl methacrylate (2-HEMA), methyl methacrylate (MMA), ethylene glycol dimethacrylate (EGDMA), triethylene glycol diacrylate (TREGDA), and 2-hydroxypropyl acrylate (2-HPA). These are the same acrylates which we had studied in our previous article on patch testing of acrylates / methacrylates in the baseline patch test series <sup>1</sup>. These preparations were then kept in syringes and in Inert Quadrate & Ideal Quick (IQ<sup>TM</sup>) test chamber unit chambers (Chemotechnique Diagnostics) for various amounts of time. All the chemistry analysis work was done in the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö, Sweden.

## **Materials and Methods**

### *Chemicals*

HPLC grade dichloromethane and HPLC grade heptane were purchased from Fisher scientific (Loughborough, UK) and ethanol 99.5% from Kemetyl (Haninge, Sweden). The heptane was degassed before use. n-Butanol was purchased from EGA-Chemie (Steinheim, Germany). 2-HEMA was purchased from Fluka (Buchs, Switzerland) while MMA, EGDMA, TREGDA and 2-HPA were purchased from Chemotechnique Diagnostics (Vellinge, Sweden). Petrolatum (snow white) Quality E was purchased from Apoteket (Gothenburg, Sweden).

### *Patch test preparations*

We prepared our patch test preparations in 2 different ways at the laboratory of the Department of Occupational and Environmental Dermatology, Skåne University Hospital, Malmö, Sweden. First, we made them in the traditional way by accurately weighing the acrylates and methacrylates listed above and diluting them into petrolatum to a final concentration of 0.10% w/w for acrylates and 2.0% w/w for methacrylates. These preparations were then put into 5ml polypropylene syringes (B. Brown Melsungen AG, Melsungen, Germany) by us. The samples in capped syringes were stored in 3 ways: in the freezer (-18 °C), refrigerator (4 °C) and under room temperature (23°C). Second, we made a MMA test preparation in the same way but while compounding, we repeatedly measured the concentration in order to get as close to 2.0% w/w as possible. This preparation was used for the IQ™ chamber investigations. A fresh commercial MMA test preparation labelled to contain 2.0% w/w and with expiry date January 2012 was purchased from one of the major test suppliers in the world to be used for the IQ™ chamber investigations.

IQ™ chambers made of polyethylene plastic, which according to information from the manufacturer was additive-free, were purchased from Chemotechnique Diagnostics (Vellinge, Sweden). Approximately 30 mg of preparation sample was applied onto each of the IQ™ chambers and the recyclable plastic protective covers provided by the manufacturer were placed onto all patches. Samples for each individual reading were applied in triplicate. These were then stored in the refrigerator and at room temperature.

#### *Sample and standard preparation for gel permeation chromatography (GPC)*

GPC analysis was used for preparations of MMA, 2-HEMA and EGDMA in all analyzed syringes. GPC was also used for the same methacrylates on IQ™ chambers when the analysis covered a time range of 0-32 days. When syringes were analyzed 100-200mg of test preparation was squeezed out of the syringes before the samples to be measured were taken. Samples from each syringe were taken in triplicate for each measurement. For IQ™ chambers, 1 sample from each of 3 chambers was taken for measurement. Approximately 10 mg of sample from the syringes or IQ™ chambers was accurately weighed into a test tube and 5.0 ml dichloromethane was added. These solutions were then filtered through Minisart SRP 15 single-use syringe filters with 0.2µm pore size diameter from Sartorius Stedim Biotech GmbH (Goettingen, Germany) into vials for analysis. Standards were prepared for each day of analysis at our department from the methacrylate chemicals mentioned above.

#### *Gel permeation chromatography*

A ThermoFinnigan system was used consisting of a P4000 quaternary pump, an ultraviolet (UV) 6000 diode array detector, an AS3000 autoinjector, an SN4000F control module, and software controlled by CHROMQUEST 4.1 and monitored by Spectral Analysis for ChromQuest (ThermoFinnigan, San José, CA, USA). The injection volume was 20 µl. We used 3 columns in series. The first column was from PSS Polymer Standards Service GmbH (Mainz, Germany). This column (4.6 mm i.d. x 250 mm) was packed with styrene divinylbenzene (SDV) copolymer, 100 Å, 5 µm. The other two columns were Shodex GPC KF-401HQ columns from Showa Denko K.K. (Tokyo, Japan). These columns (4.6 mm i.d. x 250 mm) were packed with styrene divinylbenzene (SDV) copolymer, 50 Å, 3 µm. The detector scanned the eluate in the range 200 to 300 nm and the chromatogram recorded at 240 nm was used for detection and measurement. The elution was isocratic with 100% dichloromethane and the flow rate was 0.30 ml/min. Three samples from each syringe or 1 sample from each of 3 IQ™ chambers were analysed and the mean of the 3 readings was used in the graphs.

#### *Sample and standard preparation for high-performance liquid chromatography (HPLC)*

HPLC was used to analyze 2-HPA or TREGDA preparations in syringes and on IQ™ chambers. This method was also used for preparations of MMA, 2-HEMA and EGDMA on IQ™ chambers when the analysis covered a time range of 0-24 h. 100-200mg of sample was squeezed out of each syringe before the samples to be measured were taken. Samples from each syringe were taken in triplicate for each measurement. For IQ™ chambers, 1 sample from each of 3 chambers was taken for measurement. Approximately 15-25 mg of sample was accurately weighed from the syringes or IQ™ chambers into a test tube or vial and diluted with 99% heptane + 1% ethanol or 99% heptane + 1% butanol, details are given in Table 1. Standards were freshly prepared for each day of analysis at our department from the acrylate and methacrylate chemicals mentioned above.

#### *High-performance liquid chromatography*

A ThermoFinnigan system was used consisting of a P4000 quaternary pump, an ultraviolet (UV) 6000 diode array detector, an AS3000 autoinjector, an SN4000F control module, and software controlled by CHROMQUEST 4.1 and monitored by Spectral Analysis for ChromQuest (ThermoFinnigan, San José, CA, USA). The injection volume was 20 µl for 2-HEMA and 100µl for 2-HPA and TREGDA. The column (4.6 mm i.d. x 250 mm) from Phenomenex (Torrance, CA, USA) was packed with 100 Å Luna 5 µm silica. The detector scanned the eluate in the range 200 to 360 nm and chromatograms recorded at 215 nm were used for detection and measurements. The elution was isocratic with 90% heptane + 10% ethanol for 2-HPA, 2-HEMA, TREGDA and 99% heptane + 1% butanol for MMA and EGDMA and the flow rate was 2.00 ml/min. Three samples from each syringe or 1 sample from each of 3 IQ™ chambers were analyzed and the mean of the 3 readings was used in the graphs.

We have determined the repeatability of the HPLC method by repeating the analysis ten times with a sample of each allergen in petrolatum. The coefficient of variation (CV %) was calculated for all 5 allergens and found to be in the range of 0.1-0.3.

### **Results**

The concentrations of the allergens over time from the syringes stored in the freezer, refrigerator and under room temperature are shown in Fig. 1. Those of the allergens from the IQ™ chambers in the refrigerator and under room temperature are in Fig. 2.

When analyzing the content of acrylates / methacrylates in the syringes and the IQ™ chambers, we found that the traditionally compounded test preparations contained lower concentrations than the intended, i.e. 2.0% w/w in pet. for the methacrylates and 0.1% w/w for the acrylates. The starting points for the concentrations were 1.3, 1.8, and 1.7% w/w for MMA, 2-HEMA, and EGDMA, respectively, and 0.08 and 0.09% w/w for 2-HPA and TREGDA, respectively (Figs 1-3). The MMA test preparation that was compounded by us but during repeated analysis of the concentration had a starting concentration of 2.1% w/w (Fig. 3, MMA- IQ™ (B)). The fresh commercial MMA test preparation had a starting concentration of 1.4% w/w (Fig. 3, MMA (Commercial)- IQ™). In the graphs (Figs 1-3), the line denoting 80% of the initial concentration is placed according to the start concentration actually measured.

The concentration of allergens in IQ™ chambers decreased much more rapidly than in the syringes. In general, the decrease in allergen concentration from the syringes was most rapid in room temperature, followed by refrigerator and freezer. In IQ™ chambers, the decrease in allergen concentration was more rapid in room temperature than in the refrigerator.

In almost all graphs, the first allergen to drop below 80% of its initial concentration was MMA, followed by 2-HPA, 2-HEMA, EGDMA and TREGDA. The same ranking was seen whether the allergens were in syringes or IQ™ chambers and in all storage conditions. All the allergens in syringes stored in the freezer had maintained concentrations above 80% of their initial values even after 100 days. However, the level of MMA in the syringe stored in the freezer had dropped below 80% by day 256.

## Discussion

Patch test preparations of certain allergens, e.g. isocyanates <sup>6</sup>, are not stable and these allergens tend to be detected at concentrations lower than the concentrations stated by the suppliers. According to Isaksson et al. <sup>3</sup>, producers of patch test preparations allow for a variation of  $\pm 20\%$  of the concentration given on the label before expiry. Allergens prepared in petrolatum and stored in syringes in the refrigerator at 4 °C have been thought to be stable for years <sup>7</sup>. In the past, studies regarding the diminishing concentration of allergens in patch test preparations over time have rarely been published <sup>2,3</sup>. A recent study has demonstrated that volatile allergens may disappear from petrolatum patch test preparations (8). To our knowledge, this is the first article documenting this phenomenon in acrylate / methacrylate allergens.

We knew beforehand that acrylates / methacrylates are volatile and had anticipated that the starting concentrations of the 5 preparations we were to follow could be lower than expected. However, we did not expect so much to disappear. Apparently a certain proportion of allergens had been lost while compounding the test preparations. We believe evaporation to be the major explanation of these lower than expected concentrations, but this cannot be the entire explanation. For example, TREGDA, which hardly evaporates at all, had also disappeared during compounding. This indicates that other factors may also be important, e.g. adsorption to surfaces.

Our data thus shows that we have never been within the desired concentration range with MMA and EGDMA, as the initial concentrations had been 1.3% and 1.7%, respectively (Fig. 1). Furthermore, the commercial MMA test substances which we had purchased as fresh as we could had a lower concentration than stated even though the expiry date was 2 years ahead. This only indicates that what we have seen in our investigations during compounding is not unique, and that more investigations are needed to see whether this phenomenon is common for other manufacturers.

When we subjected the 2.1% w/w MMA test preparation to storage at room temperature and in the refrigerator in IQ™ chambers for 24 hours, as expected, we had the same loss of allergen per time unit as the traditionally compounded MMA preparation we had made earlier, i.e. the slope of the curves was similar, which means that we can regard the loss of allergen per time unit as similar for the 2 preparations, regardless of whether one starts with a concentration of 2.1% or 1.3% (Fig. 3, MMA- IQ™ (A)). We have calculated that the same would apply also for the other sensitizers.

In general, we have found that stability of the allergens is inversely correlated to storage temperature. Commercial patch test suppliers usually recommend 2 years as the expiry period for fresh preparations. One of the producers has recommended that “Allergens used infrequently should be stored in a cool place protected from light” in their catalogue <sup>9</sup> and “Store dark at 5-8 °C” on the syringe labels. The other suppliers had recommended storage in the refrigerator between 2 °C and 8 °C on the syringe labels. Most patch test centres we have asked informed us that they store their allergens in the refrigerator.

We found that MMA in syringes stored at room temperature and in the refrigerator had dropped below the desirable level of 80% of the initial concentration by approximately 2 weeks. 2-HPA in room temperature had fallen below 80% after 3 weeks. In the freezer, only MMA had dropped below 80% by 6 months. The rest of the samples remained above 80% of initial level in the freezer at 6 months. In fact, EGDMA and TREGDA had remained above 80% under all 3 storage conditions.

The rate of decrease in allergen concentration is higher in the IQ<sup>TM</sup> chambers than in the syringes. This is likely because a greater surface area of the preparation is exposed to the environment. The loss of allergens may be due to evaporation and/or chemical degradation. Furthermore, there is an approximately 5mm space around each IQ<sup>TM</sup> chamber when the plastic cover is applied. This small space may allow some of the more volatile allergens to diffuse through the tape.

We have also tested the same allergens in uncapped syringes. However, these were not tested in triplicate and hence not included in our results. In general, for these uncapped syringes, we had found that the rate of decrease of allergen was greater for every allergen and in every storage condition when compared to the equivalent capped syringes.

We have also recently reported that patch test preparations sent in syringes with caps, where a negligible amount of the preparation would be exposed to the surrounding environment, have retained their allergen content much better than those sent in IQ<sup>TM</sup> chambers with covers, where a large surface area of allergen relative to volume is exposed <sup>10</sup>.

Concerning IQ<sup>TM</sup> chambers, after 1 day at room temperature or in the refrigerator, MMA and 2-HPA had dropped below 80% of the initial concentration, and by Day 2 all except TREGDA had dropped below this level. By Day 1, all samples in room temperature except TREGDA had dropped below 80% of initial concentration. TREGDA could be kept for at least 4 days in IQ<sup>TM</sup> chambers at room temperature before dropping below 80%, and for more than a month if kept in the refrigerator.

Following this, we decided to analyze the same allergens in IQ<sup>TM</sup> chambers over 24 hours (Fig. 3). Since we were especially interested in MMA, we analyzed the 3 aforementioned samples of MMA – 2 samples which we had compounded in our laboratory, as well as the commercial sample. By 4 hours, all MMA samples, except the commercial MMA sample in the refrigerator, had fallen below 80% of initial concentration. The commercial MMA sample kept in the refrigerator had retained over 80% of its allergen content beyond 8 hours. The reason for this could be that the petrolatum from the commercial sample binds to MMA a little better than the petrolatum we use. However, more investigations would be needed to prove this. Both 2-HPA samples fell below 80% by 4 hours. Both 2-HEMA and EGDMA at room temperature had fallen below 80% by approximately 8 hours, but remained above 80%

after 24 hours in the refrigerator. Only TREGDA had retained >80% of its concentration at 24 hours in both samples.

If evaporation was the main cause for the progressive decrease in concentration, a likely explanation for the different rates of loss of allergens from the samples could be related to the vapour pressures of each allergen. If we rank the different acrylate / methacrylate allergens in decreasing order of vapour pressure at room temperature: MMA > 2-HEMA > 2-HPA > EGDMA > TREGDA. In our data, the general rate of loss allergen in decreasing order was MMA, 2-HPA, 2-HEMA, EGDMA, TREGDA. Therefore the higher the vapour pressure, the more rapid the loss of allergen from the preparation, except for 2-HEMA and 2-HPA, which have very similar vapour pressures (Fig. 4). Ranking the molecular weights of these allergens in increasing order, MMA < 2-HPA = 2-HEMA < EGDMA < TREGDA. Thus, we also see an inverse correlation between rate of loss and molecular weight (Fig. 4).

Besides evaporation, another possible cause of progressive decrease in allergen concentration may be spontaneous polymerisation of the acrylate / methacrylate monomers within the syringe. This has not yet been documented in any publications. However, spontaneous polymerisation of ethyl cyanoacrylate patch test preparations in Finn chambers (Epitest Ltd Oy, Tuusula, Finland)<sup>11</sup> has been advocated to happen. In this report, experimental data strongly supported the possible explanation that aluminium, aluminium oxide or an impurity in the aluminium of the Finn chambers may have acted as a catalyst. From information obtained from one of the patch test preparation manufacturers (personal communication), we have found out that certain acrylate / methacrylate patch test preparations have had stabilizers and inhibitors added into the preparations in order to maintain stability and prevent spontaneous polymerisation in the syringes.

In order to further investigate this measured decrease in allergen concentration from patch test preparations over time, it is possible to use various chemical identification studies to detect other peaks in these preparations. This is especially so when we suspect chemical degradation, oxidation, and/or spontaneous polymerisation, rather than evaporation, as the cause. However, the methods used in our current study are inadequate for this purpose.

We started the investigations in this study with the methacrylate preparations because we knew they were detectable with the GPC method. The concentration of the acrylates in the patch test preparations, however, was 20 times lower; and we knew that we could not use GPC to detect these acrylates. While we were investigating the methacrylates, we managed to develop a method to analyze these acrylate / methacrylate allergens with HPLC. With this new method, we analyzed the acrylates. We further refined the HPLC method and now both acrylates and methacrylates can be investigated by HPLC.

In order to check for potential differences between IQ™ chambers and Finn chambers, we had also measured the concentration of an MMA preparation in Finn chambers over time and had found a graph almost identical to that of the same MMA preparation in IQ™ chambers (unpublished observations).

Our findings have implications in the practice of patch testing. For optimal stability, acrylate / methacrylate patch test preparations are best stored in the freezer, or at least in the refrigerator, rather than at room temperature. This would be even more so in tropical climates, where the average room temperature is higher. Patch test preparations of volatile allergens should only be applied to chambers immediately before application of the patches to the test

site on the skin, and not pre-loaded one day or more before, as has been the practice in some patch test centres. The manufacturer of the IQ™ chambers<sup>9</sup> has recommended that “The cover makes it possible to reattach the tape to the cover after filling the chambers with allergen preparations. This important feature facilitates and reduces significantly the time of test application because the nurse or assistant can prepare many test units in advance. The prepared unit should be stored in a plastic bag in a refrigerator and may be kept for a few days.”

Our data shows that the allergens in syringes do not stay >80% of initial concentration as long as most commercial suppliers state they should for 3 of the 5 tested acrylate / methacrylate patch test preparations, with the exception of TREGDA and EGDMA, which remained above 80% of initial concentration after 256 days. At room temperature, MMA had fallen below 80% of initial concentration by day 8, 2-HPA by day 21 and 2-HEMA by day 256. In IQ™ chambers, the fall was even more rapid, with MMA and 2-HPA falling below 80% by 1 hour, and 2-HEMA by 8 hours at room temperature.

In our study, the patch test preparations in IQ™ chambers had not been stored in plastic bags. Whether or not the additional use of a plastic bag would significantly influence the length of time it would take for the allergen concentrations to fall below 80% of initial concentration has not been determined.

To conclude, as there is a seemingly rapid decrease of acrylates / methacrylates during the manufacturing of test preparations, more investigations are needed to check the initial concentrations of acrylates / methacrylates in freshly-prepared commercial test preparations to see whether their initial concentrations are actually as stated on their labels or whether they are lower than acceptable even when purchased as freshly as possible. We recommend that patch test allergens should be stored refrigerated, replaced regularly, and freshly applied onto test patches on the day of use.

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#### **Figure legends:**

Figure 1. Concentration of acrylate / methacrylate allergens over time in syringes from samples stored in the freezer at -18°C, refrigerator (fridge) at 4°C and under room temperature (RT) at 23°C.

Figure 2. Concentration of five acrylate / methacrylate allergens over time in IQ™ chambers from samples stored in the refrigerator (fridge) at 4°C and under room temperature (RT) at 23°C.

Figure 3. Concentration five acrylate / methacrylate allergens over hours in IQ™ chambers from samples stored in the refrigerator (fridge) at 4°C and under room temperature (RT) at 23°C.

Figure 4. Chemical structures and physical characteristics of the 5 acrylate / methacrylate allergens in our study. Chemical Abstract Service (CAS) numbers, boiling point (bp), vapour pressure (vp) in torr and kilopascal (kPa), and molecular weight (MW) of the allergens used.

Table 1. Sample preparation details.

| <b>Allergen sample</b> | <b>Approx. weight</b> | <b>Diluent</b>           | <b>Volume</b> |
|------------------------|-----------------------|--------------------------|---------------|
| MMA                    | 25 mg                 | 99% heptane + 1% butanol | 20.0 ml       |
| 2-HPA                  | 25 mg                 | 99% heptane + 1% ethanol | 1.5 ml        |
| 2-HEMA                 | 25 mg                 | 99% heptane + 1% ethanol | 20.0 ml       |
| EGDMA                  | 25 mg                 | 99% heptane + 1% butanol | 20.0 ml       |
| TREGDA                 | 15 mg                 | 99% heptane + 1% ethanol | 1.5 ml        |

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

Figure 1. Concentration of acrylate / methacrylate allergens over time in syringes from samples stored in the freezer at -18°C, refrigerator (fridge) at 4°C and under room temperature (RT) at 23°C.

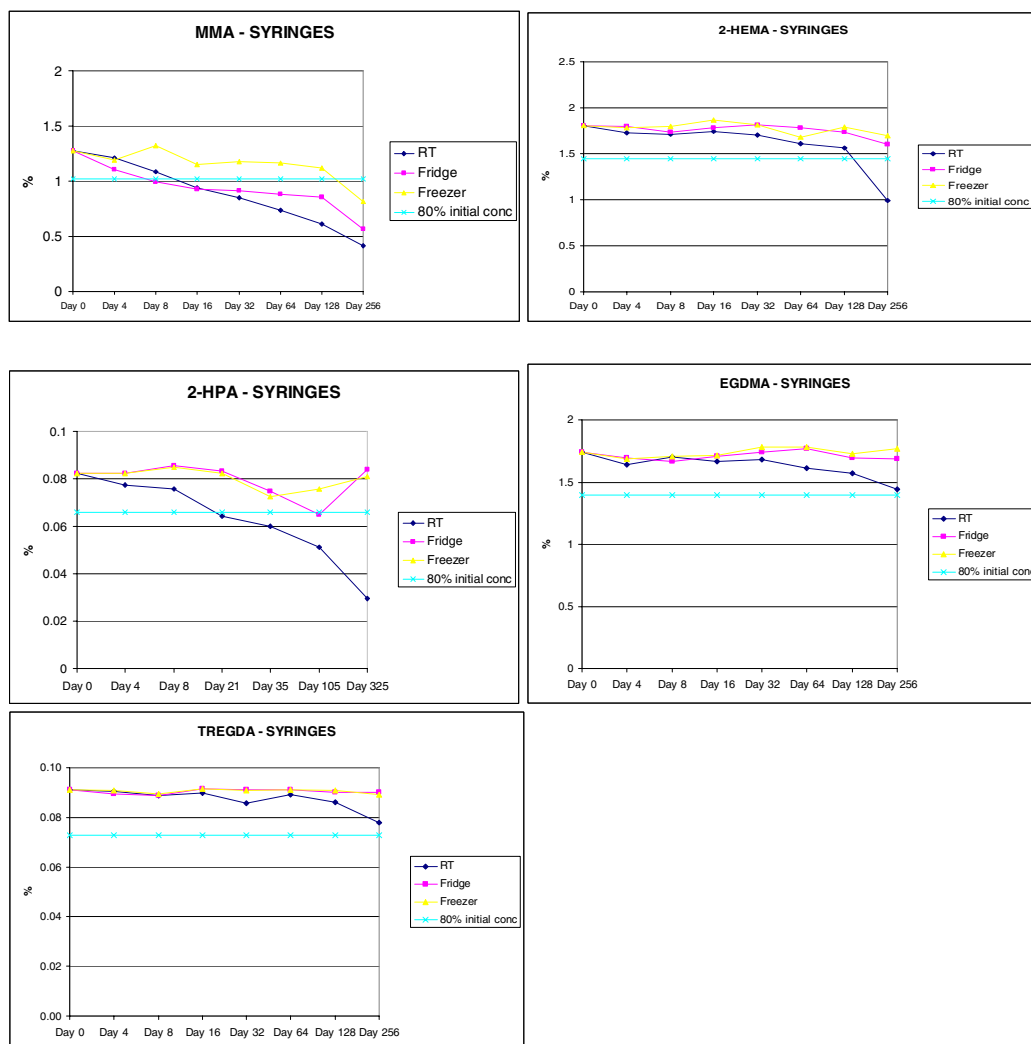


Figure 2. Concentration of five acrylate / methacrylate allergens over time in IQ™ chambers from samples stored in the refrigerator (fridge) at 4°C and under room temperature (RT) at 23°C.

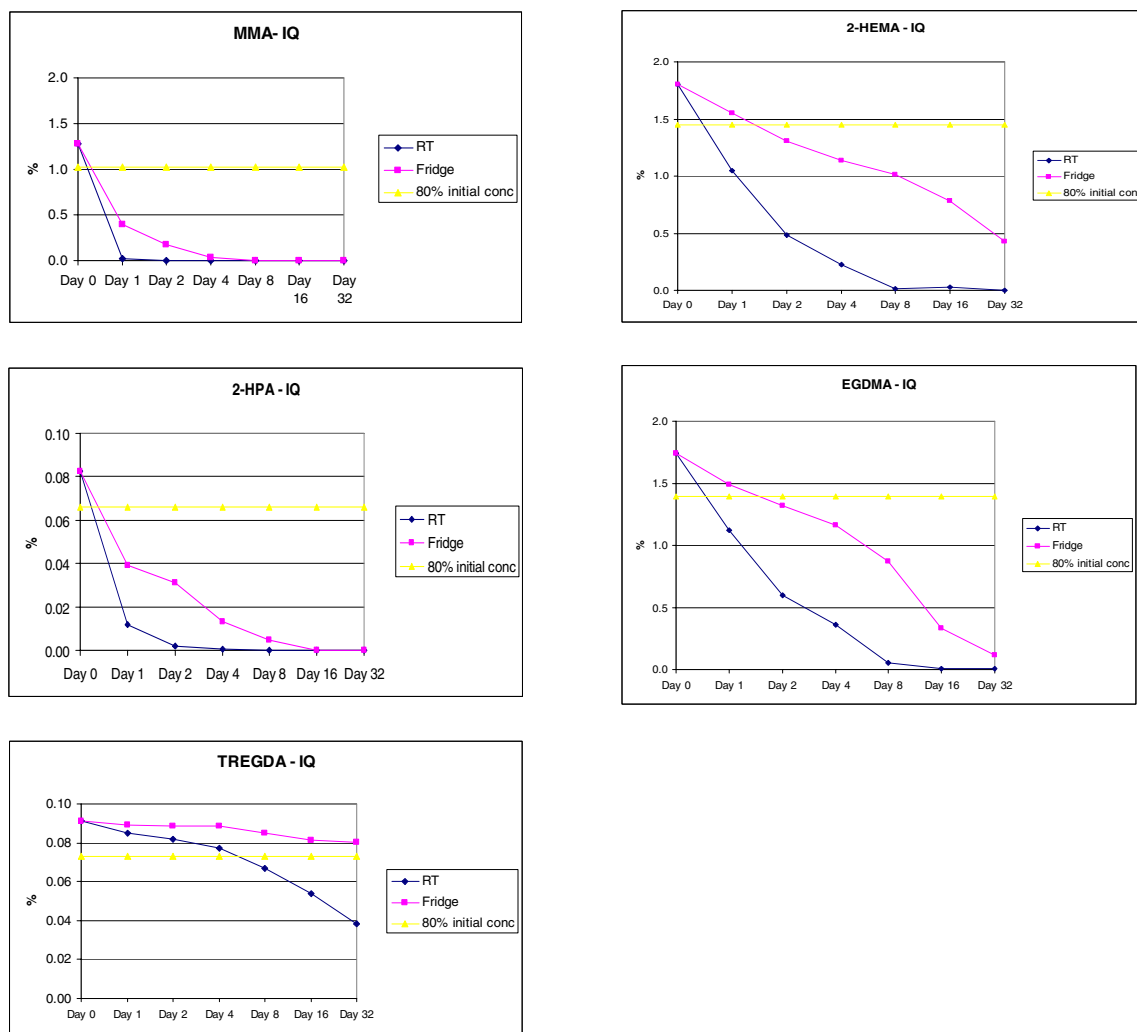


Figure 3. Concentration five acrylate / methacrylate allergens over hours in IQ™ chambers from samples stored in the refrigerator (fridge) at 4°C and under room temperature (RT) at 23°C.

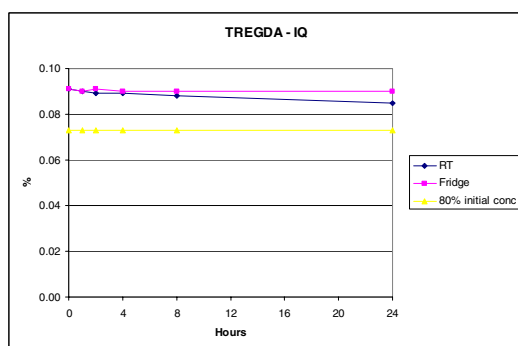
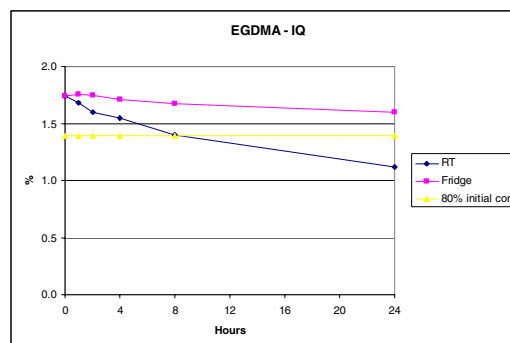
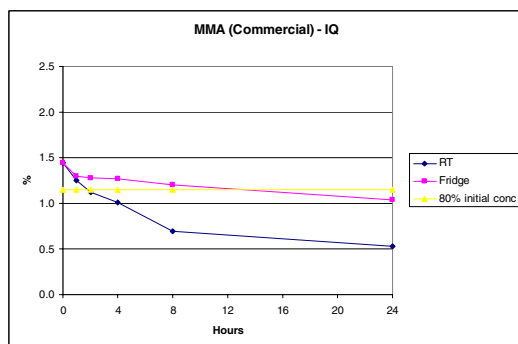
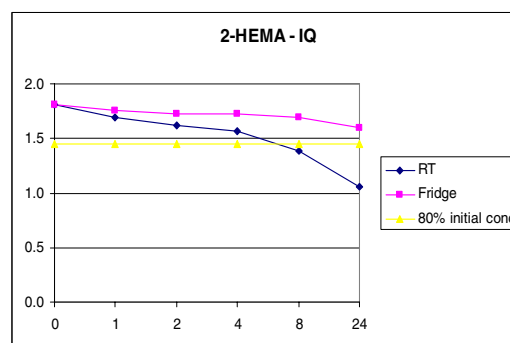
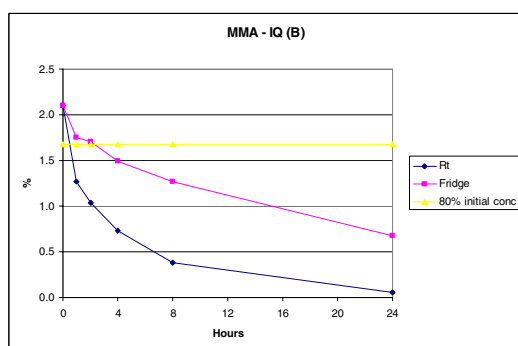
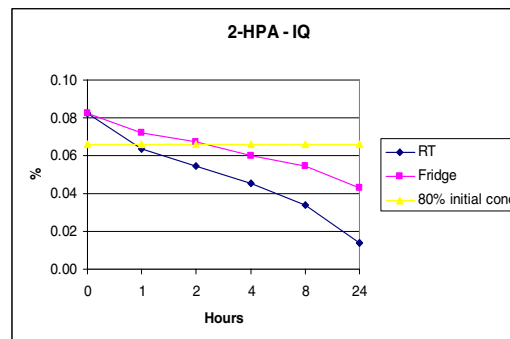
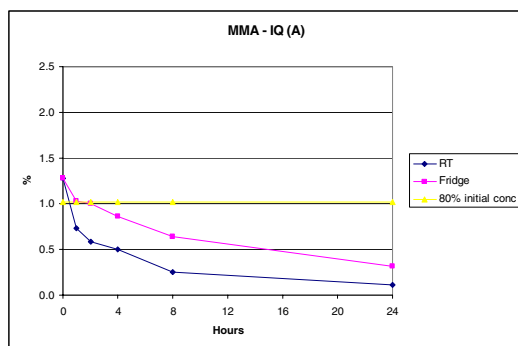
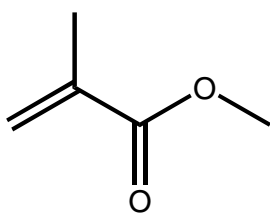


Figure 4. Chemical structures and physical characteristics of the 5 acrylate / methacrylate allergens in our study. Chemical Abstract Service (CAS) numbers, boiling point (bp), vapour pressure (vp) in torr and kilopascal (kPa), and molecular weight (MW) of the allergens used.



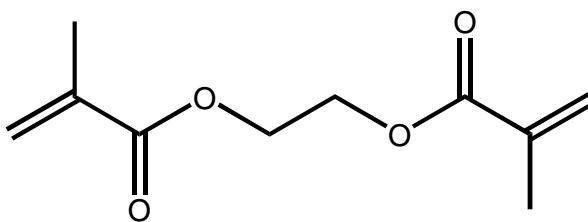
**Methyl methacrylate (MMA)**

CAS no: 80-62-6

bp: 102°C

vp: 36.9 torr = 4.9 kPa

MW: 100



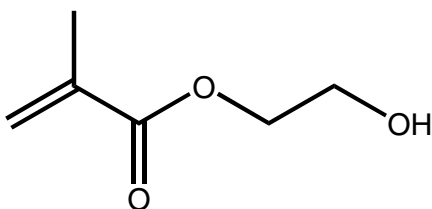
**Ethyleneglycol dimethacrylate (EGDMA)**

CAS no: 97-90-5

bp: 260°C

vp: 0.0121 torr = 0.0016 kPa

MW: 198



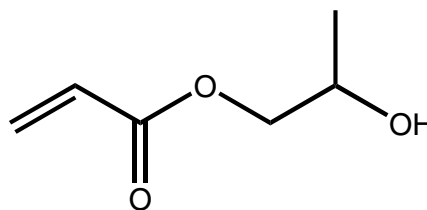
**2-Hydroxyethyl methacrylate (2-HEMA)**

CAS no: 868-77-9

bp: 189°C

vp: 0.159 torr = 0.021 kPa

MW: 130



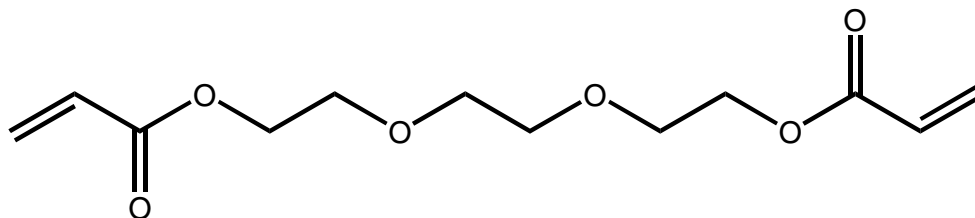
**2-Hydroxypropyl acrylate (2-HPA)**

CAS no: 999-61-1

bp: 225°C

vp: 0.115 torr = 0.015 kPa

MW: 130



**Triethyleneglycol diacrylate (TREGDA)**

CAS no: 1680-21-3

bp: 350°C

vp:  $9.5 \times 10^{-5}$  torr = 0.000013 kPa

MW: 258