

Risks and possibilities in patch testing with contaminated personal objects: usefulness of thin-layer chromatograms in a patient with acrylate contact allergy from a chemical burn

MARLÉNE ISAKSSON AND ERIK ZIMERSON

Department of Occupational and Environmental Dermatology, University Hospital MAS, Lund University, Malmö SE 205 02, Sweden

We report a case of a chemical burn from dipropylene glycol diacrylate (DPGDA) spilt on working shoes, followed by active sensitization, thus giving an occupational allergic contact dermatitis on the patient's dorsal feet. Diagnostic tests included patch testing with acetone extracts made from the different shoe layers and thin-layer chromatograms. An invisible spot on the thin-layer chromatography plate gave a test eczema and was further investigated with gas chromatography–mass spectrometry. DPGDA was detected in the spot.

Key words: chemical burn; contamination; diethylene glycol diacrylate (CAS 4074-88-8); dipropylene glycol diacrylate (CAS 57472-68-1); extracts; 2-hydroxypropyl acrylate (CAS 2918-23-2); N-isobutoxymethylacrylamide, occupational; shoe; thin-layer chromatography patch testing; triethylene glycol diacrylate (CAS 1680-21-3); tripropylene glycol diacrylate (CAS 42978-66-5).
© Blackwell Munksgaard, 2007.

Accepted for publication 5 March 2007

When investigating a suspected allergic contact reaction from a solid object, patch testing with the object 'as is' may be insufficient to trace the allergy. One way to overcome this is by patch testing with an extract made from the solid object (1). However, if the solid object is contaminated with chemicals that are potent irritants/sensitizers, patch testing with it as is may give a very strong patch test reaction, in which a patch test reader may have difficulties in distinguishing between an irritant and an allergic reaction. Also in this case, patch testing with extracts can be helpful in diagnosing the incriminating cause of the dermatitis, even if serial dilutions of the extracts may have to be done, and controls may have to be patch tested. Furthermore, the extracts can also be used for patch testing with thin-layer chromatograms, which may help to identify the substance or substances that are the cause of the dermatitis (2). The following case is a good example of the risks and possibilities in patch testing with contaminated personal objects and thin-layer chromatograms.

Case Report

A 25-year-old male worker, who had worked in one of the world's largest paint manufacturers for 5 years suddenly got a severe erythematous dermatitis with partly discharging ulcers on his dorsal feet, which rendered him 1 month sick leave and treatment with potent topical corticosteroids. At this time he worked in the industrial paint section, a small section involving much manual labour, where he handled solution-based paints. He had an anamnesis of mucosal atopy but had never experienced any skin problems before. During his sick leave, he was given new working shoes to try, as the management suspected he had spilt something on his 1-year-old shoes that had given him the dermatitis, the extension of which exactly matched the upper part of his shoes. The patient tolerated the new shoes without any problems. When back at work, his dermatitis had subsided. The company physician later sent him to us to try to elucidate the cause of the acute dermatitis.

At presentation, the dermatitis on the feet had faded and only a brownish discolouration was left. He was first tested to our standard, paint, and isocyanate series and on reading day 3 (D3) he only had reacted to *Myroxylon pereirae*. Complimentary testing was performed on D3 with his old working shoes, both as is and as an acetone extract, as we also suspected he had spilt something on them that caused the dermatitis. We cut open the upper part of the shoe, which consisted of 3 layers (leather, rubber, and cloth; Fig. 1), and tested these separate materials as is on the lower back. The patient returned already on the next day with erythematous, infiltrated bullous lesions on the lower back underneath each part of the tested shoe pieces (Fig. 2). We removed the 3 pieces and treated the dermatitis with Dermovat® (clobetasol propionate, GlaxoSmithKline, Solna, Sweden) under hydrocolloid occlusion. He returned on D7 for the second patch test reading (Table 1). The acetone extract of the shoe gave a bullous +++ reaction on D3. After additional information from the company that ultraviolet (UV)-curing acrylates were handled at work, he was later tested to our acrylate series and reacted to several substances, viz. 2-hydroxypropyl acrylate, diethylene glycol diacrylate, triethylene glycol diacrylate (TREGDA), and N-isobutoxymethylacrylamide (Table 1). To differentiate between an allergic



Fig. 1. The contaminated shoes.

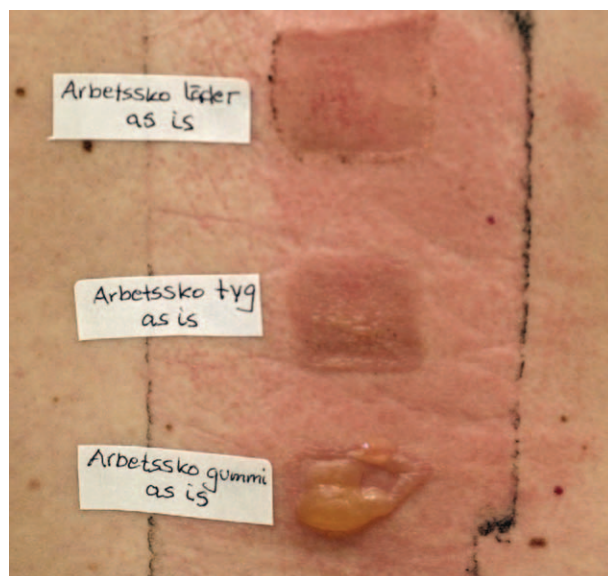


Fig. 2. Patch test results from the 3 different shoe layers tested as is. The top reaction is from the leather part (+), the middle reaction from the cloth part (+++), and the bottom reaction from the rubber part (+++).

and a toxic reaction from the acetone shoe extract, separate acetone extracts of the 3 pieces from the upper sole were made and diluted to concentrations varying from 100% down to 0.00010%. Patch testing with the extracts was performed sequentially from 0.00010% and up using incremental doses of a factor 100 until a positive reaction was seen. Thin-layer chromatography patch testing (TLC-PT) was performed with the acetone extract of the leather part (2).

Results showed that the patient reacted to a spot on the TLC plate not visible in UV irradiation (Fig. 3). A corresponding TLC plate was made, the spot that had given a positive patch test reaction (++) was scraped off the plate and extracted in ethanol and analysed by gas chromatography–mass spectrometry (GC-MS) (Micromass GCT Premier, Waters, Sollentuna, Sweden) at the Centre for Chemistry and Chemical Engineering, Lund University, Lund, Sweden. One main component was detected in the extract, dipropylene glycol diacrylate (DPGDA, CAS 57472-68-1; not present in our acrylate series) (Fig. 4), but there were also indications of minute amounts of other 3 acrylates, viz. propylene glycol diacrylate, tripropylene glycol diacrylate (TPGDA, CAS 42978-66-5), and N-isobutoxymethylacrylamide. Information from the company showed that DPGDA and TPGDA were handled by the patient. The other 2 acrylates were not present in the company, according to their chemist. Patch testing with serial dilutions of DPGDA and N-isobutoxymethylacrylamide was performed,

Table 1. Positive patch test reactions with reading days 1, 3, and 7

Substances	Concentration % w/v	Vehicle	Day 1	Day 3	Day 7
2-Hydroxypropyl acrylate	0.1	Pet.	NA	(+)	+
Diethylene glycol diacrylate	0.1	Pet.	NA	+++	++
Triethylene glycol diacrylate	0.1	Pet.	NA	++	++
N-isobutoxymethylacrylamide	1.0	Pet.	NA	++	++
Cloth as is	NA	NA	+++	NA	NA
Rubber as is	NA	NA	+++ (bullae)	NA	NA
Leather as is	NA	NA	+	NA	NA
Extract of shoe	100	Acetone	NA	+++ (bullae)	NR
Extract of leather piece	1.0	Acetone	NA	+	NR
Extract of leather piece	0.010	Acetone	NA	–	NR
Extract of cloth piece	100	Acetone	NA	+	NR
Extract of cloth piece	0.010–10	Acetone	NA	–	NR
Extract of rubber piece	1.0	Acetone	NA	+	NR
Extract of rubber piece	0.010	Acetone	NA	–	NR
DPGDA	0.10	Acetone	NA	+++	++
DPGDA	0.010	Acetone	NA	++	+
DPGDA	0.0010	Acetone	NA	–	–
N-isobutoxymethylacrylamide	1.0	Acetone	NA	?	+
N-isobutoxymethylacrylamide	0.10	Acetone	NA	–	–

DPGDA, dipropylene glycol diacrylate; NA, not applicable; NR, not read; pet., petrolatum.



Fig. 3. Patch test eczema (++) from the 'invisible' spot on the thin-layer chromatography plate. The acetone extract was made from the leather part.

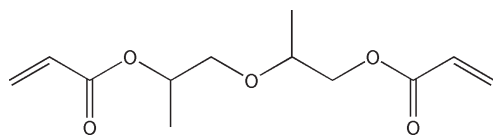


Fig. 4. Molecular structure of dipropylene glycol diacrylate.

and the patient reacted down to 0.010% w/v DPGDA (Fig. 5) and to 1.0% w/v N-isobutoxymethylacrylamide (Table 1). 20 dermatitis patients from the Department of Occupational and Environmental Dermatology in Malmö, serving as controls, were tested with DPGDA 0.010% w/v in acetone, and no one reacted. During the time it took to perform the GC-MS analysis, the patient had been transferred to another section in the

plant, now working with water-based paints, where he has stayed free of dermatitis.

Thin-layer chromatography patch testing

TLC plastic roll 500 × 200 cm (silica gel 60F₂₅₄) (VWR International, Stockholm, Sweden) was cut into 18 × 18-cm strips. Samples to be tested were deposited on one spot each from a 10-μl capillary pipette, along a straight line with at least 2 cm between each spot. Different volumes of the samples were applied on the TLC plastic roller patch, i.e. for the rubber and leather extracts 30 μl of the 10% solution and for the cloth extract 100 μl of the 100% solution. Double spots were applied for each sample that was to be investigated, one to be used as a patch test and the other to be used as a comparison and reference when reading the patch test. The TLC plastic roll chromatogram was developed in a glass tank with CHCl₃:CH₃CN 86:14 v/v as the mobile phase. The chromatograms were then examined in UV radiation, 254 nm and 360 nm. All visible spots were marked. Each originally applied spot gave rise to a band of new spots, and these bands were cut out from the chromatogram in pieces of about 2.5 × 18 cm and were then applied on the upper arm using Scanpor (Norgesplaster A/S, Vennesla, Norway) tape. The position of the TLC-PT was carefully marked. The TLC-PT was removed after 2 days and read after 1 additional day (D3) according to International Contact Dermatitis Research Group criteria. When reading the patch tests, the second chromatogram, deriving from the double sample, was used as a reference to determine to which spots the positive reactions corresponded to.



Fig. 5. Patch test results from testing with dipropylene glycol diacrylate 0.1% w/v in acetone (top, +++) and 0.01% w/v in acetone (bottom, ++).

Discussion

Patch testing with solid objects such as pieces of shoes often does not give allergic patch test reactions, which is why extracts are often made (1). However, in this case an extraordinary strong patch test reaction appeared from patch testing with the solid pieces of the shoe contaminated with acrylates. Acrylates as such are both irritating and sensitizing. In every case where you suspect a potent sensitizer or irritant has contaminated the solid object to be tested, care should be taken, so as not to get strong patch test reactions or induction of patch test sensitization. In these cases it is better to make extracts of various concentrations of the solid object and to sequentially patch test with, using the lowest concentration first, until a positive reaction is seen.

In the present case the patient worked with many hundreds of different chemicals used in the production of various industrial paints. Some were added manually and some more automated. The handling of chemicals was therefore often open, and the patient often got contaminated. However, when the dermatitis appeared, it was the first time he had ever had any skin lesions and still, he had no clue as to which chemicals could be responsible. As there were such a high number of substances used, it was practically impossible to get an idea of which substances caused the dermatitis. This caused us to patch test the shoe, and when the patient reacted to the 3 pieces so fiercely, an allergic or toxic reaction had to be ruled out. The acute partly ulcerating

dermatitis, which took 1 month to heal, even with potent topical corticosteroids used b.i.d., makes it plausible to suspect a chemical burn from an acrylate (DPGDA) followed by active sensitization to this chemical (3). When the patient reacted to the extracts, we had the possibility to prepare TLC plates for patch testing. One spot gave a corresponding test eczema verifying that the extract contained at least one sensitizer, even if this spot was not visible in UV irradiation (Fig. 3). However, even if a positive reaction is noted to a 'non-detectable' spot on a TLC-PT, the silica can then be scraped off and extracted and used for further investigations. The GC-MS analysis verified that 4 substances were present in the spot to which the patient had reacted. 2 of the substances had already been patch tested as they are present in our acrylate series, i.e. TPGDA and N-isobutoxymethylacrylamide. The former was present at work, but the patient was negative on patch testing. The latter was not present at work according to the company chemist, but the patient reacted to it anyway. Only one case of contact allergy to this acrylate has been published before, and that was in the production of binders for paints and glues, which makes it plausible that it could have been present also in the patient's work environment (4). Propylene glycol diacrylate was never tested and also not present at work. DPGDA was present at work, and the patient reacted down to 0.010% w/v in acetone. 20 control patients at the Department of Occupational and Environmental Dermatology in Malmö, Sweden, were patch tested to 0.010% w/v in acetone, and no one reacted. To our knowledge this substance has never been patch tested in a dermatitis patient before, even if the substance was implicated in a case of allergic contact dermatitis from a UV-cured lacquer in Finland. However, the Finnish patient was never tested to DPGDA *per se* but only to a product containing this acrylate (5). As DPGDA was used in the production of paints in our patient's former section and it constituted the major peak in the GC-MS analysis and the patient had a strong patch test reaction to it (reacted to 0.010% w/v), we propose that DPGDA caused the acute ulcerating dermatitis on his dorsal feet by means of a chemical burn, followed by active sensitization.

References

1. Bruze M, Trulsson L, Bendsoe N. Patch testing with ultrasonic batch extracts. *Am J Contact Dermat* 1992; 3: 133–137.
2. Bruze M, Frick M, Persson L. Patch testing with thin-layer chromatograms. *Contact Dermatitis* 2003; 48: 278–279.

3. Kanerva L, Tarvainen K, Pinola A et al. A single accidental exposure may result in a chemical burn, primary sensitization and allergic contact dermatitis. *Contact Dermatitis* 1994; 31: 229–235.
4. Gruvberger B, Bruze M, Almgren G. Occupational dermatoses in a plant producing binders for paints and glues. *Contact Dermatitis* 1998; 38: 71–77.
5. Estlander T, Jolanki R, Kanerva L. Occupational allergic contact dermatitis from UV-cured lacquer containing dipropylene glycol diacrylate. *Contact Dermatitis* 1998; 39: 36.

Address:

Marlene Isaksson, MD, PhD
Department of Occupational and Environmental Dermatology
University Hospital MAS
Lund University
Malmö SE 205 02
Sweden
Phone: +46 40 337859
Fax: +46 40 331192
e-mail: marlene.isaksson@skane.se