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REVIEW ARTICLE

Towards a perfect vehicle(s) for diagnostic patch testing: an overview

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Abstract

The correct selection of vehicles for patch testing is crucial in evaluating suspected allergic contact dermatitis. We reviewed literature by searching Pubmed and Embase for the years 1971–2011 utilizing the subjects “vehicle,” “allergic contact dermatitis” and “patch test” for advances in knowledge of vehicles. A universal optimal vehicle more efficient than petrolatum remains undiscovered. Literature provides research supporting alternate vehicles for specific allergens and insights on vehicle characteristics, such as influence on bioavailability of chemicals and subsequent percutaneous absorption or interaction with allergens. These findings should accelerate our ability to define, and thus determine, the optimum vehicle(s).

Keywords: Skin, allergic contact dermatitis, petrolatum, patch tests

Introduction

Patch testing is a standard tool used to identify allergens in allergic contact dermatitis patients. Choosing proper vehicles for patch testing is crucial for the detection of allergies since specific vehicles may confer advantages for certain substances. For example, enhanced thermodynamic activity improves the solubility of a substance in its vehicle, increasing percutaneous absorption and thus the intensity of reactions. Currently, patch testing for most substances is typically performed using petrolatum as a vehicle due to its properties of being non-sensitizing, non-irritating, and a relatively stable milieu; however, in certain cases alternate vehicles may be more suitable for specific allergens.

Cyran and Maibach (1), Lee and Maibach (2), and Tanglertsampan and Maibach (3) summarized alternate vehicles in patch testing until 1992, 2002, and 2007, respectively. We review literature subsequent to 2006, as well as earlier publications not reviewed by the referenced articles, for more information.

Materials and methods

The medical literature was searched using the medical databases Pubmed and Embase for the years 1971–2011,

utilizing the subjects “vehicle,” “allergic contact dermatitis” and “patch test”. Approximately 30 relevant publications were found.

Results

Relevant articles assessing contact allergy, vehicles, and irritants are summarized in Tables 1, 2, and 3.

Discussion

Since Cyran and Maibach (1) reviewed vehicles in patch testing in 2008, related studies have been published. Although the shortcomings of petrolatum, such as false negative results and inconsistent dosing, are recognized, no widely used substitute has been developed. However, researchers have suggested alternative vehicles for specific allergens that can be verified for the use as vehicles for other allergens. We reviewed studies by focusing on the lowest concentration of a vehicle, needed to elicit a positive patch test reaction. Greater concentration allows for more particles that may be suspended and not dissolved in the vehicle to contact the skin surface. Allergen concentration needs to be

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Table 1. Patch test literature on difference between vehicles.

Allergen	Vehicles	Dose (approx.) % or formulation [†]	Number Tested	Results	Ref.
1,3,5-Tris(2-hydroxyethyl)-hexahydrotriazine (Grotan BK®)	Petrolatum	1.0%	99	0/99 patients had positive reactions to petrolatum and 3/37 patients had positive reactions to aqueous	(7)
	Aqueous		37		
Imidazolidinylurea (IU)	Petrolatum Aqueous	2%	27183	1.96% (532 patients) had positive reactions to aqueous and 2.78% (755 patients) had positive reactions to petrolatum. The data supported petrolatum as the more sensitive vehicle. Note statistical significance was not provided	(12)
Nickel sulfate (NiSO ₄)	Petrolatum	<0.039%, 0.039%, 0.078%, 0.156%, 0.312%, 0.625%, 1.25%, 2.5%, 5.0%	53	Mean threshold concentration was 0.43% in distilled water versus 0.51% in petrolatum. Distilled water is more reliable than petrolatum as a vehicle. Advantages of petrolatum as a vehicle are apparent, however, when non-occlusive types of tapes were used	(10)
	Distilled water			No advantage in using petrolatum as a vehicle for nickel sulfate when occlusion method is employed. Note statistical significance was not provided	
Nickel sulfate	Petrolatum	2.5%	20	20/20 patients reacted to 2.5 % nickel sulfate in water vehicle versus 16/20 patients reacting to 2.5% nickel sulfate in petrolatum	(11)
	Water			Water was seen to be the more effective vehicle	
Potassium bichromate (K ₂ Cr ₂ O ₇)	Petrolatum	<0.0039%, 0.0039%, 0.0078%, 0.0156%, 0.0312%, 0.0625%, 0.125%, 0.25%, 0.5%	43	Mean threshold concentration was 0.23% in distilled water versus 0.05% in alkaline buffer (pH 12) versus 0.14% in petrolatum. The alkaline buffer with its lowest concentration threshold was distinctly the most effective vehicle. There was a tendency for petrolatum to have a lower threshold concentration when compared to water. Alkaline buffer versus petrolatum: <i>P</i> < 0.001	(8)
	Distilled water			Alkaline buffer versus distilled water: <i>P</i> < 0.001	
Potassium chromate (K ₂ CrO ₄)	Alkaline buffer (pH 12)			Petrolatum versus distilled water: 0.01 < <i>P</i> < 0.05	
	Petrolatum	<0.0039%, 0.0039%, 0.0078%, 0.0156%, 0.0312%, 0.0625%, 0.125%, 0.25%, 0.5%	47	Mean threshold concentration was 0.18% in distilled water versus 0.05% in alkaline buffer (pH 12) versus 0.22% in petrolatum. The alkaline buffer with its lowest concentration threshold was distinctly the most effective vehicle. Alkaline buffer versus petrolatum: <i>P</i> < 0.001	(8)
Thiuram mix	Distilled water			Alkaline buffer versus distilled water: <i>P</i> < 0.001	
	Alkaline buffer (pH 12)			Petrolatum versus distilled water: <i>P</i> > 0.2	
Thiuram mix	Petrolatum	0.01%, 0.1%, 1.0%	20	6/19 patients reacted to 0.005% thiuram mix in ethanol/sweat versus 2/15 patients reacting to 0.01 % thiuram mix in petrolatum	(5)
	1% Ethanol/ 99% sweat	0.005 w/v%, 0.05 w/v%, 0.5 w/v%		0.005% thiuram mix in sweat/ethanol was chosen as the dilution endpoint concentration	

[†]Concentration given in w/w, w/v, v/v not stated in original publications if unmentioned in table.

Table 2. Patch test literature on negligible difference between vehicles.

Allergen	Vehicles	Dose (approx.) % or formulation [†]	Number Tested	Results	Ref.
Cobalt chloride (CoCl ₂)	Petrolatum	<0.0156%, 0.0156%, 0.0312%,	60	Mean threshold concentration was 0.27% in distilled water versus 0.31% in petrolatum ($P > 0.2$). Distilled water is slightly more reliable than petrolatum as a vehicle, although not significantly so	(8)
	Distilled water	0.0625%, 0.125%, 0.25%, 0.5%, 1.0%, 2.0%			
Mercuric chloride (HgCl ₂)	Petrolatum	<0.0007%, 0.0007%, 0.0015%,	13	Mean threshold concentration was 0.02% in distilled water versus 0.03% in petrolatum ($0.05 < P < 0.2$). Distilled water is slightly more reliable than petrolatum as a vehicle, although not significantly so	(8)
	Distilled water	0.0031%, 0.0062%, 0.0125%, 0.025%, 0.05%, 0.1%			
Euxyl K 400	Petrolatum Ethanol (alcohol)	2.5%	2057	1.2% positive reactions (24 patients) for each vehicle. Based on the findings at 2.5% Euxyl K 400 concentration, petrolatum and ethanol are comparable vehicles	(14)

[†]Concentration given in w/w, w/v, v/v not stated in original publications.

Table 3. Patch test literature on irritation.

Irritant	Vehicle(s)	Dose (approx.) % or formulation [†]	Number Tested	Results	Ref.
MPH	Petrolatum	0.01%, 0.1%, 1%, 5%, 10%, 20%	20	The aqueous vehicle showed more doubtful irritant reactions than the petrolatum vehicle, but there were no significant true irritant or allergic response reactions on any subject at any test concentration in either vehicle. This suggests that in these vehicles, MPH up to 20% concentration does not cause irritant patch test reactions.	(17)
	Aqueous			More low-grade irritant reactions were seen with the aqueous vehicle and since aqueous vehicle is more difficult to apply to test patches, petrolatum is the preferred vehicle.	
SLS	Aqueous	10%	18	Inflammation is less intense when the water vehicle evaporates before the patch test is applied: there is a diminished reaction to SLS elicited by dry patch tests in comparison to wet ones. Complete evaporation seemed to inhibit irritation by rendering the irritant, SLS, insoluble or less able to contact the skin.	(16)
Tea tree oil	Cream base	25%	306	With cream vehicle, 25% tea tree oil had a mean irritancy score of 0. For ointment vehicle, 25% tea tree oil had a mean irritancy score of 0.0008. And for gel vehicle, 25% tea tree oil had a mean irritancy score of 0.0060.	(18)
	Ointment base			Based on findings, topical application of tea tree oil as assessed under controlled experimental conditions did not cause clinically significant irritancy of the skin.	
	Gel base				
Urea	Petrolatum	20%	17	Irritant effect is more intense when urea is dispensed in petrolatum than in water: there is significant increase in cutaneous blood flow, skin thickness, and transepidermal water loss. For clinical use where irritant effect is undesirable, urea in aqueous emulsions are preferable.	(15)
	Water				

[†]Concentration given in w/w, w/v, v/v not stated in original publications.

sufficient to deliver enough allergen to produce a reaction in truly sensitive individuals. Therefore lowest concentrations, or threshold concentrations, may identify the more effective vehicle by assuming that the lower threshold concentration of an allergen indicates easier percutaneous penetration by the allergen when administered in the given vehicle, and identifies the most biologically sensitive individual.

Kligman (4) ranked six vehicles with different physical attributes in order of descending effectiveness for patch testing of various water and lipid soluble allergens. Vehicles tested were two hydrophobic oily bases: white petrolatum and anhydrous lanolin, two emulsion type bases: hydrophilic ointment (oil in water) and Aquafor® base into which 40% water was emulsified (water in oil), a water soluble base U.S.P. Carbowax Ointment (polyethylene glycol), and water. Water soluble substances were streptomycin, nickel sulfate, penicillin G, cobaltous

sulfate, mercuric chloride, and neomycin. Lipoid soluble substances were Furacin®, monobenzyl ether of hydroquinone, tetrachlorosalicylanilide, ammoniated mercury, and Atabrine®. Kligman found that vehicle ranking was different for water and for lipid soluble allergens. For water soluble agents, petrolatum ranked first with index (indicating percent positive reactions) 87%, lanolin second with 64%, aquafor with 53%, hydrophilic ointment with 50%, water with 50%, and carbowax last with 24%. For lipid soluble agents, petrolatum is also the most effective with index 88%, hydrophilic ointment second with 71%, lanolin with 63%, carbowax with 62%, aquafor with 50%, and water vehicle excluded. The lipid soluble allergens are less influenced by the vehicle compared to water soluble agents. The great influence of the vehicle had not been fully anticipated. It was evident from the results that an inappropriate vehicle is one of the sources of low sensitivity and false negative reactions for

patch tests. Petrolatum would seem to be the vehicle of choice, but this is not always true. Interactions between the vehicle, allergen, skin and inflammatory responses is complex and vehicles should not be assumed to behave differently solely due to influence on penetration.

In experiments performed by Knudsen and Menné (5), vehicles petrolatum and 1% ethanol/99% sweat were compared at concentrations of thiuram mix. 6/19 patients reacted to 0.005% thiuram mix in ethanol/sweat versus 2/15 patients reacting to 0.01% thiuram mix in petrolatum. The lower threshold concentration for ethanol/sweat vehicle indicates that it was the most effective vehicle for thiuram mix. However, more tests should be done to determine the optimal vehicle for each chemical in the mixture.

In the study by De Groot and Conemans (6), vehicles petrolatum and PEG-400 (polyethylene glycol), along with other negative reaction vehicles, were compared at concentrations of nystatin, the patient had only a mild positive reaction for 100,000 IU/g in petrolatum while having stronger positive reactions in both 90,000 IU/g and 30,000 IU/g in PEG-400. The lower threshold concentration for PEG-400 indicates that it was the more effective vehicle. This study on one patient is a starting hypothesis for more studies with greater sample size to confirm the effectiveness of PEG-400 as an optimal vehicle for nystatin.

Geier et al. (7) compared vehicles petrolatum and aqueous, at concentration 1.0% of 1,3,5-Tris(2-hydroxyethyl)-hexahydrotriazine. Aqueous was the more effective vehicle with 3/37 positive reactions versus 0/99 positive reactions for petrolatum. Note, however, that as seen from the different sample sizes of the vehicles, different patients were tested in the groups and this may have affected the results. More studies using the same patients for both vehicles might confirm the effectiveness of aqueous as the optimal vehicle for 1,3,5-Tris(2-hydroxyethyl)-hexahydrotriazine.

Wahlberg (8,9) and, Wahlberg and Skog (10) sought an effective vehicle for metal compounds. Petrolatum, distilled water, and an alkaline buffer (pH 12) were tested as possible vehicles for various concentrations of potassium chromate and bichromate (8,9). For potassium chromate, the mean threshold concentration was 0.18% in distilled water versus 0.05% in alkaline buffer versus 0.22% in petrolatum. The difference between threshold concentrations of alkaline buffer and distilled water and the difference between threshold concentrations of alkaline buffer and petrolatum were both statistically significant with $P < 0.001$. The difference between concentrations of distilled water and petrolatum was not significant with $P > 0.2$. For potassium bichromate, the mean threshold concentration was 0.23% in distilled water versus 0.05% in alkaline buffer versus 0.14% in petrolatum. The difference between threshold concentrations of alkaline buffer and distilled water and the difference between threshold concentrations of alkaline buffer and petrolatum were both statistically

significant with $P < 0.001$. The difference between concentrations of distilled water and petrolatum showed a tendency for petrolatum to have a lower threshold concentration with $0.01 < P < 0.05$. The alkaline buffer with its lowest concentration threshold was distinctly the most effective vehicle for both potassium chromate and bichromate.

Wahlberg (8,9) also tested petrolatum and distilled water as vehicles for concentrations of other metals. For cobalt chloride (8,9), the mean threshold concentration was 0.27% in distilled water versus 0.31% in petrolatum ($P > 0.2$), for mercuric chloride (8,9), 0.02% in distilled water versus 0.03% in petrolatum ($0.05 < P < 0.2$), for nickel sulfate (10), 0.43% in distilled water versus 0.51% in petrolatum. Note that the statistical significance of the results for nickel was not provided. Distilled water with a lower threshold concentration was slightly more reliable than petrolatum as a vehicle for these metal compounds, although not significantly so.

van Ketel (11) also tested petrolatum and water as possible vehicles for nickel sulfate at 2.5% concentration. Twenty of the tested 20 patients reacted to water versus 16 patients reacting to petrolatum. The water vehicle with more positive reactions was the more effective vehicle. van Ketel hypothesizes this result was because nickel sulfate is evenly distributed in aqueous solutions whereas there is an irregular distribution for insoluble crystals of nickel in petrolatum. Note, however, that statistical significance was not provided.

In experiments performed by Rietschel et al. (12,13), vehicles petrolatum and aqueous were compared at concentration 2% imidazolidinylurea. 1.96% (532/27,183 patients) had positive reactions to aqueous and 2.78% (755/27,183 patients) had positive reactions to petrolatum. These percentages were calculated by aggregating data from each period (periods 1992–1994, 1994–1996, 1996–1998, 1998–2000, 2001–2002, and 2002–2004) provided by the literature, and seemed to support petrolatum as the more effective vehicle. Although statistical significance between time periods for only petrolatum vehicle, or only aqueous vehicle, and differences in formaldehyde sensitivity in relation to vehicles were addressed, the statistical significance of the difference between petrolatum and aqueous vehicles was not provided.

Tosti et al., (14) found that when comparing vehicles ethanol and petrolatum at concentration 2.5% of Euxyl K 400, there were 1.2% positive reactions (24 patients) for each vehicle. Based on these findings, petrolatum and ethanol are comparable vehicles for Euxyl K 400 and further studies might determine an optimal vehicle.

Irritation

Irritation should be minimized when testing for allergic contact dermatitis. Agner (15) found that when comparing vehicles petrolatum and water with concentration 20% of urea, the irritant effect was more intense in petrolatum. There was significant increase in cutaneous blood flow, skin thickness, and transepidermal water loss. For

clinical use where the irritant effect is undesirable, urea in aqueous emulsions is preferable.

Sodium lauryl sulfate (SDS) has been shown to enhance positive reaction and avoid false negative results. Dahl and Roering (16) tested SDS as a possible irritant. Two patch tests with 25 μ L 10% aqueous solutions of SLS were applied to subjects, one where the water vehicle was allowed to evaporate before the patch was applied. The degree of inflammation was less intense when the water vehicle evaporated before the patch was applied. Complete evaporation seems to inhibit irritation by rendering the irritant, SLS, insoluble or less able to contact the skin. The results showed that evaporation of water from aqueous solutions influences the irritating potential of SLS.

Fowler et al. (17), tested for the irritation threshold of methylphenidate (MPH) with aqueous and petrolatum as vehicles at concentrations and found no significant irritant reactions. This suggested that in petrolatum and aqueous vehicles, MPH up to the highest tested concentration (20%) causes low-grade irritation if any.

Aspres and Freeman (18) tested for irritation from tea tree oil in cream, ointment, and gel bases. With cream vehicle, 25% tea tree oil had a mean irritancy score of 0, for ointment vehicle, 0.0008, and for gel vehicle, 0.0060. These low irritancy scores indicate that topical application of 25% tea tree oil does not significantly irritate.

Stability

Stability is an important aspect when choosing a proper vehicle. Frick-Engfeldt et al. (19), compared the stability of petrolatum patch test preparations of diphenylmethane-4,4'-diisocyanate (4,4'-MDI) and polymeric diphenylmethane diisocyanate (PMDI) over time when stored under different conditions: at room temperature, in a refrigerator, and in a freezer. PMDI can be stable for more than a year when stored in a freezer as opposed to preparations of 4,4'-MDI. PMDI is also in liquid form at room temperature, so it is easier to disperse evenly in petrolatum, giving homogenous preparations as opposed to preparations of solid 4,4'-MDI. Thus, preparations of PMDI are more suitable for diagnosing allergy to MDI substances than preparations of 4,4'-MDI. Patch test preparations of PMDIs with a monomer content of at least 35% and test concentration of 2% in petrolatum is recommended.

Percutaneous penetration

Penetration is important to consider when choosing an effective vehicle as well. Kim et al., (20) suggested that reactions induced by significantly lower concentrations for a specific patch test system might have been due to higher skin absorption. To elicit sensitization, a compound has to penetrate the permeability barrier of the skin's large outer layer of stratum corneum (21) at some base level concentration and react with Langerhans cell surface proteins to form new chemical structures. Some molecules need to undergo metabolic processes and react with permeants on the skin surface to gain reactive,

allergenic, properties and elicit a response. Thus skin absorption and bioavailability, the rate and extent of absorption, are important factors when considering effectiveness of vehicles.

Frick-Engfeldt et al. (19), tested 4,4'-MDA and 4,4'-MDI. 4,4'-MDA is an amine, which needs to undergo metabolism to react with proteins in the skin. The skin is equipped with enzymes to catalyze these metabolic pathways. In contrast, 4,4'-MDI possess reactive properties so it does not need to undergo metabolic processes. Frick-Engfeldt et al., hypothesize that because 4,4'-MDI positive reactions appear later than 4,4'-MDA positive reactions, it is possible that 4,4'-MDI reacts with water and converts into 4,4'-MDA intermediate, ultimately forming ureas and oligoureas. 4,4'-MDI might also react with skin constituents. The ureas and conjugates from skin constituents can subsequently be hydrolyzed into 4,4'-MDA. Even after 4,4'-MDA penetrates the skin, it still needs to be metabolized to elicit an immune response.

The *in vitro* experiments performed by Gammelgaard et al. (22), compare release of chromate from petrolatum versus aqueous solution. Small amounts of chromium permeated the skin when the petrolatum preparation was applied on the skin. This is because chromium in the aqueous formulation is dissolved, while in petrolatum it is dispersed as solid crystals of varying size. Hence, petrolatum preparation results in decreased bioavailability since allergens are not fully soluble in petrolatum. Studies have shown that only small amounts of chromium, up to a concentration of 0.5%, elicit an allergic contact reaction.

Intuitively, we assume there is a relationship between the extent of penetration and clinical patch test response. Unfortunately, the experimental data is insufficient to document the veracity of this hypothesis. We believe that experiments of this type may eventually provide a more efficient approach to choosing optimal concentrations and vehicles.

Conclusion

In an attempt to utilize the world's experimental and clinical experience, we are mandated to understand the veracity of the experimental data. Benezra et al. (23), formulated a methodology for semi-quantification of the experimental data. The data available on vehicles was insufficiently in depth to utilize the Benezra system. In future experiments, with the experimental details postulated here, we hope that most of the literature will be of high quality (grade score-5).

The only generally available quantitative data on vehicles in patch testing were performed with the TRUE test-data available on the FDA website, as it was commercialized through the agencies regulatory system (24).

There is a moderately extensive literature on left-right concordance in diagnostic patch testing, as summarized by Ale and Maibach (25). Under ideal technical circumstances the concordance rate approximates 99.9%.

The bioavailability of a given allergen will depend on its thermodynamic activity and relates to solubility and allergen concentration. Unfortunately, much of the literature does not provide adequate data to make efficient interpretations.

To an initiate, diagnostic patch testing appears simple and robust; unfortunately, with experience, the opposite turns out to be the case. Many variables appear in the literature and often prevent direct comparisons from one publication to another. These include patch test chambers (FINN chamber vs. Van der Bend chamber), allergen volatility (immediate application versus one hour or more later) (26), and volumetric dosing of liquids (a drop versus a micropipette) (26). The presence or absence of a filter paper may alter the reproducibility of patch testing with liquids.

At present, petrolatum has been the preferred vehicle for a century; however, until systematic studies of the type described above are accomplished, we remain uncertain whether this tradition serves our patients as efficiently as possible. Although no definite alternative has been found for petrolatum, there has been research demonstrating the enhanced effectiveness of various alternative vehicles to petrolatum for specific allergens. These alternate vehicles require more data with greater sample size before validation for general use.

When choosing a vehicle for a particular allergen, factors such as irritancy potential, percutaneous penetration, and stability of the patch, and other traits indicated in Table 4 should be considered when evaluating the vehicle's effectiveness. Traditionally distilled water is used rather than the more variable tap water when utilizing water as a vehicle; we are not aware of experimental validation of this procedure. There may be other currently unknown biological factors that can inhibit or stimulate the effect of vehicles on the immunological system remaining to be discovered. Taken together, systematic investigation of allergen dermatopharmacokinetics in model vehicles, and mixtures thereof, should accelerate our ability to define, and thus determine, the optimum vehicle(s).

Table 4. Optimal vehicle.

Characteristic	Comments
Non-irritating	Vehicles water and petrolatum both are non-irritating
Ability to solubilize allergen	A negative aspect of petrolatum: allergens are dispersed and not fully soluble in petrolatum, thus a lower bioavailability of the allergen. A vehicle that fully solubilizes the allergen so as to obtain maximum thermodynamic activity allows for optimal penetration.
Dose proportional to percutaneous penetration	
Non-allergic	

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Declaration of interest

The authors report no declarations of interest.

References

1. Cyran C, Maibach HI. Alternate vehicles for diagnostic patch testing: an update. *G Ital Dermatol Venereol* 2008;143:91–94.
2. Lee EE, Maibach HI. Role of vehicles in diagnosing contact allergy: an update. *Exog Dermatol* 2002;1:107–111.
3. Tanglertsampan C, Maibach HI. The role of vehicles in diagnostic patch testing. A reappraisal. *Contact Derm* 1993;29:169–174.
4. Kligman AM. The identification of contact allergens by human assay. II. Factors influencing the induction and measurement of allergic contact dermatitis. *J Invest Dermatol* 1966;47:375–392.
5. Knudsen BB, Menné T. Elicitation thresholds for thiuram mix using petrolatum and ethanol/sweat as vehicles. *Contact Derm* 1996;34:410–413.
6. de Groot AC, Conemans JM. Nystatin allergy. Petrolatum is not the optimal vehicle for patch testing. *Dermatol Clin* 1990;8:153–155.
7. Geier J, Lessmann H, Becker D, Bruze M, Frosch PJ, Fuchs T et al. Patch testing with components of water-based metalworking fluids: results of a multicentre study with a second series. *Contact Derm* 2006;55:322–329.
8. Wahlberg JE. Thresholds of sensitivity in metal contact allergy. 1. Isolated and simultaneous allergy to chromium, cobalt, mercury and/or nickel. *Berufsdermatosen* 1973;21:22–33.
9. Wahlberg JE. Thresholds of sensitivity in metal contact allergy. 2. The value of percutaneous absorption studies for selection of the most suitable vehicle. *Berufsdermatosen* 1973;21:151–158.
10. Wahlberg JE, Skog E. Nickel allergy and atopy. Threshold of nickel sensitivity and immunoglobulin E determinations. *Br J Dermatol* 1971;85:97–104.
11. van Ketel WG. Patch tests: methodology, standardization and significance of positive results. *Arch Belg Dermatol Syphiligr* 1973;29:73–82.
12. Rietschel RL, Bruze M, Gruvberger B, Zug KA, Warshaw EM, Taylor JS et al. The relationship of vehicle and concentration of imidazolidinylurea, with attention to formaldehyde allergy status. *Dermatitis* 2006;17:48–49.
13. Rietschel RL, Warshaw EM, Sasseeville D, Fowler JF Jr, DeLeo VA, Belsito DV et al.; North American Contact Dermatitis Group. Sensitivity of petrolatum and aqueous vehicles for detecting allergy to imidazolidinylurea, diazolidinylurea, and DMDM hydantoin: a retrospective analysis from the North American Contact Dermatitis Group. *Dermatitis* 2007;18:155–162.
14. Tosti A, Guerra L, Bardazzi F, Gasparri F, Euxyl K 400: a new sensitizer in cosmetics. *Contact Derm* 1991;25:89–93.
15. Agner T. An experimental study of irritant effects of urea in different vehicles. *Acta Derm Venereol Suppl (Stockh)* 1992;177:44–46.
16. Dahl MV, Roering MJ. Sodium lauryl sulfate irritant patch tests. III. Evaporation of aqueous vehicle influences inflammatory response. *J Am Acad Dermatol* 1984;11:477–479.
17. Fowler JF, Warshaw EM, Squires L. Methylphenidate patch-test protocol and irritancy threshold determination in healthy adult subjects. *Dermatitis* 2009;20:271–274.
18. Aspres N, Freeman S. Predictive testing for irritancy and allergenicity of tea tree oil in normal human subjects. *Exog Dermatol* 2003;2:258–261.
19. Frick-Engfeldt M, Isaksson M, Zimerson E, Bruze M. How to optimize patch testing with diphenylmethane diisocyanate. *Contact Derm* 2007;57:138–151.

20. Kim HO, Wester RC, McMaster JA, Bucks DA, Maibach HI. Skin absorption from patch test systems. *Contact Derm* 1987;17:178-180.
21. Choy YB, Prausnitz MR. The rule of five for non-oral routes of drug delivery: ophthalmic, inhalation and transdermal. *Pharm Res* 2011;28:943-948.
22. Gammelgaard B, Fullerton A, Avnstorp C, Menné T. *In vitro* evaluation of water and petrolatum as vehicles in chromate patch testing. *Contact Derm* 1992;27:317-318.
23. Benezra C, Sigman CC, Perry LR, Helmes CT, Maibach HI. A systematic search for structure-activity relationships of skin contact sensitizers: methodology. *J Invest Dermatol* 1985;85:351-356.
24. Fischer T, Kreilgård B, Maibach HI. The true value of the TRUE Test for allergic contact dermatitis. *Curr Allergy Asthma Rep* 2001;1:316-322.
25. Ale SI, Maibach HI. Reproducibility of patch test results: a concurrent right-versus-left study using TRUE Test. *Contact Derm* 2004;50:304-312.
26. Gilpin SJ, Hui X, Maibach HI. Volatility of fragrance chemicals: patch testing implications. *Dermatitis* 2009;20:200-207.
27. Frick-Engfeldt M, Gruvberger B, Isaksson M, Hauksson I, Pontén A, Bruze M. Comparison of three different techniques for application of water solutions to Finn Chambers®. *Contact Derm* 2010;63:284-288.