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Citation for the published paper:
Bruze, Magnus and Frick-Engfeldt, Malin and Gruvberger, Birgitta and Isaksson, Marlène.

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Contact Dermatitis, 2007, Vol: 56, Issue: 1, pp. 38-42.

<http://dx.doi.org/10.1111/j.1600-0536.2007.00985.x>

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Variation in the amount of petrolatum preparation applied at patch testing

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Abstract

The elicitation of a positive patch test reaction in a given individual depends upon the dose of the sensitizer applied, the patch test technique, and the occlusion time. The dose is determined by the concentration and volume/amount of test preparation applied. If the same amount/volume of a test preparation is applied all the time with the same test technique (same area of skin) and occlusion time, it is appropriate to use concentration as a dose parameter. Most contact sensitizers are incorporated in petrolatum. With petrolatum as vehicle, it is impossible to repeatedly apply an exact volume/amount. This study was performed to investigate the inter- and intraindividual variation of petrolatum preparation applied at patch testing by 3 technicians. Weighing demonstrated that the 3 technicians had about the same precision in their petrolatum application. The investigation demonstrates that there is both an interindividual (statistically significant) and intraindividual variation in the amounts of petrolatum applied at patch testing for the 3 technicians. Presently, there is no recommendation on what amount of petrolatum preparation to apply, which merits a decision to be taken based on thorough investigations on the appropriate volumes of petrolatum preparation to be applied in various patch test systems.

Keywords: Allergic contact dermatitis; contact allergy; dose/area; Finn Chamber[®]; inter-individual; intra-individual; standard series, weighing.

The elicitation of a positive patch test reaction in a given individual depends upon: (i) the dose, i.e., the number of molecules of the sensitizer applied; (ii) the patch test technique, i.e., the vehicle used and type of occlusion; and (iii) the occlusion time (1-5). The dose is determined by the concentration and volume/amount of test preparation applied. Thus, if the same amount/volume of a test preparation is applied all the time with the same test technique (same area of skin) and occlusion time, it is appropriate to use concentration as a dose parameter. For most sensitizers, petrolatum is an appropriate vehicle as it is stable and seems to prevent/diminish degradation of the incorporated allergen as well as oxidation and polymerization (6-8). However, with petrolatum as the vehicle, it is impossible to repeatedly apply an exact volume/amount. An experienced and trained person can, however, keep the variation within a limited range (9,10). Previous measurements at our department have demonstrated that this statement was true. However, the fact that some years had passed since these measurements were made and that new technicians had been employed the last few years led us to investigate whether there was an inter- and intraindividual variation of petrolatum preparation applied at patch testing.

Materials and methods

Patch testing

For patch testing with the standard patch test series, the Finn Chamber[®] (Epitest Ltd Oy, Tuusula, Finland) technique is used implying small chambers with a diameter of 8 millimetres. The patch test preparations included the standard series of the Swedish Contact Dermatitis Group as well as additional sensitizers tested at our department. These additional sensitizers are prepared at our department, mainly in petrolatum while the standard series is purchased from Chemotechnique Diagnostics, Tygelsjö, Sweden (Table 1). The petrolatum preparations made at our department are put in the same 5 ml plastic syringes used for the petrolatum preparations from Chemotechnique Diagnostics. The application way of petrolatum preparation to the chambers varies but application as a string across the chamber predominates.

Study design

At our department 5 technicians and one nurse are participating in the patch testing procedure. One day when ordinary patch testing was carried out by 3 of them, all test strips mounted with

Finn Chambers[®], to which our standard series with 49 petrolatum preparations had just been applied, were removed from each test cubicle and refrigerated until analysed. This procedure was carried out for all 3 technicians and involved the patches intended for 3 different patients. The technicians were not informed in advance about the study. Furthermore, the technicians did not communicate with each other until the removal process was completed.

Weighing

The petrolatum preparations were applied on Finn Chambers[®] attached to Scanpor[®] tape (Norgesplaster A/S, Vennessla, Norway). To be able to weigh only the aluminium chamber with petrolatum preparation, the chamber was gently removed from the tape with a pincette and the remaining adhesive on the chamber was wiped off with a cotton swab soaked in acetone. Thereafter, the individual aluminium chambers were weighed.

To calculate the weight of an unloaded aluminium chamber, 12 unloaded Finn Chambers[®] attached to Scanpor[®] tape were handled in the same way as the ones to which petrolatum preparations had been applied.

The weight of the petrolatum preparation in an individual Finn Chamber[®] was obtained by subtracting the weight of a loaded Finn Chamber[®] with the average weight of the unloaded chambers.

Statistical calculation

All statistical analyses were carried out using SPSS version 12.0 (SPSS Inc., Chicago, USA). The interindividual variation between the 3 technicians was analyzed using One-way Anova since the data for each individual were fairly symmetric with median values close to the means (Table 1, Fig. 1) and since the number of observations for each individual was large. The intraindividual variations were assessed using descriptive data such as mean, median, standard deviation and coefficient of variation (C.V.) as well as Levene's test for homogeneity of variance.

Results

The individual weights of the 12 unloaded aluminium chambers were recorded and the calculated mean was 54.2 mg (95% CI 54.0-54.4).

The weights of the 49 petrolatum preparations are given in Table 1 for the 3 technicians.

The 3 technicians had about the same precision in their petrolatum application, i. e. they had about the same C.V. and standard deviation. This conclusion was supported by Levene's test, which could not find significant differences in the variance ($p=0.088$). One-way ANOVA analysis showed that there was a significant ($p<0.001$) interindividual variation between the three technicians. Paired comparisons between the technician (i. e. technician 1 with technician 2; technician 1 with technician 3; technician 2 with technician 3 etc.) also showed significant differences ($p<0.001$).

Discussion

With regard to contact allergy, the dose of a sensitizer per unit skin area is decisive for both sensitization and elicitation (1-5). Therefore, vehicles which enable an accurate measurement of the dose of a sensitizer applied at patch testing must be used when knowledge of the dose per unit skin area is of utmost importance, for example elicitation threshold studies in humans and animals. Knowledge of the doses used is also necessary, provided that the same patch test technique has been used, when comparing patch test data between various patch test centres and when performing multicentre studies including patch testing.

Petrolatum is the most commonly used vehicle for contact sensitizers in the standard series and additional series. Major reasons for this is that petrolatum as vehicle is practical and economical. It is easy to use and it prevents/diminishes degradation/oxidation/polymerization of sensitizers (6-8). However, with petrolatum as vehicle it is virtually impossible to apply an exact dose at patch testing. Even then, this does not disqualify petrolatum as vehicle for screening purposes of contact allergy, neither for comparisons of patch test data between departments. With petrolatum as vehicle, comparisons and multicentre studies including patch testing can be performed provided that "equivalent" doses have been used and that 2 factors are known, i.e. (i) the average dose applied at one department and (ii) the standard deviation (4).

When using the weight of various petrolatum preparations containing contact sensitizers as a parameter for the volume/weight of petrolatum applied, the density of the contact sensitizer

will affect the result. However, in this study the influence of the densities of the contact sensitizers on the weight of petrolatum preparations was considered minute and therefore ignored, as the densities of most contact sensitizers in the present investigation are close to the density of petrolatum and/or the concentrations of the contact sensitizers present in the petrolatum preparations were low. Expectedly, the results of this study demonstrate that our technicians can keep the variation of petrolatum application within a limited range. Still, for the individual technician this means that there is a factor of approximately 2 (1.9, 2.2 and 2.4, respectively) for the difference between the highest and lowest doses applied. However, although the 3 technicians have the same precision in their weighing, they have different perceptions about the correct amount to weigh since their mean differs a lot.

Surprisingly, there was a big difference in average petrolatum applications between the 3 technicians. For example, as seen in table 1, the amount between the technicians varies with a factor 2-3 for 14/48 allergens. These findings indicate that the new technicians have been taught the need of a “standardized” amount of petrolatum preparation applied at patch testing but not on what amount to apply. Currently, there is no recommendation for petrolatum while a liquid is recommended to be applied in a volume of 15 μ l with the Finn Chamber[®] technique (11).

The demonstrated intra- and inter-individual differences in petrolatum applications may have clinical implications. The significance of dose has been investigated in a previous study in a small number of nickel-hypersensitive persons (12). They were patch tested with nickel sulphate at the same concentration but with various doses; 2.5 mg, 5 mg, 10 mg and 15 mg. All doses yielded positive responses but as no information was given on the coverage of the test area, actually no conclusions on the clinical significance of dose can be drawn. For a positive reaction the minimal criteria is on infiltrated erythema covering the whole test area (4). The preservative methyldibromo glutaronitrile (MDBGN) can be used as an example of a possible clinical implication. During the past years, the optimal patch test concentration of MDBGN has been extensively discussed. However, in none of the MDBGN studies reviewed in the article by Bruze et al (13) the amount of petrolatum used for the patch testing was given. One major reason for this is that there have not been any amounts of petrolatum preparations recommended of universal acceptance for the various patch test techniques used.

From Table 2 it is obvious that also the amount of petrolatum preparation applied is important for the dose. Actually, comparing the doses applied by technician no. 1 for the MDBGN preparations at 1.0% and 0.5% with the corresponding doses for technician no. 2, the former are lower and equivalent to the doses applied by technician no. 2 for the MDBGN preparations at 0.5% and 0.3%, respectively, provided that the petrolatum preparation is not spread outside the test area.

To the best of our knowledge, this is the first time the amounts of petrolatum preparations have been investigated when routinely patch testing dermatitis patients without the technicians/patch test preparation applicators having been informed on what was planned. In a British study a nurse, being aware of the purpose of the study, prepared 25 separate Finn Chambers[®] with petrolatum preparations, where the average petrolatum weight was 11 mg, and where there was no significant weight variation (10). In a Belgian study on the amounts of petrolatum preparation applied to test chambers by 7 dermatologists on 4 consecutive days, significant weight variation occurred interindividually and also intraindividually from day to day, although they were aware of the study (14).

In summary, this investigation demonstrates that there is both an intraindividual and interindividual variation in the amounts of petrolatum applied at patch testing for the 3 technicians. The individual technician can keep the variation within a limited range while the interindividual variation necessitates steps to be taken; i.e. the amount of petrolatum preparation to be applied has to be decided, preferably by the European Society of Contact Dermatitis. Such a decision has to be based on thorough investigations on the appropriate volumes of petrolatum preparation to be applied in various patch test systems. When an appropriate volume has been decided for a particular patch-test system, technicians can be trained to weigh this amount. Thereby, “equivalent” doses are achieved which makes comparisons and multicentre studies possible provided that the average dose applied at one department as well as the standard deviation are stated (4).

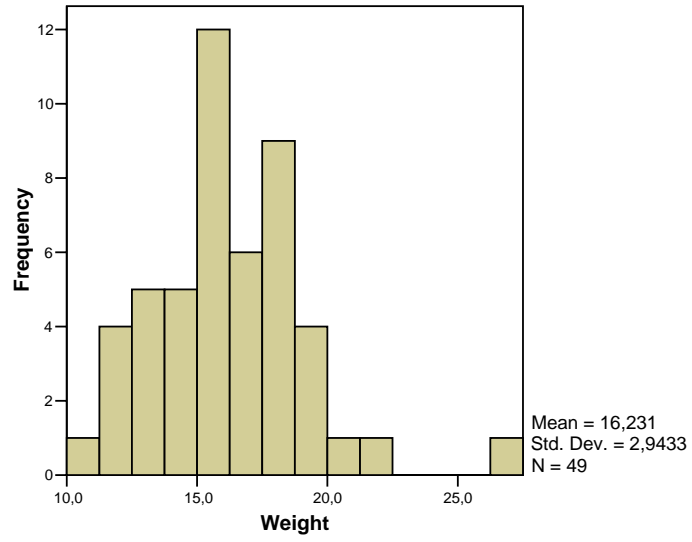
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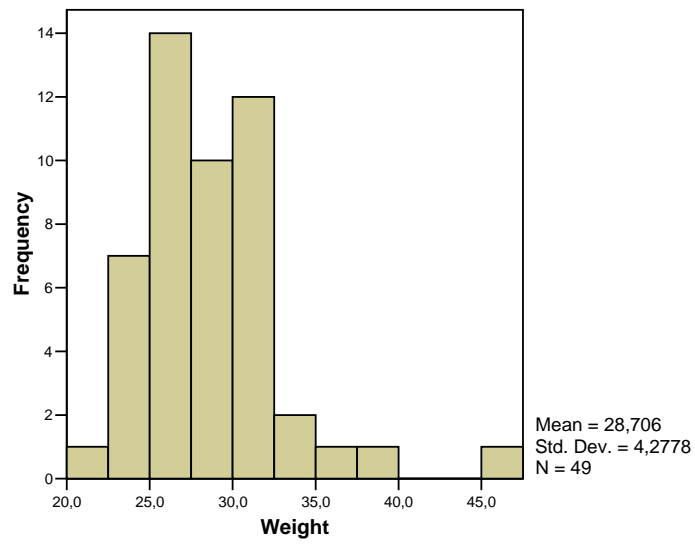
Legends

Figure 1. Histograms showing the distribution of weights in mg of petrolatum preparations applied at patch testing for 3 technicians, individually.

Technician 1



Technician 2



Technician 3

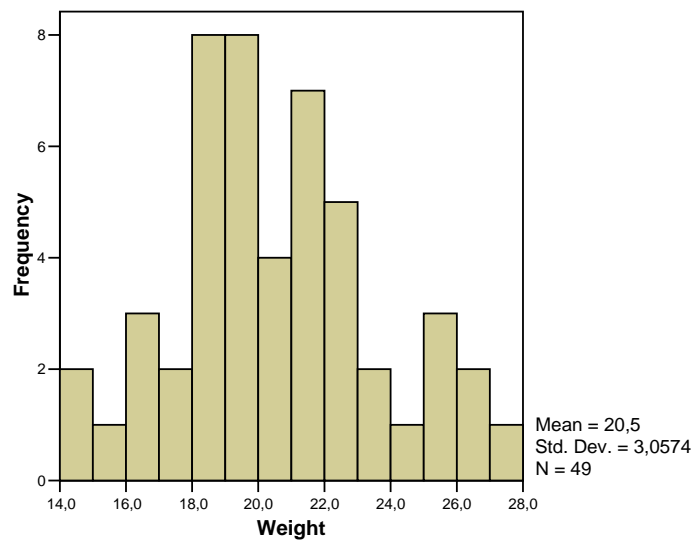


Table 1. The standard series used at the Department of Occupational and Environmental Dermatology at the time of the study as well as the weighed amount of petrolatum preparations for each lab technician.

Preparation	Conc.% w/w	Weight (g)*		
		Technician 1	Technician 2	Technician 3
Potassium dichromate	0.5	14.3	25.8	26.6
4-phenylenediamine dihydrochloride	0.94	17.7	27.8	22.3
Thiuram mix	1.0	18.1	32.6	18.0
Neomycin sulfate	20.0	19.9	29.1	21.6
Cobalt chloride hexahydrate	0.5	15.7	23.6	21.9
Benzocaine	5.0	15.6	32.2	19.2
Nickel sulfate hexahydrate	5.0	17.9	30.8	22.4
Quinoline mix	6.0	15.7	31.5	18.9
Colophony	20.0	11.1	31.7	18.7
Paraben mix	15.0	19.5	33.4	19.1
Black rubber mix	0.6	12.7	25.8	14.4
Sesquiterpene lactone mix	0.1	12.8	29.4	18.0
Mercapto mix	2.0	17.6	26.3	19.8
Epoxy resin	1.0	20.9	30.5	18.1
Balsam peru	25.0	22.0	38.0	18.5
4-tert-butylphenol formaldehyde resin	1.0	14.7	29.6	15.6
Primin	0.01	14.0	29.1	14.6
Fragrance mix	8.0	12.4	30.6	16.8
Ethylenediamine dihydrochloride	1.0	16.7	29.0	19.9
Lyril	5.0	19.8	28.3	25.6
Caine mix II	10.0	13.3	24.5	19.7
Lichen acid mix	0.3	13.5	26.4	22.2
Tixocortol-21-pivalate	0.1	12.1	31.9	23.0
Toluensulfonamid formaldehyd resin	10.0	18.4	31.1	20.3
Budesonide	0.01	14.7	26.0	21.2
Methyldibromoglutaronitrile	0.3	15.6	31.7	23.6
Qakmoss absolute	2.0	15.9	36.9	16.8
Gold sodium thiosulfate	2.0	12.2	26.1	21.9
Mercaptobenzothiazole	2.0	15.2	25.5	16.9
Quaternium 15	1.0	11.3	29.3	18.2
Thimerosal	0.1	18.3	27.2	19.5
Palladium chloride	2.0	13.1	29.7	19.5
Phenol formaldehyde resin	1.0	15.5	26.7	18.1
Bisphenol F resin (Novolac) DEN 431	0.25	15.9	25.3	17.6
Methyldibromoglutaronitrile	0.5	18.0	30.5	21.1
Methyldibromoglutaronitrile	1.0	15.3	24.1	17.5
Textile colours mix	3.2	16.8	30.3	26.1
Phenylglycidyl ether	0.25	27.0	22.5	20.3
Diphenylmethan-4,4'-diisocyanat	2.0	17.8	24.8	21.4
4,4'-Diaminodiphenylmethane	0.25	17.2	21.0	22.2
Epoxy resin, cycloaliphatic	0.5	17.9	27.1	22.0
Disperse Blue 106	0.1	16.1	26.8	26.3
Disperse Blue 124	0.1	15.2	24.3	25.7
Disperse Blue 35	0.5	17.0	28.4	24.3
Disperse Yellow 3	0.5	19.0	25.6	25.9
Disperse Orange 1	0.5	16.8	45.8	20.9
Disperse Orange 3	0.5	14.6	24.3	19.2
Disperse Red 1	0.5	15.9	25.9	27.3

Disperse Red 17	0.5	16.6	31.8	20.8
Mean	16.2	28.7	20.5	
95% Confidential interval for mean	15.4-17.1	27.5-29.9	19.6-21.4	
Median	15.9	28.4	20.3	
Standard deviation	2.94	4.28	3.06	
Coefficient of variation (%)	18.1	14.9	14.9	

Table 2. The variation in dose per area for the 3 different concentrations of methyldibromoglutaronitrile when weighed by the 3 technicians.

Preparation	Conc (%)w/w	Dose/area (mg/mm²)*		
		Technician 1	Technician 2	Technician 3
Methyldibromoglutaronitrile	0.3	0.94	1.90	1.42
Methyldibromoglutaronitrile	0.5	1.80	3.05	2.11
Methyldibromoglutaronitrile	1.0	3.06	4.82	3.50

* Area of the Finn Chambers® 50 mm²