

Effect of vehicles and elicitation concentration in contact dermatitis testing

II. Statistical analysis of data

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Data obtained in tests of several potential allergens with different vehicles and at different concentrations have been analyzed statistically.

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In Part I of this paper, Marzulli & Maibach (1976) described a series of experiments to determine the effects of different vehicles and different concentrations in tests of potential allergens carried out with human subjects. Cinnamic aldehyde and costus oil were tested in single concentrations at two skin sites with both petrolatum and 95 % ethyl alcohol as vehicles. In addition, four subjects shown to be sensitive to costus oil were tested, again at a single concentration and two skin sites, with Carbowax and hydrophilic ointment as vehicles. In another series of tests, several different concentrations of costus oil, chloracetamide, and thimerosal, all dissolved in petrolatum as vehicle, were tested on human subjects. Details of the experiments and discussion of results are presented by Marzulli & Maibach (1976). This paper describes the statistical analysis of the data obtained in the experiments and provides the rationale for ascribing significance or lack of significance to the results.

The statistical analysis follows the dis-

cussion by Steel & Torrie (1960) and additional points by Bishop et al. (1975), for cases in which two trials are applied to the same individual.

Vehicle Studies

Cinnamic aldehyde

Fifty-five individuals were exposed percutaneously to cinnamic aldehyde in 95 % ethyl alcohol and 53 to cinnamic aldehyde in petrolatum. One adverse reaction to this first exposure occurred in the group with the ethyl alcohol vehicle and none in the group with the petrolatum vehicle. The proportions of 1/55 vs 0/53 are not statistically different when analyzed by contingency analysis. The chi-square value was calculated as 0.009 where 3.84 is needed for significance at the 5 % level. In this and all subsequent chi-square values, each cell was adjusted for continuity if the expected value was less than 5. Thus there is no statistical evidence for differential action of the vehicles at first exposure (irritation). All available individuals (107 of the

original 108, since one with a first challenge of alcohol was no longer available) were then exposed to cinnamic aldehyde in a second challenge at two separate body sites. One vehicle was used at one site and the other at the other site. The results were as follows:

		Petrolatum vehicle	
		React	Not react
Alcohol	Not react	0	104
Vehicle	React	2	1

Two individuals reacted to both vehicles, and 104 to neither. These 106 data points give no information on differential reaction to the vehicle. One individual reacted to ethyl alcohol but not petrolatum; no one reacted to petrolatum and not alcohol. The chi-square value for this event is 0.25, which is not significant. Thus there is no statistical evidence for differential action of the vehicles.

Of the three who gave reactions, the average reaction score was 1.0 for the petrolatum vehicle site and 3.3 for the alcohol vehicle site. The "t" value for the paired reactions was 2.65, which is not significant for the two degrees of freedom involved. As large or larger differences in averages would be expected from chance alone 10–20 % of the time.

Costus oil

Twelve individuals were exposed to costus oil in petrolatum and 12 others to costus oil in alcohol. One adverse reaction occurred with petrolatum as vehicle and three with alcohol as vehicle. These proportions (1/12 vs 3/12) are not statistically different. Thus there is no statistical evidence for differential action due to vehicles at first challenge.

Twenty-two of the 24 individuals were available for second challenge. The two who were no longer available were both

initially exposed to the alcohol vehicle. Again, each individual was challenged on two separate sites, one with petrolatum as vehicle and the other with alcohol. The results were as follows:

		Petrolatum vehicle	
		React	Not react
Alcohol	Not react	0	16
Vehicle	React	2	4

Two individuals reacted to costus oil in both vehicles and 16 to neither. These 18 data points give no information on differential reaction to the vehicle. One individual reacted to petrolatum and not alcohol, and four reacted to alcohol but not petrolatum. The chi-square value for this is 2.25, which is not statistically significant. Thus for costus oil there is no statistical evidence for differential action at second challenge.

Of the six who reacted, the average reaction score was 1.0 for the petrolatum vehicle site and 2.67 for the ethyl alcohol site. The "t" value for the paired reactions was 2.71, which is significant at the 5 % level for the five degrees of freedom involved. Thus there is statistical evidence that among sensitized individuals the reaction is more severe when ethyl alcohol is used as the vehicle.

This experiment was repeated with 14 individuals exposed to costus oil in alcohol and 17 exposed to costus oil in petrolatum. Since no adverse reaction occurred to either agent in this first challenge, there is no statistical evidence for differential action at first challenge.

All individuals were available for the second challenge. Again, each individual was challenged on two separate sites, one with the petrolatum vehicle and the other with the alcohol vehicle. The results were as follows:

		Petrolatum vehicle	
		React	Not react
Alcohol	React	7	0
Vehicle	Not react	0	24

Seven individuals reacted to costus oil in both vehicles and 24 did not react to costus oil in either vehicle. These data points give no information on differential action. No individual reacted to the agent in alcohol and not petrolatum, and no individual reacted to the agent in petrolatum and not alcohol. Thus there is no statistical evidence for differential action at second challenge.

Of the seven who reacted, the average score was 2.00 for the petrolatum vehicle and 3.28 for the ethyl alcohol vehicle. The "t" value for paired reactions was 6.97, which is significant at the 0.1 % level for the six degrees of freedom involved. Thus there is statistical evidence that among sensitized individuals the reaction is more severe when the agent is dissolved in ethyl alcohol.

Costus oil with other vehicles

Four individuals known to be sensitive to costus oil were patch-tested at one site with costus oil dissolved in Carbowax and also at a separate site with costus oil dissolved in hydrophilic ointment. The results were as follows:

		Hydrophilic ointment vehicle	
		React	Not React
Carbowax	React	4	0
Vehicle	Not react	0	0

Since all subjects reacted to costus oil at both the Carbowax vehicle site and the hydrophilic ointment vehicle site, no information on differential activity was obtained.

The average score was 3.25 for the Carbowax vehicle and 3.75 for the hydrophilic

ointment vehicle. The "t" value for the paired reactions was 0.77, which is not significant. Thus there is no statistical evidence for difference in the severity of reaction due to these two vehicles.

Concentration Studies

Eight individuals known to be sensitive to costus oil were each patch-tested with three concentrations of costus oil in petrolatum vehicle. The results were as follows:

Costus oil Concentration %	Fraction reacting
0.100	8/8
0.010	8/8
0.001	1/8

Since the reaction ratio is 8/8 for both the 0.100 % and 0.010 % concentrations, obviously there is no statistical difference. For the two lower concentrations the results may be stated as:

		0.001 % Concentration	
		React	Not react
0.010 %	React	1	7
Concentration	Not react	0	0

One person reacted at both concentrations; no person failed to react at either concentration. These data points give no information on differential action. No person reacted at the low concentration and not at the high, and seven reacted at the high but not at the low. The chi-square value for this event is 5.14, which is statistically significant. Thus there is statistical evidence for differential action between the 0.010 % and 0.001 % concentrations.

The average reaction grade was 3.250 for the 0.10 % concentration, 2.375 for the 0.01 % concentration, and 0.250 for the 0.001 % concentration. Two-factor analysis

of variance and least significant difference calculations show that each of these averages differs significantly from the others ($P > 0.99$), and the responses are positively correlated to the dose level. Thus there is statistical evidence for a dose-response relationship.

Chloracetamide

Six individuals known to be sensitive to chloracetamide were challenged with four concentrations of chloracetamide. The results were as follows:

Chloracetamide Concentration %	Fraction reacting
2.00	4/6
0.50	1/6
0.10	0/6
0.05	0/6

For the two highest concentrations the results were as follows:

		0.50 % Concentration	
		React	Not react
2.00 %	React	1	3
Concentration	Not react	0	2

Examination of the diagonal values of 0 and 3 for statistical evidence of differential activity yields a chi-square value of 1.33, which is not statistically significant. Thus there is no statistical evidence that the response at 2.00 % is different from that at 0.50 %.

A similar matrix may be constructed for the 2.00 % and either the 0.10 or 0.50 % concentration:

		Two low concentrations	
		React	Not react
2.00 %	React	0	4
Concentration	Not react	0	2

Examination of the diagonal values of 0 and 4 for evidence of differential activity yields a chi-square value of 2.25, which is not statistically significant. Thus again there is no statistical evidence for differential activity.

The average reaction scores for these six sensitized individuals were as follows:

Concentration %	Score
2	1.83
0.5	0.50
0.1	0.00
0.05	0.00

The differences in scores among the four concentrations were statistically significant. The score for the 2 % concentration was statistically greater than the 0.1 % or 0.5 % score. No other differences could be demonstrated to be statistically significant. Thus there is statistical evidence of a concentration effect.

Thimerosal

Seven individuals known to be sensitive to thimerosal were challenged with three concentrations of thimerosal. The results were as follows:

Thimerosal Concentration %	Fraction reacting
0.050	5/7
0.010	0/7
0.005	0/7

A matrix may be constructed for the 0.050 concentration and either of the others:

		0.01 or 0.005 % Concentration	
		React	Not react
0.05 %	React	0	5
Concentration	Not react	0	2

Examination of the diagonal values 0 and 5 for evidence of differential activity yields a chi-square value of 3.2, which approaches but does not reach the required value of 3.84 for statistical significance.

The average reaction scores were:

Concentration %	Score
0.05	1.43
0.01	0.00
0.00	0.00

The average reaction score at the 0.5 % concentration was statistically greater than that for either of the two lower concentrations. The F-value with 2 and 12 degrees of freedom was 15.49.

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