

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

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Objective: To determine whether petrolatum or aqueous vehicles are more sensitive for detecting allergy to imidazolidinylurea (IU), diazolidinylurea (DU), and dimethylol dimethyl hydantoin (DM). The relationship of these allergens to formaldehyde sensitivity was also explored.

Methods: Retrospective analysis of patients patch-tested by the North American Contact Dermatitis Group. All patients were simultaneously tested to seven allergens (formaldehyde, IU in petrolatum [pet], IU aqueous [aq], DU pet, DU aq, DM pet, and DM aq). Data were analyzed in pairs with various "gold standard" definitions of "true allergy" and adjusting for correlated data.

Results: Reaction to at least one of the seven allergens occurred in 2,398 patients. In all cases except one (which just approached statistical significance), the petrolatum-based allergen was statistically significantly more sensitive than the same allergen in an aqueous base. Most of the patients allergic to the three preservatives were also allergic to formaldehyde, but most formaldehyde-allergic patients were not allergic to the IU, DU, or DM.

Conclusion: Of these two vehicles, petrolatum is significantly more sensitive than an aqueous vehicle is for detecting allergy to IU, DU, and DM.

THE NORTH AMERICAN CONTACT DERMATITIS GROUP has tested several formaldehyde-releasing preservatives: imidazolidinylurea (IU), diazolidinylurea (DU), and dimethylol dimethyl (DMDM) hydantoin (DM) in both aqueous and petrolatum vehicles since 1992. The initial goal was to identify the most sensitive vehicle. This article reviews our findings with these patch-test materials from 1992 to 2004. Because these antigens release formaldehyde, the relationship between formaldehyde patch-test reactivity and IU, DU, and DM reactivity was also examined.

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Materials and Methods

Patch Tests

We used Standards for Reporting of Diagnostic Accuracy (STARD) guidelines for reporting studies of diagnostic accuracy.¹ All patients presenting for patch testing at the North American Contact Dermatitis Group patch-test sites (ie, authors' locations) were eligible for inclusion. Finn Chambers (Epitest Ltd Oy, Tuusula, Finland) on Scanpor tape (Norgesplaster Aksjeselskap, Vennesla, Norway) were used to apply allergens from Chemotechnique Diagnostics AB (Malmö, Sweden). The patches were applied for 2 days, removed and interpreted, and reinterpreted 1 to 5 days later. Patch-test results were scored as positive if + or greater. Macular erythema was scored as doubtful and was not included in this analysis. IU was tested at 2% concentration in aqueous and petrolatum vehicles. DU and DM were tested at 1% concentration in both vehicles. Formaldehyde was tested as 1% aqueous (aq). Investigators were not blinded to the results of the other patch tests.

Data Analysis

De-identified data from participating centers were sent to a central database, manually entered, and checked for consistency. All analyses were performed with SAS software (Statistical Analysis System, SAS Institute Inc., Cary, NC). Preliminary analyses used Pearson chi-square tests to examine the association of positive reactions of specific allergens; relative risk ratios were also calculated. For the purposes of the preliminary analysis, reactions to different antigens and/or vehicles were assumed to be independent, and a significance level of .05 was used. Sensitivities for each vehicle were calculated with four different definitions of a “gold standard” to define the presence of formaldehyde sensitivity: (1) positive reaction to at least one of the seven allergens (formaldehyde, DU aq, DU in petrolatum [pet], IU aq, IU pet, DM aq, and DM pet), (2) positive reaction to at least two of the seven allergens, (3) positive reaction to at least three of the seven allergens, and (4) positive reaction to at least four of the seven allergens. Nine patients who were not tested with all seven allergens were excluded from these sensitivity analyses. The sensitivity of a specific allergen was estimated by dividing the number of participants who had a positive result for the specific allergen by the number of participants with at least one (or two) (or three) (or four) positive test results.

The sensitivities estimated for the seven diagnostic tests were compared for each allergen (by vehicle pairs) to determine the best vehicle for detecting allergy. The test statistic $(p_i - p_j) / \text{se}(p_i - p_j)$ was used to assess whether the difference in estimated sensitivities $(p_i - p_j)$ in each pair of tests (i, j) was statistically significant. Because all the diagnostic tests were performed on the same group of individuals, the sensitivities were not independent but correlated with each other. Details on how to compute this test statistic were published by Agresti in 1990.² Statistical significance at the 5% level ($\alpha = .05$) was indicated if the absolute value of the test statistic exceeded the Tukey honest significant difference critical value adjusted for multiple comparisons.³

Results

Demographics

More than 25,000 patients were enrolled in the North American Contact Dermatitis Group (NACDG) database from 1992 to 2002. A total of 2,398 patients were tested with all seven allergens and reacted to at least one allergen.

The average age of this group was 66 years (range, 7–96 years), and 59.4% (1,424) were female. Of the 2,398 patients, 91.2% (2,185) were Caucasian; 4.6% were black, 2.5% were Asian, 1.0% were Hispanic, and 0.7% were classed as “other.”

Descriptive Data for Positivity Rates to Specific Allergens

The numbers of patients who reacted to the formaldehyde-releasing preservatives in each study period are listed in Table 1. The petrolatum-based allergen preparation induced more positive reactions than the aqueous allergen did in almost every 2-year period as well as in the pooled 12-year data, but specific reactions may have been missed without the addition of the aqueous tests.

Analysis of Data for Positivity Rates by Time Period

Information from a pilot exploration of these data specifically for IU⁴ suggested that there may have been a higher proportion of patients who had positive reactions to IU pet in recent years than was seen earlier. Because of this possibility, we collapsed the data into two groups for each allergen, early (1992–1998) and late (1998–2004), and examined the difference in rates of reactions in the two periods.

For patients who were tested with IU in both vehicles and had a positive reaction to at least one, the prevalence of positive reactions to only aqueous vehicle was not significantly different between these two periods ($p = .4971$). The prevalence of positive reactions to only petrolatum was significantly lower in the early years (relative risk [RR], 0.82; 95% confidence interval [CI] 0.71–0.94; $p = .0034$). The prevalence of reactions to both vehicles, however, was 1.31 times greater in the early years than in the later years (95% CI, 1.07–1.60).

Among patients who were tested with DU in both vehicles and had positive reactions to at least one, the prevalence rates of positive reactions to aqueous vehicle only, petrolatum only, or both vehicles were not significantly different between these two periods (all p values $> .3890$).

Among patients who were tested with DM in both vehicles and had a positive reaction to at least one, the prevalence of positive reactions to only the aqueous vehicle was 1.25 times higher in earlier years than in later years (95% CI, 1.02–1.54; $p = .0341$). The prevalence rates of positive reactions to petrolatum only or to both vehicles, however, were not statistically significantly different between these two periods (p values $> .1957$).

Table 1. Formaldehyde-Releasing Preservative Allergy Data

Period	Reactive Patients			
	Total+	Both Aq+ and Pet+	Aq+ Only (% Total*)	Pet+ Only (% Total*)
Imidazolidinylurea				
1992–1994	118	39	29 (57)	50 (75)
1994–1996	132	43	38 (62)	51 (71)
1996–1998	175	51	46 (55)	78 (74)
1998–2000	205	53	62 (56)	90 (70)
2001–2002	192	43	44 (45)	105 (77)
2002–2004	182	42	37 (43)	103 (80)
Total (95% CI)	—	—	53.5% (49.4–55.6)	74.5% (71.8–77.2)
Diazolidinylurea				
1992–1994	152	58	50 (71)	44 (67)
1994–1996	160	65	46 (69)	49 (71)
1996–1998	198	69	50 (60)	79 (75)
1998–2000	233	93	58 (65)	82 (75)
2001–2002	227	76	78 (68)	73 (66)
2002–2004	218	82	46 (58)	90 (79)
Total (95% CI)	—	—	64.9% (62.2–67.6)	72.4% (69.8–74.9)
DMDM Hydantoin				
1992–1994	98	20	43 (64)	35 (56)
1994–1996	108	29	36 (60)	43 (67)
1996–1998	146	38	39 (52)	69 (73)
1998–2000	169	38	54 (54)	77 (70)
2001–2002	188	52	54 (55)	82 (71)
2002–2004	136	20	24 (49)	69 (82)
Total (95% CI)	—	—	55.6% (52.3–59.0)	70.4% (67.3–73.5)

Aq = aqueous; CI = confidence interval; DMDM = dimethylol dimethyl; Pet = petrolatum; + = positive.

*“% Total” is calculated by adding the figure in the “Only” column to the figure in the “Both” column, then dividing this sum by the figure in the “Total” column.

Associations between Reactions to Formaldehyde and IU, DU, and DM

Of patients who were tested with both formaldehyde and IU, DU, or DM, the percentage of patients allergic to each allergen who were also allergic to formaldehyde are listed in Table 2. For IU, about 50 to 70% of patients also reacted to formaldehyde. For DU, the numbers are greater (60–80%), and for DM, higher still (80–90%).

Formaldehyde Reactivity within Allergen

In evaluating the proportion of patients who reacted to formaldehyde within each allergen, there was no statistically significant difference in the proportion of patients who had positive reactions to petrolatum who were formaldehyde allergic as compared with those who had positive reactions to DM aq ($p = .9851$). There was a trend for more patients who reacted positively to petrolatum to

be formaldehyde allergic as compared to IU aq ($p = .0956$). Individuals who reacted positively to DU aq were 1.1 times more likely to also be allergic to formaldehyde than were patients who reacted positively to DU pet (95% CI, 1.04–1.19; $p = .0018$).

Formaldehyde Reactivity Comparisons between Allergens in Petrolatum

In evaluating the proportion of patients who reacted to formaldehyde between allergen groups, there were no statistically significant differences in the formaldehyde reactivity rate among IU pet–positive patients and the DU pet–positive patients ($p = .5810$). However, DM pet–positive patients were more likely than either IU pet–positive patients (RR, 1.29; 95% CI, 1.21–1.38; $p < .0001$) or DU pet–positive patients (RR, 1.26; 95% CI, 1.19–1.35; $p < .0001$) to be formaldehyde sensitive.

Table 2. Patients Allergic to Tested Allergen/Vehicles Who Were Also Allergic to Formaldehyde, by Study Period

Allergen	1992–1994	1994–1996	1996–1998	1998–2000	2001–2002	2002–2004	Pooled Data
IU aq	37/63	39/79	45/80	69/113	55/84	51/76	296/495 (59.68%)
IU pet	49/84	60/94	67/103	86/143	98/145	100/144	460/713 (64.52%)
DU aq	62/105	79/111	69/102	108/152	126/155	105/126	549/751 (73.10%)
DU pet	59/101	70/112	80/116	113/176	103/148	119/173	544/826 (65.86%)
DM aq	52/63	52/63	51/66	75/92	94/107	58/67	382/458 (83.41%)
DM pet	43/56	63/69	72/85	93/117	112/133	93/111	476/571 (83.4%)

aq = aqueous; DM = dimethylol dimethyl (DMDM) hydantoin; DU = diazolidinylurea; IU = imidazolidinylurea; pet = petrolatum.

Formaldehyde Reactivity Comparisons between Allergens in Aqueous Vehicle

In evaluating the proportion of patients who reacted to formaldehyde between allergen groups, there were statistically significant differences in the formaldehyde reactivity rate between all aqueous groups. IU aq-positive patients were 1.22 times (95% CI, 1.12–1.33; $p < .0001$) and 1.39 times (95% CI, 1.28–1.52; $p < .0001$) more likely to be formaldehyde positive than were those who reacted positively to DU aq and DM aq, respectively. DU aq-positive patients were 1.15 times more likely to be formaldehyde allergic than were DM aq-positive patients (95% CI, 1.07–1.21; $p < .0001$).

Allergy to DU, IU, and DM in Formaldehyde-Allergic Patients

As shown in Table 3, when formaldehyde-sensitive patients are checked for reactivity to DU, IU, and DM, less than a third of these formaldehyde-allergic patients are also sensitive to one of these preservatives (12 to 23% for IU, 16 to 31% for DU, 13 to 28% for DM). More formaldehyde-sensitive patients react to DU than to IU or DM.

Role of Allergen Vehicle in Detecting “True” Formaldehyde Allergy

In this 6-year period, 2,398 patients tested had positive reactions to at least one of the seven allergens (Table 4). Using this as the gold standard for “true” allergy, the sensitivities for petrolatum-based antigens ranged from 21 to 29% and were statistically significantly higher than those of the corresponding antigens in an aqueous vehicle (Table 5). More-conservative definitions of “true” allergy (of the seven, at least two, three, or four positive test results) yielded higher sensitivity rates (up to 86%). Again, for all gold standard definitions of “true” allergy (except for DU in three of the seven tests, which just approached statistical significance), the petrolatum-based allergen was statistically significantly more sensitive than the same allergen in an aqueous vehicle.

Discussion

Determination of the Best Vehicle

If only an aqueous test or only a petrolatum test is used to detect sensitivity to IU, DU, or DM, our data clearly

Table 3. Patients Allergic to Formaldehyde Who Were Also Allergic to the Tested Allergens

Allergen	1992–1994	1994–1996	1996–1998	1998–2000	2001–2002	2002–2004	Pooled Data
IU aq	37/276	39/277	45/315	69/524	55/412	51/435	296/2,239 (13.22%)
IU pet	49/275	60/277	67/309	86/526	98/408	100/434	460/2,229 (20.6%)
DU aq	62/267	79/275	69/317	108/525	126/405	105/431	549/2,220 (24.73%)
DU pet	59/272	70/278	80/314	113/534	103/407	119/433	544/2,238 (24.31%)
DM aq	52/268	52/273	51/314	75/522	94/405	58/430	382/2,212 (17.27%)
DM pet	43/273	63/274	72/313	93/527	112/404	93/432	476/2,223 (21.41%)

aq = aqueous; DM = dimethylol dimethyl (DMDM) hydantoin; DU = diazolidinylurea; IU = imidazolidinylurea; pet = petrolatum.

Table 4. Descriptive Data on Patients and Reactions Used in Sensitivity Analysis

	Definition of "True" Allergy*			
	One of Seven	Two of Seven	Three of Seven	Four of Seven
No. of Patients	2,398	1,109	731	473
No. of Reactions				
Antigen				
Formaldehyde	1,889	878	603	413
IU aq	415	348	281	229
IU pet	615	573	492	376
DU aq	619	586	495	363
DU pet	700	651	535	406
DM aq	383	352	306	233
DM pet	505	472	397	316

aq = aqueous; DM = dimethylol dimethyl (DMDM) hydantoin; DU = diazolidinylurea; IU = imidazolidinylurea; pet = petrolatum.

*The number of the seven allergens to which the patient has a positive reaction.

support petrolatum as the most sensitive vehicle for each allergen. Because there is no gold standard for "true" allergy, we used an increasing conservative definition of "true" allergy. For all of these definitions and with each allergen, we found that petrolatum-based allergens were more sensitive than the same allergen in an aqueous formulation.

Relationship of Formaldehyde-Releasing Preservatives to Formaldehyde Allergy

Most patients who are allergic to IU, DU, or DM are also reactive to formaldehyde, yet most formaldehyde-allergic patients are not reactive to the three preservatives. To understand this relationship, it is helpful to consider the amount of formaldehyde released in the patch tests and the

Table 5. Comparison of Sensitivity of Vehicles by Various "Gold Standard" Definitions

Antigen	Sensitivity (%)		z Value
	Petrolatum	Aqueous	
At Least 1 of 7 Test Results Positive ($n = 2,398$)			
IU	26	17	8.2*
DM	21	12	11.23*
DU	29	26	3.3*
At Least 2 of 7 Test Results Positive ($n = 1,109$)			
IU	52	31	10.52*
DM	43	32	5.73*
DU	59	53	2.85*
At Least 3 of 7 Test Results Positive ($n = 731$)			
IU	67	38	12.02*
DM	54	42	5.01*
DU	73	68	2.19
At Least 4 of 7 Test Results Positive ($n = 473$)			
IU	79	48	6.43*
DM	67	49	6.21*
DU	86	77	3.41*

DM = dimethylol dimethyl (DMDM) hydantoin; DU = diazolidinylurea; IU = imidazolidinylurea; n = number of patients meeting this "gold standard" definition.

Tukey honest significant difference critical value = 2.85.

* $p < .05$.

amount to which patients are exposed when actually using products containing these preservatives.

It is also useful to remember the threshold concentration for the elicitation of reactions that has been found for formaldehyde. Flyvholm and colleagues found that at less than 250 parts per million (ppm), reactions to formaldehyde were not observed.⁵ And in occluded patch tests, only 3 of 20 subjects reacted to concentrations of 500 ppm or less. More recently, the “no reaction” level for DU in product use testing was established at 0.05%, which is 500 ppm (with a formaldehyde release of 130 ppm).⁶ As shown in Table 6,^{6–8} when a patient has positive patch-test reactions to both formaldehyde and DM, the patient would have to have a sensitivity to formaldehyde at the 200 ppm threshold for formaldehyde allergy to explain both reactions. Likewise, if the patient shows positive reactions to IU and formaldehyde, that patient would have to have a sensitivity to formaldehyde at as little as 90 ppm for formaldehyde allergy to explain both reactions. Since that level of reactivity is exceedingly rare (having been reported only once, by Jordan and colleagues in a test-retest setting that found a formaldehyde reaction to 30 ppm),⁹ it would be more accurate to say that true sensitivity to both compounds exists and was environmentally acquired. The same cannot be said of DU because the patch test exposes the patient to an amount of formaldehyde sufficient for formaldehyde allergy alone to possibly explain the reaction to DU (2,500 ppm). The concentration of formaldehyde in a DU patch test would have triggered a reaction in one-quarter of Flyvholm’s subjects.⁵ That figure parallels our data showing that 23 to 25% of formaldehyde-sensitive patients also react to DU

(see Table 3). Given these facts, one would expect that more formaldehyde-sensitive patients would react to DU than to either IU or DM. Our findings support this (see Table 3).

Statistical Relationship between Formaldehyde Allergy and Formaldehyde Releasers

Because we found no detectable formaldehyde release from petrolatum-based IU as compared to aqueous material,⁴ we investigated whether there was a difference between the number of patients who react positively to either IU aq or pet and who are also identified as formaldehyde positive. We did the same for DU and DM. We found no statistically significant difference in the number of patients who were simultaneously positively reactive to IU aq or pet and to formaldehyde ($p = .09$), nor was there a difference between patients reactive to DM aq and pet who were simultaneously reactive to formaldehyde ($p = .98$). This might have been expected because the amount of formaldehyde released by both of these items in a patch test does not exceed the common threshold for eliciting formaldehyde sensitivity. However, patients allergic to DU aq were significantly more likely to be formaldehyde sensitive ($p = .0018$) than were patients who were allergic to DU pet. This fits well with the notion that petrolatum-based materials do not facilitate formaldehyde release whereas those based in aqueous vehicles do.

Considering the amount of formaldehyde released by the three preservatives, it would be expected that IU-positive patients would be least likely to also react to

Table 6. Patch-Test and Cosmetics Concentrations of Formaldehyde-Releasing Preservatives and the Amount of Formaldehyde Released

Allergen	Patch-Test Concentration		Released in Patch Test (ppm)
	%	ppm	
IU*	2	20,000	90
DU†	1	10,000	2,500
DM*	1	10,000	200
	Cosmetics Concentration		Released in Cosmetics (ppm)
	Range (%)	ppm	
IU*	0.03–0.2	300–2,000	1.35–9.00
DU†	0.1–0.5	1,000–5,000	246–1,233
DM*	0.1–1.0	1,000–10,000	20–200

DM = dimethylol dimethyl (DMDM) hydantoin; DU = diazolidinylurea; IU = imidazolidinylurea; ppm = parts per million.

*Information from Fransway AF.⁷

†Information from Zachariae C et al⁶; Karlberg AT et al.⁸

formaldehyde. That is the case; IU-positive patients were significantly less likely to be formaldehyde sensitive than were both DU- and DM-positive patients ($p < .0001$). As DM releases less formaldehyde than DU, it might be expected that fewer patients sensitive to DM would be formaldehyde sensitive as compared to those sensitive to DU, and that was the case ($p < .0001$).

Environmental Exposure to Formaldehyde from Formaldehyde Releasers

Table 6 shows the amount of formaldehyde released from typical cosmetic formulations. The amounts range from a low of less than 10 ppm from IU to a high of 1,200 ppm from DU; the latter is above the threshold for formaldehyde elicitation. A recent study found that the safe concentration for DU was 0.05%, which is 500 ppm DU and releases 130 ppm formaldehyde.⁶ This study involved use tests rather than patch tests to establish the safe values

Studies of another sensitizer, methylchloroisothiazolinone/methylisothiazolinone (MCI/MI), found that the lower limit of detectable allergy with patch tests was 30 ppm; yet, use tests established that a product containing 15 ppm could cause allergic contact dermatitis in individuals who were patch test negative at 200 to 300 ppm MCI/MI.¹⁰ Because use tests involve many exposures over a 1-, 2-, or even a 4-week period on normal skin, we can conclude that the low levels of exposure to formaldehyde that occur below the patch-test threshold of reactivity can still result in clinical allergy. Because 49 to 69% of IU-sensitive patients also react to formaldehyde, we might question whether exposures of less than 10 ppm are problematic. However, it would be unreasonable to assume that users of cosmetics would have been exposed only to IU-containing cosmetics. It is more likely that they would have been exposed to numerous products that release formaldehyde (such as DU-preserved items) and that might provide an exposure that results in clinical allergy. Zachariae and colleagues provided a target for industry to shoot for, namely, no more than 130 ppm of formaldehyde released by a preservative system.⁶

There are several limitations to this study. First, doubtful reactions have been found to have relevance based on repeated open application testing (ROAT) in up to 44% of cases.¹¹ The same study found an 80% relevance to + or ++ reactions. For this analysis, we limited our consideration to the higher standard of at least a + reaction for inclusion as a true positive since ROAT testing was not done. Therefore, we may have missed relevant positives.

Second, we used sensitivity as our unit of effectiveness. This requires a gold standard for defining allergy. Because no gold standard exists and because we did not determine the reproducibility of reactions, we used a range of definitions that in many cases included the test in question. Although this could confound results, we feel that given the large numbers of patients and similar results in descriptive and pairwise analyses, our conclusions supporting petrolatum as the best vehicle are justified. Third, a value judgment must be made as to whether a single test is sufficient for clinical work. Previously we called attention to a shift in our data.⁴ We reported a trend that supported the use of IU pet over IU aq (see Table 1, 2002–2004 data).⁴ A chemical analysis of the IU aq and IU pet syringes from this study period found the aqueous vehicle to be correctly formulated at 2%, but the petrolatum-based material was formulated at 3%. This raises the possibility that a higher concentration of allergen in petrolatum might be able to overcome the need for testing with both vehicles. An investigation of that possibility has not yet been undertaken. It is possible that some variation in our results could be due to differences in the amount of aqueous and petrolatum test material applied to the test patches. However, considering the number of study centers and the number of patch tests performed, it is unlikely that there would be any consistent greater or lesser amount of allergen material at any specific site that could have influenced the results.

More research is needed on the relationship between different formaldehyde-releasing agents and relevance. A study of the relationship between patch-test data obtained from dilution series and 28-day use tests would help establish the relationship between the patch-test vehicle and concentration chosen for clinical diagnostic work and allergic contact dermatitis. Actual measurement of formaldehyde release upon skin contact from different vehicles could also help clarify the relationship between formaldehyde and the preservatives IU, DU, and DM.

Conclusion

Petrolatum is a significantly more sensitive vehicle than is water (aqueous vehicle) for determining allergy to imidazolidinylurea, diazolidinylurea, and dimethylol dimethyl (DMDM) hydantoin.

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