MEMORANDUM

To: Judy Facey, EPA; Alexander Kliminsky, EPA; Lori Brunsman, EPA; Colleen Rossmeisl, EPA; Elizabeth Donovan, EPA; John Allran, EPA; Michelle Arling, EPA

From: Sorina Eftim, ICF; Melissa Miller, ICF

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Re: Statistical Review of Data from Two Formaldehyde Human Patch Test Studies: Flyvhom et al. (1997) and Fischer et al. (1995)

1. Introduction and Summary

ICF was asked with reviewing of the statistical methods, and identify additional statistical reanalysis of the data provided in the Flyvhom et al. (1997) and Fischer et al. (1995) formaldehyde human patch test studies, as follows:

Fischer T, Andersen T, Bengtsson U, Frosch P, Gunnarsson Y, Kreilgård B, Menné T, Shaw S, Svensson L, Wilkinson J. (1995). Clinical Standardization of the TRUE Test™ Formaldehyde Patch. Book chapter : Surber C, Elsner P, Bircher AJ (eds): Exogenous Dermatology: Advances in Skin-Related Allergology, Bioengineering, Pharmacology and Toxicology, Basel, Karger, v122, pp 24-30.

Flyvholm MA, Hall BM, Agner T, Tiedemann E, Greenhill P, Vanderveken W, Freeberg FE and Menné, T. (1997) Threshold for occluded formaldehyde patch test in formaldehyde-sensitive patients. Relationship to repeated open application test with a product containing formaldehyde releaser. Contact Dermatitis. 36(1):26-33. doi: 10.1111/j.1600-0536.1997.tb00918.x. PMID: 9034684

EPA's attempts to obtain the raw data from the study authors was unsuccessful. For each of these two studies, ICF reviewed and attempted to reproduce the statistical analyses described in the studies, or to reanalyze data where feasible to confirm findings. Each section of this memorandum briefly summarizes the studies discussed here, and summarizes the statistical review, accompanied by relevant figures, tables, and supplemental material from the two studies. No additional statistical were feasible for either of the two studies, thus no statistical code accompanies this memorandum.

2. Fisher et al., 1995

This study was conducted in order to develop the TRUE Test formaldehyde patch, using N-hydroxymethylsuccinimide (HMS) as a pro-allergen candidate for the patch. The patches are

formaldehyde releasers with the amounts formaldehyde equivalent to 0.01, 0.02, 0.03, 0.04, 0.08, 0.10, 0.12, 0.15, 0.19, 0.26, 0.33, 0.57 and 1.12 mg/cm^2. For comparison, control patch tests were performed with formaldehyde 1 % in water and dilutions thereof.

Healthy volunteers without known sensitivity to formaldehyde, consecutive patients with contact dermatitis, and patients with previous patch tests to formaldehyde were included in the study. There were 5 experiments conducted, described briefly below.

Group 1 (*First irritant study)*: Nine healthy volunteers (3 women, 6 men) with no known skin disease and not sensitive to formaldehyde were patch tested with TRUE test HMS patches with 0.12, 0.57, and 1.12 mg/cm^2 formaldehyde.

Group 2 (*First Dose-Response Study in Formaldehyde-Sensitive Patients*): 25 patients with previous positive patch test reactions to formaldehyde were rechallenged with a) TRUE test HMS patches with 0.02, 0.03, 0.04, 0.08, 0.12, and 0.15 mg/cm^2 formaldehyde, and b) formaldehyde aqueous tests at 0.015, 0.032, 0.063, 0.13, 0.25, 0.5 and 1.0%.

Group 3 (*First Dose-Response Study on Consecutive Eczema Patients*): 120 patients with contact dermatitis were tested with a) TRUE test HMS patches with 0.01, 0.02, 0.04, 0.08, 0.12, and 0.15 mg/cm2 formaldehyde and b) formaldehyde aqueous test at 1.0%.

Group 4 (*Second Dose-Response Study in Formaldehyde-Sensitive Patients*): 24 patients with previous positive patch test reactions to formaldehyde were tested with a) TRUE test HMS patches with 0.15, 0.20, 0.26, and 0.33 mg/cm^2 formaldehyde, and b) formaldehyde aqueous tests at 0.1, 0.3, and 1.0%.

Group 5 (*Second Dose-Response Study on Consecutive Eczema Patients*): 255 consecutive patients (96 males and 159 females) with contact dermatitis were tested with a) TRUE Test HMS patches with 0.11, 0.19, 0.26 and 0.33 mg/cm^2 formaldehyde; b) formaldehyde aqueous tests 1.0%; c) formaldehyde standard test as used at the different clinics either in aqueous or petrolatum vehicle and placebo patches, and (d) vehicles PVP, and succinimide equivalent to the amount included in the active HMS-TRUE Test patches.

Results

Authors state that “descriptive methods were used”. Only for Group 5 there is a “Statistical results” section. Methods and software used are not described in the study.

Group 1 (*First irritant study)*: Five out of nine patients had irritant reactions to the 1.12 mg/cm^2 patch, and two of them also had irritant reactions to the 0.57 mg/cm^2 patch. No irritant reactions occurred with the 0.12 mg/cm^2 patch.

Data is not shown; hence the statement cannot be confirmed, and no statistical analyses were feasible.

Group 2 (*First Dose-Response Study in Formaldehyde-Sensitive Patients*): 17 of 25 patients had positive reactions with both methods; 2 patients had positive reactions to TRUE Test formaldehyde patches only; 5 patients had positive reactions to formaldehyde aqueous tests only. Data shown in Table 1. Comparisons of the two methods indicate that a TRUE Test formaldehyde patch with 0.15 mg/cm^2

formaldehyde induces reactions equivalent to formaldehyde aqueous tests in a concentration between 0.5 and 1%.

Minimal Finn Chamber formaldehyde concentration, %	Minimal TRUE Test formaldehyde dose, mg/cm^2							
	neg	0.15	0.12	0.08	0.04	0.03	0.02	0.01
neg	1[a]	1		1				
1.0	1	1			1			
0.5			1	1				
0.25	3			1		4		
0.13	1			2		1		
0.063						1		2
0.032								1
0.015				1				

[a] Questionable to both methods.

Table 1. Minimal dose/concentration for positive reactions in patients tested with TRUE Test formaldehyde patches and with Finn Chambers dosed with 15 ~I formaldehyde in water (copied from Fisher et al., 1995)

The statement of interest is qualitative, showing that 5 formaldehyde-sensitive patients had positive reactions to formaldehyde aqueous tests only. As illustrated in Table 1, patients not exposed to TRUE test HMS patches had positive reactions in formaldehyde aqueous tests at 1.0% (n=1), 0.25% (n=3) and 0.13% (n=1).

Group 3 (*First Dose-Response Study on Consecutive Eczema Patients*): Three of 120 participants had "positive and relevant allergic reactions" to the patch and aqueous test; no participants had irritant reactions.

Data is not shown; hence statement cannot be confirmed, and no statistical analyses are feasible.

Group 4 (*Second Dose-Response Study in Formaldehyde-Sensitive Patients*): 13/24 participants had positive reactions to both the patch and the aqueous test. Comparison of the two methods showed that TRUE Test formaldehyde patches with 0.20 and with 0.26 mg/cm2 formaldehyde gives an equivalent test response as a formaldehyde aqueous 1% test.

Data is not shown; hence statement cannot be confirmed, and no statistical analyses are feasible.

Group 5 (*Second Dose-Response Study on Consecutive Eczema Patients*): All four TRUE Test formaldehyde patch concentrations elicited reactions in at least one participant. Nine patients (of 255 tested, 3.5%) had positivity to formaldehyde: 4 to both methods, 2 to formaldehyde aqueous tests with IR/? reactions on TRUE Test patches, and 3 to TRUE Test formaldehyde patches with IR/? reaction for 1 and negative for 2 on the formaldehyde aqueous tests.

Calculation of optimal dose for TRUE Test patch to illicit irritation (*Statistical Results)*: The acceptable frequency of irritation in group 5 was set to at most 10% and based on assumed point estimate of 5% for the recommended dose with n=255.The upper 95% confidence limit for this frequency was about 7.5% and indicates that the HMS dose equivalent to 0.18 mg/cm^2 formaldehyde is acceptable.

The data is available in table 2. Although a power calculation can be reproduced, since irritation was not the focus of the review, no further analyses were needed.

Patient No.	Reactions to TRUE Test HMS patches formaldehyde, mg/cm^2				Control formaldehyde 1% aq.
	0.33	0.26	0.19	0.11	
Positive reactions					
106	++	IR/?	+	–	+
123	++	+	–	–	IR/?
144	+	+	+	IR/?	+
228	++	++	–	+	–
250	++	++	++	++	–
347	+	–	–	+	++
519	IR/?	IR/?	IR/?	IR/?	+
529	IR/?	IR/?	IR/?	–	+
550	+	+	+	+	+
Irritant reactions					
112	IR/?	IR/?	IR/?	–	IR/?
146	IR/?	IR/?	IR/?	–	IR/?
212	IR/?	–	–	–	–
224	IR/?	–	–	–	–
244	IR/?	–	–	–	–
344	–	–	–	–	IR/?
439	IR/?	IR/?	–	–	–
510	IR/?	IR/?	–	–	–
516	IR/?	IR/?	IR/?	–	–
526	IR/?	IR/?	IR/?	–	IR/?

Table 2. Reactions to TRUE Test HMS patches and formaldehyde 1% control Finn Chamber patches in 255 consecutive patients (coped from Fisher et al., 1995)

3. Flyvholm et al. 1997

Twenty formaldehyde-sensitive patients and a control group of 20 healthy volunteers were included in the study. Occluded patch tests were conducted with formaldehyde solutions in concentrations of 0, 25, 50, 250, 500, 1,000, 5,000 and 10,000 ppm (equivalent to 0, 0.0025 %, 0.0050 %, 0.025 %, 0.050 %, 0.1 %, 0.5 %, and 1 %). Non-occluded patch tests were conducted with formaldehyde solutions in concentrations of 0, 25, 50, 100, 250, 500, 1,000, 5,000 and 10,000 ppm and repeated open application test (ROAT) for one week with a leave-on cosmetic product containing on average 300 ppm (equivalent to 0.03 %) formaldehyde, were carried out simultaneously on each subject.

Results

Occluded patch test: 19 of the 20 patients reacted to 10,000 ppm formaldehyde as the lowest sensitization level, 9 reacted to 5,000 ppm, 3 reacted to 1,000 ppm, 2 reacted to 500 ppm and 1 reacted to 250 ppm (Figure 2 in Flyvholm 1997, shown below) . The study concluded that the threshold concentration for occluded patch test to formaldehyde in formaldehyde-sensitive patients was 250 ppm.

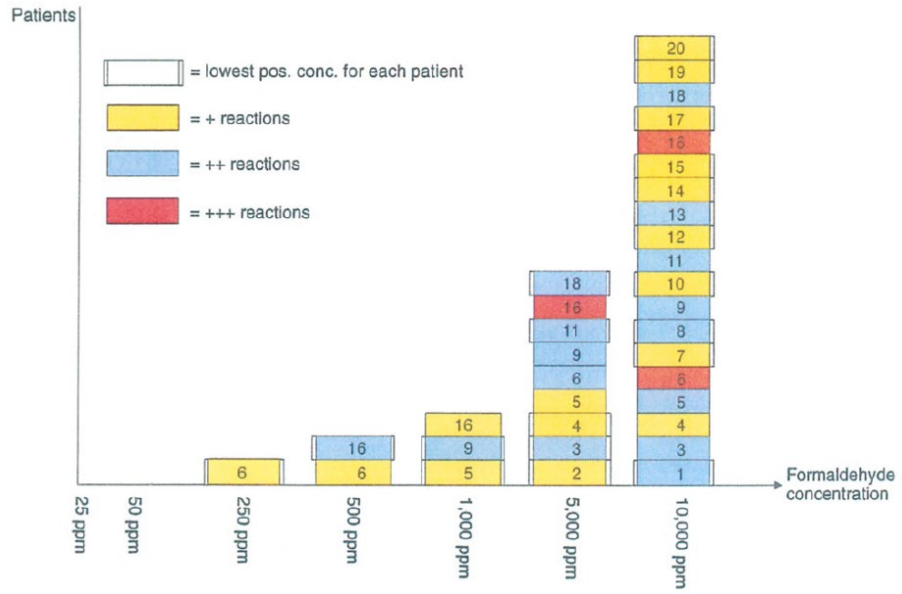

Figure 1 Positive patch test reactions to formaldehyde in occluded patch testing or in diagnostic patch testing (10,000 ppm) among 20 formaldehyde-sensitive eczema patients. N.B. The number in each box is the patient number (copied from Flyvholm et al. 1997).

Strength of reaction to the 10,000-ppm formaldehyde test by lowest dose to elicit a reaction in the diagnostic patch test: One patient had a + reaction to 10,000 ppm formaldehyde in the diagnostic patch test but reacted to less than 10,000 ppm formaldehyde in the occlusion patch test. All participants that reacted to lower formaldehyde concentrations in the diagnostic patch test had ++ or +++ reactions to 10,000 ppm formaldehyde.

Four patients reacted to low formaldehyde concentrations (1 ,000 ppm and lower). Clear dose-response with negative and/or follicular reactions to the lowest concentrations and + + or + + + to the highest concentrations was observed for these patients. (**Table 3**)

	Dilution series						Standard series 1% 10,000 ppm
Patient number	25 ppm	250 ppm	500 ppm	1,000 ppm	5,000 ppm	10,000 ppm	
#5	2 foll.	–	–	+	+	++	++
#6[a)]	–	+	+	foll.[b)]	++	++	+++
#9	–	3 foll.	foll.	++	++	++	++
#16	–	foll./+ ?[c)]	++	+	+++	+++	+?

[a)] Retest 1 year later with 50 ppm, 100 ppm and 250 ppm was negative.
[b)] Follicular reactions: more than 3 follicular papules. All follicular reactions were considered negative.
[c)] Even redness and follicular papules in the test area.

Table 3. Patch test reactions for 4 patients reacting to low formaldehyde concentrations (1,00 ppm and lower) in the occluded patch test (copied from Flyvholm et al. 1997)

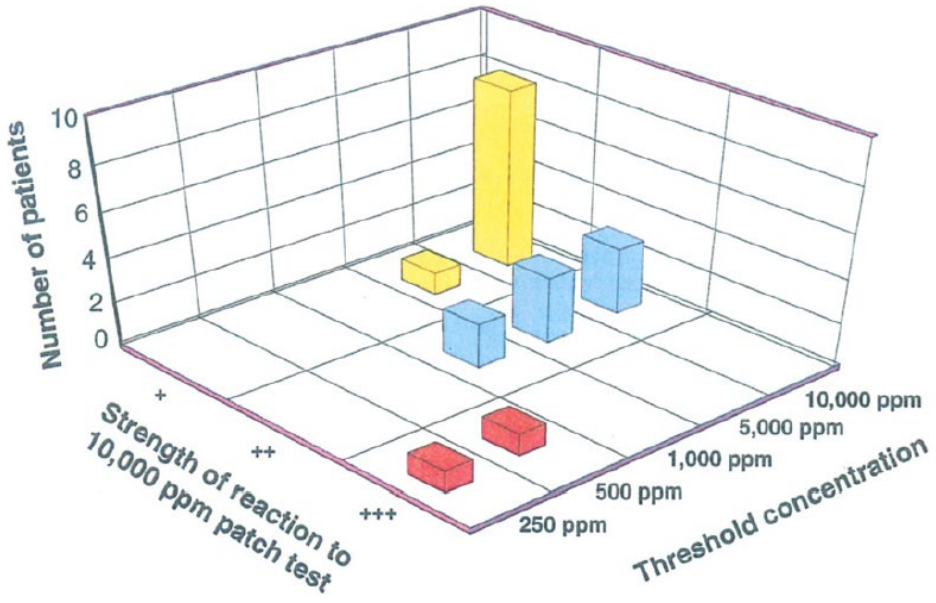

Figure 2 Lowest formaldehyde concentrations giving positive reactions in occluded patch testing, compared to the strength of the reactions in the diagnostic patch testing (10,000 ppm) among 19 formaldehyde-sensitive eczema patients (copied from Flyvholm et al. 1997).

Summary data is available in text, and in figures 1 and 2, and Table 3. Given the very small number of patients reacting to low formaldehyde concentrations, it is not possible to formally quantify a dose-response model due to low statistical power.

Non-occluded patch test: No positive reactions were observed. Six out of 20 patients had weak reactions showing erythema without infiltration or follicular reactions (not meeting the criteria for positive reactions).

Data is not shown; hence statement cannot be confirmed, and no statistical analyses are feasible.

ROAT: No positive reactions were observed. Five out of 20 patients had weak reactions showing erythema without infiltration or follicular reactions (not meeting the criteria for positive reactions).

Data for the 5 patients with reactions in ROAT is available in Table 4. However, since no positive reactions were observed. Formal comparison of the occluded vs. the ROAT are not possible for all the patients given the lack of raw data.

No positive reactions were observed in the control group to any of the test procedures.

Data is not shown; hence statement cannot be confirmed, and no statistical analyses are feasible.

Table 3. Reactions in the ROAT test and lowest positive formaldehyde concentrations in the occluded patch test for patients with follicular reactions in the ROAT

	Occluded patch test		Repeated open application test (ROAT)		
Patient number	Lowest positive concentrations (ppm)	$\mu g/cm^2$	Reactions to the ROAT-cream	conc.[a)] (ppm)	dose ($\mu g/cm^2$)
#7	10,000 (+)	0.30	1 foll. (day 5, 6)	291	0.71
#9	1,000 (++)	0.03	foll. (day. 7)	280	2.92
#10	10,000 (+)	0.30	2 foll. (day. 2)	258	0.84
#13	10,000 (++)	0.30	2 foll. (day 3)	289	1.12
#18	5,000 (++)	0.15	5 foll. (day 7)	367	1.81

[a)] The cream used by the individual patients was analyzed for free formaldehyde.

Table 4. Reactions in the ROAT test and lowest positive formaldehyde concentrations in the occluded patch test for patients with follicular reactions in the ROAT (copied from Flyvholm et al. 1997).

References

Fischer T, Andersen T, Bengtsson U, Frosch P, Gunnarsson Y, Kreilgård B, Menné T, Shaw S, Svensson L, Wilkinson J. (1995). Clinical Standardization of the TRUE Test™ Formaldehyde Patch. Book chapter : Surber C, Elsner P, Bircher AJ (eds): Exogenous Dermatology: Advances in Skin-Related Allergology, Bioengineering, Pharmacology and Toxicology, Basel, Karger, v122, pp 24-30.

Flyvholm MA, Hall BM, Agner T, Tiedemann E, Greenhill P, Vanderveken W, Freeberg FE and Menné, T. (1997) Threshold for occluded formaldehyde patch test in formaldehyde-sensitive patients. Relationship to repeated open application test with a product containing formaldehyde releaser. Contact Dermatitis. 36(1):26-33. doi: 10.1111/j.1600-0536.1997.tb00918.x. PMID: 9034684