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OPPT/Existing Chemicals Risk Assessment Division/RAB 6 **Date**: _____

DRAFT DATA EVALUATION RECORD

STUDY TYPE: Skin Sensitization non-guideline (Repeat Insult Patch Test) - Human

PC CODE: 043001 DP BARCODE: N/A

TASK GROUP No.: NA

TEST MATERIAL (PURITY): Formaldehyde, N-hydroxymethylsuccinimide (HMS) (% purity not provided)

SYNONYMS: None

CITATIONS: Fischer, T; Andersen, K; 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. In Exogenous Dermatology: Advances in Skin-Related Allergology, Bioengineering, Pharmacology and Toxicology. Current Problems in Dermatology Edited by Surber C and Elsner P. volume 22:24-30. Basel: S Karger, AG. DOI: https://doi.org/10.1159/isbn.978-3-318-03459-2

EXECUTIVE SUMMARY:

This publication presents the clinical data used to develop the TRUE Test™ formaldehyde patch test system, a "ready to use system with a high degree of standardization" (Fischer et al, 1995, p. 25). Because formaldehyde is a highly reactive water-soluble gas, the incorporation of formaldehyde into a dry patch test can be challenging. Therefore, the study authors developed the TRUE Test ™ formaldehyde patch, which utilizes the pro-allergen N-hydroxymethylsuccinimide (HMS). Upon exposure to moisture in the skin, HMS in the TRUE Test dermal patch test undergoes hydrolysis to form formaldehyde and succinate. The study assessed the dose response of the TRUE Test system versus standard formaldehyde in aqueous solution patch tests (Finn Chamber system) in a range of concentrations for formaldehyde-sensitive individuals as well as those with contact dermatitis. The study aim was to correlate results of the TRUE Test with standard patch testing, as well as establish guidance for concentrations for standard allergen testing with the TRUE Test system to detect contact allergy

without inducing irritation. Five different groups were utilized to determine levels at which irritation versus sensitivity occur, as well as a comparison of positive reactions to the TRUE Test system compared to aqueous formaldehyde patch tests at a range of test concentrations. The focus of this data evaluation record (DER) is on Group 2, where a dilution series was tested with both the TRUE Test and formaldehyde 1% aqueous patch test systems in formaldehyde-sensitive subjects, as this provides information that may inform on the minimum elicitation threshold for formaldehyde. Although Group 4 also tested a dilution series for the TRUE Test system, very limited information was provided in the results section and was therefore of limited utility.

Group 2 testing for each system was conducted at 0.02, 0.03, 0.04, 0.08, 0.12 and 0.15 mg/cm^2 for the TRUE Test system and at 0.015, 0.032, 0.063, 0.13, 0.25, 0.5 and 1.0 % (equivalent to 0.0045, 0.0096, 0.019, 0.039, 0.075, 0.15 and 0.3 mg/cm^2) in the Finn Chamber system with standard formaldehyde in aqueous solution. Output across the varying doses from the two test systems gave consistent results. The lowest dose for positive reaction from the Finn Chamber was 0.015 % or 0.0045 mg/cm^2 (4.5 µg/cm^2) versus 0.01 mg/cm^2 (10 µg/cm^2) from the TRUE Test system, reflecting the lowest concentration tested for each system. The LOAEL value from this study is 0.015 % (equivalent to 0.0045 mg/cm^2 or 4.5 µg/cm^2); no NOAEL value is established.

This study is classified as **acceptable/non-guideline.** It was not submitted by the registrant for fulfillment of a guideline requirement. The study provides quantitative information on elicitation thresholds for formaldehyde in humans and can be considered as part of endpoint selection and POD derivation for elicitation of dermal sensitization from dermal exposure.

COMPLIANCE: This is a published study and as such, does not contain statements of compliance or confidentiality.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Materials: Formaldehyde, N-hydroxymethylsuccinimide (HMS)
   Description: Not provided
   Lot/Batch #: Not provided
   Purity: Not provided

   CAS # of TGAI: 50-00-0, 5146-68-9

2. Sample preparation, storage, and analysis: No information was provided on the analytical grade of formaldehyde. The study authors reported that the TRUE Test ™ patches were stable for at least 24 months at 8°C; however, no data were reported. A positive control was not used in this study. It is important to ensure the carrier succinimide does not cause toxicity. The study authors reported that: "succinimide has been tested with 20% topical applications and 5% intradermal injections in guinea pigs, resulting in no skin irritation; guinea pig maximization tests with succinimide showed no allergic potential; and its clinical use in the treatment of nephrolithiasis and epilepsy at 10 g/day showed no adverse effects other than decreased serum levels of vitamin B_6" (Fischer, et al., 1995, p. 25).

TRUE test patches were formulated with the proallergen HMS in the vehicle polyvidone (PVP). Additional carriers or vehicles discussed in Fischer without further details provided are water and petrolatum.

B. STUDY DESIGN and METHODS:

Study Participants

Regarding subjects included in the study, the publication states: "Healthy volunteers without known sensitivity to formaldehyde, consecutive patients with contact dermatitis, and patients with previous patch tests to formaldehyde were included after informed consent" (Fischer et al., 1995, p. 26).

Institutional Review Board Approval and Informed Consent

The publication states that "[t]he studies were approved by ethical committees" (Fischer et al., 1995, p. 26). No further information was provided in the study regarding this review.

Experimental Design

The following methodology on patch testing with TRUE Test system and formaldehyde 1% in water is reproduced from the publication (Fischer et al. 1995, pp. 25-26):

"Patches were formulated from the proallergen HMS in the vehicle polyvidon (PVP) by printing it on flexible polyester carrier foil sheets. After drying, the printed sheets were cut in patches, mounted on hypoallergenic tape and covered with protective siliconized polyethylene foil. The test strips were packed with desiccant paper in an air-tight laminated envelope keeping the patch absolutely free from water.

HMS in PVP was formulated as TRUE Test patches with the amounts of 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; analytical values obtained with colorimetric method after hydrolysis. A TRUE Test patch with 0.81 mg/cm^2 HMS contains 0.19 mg/cm^2 of formaldehyde and will expose the skin to the same amount of formaldehyde as a Finn Chamber test with 15 µL 1% formaldehyde solution. The area of the TRUE Test patch is 0.81 cm^2.

For the purpose of comparison, control patch tests were performed with formaldehyde 1% in water and dilutions thereof (Chemotechnique Diagnostics AB, Malmo, Sweden). 15 µL of the formaldehyde preparations were applied in Finn Chambers (EpiTest, Helsinki, Finland). The patch test series were applied on the upper back. Left/right application varied at random. With few exceptions, the test strips remained on the back for 48 h and were evaluated after 72 or 96 h. The tests were evaluated according to the ranking scale recommended by International Contact Dermatitis Research Group (ICDRG)." (Fischer et al. 1995, p. 25-26)

Five different study groups were evaluated for the test, as outlined in **Table 1**.

Table 1. Experimental design for 5 study groups.

Group Number	Number of participants and subject population (male/female breakdown, if available)	TRUE Test formaldehyde concentrations (mg/cm^2)	Formaldehyde aqueous test concentrations (%)	Formaldehyde aqueous test concentrations (mg/cm^2)[1]
1	9 healthy individuals; 3 women/6 men	0.12, 0.57 and 1.12	NA	NA
2	25 formaldehyde sensitive individuals	0.02, 0.03, 0.04, 0.08, 0.12 and 0.15	0.015, 0.032, 0.063, 0.13, 0.25, 0.5 and 1.0	0.0045, 0.0096, 0.019, 0.039, 0.075, 0.15 and 0.3
3	120 eczema/contact dermatitis individuals	0.01, 0.02, 0.04, 0.08, 0.12 and 0.15	1.0	0.3
4	24 formaldehyde sensitive individuals	0.15, 0.20, 0.26 and 0.33	0.1, 0.3 and 1.0	0.03, 0.09 and 0.3
5	255 eczema/contact dermatitis individuals; 96 male/159 female[2]	0.11, 0.19, 0.26 and 0.33	1.0	0.3

Blue indicates focus of this DER. This Group provides information that may inform on the minimum elicitation threshold for formaldehyde.

[1] Concentrations presented as % in study; converted to mg/cm^2 for easier comparison based on 15 µl applied in Finn Chamber system (0.8 cm diameter)
[2] Group 5 notes testing "A comparative study on 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." (pg. 26 of Fischer et al. 1995)

Evaluations

The study indicated patch tests were scored using "ranking scale recommended by ICDRG" (Fischer et al. 1995, pg. 26), shown in **Table 2** below. Although the study reference provided was not obtained, other publications have cited these criteria, which are summarized below.

Table 2. Patch test reading designations and descriptions (summarized from Fregert, S., 1981)

Patch test reading	Description
+?	doubtful reaction; faint erythema only
+	weak positive reaction; erythema, infiltration, possibly papules
++	strong positive reaction; erythema, infiltration, papules, vesicles
+++	extreme positive reaction; intense erythema and infiltration and coalescing vesicles
-	Negative reaction
IR	Irritant reaction of different types

Statistical Analysis

Regarding statistical analyses, the study indicated "Descriptive methods were used." (Fischer et al., 1995, p. 26). EPA, in consultation with the ICF statistics contractor determined additional statistical analyses or dose response modeling was not feasible (ICF, 2023, memorandum).

II. RESULTS

Results for each test group are summarized below.

Group 1 (*First irritant study*): Five out of nine subjects had irritant reactions to the 1.12 mg/cm2 patch, and two of them also had irritant reactions to the 0.57 mg/cm2 patch. No irritant reactions occurred with the 0.12 mg/cm2 patch.

Group 2 (*First Dose-Response Study in Formaldehyde-Sensitive Subjects*): 17 of 25 subjects had positive reactions with both methods; 2 subjects had positive reactions to TRUE Test

formaldehyde patches only; 5 subjects had positive reactions to formaldehyde aqueous tests only. 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%.

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

Group 4 (*Second Dose-Response Study in Formaldehyde-Sensitive Subjects*): 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/cm^2 formaldehyde gives an equivalent test response as a formaldehyde aqueous 1% test.

Group 5 (*Second Dose-Response Study on Consecutive Eczema Subjects*): All four TRUE Test formaldehyde patch concentrations elicited reactions in at least one participant. Nine subjects (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.

This DER is focused primarily on results from Group 2, and as such further analysis from that group is provided below. **Table 3** below is excerpted from the publication (Fischer et al. 1995, pg. 27).

Table 3. Minimal dose/concentration for positive reactions in subjects tested with TRUE Test formaldehyde patches and with Finn Chambers dosed with 15 µL formaldehyde in water (Fischer et al. 1995, Table 1, pg. 27).

Minimal Finn Chamber formaldehyde concentration, %	Minimal TRUE Test formaldehyde dose, mg/cm²							
	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.

This data is also reflected in **Table 4** below, summarized by the EPA reviewer for each test group separately. A graphical depiction of these results is provided in **Figure 1**.

Table 4. Finn Chamber and TRUE Test response rates at varying doses.[1]

Finn chamber formaldehyde concentration (%)/ dose (mg/cm²)	**Positive results in Finn Chamber (number affected/total number)[2]**	**TRUE Test formaldehyde dose (mg/cm²)**	**Positive results in TRUE Test (number affected/total number)[2]**
1 / 0.3	22/25	0.15	19/25
0.5 / 0.15	19/25	0.12	17/25
0.25 / 0.075	17/25	0.08	16/25
0.13 / 0.039	9/25	0.04	10/25
0.063 / 0.019	5/25	0.03	9/25
0.032 / 0.0096	2/25	0.02	3/25
0.015 / 0.0045	1/25	0.01	3/25

[1] Finn Chamber aqueous concentrations were provided in %, conversion to dose in mg/cm² based on 15 µl applied in Finn Chamber system (0.8 cm diameter). (e.g., the Finn Chamber concentration at 1% was converted to an equivalent dose mg/cm² as follows: 1% = 10,000 ppm = 10,000 mg/L; and (10,000 mg/L)(15 µL/Π(0.4)²)(1 L/10⁶ µL) = 0.3 mg/cm²)

[2] Total number of positive results based on assumption those reacting at lowest concentration also react to higher test concentrations.

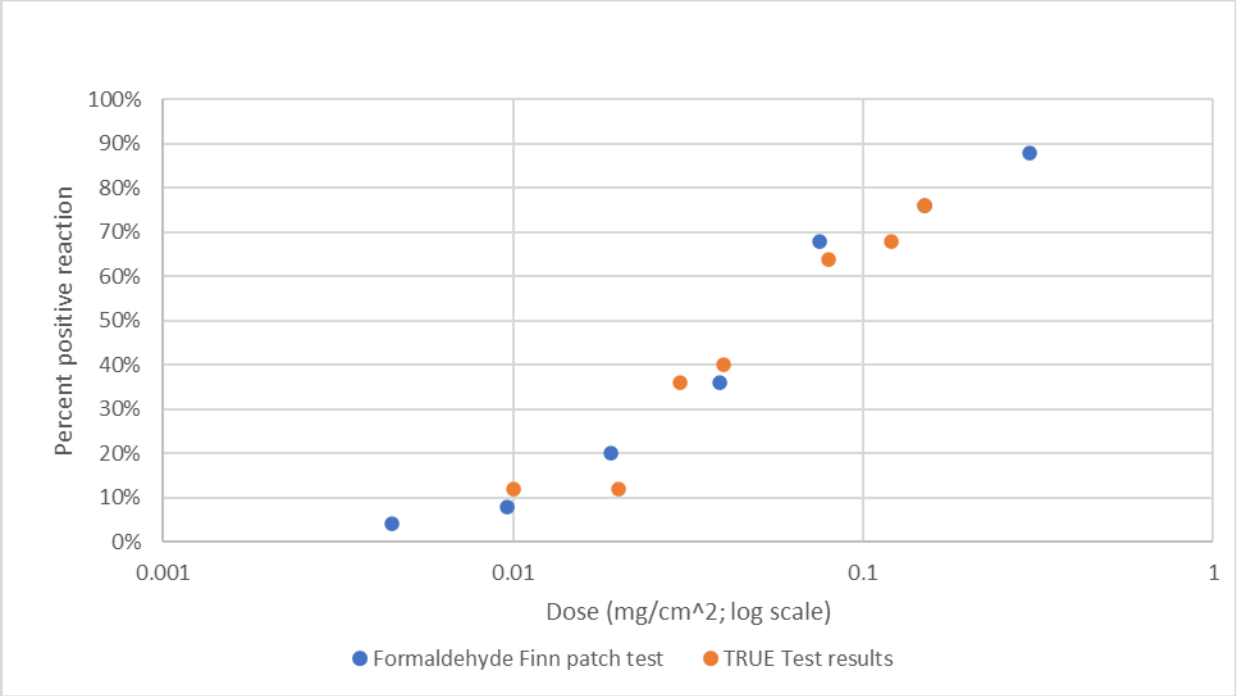

Figure 1. Percent positive reaction versus dose in formaldehyde testing with Finn Chamber and TRUE Test system.

Relatively limited information was provided on the remainder of the test results other than that summarized from the study for each group above. For Group 5, additional details were provided on subjects that had positive reactions or irritant reactions for each test system and are provided in **Table 5** below, from Fischer et al. 1995, pg. 28.

Table 5. Reactions to TRUE Test HMS patches and formaldehyde 1% control Finn Chamber patches in 255 consecutive subjects (Fischer et al. 1995, Table 2, pg. 28)

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/?

III. REVIEWER'S CONCLUSIONS:

The study aim was to correlate results of the TRUE Test with standard patch testing, as well as establish guidances for concentrations for standard allergen testing with the TRUE Test system to detect contact allergy without inducing irritation reactions. The focus of this review was on Group 2 where a dilution series was tested with both the TRUE test and formaldehyde 1% aqueous patch test systems in formaldehyde-sensitive subjects, in order to provide information for points of departure for human health risk assessment for skin sensitization.

Based on the comparative analysis of subjects tested with formaldehyde in the Finn Chamber system versus the TRUE Test system at varying concentrations/doses (Group 2), there was alignment of the test results for each system when results were normalized to a dose in mg/cm^2. The lowest dose for positive reaction from the Finn Chamber was 0.0045 mg/cm^2 (4.5 µg/cm^2) versus 0.01 mg/cm^2 (10 µg/cm^2) from the TRUE Test™ system, reflecting the lowest concentration tested for each system. Results from both tests followed reasonable dose response behavior with more individuals reacting at higher test concentrations. The LOAEL value (based on positive response to formaldehyde in aqueous solution tested in the Finn chamber system) is

0.015%. This is equivalent to a 0.0045 mg/cm^2 (4.5 µg/cm^2) based on the area and amount of solution applied to the Finn chamber system. No NOAEL values are established.

This study is classified as **acceptable/non-guideline**. It was not submitted by the registrant for fulfillment of a guideline requirement. The study provides quantitative information on elicitation thresholds for formaldehyde in humans and can be considered as part of endpoint selection and POD derivation for elicitation of dermal sensitization from dermal exposure.

As this study was obtained from the peer reviewed open scientific literature, the OPP guidance document "Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment" (USEPA, 2012) is also applicable when considering the use of open literature studies for risk assessment purposes. This guidance document presents criteria for screening of studies, and criteria for whether the study is of sufficient quality to be used quantitatively. Screening criteria include the following:

1. The toxic effects are related to defined chemical exposure;
2. The toxic effects are on an appropriate test animal species;
3. The presence or absence of toxicological effects is observed;
4. A chemical concentration/dose or application rate is reported;
5. An explicit duration of exposure is included;
6. Toxicology information is reported for the chemical of interest or its structural analog;
7. The article is available in the English language;
8. The study results are presented as a full article (i.e., not an abstract);
9. The paper is a publicly available document;
10. The paper is the primary source of the data;
11. Treatment(s) are compared to acceptable controls;
12. The location of the study (e.g., laboratory vs. field) is reported;
13. Adequate data are provided on the chemical tested (i.e., test article characterization);
14. Adequate data are provided on the species tested;
15. The study results (findings) are adequately reported; and
16. The study findings are relevant to assessing human health risks

The current study does not fully meet all of the screening criteria as outlined below:

- Criterion #11 was not fully met (there was not a true control). In this study, formaldehyde in the Finn chamber was the control to compare to the results of the TRUE Test system for validation of the test and a vehicle control was not simultaneously tested. However, in Group 5 the study states testing was also conducted with "(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." In reporting the results, although control results were not expressly reported, only positive or irritant effects were reported in the formaldehyde 1% in water or TRUE Test patch dilution series.
- Criterion #13 was not fully met (there was little information on the test article characterization). While not all characteristics are provided for the test article, there was

sufficient information to draw some conclusions about the article as a whole. The study authors reported that the TRUE Test patches were formulated with formaldehyde at reported concentrations ranging from 0.01 to 1.12 mg/cm^2, and that analytical concentrations were verified by a colorimetric method detecting formaldehyde release after hydrolysis. However, no details were provided on the colorimetric method including any data on the limit of detection or quantification. It is not clear if the values reported are nominal or analytical.

Although some uncertainties exist in this study, it is concluded that the study is appropriate for quantitative use and can be considered as part of endpoint selection and POD derivation for elicitation of dermal sensitization from dermal exposure. This is concluded based on the interpretation of the criteria as established in the guidance (USEPA, 2012) as follows:

• The dose from the open literature study is lower (i.e., more sensitive) than the lowest dose from a registrant-submitted or other open literature studies – this criterion is not met as the study did not show the lowest 'dose' in comparison to the Flyvholm et al. 1997 study, but represents a dose between the NOAEL and LOAEL from that study.
• The open literature data are reported in (or have the ability to be converted to) units that can be compared to other study results- results are reported in or can be converted to μg/cm^2, which can be compared to other studies – this criterion is met.
• Sufficient information is provided in the open literature to substantiate whether the study conclusions/endpoints/doses are accurate, reliable, and reasonable, and a judgement can be made that the study findings could potentially be replicated – it is the judgement of the reviewer that this criterion has been met.

Study limitations or weaknesses are outlined below.

Limited information was provided on the test substance, including the purity or source of formaldehyde. Formaldehyde in aqueous solution is commonly formulated with stabilizers given the relative instability of the chemical in water. Methanol is one of the common stabilizers used with formaldehyde. While methanol is an irritant, it is not a known dermal sensitizer, which is considered in weighing the impact this uncertainty may have on the study results. It was unclear when control groups were analyzed, and data was not specifically provided on the control results from Group 5.

IV. REFERENCES

Flyvhom, MA, Hall, BM, Agner, T, Edemann, ET, Greenhill, P, Vanderveken, W, Freeberg, FE And T Menne. 1997. Threshold for occluded formaldehyde patch test in formaldehyde-sensitive patients. Contact Dermatitis. 36, 26-33.

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