HSRB Human Studies Review Board

November 30, 2023

H. Christopher Frey, Ph.D.
Assistant Administrator, Office of Research and Development
United States Environmental Protection Agency
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Subject: October 11-12, 2023, EPA Human Studies Review Board Meeting Report

Dear Dr. Frey:

The United States Environmental Protection Agency (EPA) requested that the Human Studies Review Board (HSRB) provide scientific and ethics review of two research articles.

On October 11, 2023, the HSRB considered the research article by Flyvholm et al. (1997): ”Threshold for occluded formaldehyde patch test in formaldehyde-sensitive patients” published in *Contact Dermatitis*. Briefly, the research article summarizes an investigation of the eliciting threshold concentration of formaldehyde in formaldehyde-sensitive individuals in occluded and non-occluded patch tests, and to evaluate the relationship to repeated open application test (ROAT) with a product containing a formaldehyde releaser.

On October 12, 2023, the HSRB considered the research article by Fischer et al. (1995): “Clinical standardization of the TRUE Test™ formaldehyde patch” published in *Exogenous Dermatology: Advances in Skin-related Allergology, Bioengineering, Pharmacology and Toxicology. Current Problems in Dermatology, Edited by Surber C and Elsner P*. Briefly, the research article summarizes clinical studies of the development of a patch test using formaldehyde – the TRUE Test™ formaldehyde patch.

The HSRB’s responses to the charge questions for the two studies presented at the meetings on October 11 and 12, along with detailed comments and recommendations for the EPA to consider are provided in the enclosed final meeting report.

Sincerely,

Lisa Corey

Julia A. Sharp

Lisa Corey, Ph.D.
Co-Chair, HSRB

Julia Sharp, Ph.D.
Co-Chair, HSRB

Report of the U.S. Environmental Protection Agency Human Subjects Review Board

HSRB Board Members

EPA Contact

Tom Tracy, Designated Federal Officer

Disclaimer Text: This report is a consensus report written by the Human Studies Review Board (HSRB), a public advisory committee chartered under the Federal Advisory Committee Act (FACA) that provides external advice, information, and recommendations to the U.S. Environmental Protection Agency (EPA). HSRB members represent themselves, and opinions are not the views of their employer. Mention of trade names or commercial products does not constitute a recommendation for use.

Contents

List of Acronyms – Flyvholm et al. (1997)

EPA	Environmental Protection Agency
HCHO	Formaldehyde
HSRB	Human Studies Review Board
LOAEL	Lowest Observed Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
ROAT	Repeated open application test

HSRB Meeting Report – Flyvholm et al. (1997)

Flyvholm, MA, Hall, BM, Agner, T, Tiedemann, E, Greenhill, P, Vanderveken, W, Freeberg, FE and T Menné. (1997). Threshold for Occluded Formaldehyde Patch Test in Formaldehyde-Sensitive Patients. Contact Dermatitis. 36: 26-33.

Introduction

On October 11, 2023, the Human Studies Review Board (HSRB) considered the research article by Flyvholm et al. (1997): "Threshold for occluded formaldehyde (HCHO) patch test in HCHO-sensitive patients" published in *Contact Dermatitis*. Briefly, the research article summarizes an investigation of the eliciting threshold concentration of HCHO in HCHO-sensitive individuals in occluded and non-occluded patch tests, and to evaluate the relationship to repeated open application test (ROAT) with a product containing a HCHO releaser.

Review Process

The Board conducted a public meeting on October 11, 2023. Advance notice of the meeting was published in the *Federal Register* as "Human Studies Review Board; Notification of a Public Meeting" (EPA, FRL-10408-01-ORD). This Final Report of the meeting describes the HSRB's discussion, recommendations, rationale, and consensus in response to the charge questions on ethical and scientific aspects of the research.

For each agenda item, the Agency staff presented their review of the scientific and ethical aspects of the research. Each presentation was followed by clarifying questions from the Board. The HSRB solicited public comments and then proceeded to address the charge questions under consideration. The Board discussed the science and ethics charge questions and developed a consensus response to each question. For each of the charge questions, the Chair called for the Board to vote to confirm concurrence on a summary statement reflecting the Board's response.

For their evaluation and discussion, the Board considered materials presented at the meeting, research articles, and related materials, the Agency's science and ethics reviews of the research studies, the Agency's statistical analysis of the research data, comments from the Public, and oral comments from Agency staff during the HSRB meeting discussions. A comprehensive list of background documents is available at https://www.epa.gov/osa/hsrb-october-11-13-2023.

Charge Questions and Context

Charge to the Board – Science

Is the research described in the published study *"Flyvholm, MA, Hall, BM, Agner, T, Tiedemann, E, Greenhill, P, Vanderveken, W, Freeberg, FE and T Menné. (1997). Threshold for Occluded Formaldehyde Patch Test in Formaldehyde-Sensitive Patients. Contact Dermatitis. 36: 26-33"* scientifically sound, providing reliable data for consideration as part of endpoint selection and derivation of a point of departure for elicitation of dermal sensitization from dermal exposure?

HSRB Response

The research described in the published study *"Flyvholm, MA, Hall, BM, Agner, T, Tiedemann, E, Greenhill, P, Vanderveken, W, Freeberg, FE and T Menné. (1997). Threshold for Occluded Formaldehyde Patch Test in Formaldehyde-Sensitive Patients. Contact Dermatitis. 36: 26-33"* could be used as part of endpoint selection and derivation of a point of departure for elicitation of dermal sensitization from dermal exposure, given the limitations and recommendations provided by the HSRB are taken into account.

Science Review

This study was designed to investigate the eliciting threshold concentration of HCHO in HCHO-sensitive individuals in the occluded and non-occluded patch test, and to evaluate the relationship to ROAT with a product containing a HCHO releaser.

The study recruited 20 HCHO-sensitive volunteers (14 women, 6 men; age range: 32-71) as the treatment group. A volunteer was considered HCHO-sensitive if the volunteer had a positive patch test response to HCHO (1% aq.) and negative patch test responses to paraben mix, Germall 115, and rubber. The study recruited another 20 healthy volunteers (12 women, 8 men; age range: 22-54) as the control group. The volunteers in the control group had negative patch test responses to HCHO, parabens, Germall 115, and rubber.

The occluded patch test, non-occluded patch test, and ROAT were carried out simultaneously on each volunteer of the treatment group. The occluded HCHO solutions (with parabens, Germall 115, and finger cot rubber) were applied to the upper back utilizing 0.8 cm diameter Finn Chambers on Scanpor tape. The non-occluded exposures were comprised of 15 µL applied to 1 cm^2 area of the upper arm. The HCHO solutions in the occluded test were 0, 25, 50, 250, 500, 1,000, 5,000, and 10,000 ppm and in the non-included test were 0, 25, 50, 100, 250, 500, 1,000, 5,000, and 10,000 ppm. Grading of the response was performed at 2, 3, and 6-9 days. The ROAT was carried out using a leave-on cosmetic product that contained parabens and Germall 115 (HCHO releaser). The product was an oil-in-water emulsion formulation. Around 0.1 mL of the product was applied to a 5×5 cm area at the flexor mid-aspect of the left upper arm of each volunteer. The product was applied twice per day and for a maximum of 1 week. Readings were performed 1 week after the initial application unless a positive reaction was observed before that.

The response to the occluded and non-occluded patch test was graded using the following scale: (-), negative reaction; (IR), irritant reaction of different types; (+?), doubtful reaction with faint erythema only; (+), weak positive reaction with erythema, infiltration and possibly papules; (++), strong positive reaction with erythema, infiltration, papules and vesicles; (+++), extreme positive reaction with intense erythema, infiltration and coalescing vesicles. For the ROAT test grading any changes in the skin test area was as follows: (i), slight dryness and scaling without redness; (ii) slight uneven redness without infiltration (edema); (iii) popular, follicular reaction; (iv) even redness, infiltration (edema) and scaling. Of note, only (iv) was defined as a positive reaction.

In the occluded patch test, 9 out of 20 participants responded at concentrations ≤ 5,000 ppm (3 out of 20 to 1,000 ppm, 2 out of 20 to 500 ppm and 1 out of 20 to 250 ppm (Flyvholm et al. (1997), Figure 2, p. 28)). The degree of response was associated with sensitivity (Flyvholm et al. (1997), Figure 3, p. 29). No positive reactions were observed in the non-occluded or ROAT exposure groups. The study concluded that the threshold HCHO concentration for occluded patch test in HCHO-sensitive population was 250 ppm.

Statistical Review

Except for very basic descriptive statistics described in detail below, there are no statistical analyses and/or related conclusions presented in this study.

The descriptive statistical analyses are reported as the percentage of participants agreeing to participate, the number of total participants, and the classification of strength of the dilution series test reaction (nominal as +, ++, +++). Additionally, summaries in the tables included age and sex/ratio characterization of the participants involved in the study (Flyvholm et al. (1997), Table 1), counts of positive reactions to HCHO concentrations in four HCHO-sensitive participants exposed to the occluded patch test (Flyvholm et al. (1997), Table 2), and counts of lowest positive concentrations in five HCHO sensitive participants positive to occluded patch test and ROAT (Flyvholm et al. (1997), Table 3).

Comments

The HSRB notes a few limitations of the Flyvholm et al. (1997) study and manuscript, which are discussed in detail below.

1) Information on the test materials and the HCHO concentrations in the test materials were not clear. According to Sections "Test materials" (Page 28) and "Occluded patch test" (Page 29), it is unclear in the manuscript whether the occluded patch test was carried out using a mixture of "15 µl of the formaldehyde solutions, formaldehyde, paraben mix, Germall 115 (imidazolidinyl urea), and rubber from finger cots used for ROAT" (Page 29) or if a single HCHO solution was used in the occluded patch test.
2) This manuscript lacks a few details that are critical for data interpretation:
    a. It is unclear why the 100-ppm exposure was included in the non-occluded study but not the occluded. This could be important given the No Observed Adverse Effect Level (NOAEL) of 50 ppm. There was only one subject responding at 250 ppm (see next bullet) and that was determined to be the Lowest Observed Adverse Effect Level (LOAEL). If the 100-ppm exposure had been conducted, it is possble that this would have been the NOAEL.
    b. It is unclear why all three procedures were done simultaneously. It is unknown whether this is acceptable for a dermal sensitization test. It is also unknown whether or not including follicular reactions is accepted in such studies.
    c. The solvent used for the HCHO solutions was unknown. If it is not water, it is unclear whether the study volunteers have been tested for their sensitivities to this solvent.
    d. According to Sections "Occluded patch test" and "Non-occluded patch test" (Flyvholm et al. (1997), Page 5), it appears reaction readings were performed at different time intervals (e.g., 2, 3 and 6-9 days after the initial exposure). However, it is unclear at what interval the readings presented in this paper (such as in Flyvholm et al. (1997), Figure 2 and Table 2) were obtained. It is also unknown for each volunteer whether there was any change of responses between readings at different intervals. A report on the time-dependent appearance of patch test reactions would have strengthened the study.
    e. This study does not include an unexposed control group. The controls in this study appear to have been exposed to the test materials but were considered controls because they did not have a reaction. For volunteers in the control group, the manuscript is unclear what test was conducted and whether they were exposed to any test materials based on the statement that "...control group….were tested with the same procedures and test materials" (Page 27).
    f. A detailed analysis of the purity of the reagents used, including HCHO, was not provided.
3) Some inconsistencies were noticed in this paper and the study results. These inconsistencies hamper the understanding of study findings:

a. According to the footnote of Table 2, Volunteer #6 was retested 1 year later after the initial testing. Volunteer #6 is the participant that showed the lowest threshold concentration at 250 ppm. However, during the retesting, a positive reaction was not observed at 250 ppm. EPA does make the case that since they did not test higher concentrations to establish a positive response the cautious approach would be to keep the LOAEL at 250 ppm. The HSRB concurs with EPA's decision.
b. It is the HSRB's understanding that volunteers with low threshold concentrations in the occluded patch test were more likely to develop reactions to the ROAT cream. However, while comparing the results between Flyvholm et al. (1997), Tables 2 and 3, it appears most volunteers with low threshold concentrations in the occluded patch test (such as Volunteer #5, 6 and 16, Table 2) did not show reactions to the ROAT cream (Table 3). There was no discussion included to explain this inconsistency. As the amount of ROAT cream applied by each volunteer was not provided, it is unknown whether this is due to different amounts of ROAT cream applied by different volunteers.

4) The authors state that free HCHO concentration in the ROAT cream is around 300 ppm. However, it is unknown how the 300 ppm was determined. As stated in Section "Test materials," HCHO in the ROAT cream was from Germall 115 (HCHO releaser). According to the footnote in Flyvholm et al. (1997), Table 3, it appears the 300-ppm value represents the free HCHO concentration in the ROAT cream. However, it is unclear when this measurement was conducted (before or after the exposure). It is unknown whether there was any change of free HCHO concentrations during the 1-week exposure due to loss (such as volatilization and degradation) and release from Germall 115. As the authors note, the failure of a positive reaction in the ROAT study (300 ppm) could be due to rapid evaporation (dependent upon other components) or an insufficient exposure period. There was also wide variability in the actual self-administered dose (minimum range of 0.71-2.92 $\mu g/cm^2$, Flyvholm et al. (1997), Table 5, dose range of five volunteers in the ROAT).
5) It appears more women than men were recruited for both the treatment and control groups. The age range of each gender in each group was unknown. Summary statistics of gender and age range for the treatment and control groups were provided. Information on the gender and age of each volunteer was not provided for each volunteer, so it is unclear whether gender or age was a covariate that might affect the responses.
6) The authors reported no difference in the degree of sensitivity to HCHO among participants and non-participants, but no data was provided to support the conclusion.
7) The authors reported a relationship between the degree of patch test reactivity and HCHO concentrations without supported statistical analyses. Similarly, the authors state that the HCHO threshold concentration in known HCHO-sensitive individuals was 250 ppm, without supported disclosed statistical evidence. The ROAT testing reported no positive reaction to 300 ppm free HCHO, however material provided in the discussion indicates that the results cannot be used unless further evaluated by prolonged studies.
8) The study results related to dermal sensitization due to exposure to HCHO might not be generalizable in the general population. The study focuses on threshold concentration of HCHO in already sensitized individuals (cases) with little information on the control group of healthy volunteers, (e.g., except sex ratio and age-related descriptive statistics). Additionally, no definite positive reaction is observed or reported in this control group to any of the described tests and/or procedures.
9) Flyvholm et al. (1997) reported that the study design was a 1:1 case-control study (cases: HCHO-sensitive participants; controls: healthy volunteers). This setting can be useful to assess differences in the severity of reactions and/or specific concentrations of HCHO between already known HCHO-sensitive participants and the general population if that is the main topic of the research.
a. It is unclear if the study sample size is appropriate for this design.

b. For this design, a proper matching is required. The authors do not report information on the similarities between the HCHO-sensitive participants and the general population.
c. Statistical analyses for this type of design would adjust for group differences. If the study design focuses on repeated measures of the severity of reaction on different HCHO doses on a cohort including HCHO-sensitive and non-sensitive participants, the required statistical approach is even more sophisticated (e.g., assessing correlation structures, addressing the timeframe of the study of 2 years, etc.).

Recommendations

- When using this study, the HSRB recommends that EPA include discussion to clarify their interpretation of the description in the paper (Pages 28 and 29) regarding how the concentration range (25-10,000 ppm) of the final test mixtures was determined and what the occluded patch test concentrations were.
- The HSRB recommends that EPA clarify their interpretation of the meaning of the statement that the "…control group… were tested with the same procedures and test materials) (p. 29).
- The HSRB recommends that EPA should include a description of the patch test grading system along with pictorial depictions of what each grading level signifies.
- As the toxicity endpoint derived from this study is based on the LOAEL (250 ppm) from one subject in the occluded test, the HSRB recommends the study be considered as part of quantitative evidence to select a point of departure for elicitation of dermal sensitization from dermal exposure.
- Additionally, the HSRB recommends caution in using the study results due to differences between the study methods (e.g., reading criteria) and new guidelines for diagnostic patch testing best practices recommended by the European Society of Contact Dermatitis since 2015[1].
- The HSRB recommends that, from a statistical perspective, this research should be used with caution for the endpoint selection and/or derivation of a point of departure for dermal sensitization from dermal exposure to HCHO.

Charge to the Board – Ethics

- Does available information support a determination that the conduct of the research was not fundamentally unethical?
- Does available information support a determination that the research was not deficient relative to the ethical standards prevailing at the time the research was conducted or conducted in a way that placed participants at increased risk of harm or impaired their informed consent?

HSRB Response

Based on its review of "*Threshold for Occluded Formaldehyde Patch Test in Formaldehyde-Sensitive Patients*" (1997) by Flyvholm et al. and associated materials provided by EPA, the HSRB has determined that the available information supports the determination that there is no clear and convincing evidence that the research was conducted unethically or deficient relative to ethical standards at the time the study was performed. Participants were not placed at increased risk and no study activities impaired their informed consent.

[1] Johansen *et al*. 2015. European Society of Contact Dermatitis guideline for diagnostic patch testing – recommendations on best practice. *Contact Dermatitis, 73, 195–221*

Furthermore, based on its review of "*Threshold for Occluded Formaldehyde Patch Test in Formaldehyde-Sensitive Patients*" (1997) by Flyvholm et al. and associated materials provided by EPA, the HSRB has determined that the conduct of the research was not fundamentally unethical. Based on the provided information, the research was not deficient relative to the ethical standards prevailing at the time the research was conducted and was not conducted in a way that placed participants at increased risk of harm or impaired their informed consent.

Ethics Review

Subject Selection and Recruitment

40 subjects were enrolled in the study. 20 subjects (14 female, 6 male) were sensitive to HCHO and 20 (12 female, 8 male) were healthy controls. Participants ranged in age from 22 to 71. HCHO-sensitive participants were recruited from the Department of Dermatology at Gentofte Hospital in Copenhagen, Denmark. There is no information provided about how the control participants were recruited.

Inclusion criteria for the HCHO-sensitive subjects included being a patient at the Department of Dermatology with a positive patch test to HCHO and negative patch tests to Germall 115, parabens, and rubber. Sensitive subjects were excluded if they had dermatitis or other skin diseases at or near the skin sites used for testing, and/or diseases, exposure, or use of medications that would interfere with the testing. The controls were included if they had a negative patch test for HCHO, Germall 115, parabens, and rubber. Four potential subjects were excluded because their sensitivity to HCHO could not be confirmed and a further three were excluded due to positive reactions to Germall 115.

There is no information provided about participation incentives.

Informed Consent Process

All participants provided written informed consent after receiving both oral and written information about the study. Otherwise, no information is provided regarding the informed consent process as relates to timing or personnel.

Risks and Benefits

Risks were minimized via the screening procedures, the informed consent process, verification of eligibility via inclusion and exclusion criteria and by conducting the study at the dermatology clinic under supervision of medical personnel. While HCHO is a known irritant, subjects were only exposed to concentrations standard in diagnostic testing. No information is included about any potential risks in being exposed to Germall 115, parabens, or rubber.

There were no direct benefits to participating subjects.

Participant privacy and data confidentiality appear to have been protected: no participants are identified in the article; demographics and/or characteristics are only reported in aggregate and only include sex and age.

As indicated in the manuscript, 16 potential subjects declined to participate showing voluntariness of participation and seven were excluded for safety reasons.

Independent Ethics Review

No specific information related to the review process is included, but the manuscript states that the study was reviewed and approved by the Ethical Committee of the Copenhagen Municipality.

Review Summary

Based on the HSRB review of the provided documents, including the published article and the provided EPA science and ethics review, there is no evidence to suggest that this study was conducted

unethically. No children were enrolled and there is no indication that pregnant or nursing subjects were enrolled in the study. Risks were adequately minimized through the inclusion and exclusion criteria, study procedures, and by utilizing an accepted threshold of HCHO exposure. Informed consent was obtained, and the study was reviewed and approved by an independent ethics committee. While important and specific information is lacking from the article related to the ethical conduct of this study, there is no evidence that it was conducted unethically or deficient relative to the standards of the time. No study procedures would invalidate or impair the participants' informed consent.

List of Acronyms – Fischer et al. (1995)

EPA	Environmental Protection Agency
HCHO	Formaldehyde
HMS	N-hydroxy methylsuccinimide
HSRB	Human Studies Review Board
LOAEL	Lowest Observed Adverse Effect Level
POD	Point of Departure

HSRB Meeting Report – Fischer et al. (1995)

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

Introduction

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Review Process

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Charge Questions and Context

Charge to the Board – Science

Is the research described in the published study *"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*" scientifically sound, providing reliable data for consideration as part of endpoint selection and derivation of a point of departure for elicitation of dermal sensitization from dermal exposure?

HSRB Response

The research described in the published study *"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*" is scientifically sound for comparing HCHO dermal testing methods. The data from this study, in particular from the Finn Test used in Group 2, could be used to corroborate results of studies that were specifically designed to identify a HCHO dermal sensitization elicitation threshold from dermal exposure, given the limitations and recommendations provided by the HSRB are taken into account.

Science Review

Fischer et al. (1995) present efforts to develop an improved approach for dermatological patch testing of HCHO. The study is a comparative study that documents reactions to dermal exposure of HCHO from two dosage forms at various concentrations: the TRUE Test™ patch that used an innovative prodrug approach where the patch incorporates N-hydroxy methylsuccinimide (HMS), which disassociates "instantly" into HCHO and succinimide upon contact with the humidity of the skin. The second dosage form consisted of dermal application of aqueous solution of HCHO (aqueous patches, Finn Chamber studies). Although the objective of this study was not to identify a threshold for elicitation of reaction to HCHO, the study did test multiple doses of both HMS and aqueous HCHO, in an attempt to correlate subject response to these different test methods. The data provided make it possible to infer a dose response pattern.

Five different groups of subjects were studied: healthy individuals with no skin disease and no HCHO-sensitivity (Group 1), HCHO-sensitive patients (Groups 2 and 4) and patients with eczema (Groups 3 and 5). Groups 4 and 5 represented a second set of trials with new subjects. The most detailed dose response data, and the data that EPA has focused on, are from Group 2, where 25 patients who were previously patch-test-positive to HCHO were exposed to TRUE Test™ HMS doses with effective HCHO concentrations of 20, 30, 40, 80, 120, and 150 μg/cm^2 HCHO and aqueous HCHO applied *via* Finn chambers at concentrations of 0.015, 0.032, 0.063, 0.13, 0.25, 0.5 and 1% (equivalent to 4.5, 9.6, 19, 39, 75, 150 and 300 μg/cm^2). The other test groups had a fewer number of doses (e.g., Group 5 had four TRUE Test™ doses and only one aqueous HCHO dose). Individual subject data were only provided for Group 2 (Table 1) and Group 5 (Table 2). Group 5 had higher HCHO doses than Group 2 and was therefore less useful for EPA to derive a threshold. Thus, EPA's focus on data from Group 2 is reasonable.

Statistical Review

Descriptive statistics, various cross-tabulation tables, and qualitative outcomes were provided.

Limited data were provided for Groups 1, 3, 4, and 5. For Group 1, irritant reactions occurred in 2 out of 9 participants (22.2%) at 0.57 mg/cm^2 and 5 out of 9 (55.6%) at 1.12 mg/cm^2. For Group 3, allergic reactions in 3 out of 120 participants (2.5%) in both TRUE Test™ and aqueous preparations. For Group 4, positive reactions occurred for 13 out of 24 participants (54.2%) in both TRUE Test™ and aqueous preparations, with 1 out of 24 participants (4.2%) reacting only to the TRUE Test™ patch. Additionally, it is stated that 0.20 and 0.26 mg/cm^2 TRUE Test™ patches provide an equivalent response as 1% aqueous preparations. For Group 5, reactions were shown in all four TRUE Test™ patch doses. Specifically, 9 out of 255 participants (3.5%) showed positive reactions and 10/255 participants (3.9%) showed irritant reactions. A statement is made concerning the accepted irritation rate for Group 5 based on the upper limit of the 95% confidence limit resulting in an HMS dose equivalent of 0.18 mg/cm^2.

Group 2 results provide potential data to directly compare TRUE Test™ doses and aqueous preparations. Varying reactions were reported for 17 out of 25 participants (68%) for both TRUE Test™ patches and aqueous preparations; 2 out of 25 participants (8.0%) for TRUE Test™ patches only; and 5 out of 25 participants (20.0%) for aqueous preparations only. Importantly, it was noted that the lowest dose giving a positive elicitation response for the TRUE Test™ system was 10 $\mu g/cm^2$ whereas the lowest elicitation dose obtained with the Finn chambers and aqueous HCHO was 4.5 $\mu g/cm^2$. One subject had a positive reaction at this aqueous HCHO dose, but the magnitude of response was not mentioned. For the TRUE Test™, six of the HCHO sensitive subjects had negative reactions; for the Finn chamber test with aqueous HCHO, three subjects did not have positive reactions at any dose. Because one subject reacted at the lowest Finn chamber dose (4.5 $\mu g/cm^2$) and two subjects reacted at the lowest TRUE Test™ result (10 $\mu g/cm^2$), there was No Observed Adverse Effect Level established.

Comments

In general, we agree with EPA's conclusion that the study may provide useful data for risk assessment related to elicitation of reactions in HCHO-sensitized individuals. The methodology used in the patch testing follows standard practice in the field of dermatology (e.g., patch test sizes and placement, times of patch reading, scoring rubric). This lends confidence to the study. However, because the study was not designed to investigate threshold but was rather intended to validate a test system, substantial information is lacking. Below are additional comments that EPA should consider.

1) The study does not provide any information for Group 2 on the strength of reactions (+++, ++, +, +?). This would be helpful in interpreting the dose response. Can EPA obtain this information? This may not be necessary if the study is used in only a corroborating fashion. What were the instructions given to the volunteers for determining a "+" vs a "++" reaction (Table 2)? Though test reading designations are presented in Table 2, US EPA DER (p. 5), the procedural details remain unknown.
2) The difference between positive and irritant reactions is unclear (Table 2).
3) Statistical analyses used were casually dismissed by the authors with the statement "[d]escriptive methods were used." No citations were provided. While the efforts by EPA to address this omission is applauded, the statement that "additional statistical analyses or dose response modeling was not feasible (DER, p.5)," raises concerns regarding the validity of the information from the Fischer et al. (1995) paper to the charge question posed, which is quantitative (or semi-quantitative) in nature.
4) It would be interesting to examine the Finn Chamber data (Fischer et al. column 1, Table 3, EPA DER, p.7) in the same units as for the TRUE Test™ patches (mg/cm^2). Conversion information is provided (a TRUE Test™ patch containing 0.81 mg/cm^2 of HMS (0.19 mg/cm^2 of HCHO) is equivalent to the amount of HCHO in a Finn Chamber test with 15 µl 1% HCHO solution).
5) We appreciate EPA preparing Table 4 and Figure 1, which illustrate the dose-response pattern observed in this study much clearer than in Fischer et al. (1995), Table 1.
6) On page 10 of their report, EPA comments on the lack of an acceptable control. We agree there is no negative (vehicle) control which would be helpful to judge whether individual reactions were spurious. Regarding a positive control, to some extent the testing of HCHO at high concentrations (1%) is a positive control of the assumption that the subjects were HCHO-sensitized based on prior testing. The fact that three of the subjects were negative at all concentrations of HCHO suggests either a change in sensitization status or that the original assignment was mistaken.
7) Given the development of tolerance ("sensitization") to prior HCHO exposure and the high degree of irreproducibility (discussion section, Fischer et al. (1995), Page 29, third paragraph from the bottom), what should the guidelines be for appropriate volunteer selection?
8) Do skin conditions (e.g., dermatitis) influence reactions to HCHO? The authors did not address this issue though this and several volunteer groups were studied.

9) Sample sizes for the various groups are quite different among the experiments, and female to male ratios are only provided for some of the groups. It appears that the best quantitative information comes from Group 2 with a sample size of n = 24. It is unknown if there are differences between female and male subjects, and this is not addressed by the authors and therefore cannot be evaluated.
10) Table 1 provides results of a cross-tabulation table of TRUE Test™ vs. aqueous preparations. If this is based on the Group 2 study, the 0.01 mg/cm^2 dose results for TRUE Test™ patches are surprising given that it is not listed as a dose in the "Experimental Design" section (Fischer et al., 1995, Page 26). This dose was only reported for Group 3 experiments, but these were only tested against aqueous preparations of 1.0%. Therefore, the last column of Table 1 in Fischer et al. (1995) for the TRUE Test™ HCHO patches is confusing.
11) The draft EPA Science Review Document ("FORMALDEHYDE/043001 [Fischer, et al. 1995]"; Page 7 of 12) provides an ad hoc re-evaluation of the data from Group 2 based on positive results for both TRUE Test™ patches and aqueous preparations under the assumption that subjects who react at a given dose would also react at a higher dose. Based on the presumed alignment of TRUE Test™ patches and aqueous preparations, the science reviews suggest a Lowest Observed Adverse Effect Level (LOAEL) value of 0.015% (0.0045 mg/cm^2). Though the basis for this assessment is interesting, absence of raw data is unfortunate.
12) EPA acknowledges in their review ("FORMALDEHYDE/043001 [Fischer, et al. 1995]"; Pages 10-11 of 12) that the study did not fully meet all the screening criteria. Most critically, Criterion #11 ("Treatment(s) are compared to acceptable controls") and Criterion #13 (Adequate data are provided on the chemical tested (i.e., test article characterization)); however, after acknowledging these limitations, they conclude that the study "is appropriate for quantitative use and can be considered as part of endpoint selection and Point of Departure (POD) derivation" (Page 11 of 12).

Recommendations

- The HSRB recommends that this study only be used in a supporting fashion, i.e., as corroborating the results of studies that were specifically designed to identify an elicitation threshold for HCHO.
- The relevant information for determining the LOAEL value is based on 24 subjects of unknown sex. The HSRB recommends that EPA acknowledge this lack of information as a critical limitation of the study if the results are to be used for POD determination.
- It is not clear how long a time interval occurred between the initial Group 2 evaluation, *i.e.*, "previously positive patch test reactions to formaldehyde" and the subsequent testing that is the subject of the article. There is some evidence that exposure to an allergen heightens sensitivity to simultaneous or subsequent testing (*i.e.*, excited skin syndrome) [2]. Patch testing with a range of HCHO concentrations (more than two orders of magnitude) could have heightened responses to the lowest concentrations. The HSRB recommends that EPA comment on the potential for excited skin syndrome to have artificially lowered the actual threshold (which as noted below is based on a single individual).
- This is a study with a limited sample size (i.e., 22 individuals who reacted to HCHO with the Finn chambers, 19 who reacted with the TRUE Test™ chambers), and the "threshold" is based on the reaction of a single individual for the Finn chamber/aqueous HCHO method. The HSRB has reservations about using this single data point as the basis for risk assessment. There are

[2] See for example, Duarte *et al*. 2002. Excited skin syndrome: study of 39 patients. *Am J Contact Dermat*. 13(2):59-65 and Maibach *et al*. 2002. Quantification of the excited skin syndrome (the "angry back"). Retesting one patch at a time. *Contact Dermatitis* 8(1):78.

a number of other papers reporting the results of closed patch testing with HCHO. The HSRB recommends that EPA consider aggregating this data and using something like a benchmark dose approach to arrive at a more robust point of departure. As an example, the HSRB provides a Society of Toxicology poster by Lewandowski *et al*. which showed how this could be done for nickel and chromium[3].

- The HSRB recommends that EPA provide additional justification for how a study presenting largely qualitative information can or should be used for derivation of a POD.
- The ICF document ("Statistical Review of Data from two Formaldehyde Human Patch Test Studies: Flyvholm et al. (1997) and Fischer et al. (1995)") states "No additional statistical [sic] were feasible for either of the two studies, thus no statistical code accompanies this memorandum" (Page 1). The HSRB recommends that EPA provide additional justification for the usefulness of their reanalysis of the data given the analytical non-feasibility statement in their statistical review.
- The study involved individuals with already demonstrated sensitization to HCHO. This is not consistent with the general population (although HCHO is one of the more common chemical allergens). The HSRB recommends that EPA keep the use of this highly sensitive population in mind if using this study for risk assessment. This appears to be the case in that EPA refers to "POD derivation for elicitation" on page 2 of their report, but the HSRB believes it is important that the distinction between threshold for induction and elicitation not be lost.

Charge to the Board – Ethics

- Does available information support a determination that the conduct of the research was not fundamentally unethical?
- Does available information support a determination that the research was not deficient relative to the ethical standards prevailing at the time the research was conducted or conducted in a way that placed participants at increased risk of harm or impaired their informed consent?

HSRB Response

Based on its review of "Clinical Standardization of the TRUE Test™ Formaldehyde Patch" by Fischer et al. (1995) and associated materials provided by EPA, the HSRB has determined that the conduct of the research was not fundamentally unethical.

Furthermore, based on its review of "Clinical Standardization of the TRUE Test™ Formaldehyde Patch" by Fischer et al. (1995) and associated materials provided by EPA, the HSRB has determined that the research was NOT deficient relative to the ethical standards prevailing at the time the research was conducted and was NOT conducted in a way that placed participants at increased risk of harm or impaired their informed consent.

Ethics Review

This publication describes and summarizes clinical studies carried out to develop the TRUE Test™ HCHO patch. The studies verify the efficacy of the TRUE Test™ for determining HCHO allergy in humans, and further determine that an optimal dose is 0.18-0.20 mg formaldehyde/cm².

Subject Selection and Recruitment

[3] Lewandowski and Cohen. 2017. Nickel and trivalent chromium thresholds for skin sensitization. Toxicologist 156 (1): 284.

Little information is included regarding subject selection. The publication simply states that participants consisted of "[h]ealthy volunteers without known sensitivity to formaldehyde, consecutive patients with contact dermatitis, and patients with previous patch tests to formaldehyde" (26).

Informed Consent Process and Independent Ethics Review

The authors note that informed consent was obtained from participants, and that the studies were approved by "ethical committees," but do not provide further information regarding the processes of selecting participants or approving the studies. Based on the information provided in the publication, it appears that patient confidentiality was maintained across the studies described therein.

Review Summary

Based on the HSRB review of the provided documents, including the published article and the provided EPA science and ethics review, there is no evidence to suggest that this study was conducted unethically. No children were enrolled and there is no indication that pregnant or nursing subjects were enrolled in the study. Risks were adequately minimized through the inclusion and exclusion criteria, study procedures, and by utilizing an accepted threshold of HCHO exposure. Informed consent was obtained, and the study was reviewed and approved by an independent ethics committee but did not provide further information regarding the processes of selecting participants or approving the studies. While important and specific information is lacking from the article related to the ethical conduct of this study, there is no evidence that it was conducted unethically or deficient relative to the standards of the time. Further, there is no evidence to indicate deficiency relative to ethical standards of that time (1993-1995), including the 1989 Declaration of Helsinki. No study procedures would invalidate or impair the participants' informed consent.

Recommendations

No specific recommendations based on the ethics review.

Recommendations for Future Studies

None