March 31, 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 25, 2022, 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 three research articles and one study report involving human participants.

On October 25, 2022, the HSRB considered Chapter 14 in *Formaldehyde Toxicity* by Andersen and Mølhave (1983): "Controlled human studies with formaldehyde: A five-H exposure study" (James E. Gibson ed. Hemisphere Publishing Group, Washington, D. C.). Briefly, the manuscript reports findings of a study to examine formaldehyde vapor exposure effects on nasal mucociliary flow, nasal airflow resistance, forced expiratory vital capacity, and irritation threshold. EPA proposes to use the results of the study as part of a weight-of-evidence in determining a point of departure for acute inhalation exposure to formaldehyde (HCHO) in the human population.

On October 26, 2022, the HSRB considered the research article by Kulle et al., "Formaldehyde dose-response in healthy non-smokers" (*JAPCA*. Volume 37, pp. 919–924) and by Kulle, "Acute odor and irritation response in healthy nonsmokers with formaldehyde exposure" (*Inhalation Toxicology*. Volume 5. Issue 3. pp. 323–332). Briefly, the two manuscripts report findings of a study to examine pulmonary function and irritant symptoms over a range of HCHO exposures at rest and with exercise. EPA proposes to use the quantitative results of the study as part of a weight-of-evidence in determining a point of departure for acute inhalation exposure to HCHO in the human population.

On October 27, 2022, the HSRB considered the Study Report MIM-006, *Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray Against Mosquitoes*. Briefly, the study reports the results of the complete protection time of MIMIKAI Lilly Pilly mosquito repellent based on field studies using human subjects. The results of the study will be used to inform the registration and labeling requirements.

The HSRB's responses to the charge questions for the three reports presented at the meeting on October 25, 2022, along with detailed rationale and recommendations for their conclusions are provided in the enclosed final meeting report.

Sincerely,

Lisa Corey

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

Julia A. Sharp

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

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

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List of Acronyms – Andersen and Mølhave Study

ANOVA	analysis of variance
BMD	benchmark dose
EPA	Environmental Protection Agency
FEV_{10}	forced expiratory volume in 10 s
$FEF_{25–75}$	forced expiratory flow at 25% and 75% of the pulmonary volume
FVC	forced expiratory vital capacity
HCHO	formaldehyde
HSRB	Human Studies Review Board
ORD	Office of Research and Development
POD	point of departure

HSRB Meeting Report – Andersen and Mølhave Study

Andersen and Mølhave (1983): Chapter 14: Controlled Human Studies with Formaldehyde. In: Formaldehyde Toxicity, James E. Gibson ed. Hemisphere Publishing Group, Washington D.C. Open literature study.

Introduction

On October 25, 2022, the Human Studies Review Board (HSRB) considered Chapter 14 in Formaldehyde Toxicity by Andersen and Mølhave (1983): "Controlled human studies with formaldehyde: A five-H exposure study" (James E. Gibson ed. Hemisphere Publishing Group, Washington, D. C.). Briefly, the manuscript reports findings of a study to examine formaldehyde (HCHO) vapor exposure effects on nasal mucociliary flow, nasal airflow resistance, forced expiratory vital capacity (FVC), and irritation threshold. EPA proposes to use the results of the study as part of a weight-of-evidence in determining a point of departure (POD) for acute inhalation exposure to HCHO in the human population.

Review Process

The Board conducted a public meeting on October 25, 2022. Advance notice of the meeting was published in the *Federal Register* as "Human Studies Review Board; Notification of a Public Meeting" (EPA, FRL-9328-01-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 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/october-25-2022-hsrb-meeting.

Charge Questions and Context

Charge to the Board – Science

Is the research described in Formaldehyde Toxicity, Chapter 14 (Controlled Human Studies with Formaldehyde) by Ib Andersen and Lars Mølhave, under the section titled "A Five-H Exposure Study" scientifically sound, providing reliable data for use in a weight-of-evidence to determine a POD for acute inhalation exposures to formaldehyde?

HSRB Response

The research described in Formaldehyde Toxicity, Chapter 14 (Controlled Human Studies with Formaldehyde) by Ib Andersen and Lars Mølhave, under the section titled "A Five-H Exposure Study" provides scientifically sound data for qualitative use in a weight-of-evidence to support the determination of a POD for acute inhalation exposure to formaldehyde, given the limitations and recommendations provided by the HSRB are considered.

The HSRB also has specific comments, recommendations, and additional minor points, which are described in the discussion below.

Science Review

The publication cited is a book chapter that reviews the results of several studies, including a 5-hour study performed by one (or both) of the book chapter authors. EPA noted that it was only reviewing and requesting feedback on the 5-hour study for use in risk assessment.

The study involved 16 volunteers, 5 women and 11 men, all young (ranging from 20 to 33 years of age) and healthy. Five of the volunteers were smokers (but data were not analyzed separately for the smokers).

The participants were exposed in groups of four to 0.3, 0.5, 1.0, and 2.0 mg/m^3 formaldehyde, equivalent to 0.24, 0.4, 0.81, and 1.62 ppm. Concentration exposures were randomized. The participants were not engaged in exercise during the exposures. HCHO was "generated by passing air through an oven [containing] paraformaldehyde heated to about 80 degrees C. The paraformaldehyde had been previously baked at 120 degrees for 5 h to stabilize its degassing of formaldehyde." Concentrations of HCHO were within ± 20% of target concentrations. Inhaled air was maintained at the following conditions: a temperature of 23 ± 0.5°C, 50 ± 5% relative humidity, air velocity of 10 ± 3 cm/s, and air supply rate of 500 cubic meters/hour. HCHO exposure was assessed using 1-hour air samples collected during the exposures.

Each experimental day consisted of three "identical series of measurements": during the control phase (0 mg/m^3 HCHO) and after exposure for durations of 2-3 hours and 4-5 hours, in separate experiments. Measurements included determination of nasal mucociliary flow using a tracer particle method, followed by nasal air flow resistance and several standard lung function tests (FVC, forced expiratory volume in 10 s [FEV_{10}], forced expiratory flow at 25 and 75% of the pulmonary volume [FEF_{25-75}]). Degree of airway irritation was self-assessed on a scale of 1–100, with 1 representing "complete comfort" and 100 indicating "severe discomfort." The volunteers also participated in mathematical (adding and multiplication) and dexterity (card punching) tests. A Latin square study design was used and the statistical methodology involved nonparametric tests and analysis of variance (ANOVA) at a confidence level of $p = 0.05$. Additional statistical analyses of the Andersen and Mølhave study were performed by Dr. Jonathan Cohen and Rachel O'Neal of ICF and were provided in a separate memo to the HSRB.

The results of this study were somewhat unusual as a clear dose-response pattern was not observed for some endpoints. Mucociliary flow was affected by low-dose HCHO exposure but was unchanged or appeared to recover slightly at higher exposures. Significant decreases in mucociliary flow were observed only after exposure to an air HCHO concentration of 0.5 mg/m^3. The decrease was observed only in the most anterior of the three nasal sites studied in this segment; flow was not affected at higher concentrations of HCHO or at the other nasal sites (e.g., posterior) studied. There appeared to be no

changes in respiratory measurements as a result of exposure. The perception of ethyl valerate odor was affected at 2 mg/m^3 (1.62 ppm) but not at lower concentrations. The rationale, significance, and experimental details for the use of ethyl valerate in this study were not discussed and were therefore not presented in the review of the study. Regarding discomfort ratings, the participants never reported the effects as being greater than "slight discomfort." The authors reported that during the first 2 hours of exposure, no increase in subjective feelings of discomfort was reported at 0.3 or 0.5 mg/m^3 (0.24 and 0.4 ppm), but increased discomfort was reported at higher concentrations (0.81 and 1.62 ppm). With longer periods of exposure, discomfort was reported at the two lowest concentrations, although no dose response was apparent, as greater effects were observed at 0.3 mg/m^3 than at 0.5 mg/m^3 (Figure 7). The highest discomfort rating was observed with the 2 mg/m^3 concentration of HCHO, with a drop in discomfort scores after 2 hours, suggesting tolerance (Figure 7, Chapter 14). An unexpected observation was noted from review of the data: following exposure to 0.3 and 0.5 mg/m^3 of HCHO for more than 2 hours, the lower air concentration of HCHO was reported to be “more uncomfortable.” This might be an artifact since no statistical analyses of the data were provided. In addition, the perception of discomfort occurred sooner at 0.3 mg/m^3 than at 0.5 mg/m^3 (approximately 2.5 *vs.* 4 hours in Figure 7). The authors further noted that conjunctival irritation and dryness of the nose and throat were reported by 3, 5, 15, and 15 participants after 5 hours of HCHO exposure at 0.3, 0.5, 1, and 2 mg/m^3, respectively. The authors reported that HCHO exposure had no effect on performance on the math or card punching tests.

Comments

Confidence in this study is limited. It is unclear if the study underwent peer review because it was published as a chapter in the authors’ book. In addition, the HCHO exposures were only assessed *via* hourly average samples. The study can be used as supportive information in a weight-of-evidence approach but should not be the primary basis for a POD. This appears to be consistent with the EPA position. A few points to note:

- The study lacks key experimental, quantitative, and statistical details, which raise questions regarding the reliability of the results presented. In particular, results were presented only as graphs and raw data could not be obtained, limiting the statistical analyses.
- The sample size was small, and the sample was biased toward healthy young adults. All participants had essentially normal lung function and no history of allergies or asthma. Although both sexes were included, the study population was weighted more heavily toward men. The study included smokers, who may be less sensitive to the irritant effects from HCHO inhalation exposure. Thus, issues of sensitive subpopulations should be considered by the Agency.
- Symptoms were assessed using a dial turning approach with a score from 1 to 100. However, none of the participants rated their comfort level higher than 18 (based on Figure 7), so the precision in differentiating differing levels of slight discomfort is unclear. The authors did not indicate the amount of training provided on the dial turning approach.
- The lack of an apparent dose-response for the discomfort findings is concerning (Figure 7).
- Figure 7 shows the mean discomfort vote at different concentrations over time. What is the variation in votes? It would be useful to have error bars on this figure (although it would likely be quite complicated). In this particular case, it would be very helpful to review the raw data if that can be arranged.

- The study was conducted in 1970 and raised issues related to the potential limitations of current methodologies and technologies available to conduct HCHO exposure studies. The limited quantitative information provided in acute exposure studies on the toxicity of HCHO discussed in this section of the chapter by Andersen and Mølhave makes it difficult to find definite answers to the questions posed.

Statistical Review

The experimental design of the Andersen & Mølhave (1983) study was likely acceptable at the time it was completed. However, the ratio of women/men participants (5:11), the broad range of ages (20-33), and the inclusion of smokers (n = 5, with n = 1 classified as a "heavy" smoker) may lead to results contaminated by confounding effects in such a small study in which differential results could not be adequately evaluated. In fact, in the included review by Pettersson & Rehn (in cit.), the reviewers found that smokers were "significantly less sensitive than were nonsmokers" (p. 156); however, confounding effects based on participant age and sex were not found to lead to different results. Hence, the inclusion of smokers in the present study is questionable. No information is provided about sample size or power determinations.

Andersen & Mølhave (1983) indicated that they analyzed the data using "nonparametric tests…[and] ANOVA" (p. 159). The authors performed a data transformation to use the ANOVA but did not clarify why they used both nonparametric and parametric procedures on the data. Additionally, insufficient reporting of these statistics in the Results (starting p. 160) makes it difficult to ascertain exact findings, and the data visualizations largely provided group results that introduced difficulties in understanding the actual magnitude (i.e., effect size) of the statistical results. Further, no information was provided about the effects of using the same subjects and assuming the measurements were independent.

We generally agree with the evaluation submitted by Cohen & O'Neal (ICF Memo to Facey, McMahon, Kliminsky & Burgin of EPA, dated September 05, 2022) on the Andersen & Mølhave (1983) study. Specifically, the results concerning nasal mucus flow, airway resistance, and odor threshold cannot be validated based on the information provided. The reanalysis of discomfort and irritation is interesting. However, as with the Andersen & Mølhave (1983) study, the Cohen & O'Neal (2022) report makes a broad and limiting assumption that the reported data are independent and a strong assumption of the statistical tests and benchmark dose (BMD) software modeling performed. The authors described this assumption and its implications on their analyses in their report. Because data collected on the same individuals over time were analyzed as independent samples and correlation among measurements of the same individuals were not provided, the results are speculative from a statistical perspective. It is noted the resampling procedures were appropriate as reported by Cohen & O'Neal (2022).

Based on review of the available information and subsequent analyses, quantitative evidence for POD determination for acute/short-term inhalation exposures to HCHO is not strongly supported. However, reanalysis of the data by Cohen & O'Neal (2022) provides an interesting set of results under the limiting assumption of statistical independence. Given the small data set, likely non-independence of data, and potential issues with the inclusion of smokers in the study without subgroup analyses, the quantitative results are generally not compelling.

Recommendations

Based on the review of the charge document and the other supporting documents provided, including the EPA Science Review, the HSRB recommends that EPA specifically state that:

- The limiting assumption of statistical independence is not satisfied, and hence the analyses in this study are of limited quantitative value for POD determination.
- The inclusion of potential confounders in the data (e.g., smokers, sex, broad age range, and exposure tolerance) may impact the quantitative results.
- The statistical analyses are limited owing to the absence of individual-level data as well as absence of specific statistical results in the chapter and therefore should be interpreted with caution.
- The limitations described by the HSRB should be presented by EPA in its report and the results of this study used qualitatively to support the weight of evidence.

Charge to the Board – Ethics

Does available information support a determination that there is no clear and convincing evidence that the conduct of the research was fundamentally unethical or deficient relative to the ethical standards prevailing at the time the research was conducted in a way that placed participants at increased risk of harm or impaired their informed consent?

HSRB Response

The Board does not believe that the research described in Formaldehyde Toxicity, Chapter 14 (Controlled Human Studies with Formaldehyde) by Ib Andersen and Lars Mølhave, under the section titled "A Five-H Exposure Study" provides clear and convincing evidence that the conduct of the research was fundamentally unethical or deficient relative to the ethical standards prevailing at the time the research was conducted in a way that placed participants at increased risk of harm or impaired their informed consent.

Ethics Review

Participant Recruitment and Selection

There is no direct information related to the method used for participant recruitment or selection. However, there is no indication that the process was inequitable or coercive. All 16 participants were over the age of 18 (20–33 years old). Eleven were men, and five were women.

Specific inclusion and exclusion criteria were not delineated in the chapter; however, all participants were reported to be "healthy students," with "apparently" healthy upper airways. None had been exposed to HCHO and none had chronic or acute respiratory disease, though six were reported to be smokers (one "heavy" smoker) (p. 158). There is no information about compensation or other incentives for participation.

Informed Consent Process

The study chapter provides no information regarding the informed consent process, including documentation, whether it was obtained in writing or verbally, and how participant comprehension was assessed, if at all.

Risks and Benefits

The risks to participants were adequately minimized. The risks posed by HCHO exposure in humans include eye, nose, and throat irritation at low concentrations and rashes, shortness of breath, wheezing,

and changes in lung function at higher concentrations. HCHO degassing was controlled and maintained at a constant and controlled rate, and the concentration was ensured through air sample analyses. The HCHO exposure threshold was set lower than the accepted limit at the time, and as mentioned previously, only healthy volunteers participated. Reactions to the exposure included increasing discomfort levels over time, but the average level never exceeded 18 units, which investigators report is in the middle of the "slight discomfort" range (p. 162). Indeed, at the end of the exposure day, 6 of the 16 participants reported no discomfort at the highest exposure level. Most participants complained of irritation and dryness in the nose and throat, but all symptoms had ceased by the following day. Based on the study discussion, these symptoms should be expected when humans are exposed to HCHO. Otherwise, no adverse events are reported in the study chapter.

No information is provided regarding participant confidentiality or data security. However, in the published study, participants are only referred to by number and no identifying information is otherwise provided.

There were no direct benefits to participants, though establishing HCHO exposure limits could be considered a benefit to society.

Independent Ethics Review

There is no information regarding an independent ethics review in the study chapter.

Review Summary

Based on the limited information provided in the study chapter, this study appears to have been conducted ethically. Only healthy adults were enrolled (ages ranged from 20 to 33), there is no indication that pregnant or lactating women were included, and procedures were put in place to minimize the risk to participants. Irritation due to exposure was self-reported by participants and was consistently at the low end of the provided discomfort spectrum, with some participants reporting no discomfort at all. While the study chapter is lacking specific and detailed information regarding the informed consent process, the safeguarding of participant data and identity, and independent ethics review, there is no evidence in the chapter that the study was conducted unethically. Risks to participants were minimized and none of the study procedures would invalidate or impair the ability to provide informed consent.

List of Acronyms – Kulle et al. Study

ANOVA	analysis of variance
BMD	benchmark dose
EPA	Environmental Protection Agency
FEV_{10}	forced expiratory volume in 10 s
$FEF_{25–75}$	forced expiratory flow at 25% and 75% of the pulmonary volume
FVC	forced expiratory vital capacity
HCHO	formaldehyde
HSRB	Human Studies Review Board
POD	point of departure
SGaw	specific airway conductance

HSRB Meeting Report – Kulle et al. Studies

Kulle, T., Sauder, L., Hebel, J. R., Green, D. J, and Chatham, M. (1987). Formaldehyde dose-response in healthy nonsmokers, *JAPCA*, 37: 919-924.

Kulle, T.J. (1993). Acute Odor and Irritation Response in Healthy Nonsmokers with Formaldehyde Exposure. Inhalation Toxicology 5(3): 323-332. DOI: 10.3109/08958379308998389

Introduction

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Review Process

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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 as well as oral comments from Agency staff during the HSRB meeting discussions. A comprehensive list of background documents is available at https://www.epa.gov/osa/october-25-2022-hsrb-meeting.

Charge Questions and Context

Charge to the Board – Science

Is the research by Thomas J. Kulle et al. described in the 1987 publication "Formaldehyde dose-response in healthy nonsmokers" and the 1993 publication "Acute Odor and Irritation Response in Healthy Nonsmokers with Formaldehyde Exposure" scientifically sound, providing reliable data for use in a weight-of-evidence to determine a POD for acute inhalation exposure to formaldehyde?

HSRB Response

The research described in the 1987 publication "Formaldehyde dose-response in healthy nonsmokers" and the 1993 publication "Acute Odor and Irritation Response in Healthy Nonsmokers with Formaldehyde Exposure" is scientifically sound, providing reliable data for use in a weight-of-evidence to determine a POD for acute inhalation exposure to HCHO provided that the recommendations provided by the HSRB are considered.

The HSRB also had specific comments, recommendations, and additional minor points, which are described in the discussion below.

Science Review

The two publications are based on one single study, which investigated dose-response relationships in irritation symptoms and pulmonary functions from HCHO exposures in a controlled environmental chamber. The 1987 publication provides details of the study methods. Nineteen healthy nonsmoking human volunteers participated in the study and were divided into two groups. The first group included ten participants (four men and six women), who received five 3-hour exposures to HCHO or control air. Exposures in the first group were 0.0, 0.5, 1.0, and 2.0 ppm at rest and an additional 2.0 ppm exposure with exercise (an 8-minute bicycle ride every 30 minutes). The second group of nine individuals (six men and three women) received four exposures at 0.0, 1.0, 2.0, and 3.0 ppm at rest and an additional 2.0 ppm with exercise. Exposures were randomized and separated by 1 week to avoid possible carryover effects from previous exposures. Each participant was exposed to HCHO at the same time each day to avoid possible diurnal effects.

To evaluate irritation symptoms from the HCHO exposures, questionnaires on the presence and severity of odor sensation, nose and throat irritation, eye irritation, chest discomfort and tightness, cough, headache, heart palpitations, and double vision were collected before and after (t = 180 min) each exposure as well as at 24 hours post-exposure. To evaluate the dose response in pulmonary functions, spirometric measurements (forced vital capacity (FVC), forced expiratory volume in 10 s (FEV1), and forced expiratory flow at 25% and 75% of the pulmonary volume ($FEF_{25-75\%}$)) were collected immediately before and during each exposure, every 30 min (t = 0, 30, 60, 90, 120, 150, and 180 min), and at 24 hours after exposure to 3.0 ppm (at rest) and 2.0 ppm (with exercise) HCHO. Airway resistance, measured as specific airway conductance (SGaw), and thoracic gas volume (functional residual capacity) were measured immediately before (t = 0 min) and after (t = 180 min) each exposure as well as at 24 hours post-exposure. Nonspecific airway reactivity (log PD35SGaw) was measured after (t = 180 min) each exposure as well as at 24 hours post-exposure. Nasal resistance was determined before and after (t = 180 min) at-rest exposures to 2.0 and 3.0 ppm HCHO. Review and discussion of the statistical analysis in both publications will be provided in detail in the statistical review. In general, analysis of variance was used in Kulle et al. (1987) to assess dose-response relationships for reported symptoms and measured pulmonary parameters.

This study reported dose-response relationships for odor sensation and eye irritation. Mild nose and throat irritation was reported by 37% (7 out of 19) and 22% (2 out of 9) of the participants at 2 and 3 ppm, respectively, but the reporting percentage was not significantly different from that at control concentrations (clean air). There were no significant decrements in pulmonary functions (FVC, FEV1, $FEF_{25-75\%}$, SGaw) at the tested exposure levels.

Kulle subsequently reanalyzed the data using different statistical tests (the McNemar 2 × 2 test) (Kulle, 1993). The stated aim of the reanalysis was to define a threshold for the onset of irritation symptoms. After the reanalysis, Kulle stated that the threshold for eye irritation was estimated to be in the neighborhood of 0.5–1.0 ppm HCHO, which is consistent with the prior finding of no effect at 0.5 ppm and slight irritation at 1 ppm. Kulle also stated that the threshold for nose and throat irritation was estimated to be around 1.0 ppm HCHO, again consistent with the prior finding of marginally significant dose-response with effects starting at 1 ppm. Overall, the reanalysis found similar effect levels as the original study.

Comments

In general, the study methods and results presented in these two publications provide scientifically reliable data. We agree with the conclusion provided in the U.S. Environmental Protection Agency (EPA) review and consider this study acceptable as part of the weight-of-evidence to support the acute inhalation risk assessment for HCHO.

Specific concerns with these studies are as follows:

- All participants in this study were healthy nonsmoking, young adults (average age of 26.3 [± 4.7 standard deviation] years). The data therefore do not include sensitive populations such as individuals with preexisting conditions (e.g., asthma). This should be addressed in EPA's risk assessment. For example, whether existing health status (e.g., asthma) affects the particular endpoint used as the POD (e.g., eye irritation versus respiratory function) should be considered.
- Advantages of the study included the balanced sex ratio and the exclusion of smokers, who may be habituated to HCHO exposure. Exposure appears to have been well characterized with the two methods used (continuous gas monitors and impingers).
- Symptom responses from different individuals were summarized to calculate the reporting percentage at different HCHO levels. If it can be obtained, EPA should examine the raw data to analyze the response change or increase to increasing HCHO level for each participant. The lack of raw data and the impact on validation and further analysis were noted in the statistical review document provided by EPA from ICF.
- Symptom scores (which yielded the most sensitive measures of effect) were collected through participant self-reporting (questionnaire) without interviews. Again, examination of the raw data may be useful to determine if there are outliers and to understand variability between participants.
- This study measured functional residual capacity but the results were not present in either publication.

Statistical Review

The study was conducted with healthy young nonsmokers without allergies, asthma, hay fever, or upper respiratory infection 6 weeks prior the study. The experimental protocol was a randomized block design with participants serving as their own controls. Each participant received five exposures in the same controlled environment. The experiment controlled for exposure time (3 hours, 1 week apart), potential carryover effects, potential diurnal variations in response, and also used homogeneous standardized measurements. Five separate exposures/measurements were reported for every participant. The authors indicated that randomization was performed relative to the HCHO dose and for every visit, but the description is unclear.

In the original statistical analyses (Kulle et al. 1987), three analysis of variances (ANOVAs) were conducted: 1 for 10 participants (0.0, 0.5, 1.0, and 2.0 ppm exposures), 1 for 9 participants (0.0, 1.0, 2.0, and 3.0 ppm), and 1 for all 19 participants with common exposures (0.0, 1.0, and 2.0 ppm). A log-linear dose response was used in the ANOVA for 10 participants due to unequal spacing in doses. These ANOVAs were conducted for each specific time point (7 time points × 3 participant groupings = 21 total ANOVAs for each response variable). Tukey adjusted follow-up tests were conducted when the overall ANOVA F test was significant. A contrast was used to examine the monotonic trend with increasing dose. Symptomatic responses were assigned severity levels of 0 = none, 1 = mild, 2 = moderated, and 3 = severe.

In the presented analyses, it is unclear whether a block (participant) was included in the model. If a block was not included, the F test indicating no statistical significances related to different ppm HCHO concentrations might be erroneous due the independence assumption of the one-way ANOVA (i.e., there are repeated measures of the same 19 participants). A mixed model with participant included as a random effect would account for the repeated measurement of the same participants.

Kulle (1993) reexamined the data to compare the symptomatic responses and quantify threshold concentrations. The symptomatic response was the difference between the score at completion of the exposure and the score prior to exposure (t180–t0). McNemar's test was used to analyze symptomatic disagreements for individuals before and at the completion of exposure at each exposure concentration (0.0, 0.5, 1.0, 2.0, and 3.0 ppm). Tables 2, 3, and 4 report odor sensation, eye irritation, and nose/throat irritation symptoms of HCHO exposures at the five concentrations. Because the tables do not indicate the individual participants in each exposure reporting symptoms, the analysis is not reproducible.

EPA, specifically Dr. Jonathan Cohen and Rachel O'Neal, conducted additional statistical analyses of the data. Benchmark dose analyses were conducted for the odor sensation and sensory irritation rates. These analyses were then compared with those reported in the Integrated Risk Information System report. Fisher's Exact Tests were used to analyze symptomatic responses at different dose levels and Cochran-Armitage tests were used to examine trends in response rates. Dr. Cohen reported that the assumption of independence for these tests may not be satisfied since the participants were exposed to multiple doses. Dose-response models were then used to fit and plot dose-response models and to estimate the benchmark dose (BMD) as the dose at which there was a 10% extra risk above an assumed 0% risk for unexposed individuals.

Without the raw subject-level data, the analyses presented in the manuscripts are not reproducible and further analyses are limited. Analyses that rely on the assumption of independence during multiple exposures like Fisher's Exact Test and Cochran-Armitage test should be interpreted with caution as the underlying correlation between observations cannot be accounted for. This could lead to increased Type I errors.

Recommendations

- EPA should consider two publications from Pazdrak et al. (1993) and Krakowiak et al. (1998) when assessing acute inhalation risks. Both studies included human subjects with potentially increased sensitivities to HCHO exposures (humans with asthma or preexisting skin sensitization). Both studies monitored changes in nasal lavage, which may serve as a more responsive health effect indicator from acute HCHO inhalation exposure.
- On page 5 of the EPA review and in the paragraph above the section "*RESULTS,*" BMD is mentioned and used to "*fit and plot dose-response models.*" Please include this analysis as an attachment to the final report.
- It is unclear what symptom responses were used in the BMD analysis. Odor sensation is not suitable to be considered as a toxicity endpoint here, and eye irritation may be caused by direct eye contact with HCHO in air instead of inhalation. If EPA plans to use BMD, further discussion of the modeling process is needed.
- Page 2 of the EPA review states "*At the 1.0 ppm formaldehyde exposure concentration, 3 of 19 subjects reported mild eye irritation and 1 reported moderate eye irritation.*" According to Table 3 in Kulle (1993) (the same table cited on page 7 of the EPA review), at 1 ppm, four participants (two men and two women) reported mild eye irritation. Please update if necessary.
- There is a page number error in the EPA review; there are two on page 2. There is also a redundant blank row on page 2, above the section "*COMPLIANCE.*"
- EPA should specify the block used in the original study, as this is not clear either in the original manuscript or in EPA's review.
- EPA should note that a mixed model would be most appropriate to analyze the original data, but that this model could not be considered currently due to a lack of access to the raw data.
- On page 4, there is a missing 'the' in the quote from the 1993 paper. Change to: "….was determined as the difference between [the] score at the completion…"
- EPA should note that the additional tests (Fisher's Exact Test and Cochran-Armitage test) should be interpreted with caution given the assumption of independence is likely not satisfied.
- On page 5, clarification is needed for these statements: "For odor sensation and eye irritation, statistically significant differences and trends were found in the response rates at the 5% level. For nose/throat irritation, no statistically significant differences were found in the response rates at the 10% level, but statistically significant trends in the response rates were observed at the 10% level."
    - It is unclear what the statistically significant differences and trends were. Please add this discussion to the final document.
    - "5% level" and "10% level" should be changed to add the word 'significance': "5% significance level"
    - The second sentence is unclear without clarification of the differences and trends considered. Recommend breaking these sentences into multiple sentences and describing the differences and trends found.

Charge to the Board – Ethics

Does available information support a determination that there is no clear and convincing evidence that the conduct of the research was fundamentally unethical or deficient relative to the ethical standards prevailing at the time the research was conducted in a way that placed participants at increased risk of harm or impaired their informed consent?

HSRB Response

The Board does not find that the research described in the 1987 published article "Formaldehyde dose-response in healthy nonsmokers" and the 1993 publication "Acute Odor and Irritation Response in Healthy Nonsmokers with Formaldehyde Exposure" provides clear and convincing evidence that the conduct of the research was fundamentally unethical or deficient relative to the ethical standards prevailing at the time the research was conducted in a way that placed participants at increased risk of harm or impaired their informed consent.

Ethics Review

Nineteen adults participated in the study, 10 men and 9 women. Inclusion and exclusion criteria are not explicitly delineated in either article, but the participants underwent a screening examination, which included their medical history, a physical exam, ECG, pulmonary function tests, and nonspecific airway reactivity through the optional methacholine challenge test (p. 919, 1987). Participants were healthy and nonsmoking, denied a history of allergy, asthma, hay fever, or upper respiratory infection in the 6 weeks prior to the study, and underwent an exercise test on the screening day. The 1993 article reports that "all pulmonary and airway reactivity determinations for this healthy, nonsmoking group of ten men and nine women were within normal ranges" (p. 327). Some of the exclusion criteria can therefore be deduced: smoking status, recent history of allergy, asthma, or URI, and potentially disqualifying elements of medical history related to physical examination, ECG, and pulmonary functioning.

Participants were financially incentivized with an undisclosed amount, but otherwise no information is provided about recruitment methods. However, there is no evidence to indicate that recruitment, participant selection, and/or screening was unethical or inequitable.

Informed Consent Process

Written ("signed" 1993, p. 325) informed consent was obtained for all study participants, but neither article provides further information on the process, timing, or documentation of consent.

Risks and Benefits

Specific study risks are not detailed in either article, but investigators minimized risks to participants by selecting HCHO exposure concentrations in line with accepted Occupational Safety and Health Administration standards of the day. The 1993 article specifically discusses participant symptoms following HCHO exposure, which include irritation of the eyes, nose, and throat. Risks were also minimized through the screening procedures and by only recruiting healthy, nonsmoking adults.

Participant privacy and data confidentiality appear to have been protected: no participants are identified in either article; demographics and characteristics are only reported in aggregate.

There is no direct benefit to study participants, but there is a potential benefit to society by establishing safe and acceptable levels of HCHO exposure.

Independent Ethics Review

The study was reviewed and approved by the Human Volunteers Research Committee at the University of Maryland. No further information is provided about the protocol or review process.

Review Summary

Based on our review of the provided documents, including both published articles, the HSRB does not find any evidence that this study was conducted unethically. Only healthy, nonsmoking adults were enrolled. No children were enrolled and there is no indication that pregnant or lactating individuals were enrolled in the study. Risks were adequately minimized through screening procedures and by adopting an accepted threshold of HCHO exposure. Informed consent was obtained, and the study was reviewed and approved by an independent ethics committee. No study procedures would invalidate or impair the participants' informed consent. While important and specific information is lacking from both articles related to the ethical conduct of this study, there is no evidence that it was conducted unethically or was deficient relative to the standards of the time.

List of Acronyms – MIM-006 Study

CFR	Code of Federal Regulations
CI	confidence interval
CPT	complete protection time
EPA	Environmental Protection Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
HCHO	formaldehyde
HSRB	Human Studies Review Board
IRB	Institutional Review Board
mCPT	median complete protection time
POD	point of departure
SACHRP	Secretary's Advisory Committee for Human Research Protections
UC	University of California

HSRB Meeting Report – MIM-006

Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray Against Mosquitoes. Study Report MIM-006. May 12, 2022.

Introduction

On October 26, 2022, the Human Studies Review Board (HSRB) considered the research article by Kulle et al., "Formaldehyde dose-response in healthy non-smokers" (*JAPCA*. Volume 37, pp. 919–924) and by Kulle, "Acute odor and irritation response in healthy nonsmokers with formaldehyde exposure" (*Inhalation Toxicology*. Volume 5. Issue 3. pp. 323–332). EPA proposes to use the quantitative results of the study as part of a weight-of-evidence in determining a point of departure (POD) for acute inhalation exposure to formaldehyde (HCHO) in the human population.

Review Process

The Board conducted a public meeting on October 27, 2022. Advance notice of the meeting was published in the *Federal Register* as "Human Studies Review Board; Notification of a Public Meeting" (EPA, FRL-9328-01-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.

The Agency staff presented their review of the scientific and ethical aspects of the research, with each presentation followed by clarifying questions from the Board. The HSRB considered public comments presented by the sponsor and then proceeded to address the charge questions. 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, 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/october-25-2022-hsrb-meeting.

Charge Questions and Context

Charge to the Board – Science

Did the research summarized in "Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray Against Mosquitoes" generate scientifically reliable data, useful for estimating the amount of time the product tested repels mosquitoes?

HSRB Response

The research summarized in "Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray Against Mosquitoes" generated scientifically reliable data that is useful for estimating the amount of time the product tested repels mosquitoes provided the recommendations provided by the HSRB are considered.

The HSRB also has specific comments, recommendations, and additional minor points, which are described in the discussion below.

Science Review

The objective of this study was to determine the complete protection time (CPT) of MIMIKAI Lilly Pilly mosquito repellent, which is a formulation of 11% Oil of Lemon Eucalyptus and 7.75% 2-undecanone (methyl nonyl ketone). Both ingredients are already approved for use under the FIFRA.

Twenty-six volunteers were tested in two different habitats in Sacramento Valley, CA (Site 1: 7 women, 6 men; Site 2: 6 women, 7 men). The arms of the volunteers were treated with 0.5 g/600 cm^2 and exposed for 5 minutes every 30 minutes until product failure or cessation of testing. Product failure was defined as the first confirmed landing, which is a landing followed by a second landing within the next 30–60 minutes. There were seven protocol deviations. These were all described, including the potential impact of each on the study.

The study resulted in "substantial and prolonged protection" against *Aedes melanimon*, *Ae. vexans*, *Ae. nigromaculis*, *Culex tarsalis*, and *Anopheles freeborni*. The median CPT (mCPT) was 8.6 hours for Site 2 and >9 hours at Site 1, when the study was ended due to low landing pressure. The study locations did not have *Ae. albopictus* and *Ae. Aegypti,* which are the primary vectors of Zika, Dengue, and Chikungunya. Therefore, the submitted data do not support efficacy claims against vectors of Zika, Dengue, and Chikungunya on the product label.

Comments

We agree with the conclusions by EPA that this study provides reliable, scientifically sound data to support CPT. We appreciate the revisions by EPA and the sponsor prior to the study and this existing report, and issues that might have been of concern have already been addressed. Although there were protocol deviations, these do not appear to undermine the conclusions of the study. We further agree with EPA that claims of efficacy against vectors of Zika, Dengue, and Chikungunya cannot be made on the label. We would also agree with the considerations for future studies

Specifics concerning these studies are as follows:

1) There continues to be an inconsistent use of scientific references to support statements. For example,
    - Section 1.2. "Traditional DEET-based repellents are highly effective but are often cosmetically mediocre and may produce mild to serious side effects." (Additionally, what does "cosmetically mediocre" mean?)
    - Section 1.3. Discussions of Zika, Dengue, and other diseases.
    - Section 2. Sources of NOAELs/LOAELs
    - Section 3. Has references (as footnotes).
2) P. 34 "landing when the ambient pressure dropped off at ca. 9 hours post-application." Change to landing pressure, rather than ambient.

Statistical Review

The Agency reviewed the study titled, *Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray Against Mosquitoes*. The registrant provided clarification on the methods requested by the Agency. The Agency accepted the study methods after reviewing the registrant's clarifications.

Recruitment yielded a single pool of candidates, which was subsequently separated into two pools. Therefore, two pools of participants from a single pool of candidates were randomly assigned to the treated, control, and alternate groups within each test day. Treated individuals were randomly assigned by gender. One man and one woman participant were randomly selected as untreated controls, and five participants were randomly selected as alternates. A protocol deviation is not likely to compromise results because statistical analysis was performed within each test day.

Thirteen individuals per treatment group were used in the study. The test was performed in adjacent Sacramento Valley counties.

Kaplan-Meier Survival Analyses were used to calculate the mCPT and 95% confidence intervals (95% CI) for landings data collected at Site 2. The Agency used statistical methods recommended in the protocol, providing a 95% CI that differed from the 95% CI reported for Site 2, due to default transformation methods in the statistical programs used. The researcher used a log transformation, and the Agency used a log-log transformation as recommended in the study protocol. The study results were similar. The mCPT at Site 2 was estimated as 519 minutes in both statistical methods.

In the study, the proposed statistical methods are appropriate to answer the research question. Therefore, the protocol generates reliable data.

Recommendations

There are no additional recommendations based on the statistical review.

Charge to the Board – Ethics

Does the available information support a determination that the research was conducted in substantial compliance with the applicable requirements of 40 Code of Federal Regulations (CFR) part 26, subparts K and L?

HSRB Response

Based on its review of the materials provided by EPA and subject to any limitations or recommendations of EPA or HSRB, the HSRB concludes that the "Field Efficacy Test of an Oil of Lemon Eucalyptus and Methyl Nonyl Ketone-Based Repellent Spray Against Mosquitoes" was conducted in substantial compliance with the applicable requirements of 40 CFR part 26, subparts K and L.

Ethics Review

The EPA ethics review provides the applicable ethical standards on page 8 of that review and these have been reproduced below.

Applicable Ethical Standards

The following provisions of 40 CFR 26 Subpart Q define the applicable ethical standards, which read in pertinent part:

> §26.1705: Except as provided in §26.1706, EPA must not rely on data from any research subject to this section unless EPA determines that the research was conducted in substantial compliance with all applicable provisions of subparts A through L of this part.
>
> In addition, §12(a)(2)(P) of the FIFRA applies. This passage reads:

In general, [i]t shall be unlawful for any person . . . to use any pesticide in tests on human beings unless such human beings (i) are fully informed of the nature and purposes of the test and of any physical and mental health consequences which are reasonably foreseeable therefrom, and (ii) freely volunteer to participate in the test.

Comments regarding §26.1703:

This study had a lower age limit of 18 years of age for participants. The youngest enrolled/consented participant was 19 years of age, which meets the requirement that the study not include children.

This study excluded pregnant women as part of the consent process. The authors confirmed the candidates were not pregnant during screening and further included extensive pregnancy testing (with appropriate maintenance of confidentiality) throughout the study, including before testing sessions. None of the individuals who participated in testing were pregnant, which meets the requirement that the study not include pregnant women.

Comments regarding §26.1705:

For practical purposes, this section of the regulations references the Federal government's regulations found at 40 CFR 26 subparts K and L which is similar to the Common Rule for the protection of human research subjects (also referred to as the U.S. Department of Health and Human Services regulations 45 CFR 46). Essentially the Common Rule requires that a study such as this one that involves human subjects address the following (this list incorporates only the requirements relevant to this study):

1) The research must be scientifically sound (covered separately in the HSRB science review).
2) The study protocol, informed consent document, and all recruitment materials must be reviewed and approved by an appropriately constituted institutional review board (IRB).
    a. The regulatory requirements for IRB membership, functions and operations, review of research, record keeping and communications with the investigator are specified in the Common Rule.
3) For a study such as the one being reviewed, the IRB may approve research covered by the Common Rule if it determines that all of the following requirements are satisfied:
4) Risks to subjects are minimized:
    a. By using procedures that are consistent with sound research design and that do not unnecessarily expose subjects to risk, and
    b. Whenever appropriate, by using procedures already being performed on the subjects for diagnostic or treatment purposes.
5) Risks to subjects are reasonable in relation to anticipated benefits, if any, and the importance of the knowledge that may reasonably be expected to result. In evaluating risks and benefits, the IRB should consider only those risks and benefits that may result from the research (as distinguished from risks and benefits of therapies subjects would receive even if not participating in the research). The IRB should not consider possible long-range effects of applying knowledge gained in the research (e.g., the possible effects of the research on public policy) as among those research risks that fall within the purview of its responsibility.
6) Selection of subjects is equitable. In making this assessment the IRB should consider the purposes of the research and the setting in which the research will be conducted. The IRB should be particularly cognizant of the special problems of research that involves populations vulnerable to coercion or undue influence, such as children, prisoners, individuals with impaired decision-making capacity, or economically or educationally disadvantaged persons.
7) Informed consent will be sought from each prospective subject or the candidate's legally authorized representative, in accordance with, and to the extent required by, § 26.116.

8) Informed consent will be appropriately documented or appropriately waived in accordance with § 26.117.
9) When appropriate, the research plan makes adequate provision for monitoring the data collected to ensure the safety of subjects.
10) When appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of data.

At the conclusion of the study, the study report and associated materials, including protocol deviations, are reviewed against these same standards.

As noted in the EPA ethics review, the protocol for this study, informed consent, and study recruitment materials as well as all similar materials for amendments were reviewed and approved by Advarra IRB. Advarra is a commercial IRB that meets the Common Rule regulatory requirements for membership, functions, operations, and review of research, record keeping, and communications with the investigator.

In addition to Advarra reviewing and approving this study protocol, informed consent, and subject recruitment materials, both EPA and the HSRB reviewed the materials, and the sponsor largely addressed the EPA and HSRB recommendations prior to the conduct of the study. These changes were reviewed and approved by the Advarra IRB.

Risks to participants were minimized in this study in multiple ways, including via the exclusion criteria in the protocol if participation in the research might pose a risk to a candidate's health (for example, via an allergic reaction), the use of lab-raised mosquitoes, via testing mosquitoes for viral vectors prior to testing sessions, via the use of barriers to shield skin outside the testing area, etc. Participants were monitored during the study for adverse events associated with mosquito bites, as well as other risks due to the environment, such as heat exhaustion. The risks associated with participation in this study were reasonable given the knowledge that would be gained (about the efficacy of the tested product). There were seven deviations from the protocol, none of which impacted the safety or welfare of the study subjects.

Research participants were required to speak and read English. Given that this study was conducted in Northern California, it seems likely that some potential participants may have limited English abilities and preferred Spanish, but a Spanish language consent form was not provided. However, it also seems likely that since this study was conducted in Northern California, some individuals whose preferred language was Spanish also spoke and read sufficient English to qualify for the study. Although the protocol mentions tracking ethnicity, there are no summary statistics regarding ethnicity or minorities for the group of individuals that enrolled/consented, so it is not possible to determine if the selection of participants is equitable in this regard, particularly with respect to the local communities in Northern California where this study was conducted. Of particular concern, during the HSRB review of this protocol in April 2021, questions were raised regarding the diversity and representativeness of the participants, and the HSRB recommended "that additional information be provided on how subjects from a variety of ethnicities will be recruited either by recruiting within targeted communities or other methods" (page 378 of study report). This does not appear to have been done, and the protocol includes a scientific (but not ethical) discussion or why ethnicity is not important to the study (page 51 of study report). Additionally, as noted, data on ethnicity were not provided in the study report.

The study report does indicate that of the 46 individuals that enrolled/consented, 20 were men and 26 were women and efforts were made via stratified randomization of treatment to maintain an approximate gender balance during testing sessions. This was done despite the fact that more female than male candidates volunteered for the study, and this would presumably be reflected in the

demographics of the 57 individuals who responded to the interview if this information was available. The gender balance makes this a situation in which the selection of subjects was not equitable based on gender.

Participants ranged in age from 19 to 54, but details of the spread of participants across the age range is not provided. Nor is the age distribution of the 57 individuals who were recruited. So again, it difficult to assess whether the selection of participants was equitable with respect to age.

One of the methods of recruitment was the University of California (UC) Davis ECOSOCIAL list serve, and another was the UC Davis Entomology Club email newsletter (see next section). This suggests that some of the participants may be students, a group that in some cases may be subject to coercion (for example, if their faculty members are involved in the testing study in some way). This study was conducted by Carroll-Loye Biological Research of Davis, California, but there was no indication of whether individuals listed in the study report also had a connection with UC Davis. It would be helpful to have such information if this is the case.

The above observations regarding uncertainties concerning the equitable selection of subjects could be improved by requiring future study reports to contain more information regarding demographics of the individuals selected for the study, including breakdowns according to age, gender, and minorities and ethnicities (while not ideal, the census categories are a good starting point). In some cases, this may be important for purposes of scientific analyses, but in all cases, it is necessary to adequately evaluate whether the selection of subjects is equitable as required by the justice principle in ethics (regarding the importance of this principle, see the Belmont report and also a recent Secretary's Advisory Committee for Human Research Protections (SACHRP) recommendation on the topic: Consideration of the Principle of Justice 45 CFR part 46, https://www.hhs.gov/ohrp/sachrp-committee/recommendations/attachment-a-consideration-of-the-principle-of-justice-45-cfr-46.html).

Informed consent is discussed under the FIFRA requirements in the following section and will not be covered here.

To maintain the privacy and confidentiality of participants, they were assigned a study subject number that was used instead of their names in all study documents and communications. Pregnancy tests were conducted in private and were communicated confidentially to a female member of the study staff to confirm eligibility of female candidates to participate in the testing.

Provided the scientific review finds this study scientifically acceptable, the above review of the materials provided indicates that this study was reviewed, approved, and conducted in substantial compliance with the regulations found at 40 CFR 26 subparts K and L, which are similar to the Common Rule required by §26.1705. The one limitation is that due to insufficient information in the report, a thorough evaluation of the equitable selection of participants was not possible.

Comments regarding §12(a)(2)(P) of the FIFRA:

> Subjects were recruited from the local communities in Northern California via Craigslist, the UC Davis ECOSOCIAL list serve, the UC Davis Entomology Club email newsletter, and word of mouth.

This study, including its informed consents and subsequent amendments, was reviewed and approved several times by Advarra IRB. The final approval of the protocol and informed consent document was for amendment 3, the last amendment, which occurred on September 11, 2021. While the study was initiated with the IRB on February 17, 2020, the actual conduct of the field study occurred on September 26, 2021, and October 3, 2021, and utilized the amendment 3 protocol and informed consent. The study completion date was October 28, 2021, and the study was closed by the IRB on November 16, 2021. The IRB-approved informed consent includes "the nature and purposes of the test and of any physical and

mental health consequences which are reasonably foreseeable therefrom," thus meeting the regulatory requirement. In addition, the consent process included demonstrations of the repellent application, attractiveness testing, and aspirator use, further addressing the regulatory requirements.

Participants received $25 per hour for each hour of participation in each phase (consent, training, test day) rounded up to the next hour. Participants were paid in person at the end of each visit. Compensation was consistent with the IRB-approved protocol and informed consent. Given the time spent in testing and the nature of the testing, the compensation amount was reasonable and is not expected to have unduly influenced the recipients to participate in the study.

All individuals who participated in the testing had signed the amendment 3 informed consent document, either during an in-person outdoor meeting or, for those attending an on-line consent meeting, when they presented for their first testing session. The consent document specifically indicated that participation was voluntary and that they could withdraw at any time. The candidates were also informed of this verbally prior to testing and were not coerced. All of these indicate that the candidates freely volunteered to participate in the testing. No participants withdrew or were removed from participation in this study.

Recommendations

EPA should note that information on demographics was not provided, therefore it is unclear whether there was equitable subject selection. It is necessary to adequately evaluate whether the selection of subjects is equitable as required by the justice principle in ethics (regarding the importance of this principle, see the Belmont report and also a recent SACHRP recommendation on the topic: Consideration of the Principle of Justice 45 CFR part 46, https://www.hhs.gov/ohrp/sachrp-committee/recommendations/attachment-a-consideration-of-the-principle-of-justice-45-cfr-46.html). As noted above, this may apply to language restrictions, sex, and age distribution.

Recommendations for Future Studies

In addition to the specific recommendations for this study, the Board has several recommendations for future studies:

- The above study was performed in adjacent Sacramento Valley counties. EPA requires distinct ecosystems with distinct mosquito populations for testing sites. Although the proximity of the two test sites was less than the known range (<15 km) of the species of mosquitoes being evaluated, data supporting classification as two distinct ecosystems were provided and acknowledged. To avoid future issues related to this question, selected sites should be spatially separated as well. EPA could for example require sites be selected in at least two geographical regions of the United States (e.g., Southeastern and Pacific NW).
- The HSRB recommends that EPA require all future study reports to contain more information regarding demographics of the subjects selected for the study, including breakdowns according to age, gender, race, and ethnicities (while not ideal, the census categories are a good starting point) and potentially other characteristics in order to improve EPA's and HSRB's ability to evaluate the equitable selection of participants.
- We recommend that EPA provide guidance to sponsors for a standardized report format to increase readability and presentation of information. It might help for EPA to develop a general outline for study reports of this sort, perhaps using a scaled down version of the ICH E3 guidance.