

March 13th, 2017

EPA-HSRB-17-1

Robert J. Kavlock, Ph.D.
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Office of the Science Advisor
1200 Pennsylvania Avenue, NW
Washington, DC 20460

Subject: January 25-26, 2017 EPA Human Studies Review Board Meeting Report

Dear Dr. Kavlock,

The United States Environmental Protection Agency (EPA or Agency) requested that the Human Studies Review Board (HSRB) provide scientific and ethics reviews of five items:

- A published study, **Cimetidine-Carbaryl Interaction in Humans: Evidence for an Active Metabolite of Carbaryl**, authored by **D. Gail May, Rebecca J. Naukam, J. Reddy Kambam, and Robert A. Branch. Journal of Pharmacology Exposure Therapy (1992) 262(3), 1057-1061;**
- An unpublished study: **A randomized double blind study with malathion to determine the residues of malathion dicarboxylic acid (DCA), malathion monocarboxylic acid (MCA), dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), and dimethyl dithiophosphate (DMDTP) in human urine;**
- A published study, **Methylisothiazolinone contact allergy and dose-response relationships**, authored by **Michael D. Lundov, Claus Zachariae, and Jeanne D. Johansen. Contact Dermatitis (2011) 64, 330-336.**
- A published study, **Methylisothiazolinone in rinse-off products causes allergic contact dermatitis: a repeated open-application study**, authored by **K. Yazar, M. D.**

Lundov, A. Faurschou, M. Matura, A. Boman, J. D. Johansen, and C. Lidén. *British Journal of Dermatology* (2015) 173, 115-122.

- **A published study, *An evaluation of dose/unit area and time as key factors influencing the elicitation capacity of methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) in MCI/MI-allergic patients, authored by Claus Zachariae, Anne Lerbaek, Pauline M. McNamee, John E. Gray, Mike Wooder, and Torkil Menné. *Contact Dermatitis* (2006) 55, 160-166.***

The charge questions posed to the Board included assessing scientific and ethical aspects of these studies viewed individually, as well as the proposed combined use of the three studies related to methylisothiazolinone for a weight of evidence approach to risk assessment.

The Board's responses to the charge questions and detailed rationale and recommendations are provided in the enclosed final meeting report.

Signed,

A handwritten signature in black ink, appearing to be 'Liza Dawson', with a long horizontal line extending to the right.

Liza Dawson, PhD

Chair

EPA Human Studies Review Board

INTRODUCTION

On January 25-26, 2017, the United States Environmental Protection Agency's (EPA or Agency) Human Studies Review Board (HSRB or Board) met to address the scientific and ethical charge questions related to five items:

- A published study, **Cimetidine-Carbaryl Interaction in Humans: Evidence for an Active Metabolite of Carbaryl**, authored by **D. Gail May, Rebecca J. Naukam, J. Reddy Kambam, and Robert A. Branch**. **Journal of Pharmacology Exposure Therapy (1992) 262(3), 1057-1061;**
- An unpublished study: **A randomized double blind study with malathion to determine the residues of malathion dicarboxylic acid (DCA), malathion monocarboxylic acid (MCA), dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), and dimethyl dithiophosphate (DMDTP) in human urine;**
- A published study, **Methylisothiazolinone contact allergy and dose-response relationships**, authored by **Michael D. Lundov, Claus Zachariae, and Jeanne D. Johansen**. **Contact Dermatitis (2011) 64, 330-336.**
- A published study, **Methylisothiazolinone in rinse-off products causes allergic contact dermatitis: a repeated open-application study**, authored by **K. Yazar, M. D. Lundov, A. Faurschou, M. Matura, A. Boman, J. D. Johansen, and C. Lidén**. **British Journal of Dermatology (2015) 173, 115-122.**
- A published study, **An evaluation of dose/unit area and time as key factors influencing the elicitation capacity of methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) in MCI/MI-allergic patients**, authored by **Claus Zachariae, Anne Lerbaek, Pauline M. McNamee, John E. Gray, Mike Wooder, and Torkil Menné**. **Contact Dermatitis (2006) 55, 160-166.**

In addition, EPA requested that the HSRB respond to an additional charge question related to the use of the three MI studies in a weight of evidence approach to establishing a point of departure for risk assessment.

REVIEW PROCESS

The Board conducted a public meeting on January, 25-26, 2017. Advance notice of the meeting was published in the *Federal Register* as “Human Studies Review Board; Notification of a Public Meetings” (EPA, December 19, 2016, 95982, Vol. 81, No.250).

Following welcoming remarks from Agency officials, the Board began its consideration of the materials presented for review. On day one of the meeting, EPA staff began by presenting an overview of physiologically - based pharmacokinetic (PBPK) modeling and its intended uses by the Agency prior to the review of specific studies of cimetidine/carbaryl and malathion. On day two of the meeting, Agency staff presented an overview of repeated open application test (ROAT) studies prior to the review of the dermal exposure studies related to methylisothiazolinone, as well as a description of the use of ROAT studies by the Agency for a point of departure for risk assessment. These informational sessions provided the Board with context for the scientific and ethical review of each study and help Board members understand how the data would contribute to a larger process of risk assessment by the Agency.

On each day, the Board then heard presentations from EPA for each agenda item in sequence, consisting of the Agency’s review of scientific and ethical aspects of the studies, followed by discussion from members of the HSRB who reviewed each of the studies for scientific and ethical soundness. This Final Report of the meeting describes the HSRB’s discussion, recommendations, rationale and consensus in response to each charge question for each of these items.

For each agenda item, Agency staff first presented their review of the science and the Board asked the Agency presenters clarifying questions. The staff then described their review of the ethical aspects and the Board asked clarifying questions about those. The HSRB solicited public comments at that point. Following public comments, the Board read the Charge Questions under consideration. The Board addressed the science charge questions first, followed by the ethics charge questions. The Chair then called for a vote to confirm concurrence on a summary statement in response to each charge question.

For their evaluation and discussion, the Board considered the published papers, Agency presentations on intended uses of the data and information from authors of the published articles, the Agency's science and ethics reviews of the studies, other published papers and guidelines relevant to the field, and public comments made at the meeting.

On day one, prior to the review of the first two items, EPA staff made a detailed presentation to the board about the planned uses of data in PBPK modeling. Briefly, PBPK modeling allows risk assessors to convert an externally applied dose from an animal toxicity study into an internal dose at the target organ in humans. The models use biological and chemical properties that determine the pharmacokinetic processes that take place in the human body: absorption, distribution, metabolism and excretion. The aim of PBPK modeling is to make predictions about human health risk based on the prediction of a tissue response from a specific dose.

Both of the pesticides discussed in the meeting, carbaryl and malathion, are AChE inhibitors, but they have a different mechanism of action. The Agency plans to use plasma carbaryl concentrations and AChE inhibition data from the carbaryl study to evaluate PBPK models. The malathion study did not demonstrate AChE inhibition, so therefore AChE inhibition data from the malathion study will not be used. The malathion study demonstrates a no observed adverse effect level (NOAEL) which is used as a point of departure for risk assessment. The pharmacokinetic (PK) data from the malathion study will be used, including the urinary metabolites monocarboxylic acid (MCA) and dimethyl phosphate (DMP), and plasma malathion and malaaxon. The plasma PK data will be used for making model predictions, and the urinary metabolite data will be used for validation of the model. The PBPK models will be presented to the FIFRA Scientific Advisory Panel for external peer review later in 2017.

HSRB review of a published article: Cimetidine-Carbaryl Interaction in Humans: Evidence for an Active Metabolite of Carbaryl, authored by D. Gail May, Rebecca J. Naukam, J. Reddy Kambam, and Robert A. Branch. Journal of Pharmacology Exposure Therapy (1992) 262(3), 1057-1061

CHARGE TO THE BOARD AND BOARD RESPONSE

Charge to the Board:

Is the research described in the published article “Cimetidine-Carbaryl Interaction in Humans: Evidence for an Active Metabolite of Carbaryl” scientifically sound, providing reliable data?

Board Response:

The Board concluded that the research described in the published article “Cimetidine-Carbaryl Interaction in Humans: Evidence for an Active Metabolite of Carbaryl” is scientifically sound and provides data that are reliable for the purposes proposed by EPA.

HSRB Detailed Recommendations and Rationale:

This study, performed in the early 1990’s, involved in vitro tests of carbaryl, alone or together with the histamine-2 (H2) receptor antagonist, cimetidine, using erythrocyte acetylcholinesterase (AChE) activity as a pharmacodynamics endpoint of carbaryl-related bio-activity. In vivo study in this work, in four male subjects, followed plasma carbaryl and erythrocyte AChE activity over time following a single, oral dose. Subsequently the impact of pretreatment with a known cytochrome P450 inhibitor on pharmacokinetics and pharmacodynamics in those same study participants was assessed by pretreating them with 600 mg of cimetidine (divided into three, 8-hour doses) daily for three days, followed by a single carbaryl oral dosing. The investigators found that when cimetidine was pre-administered, that carbaryl peak plasma concentration was higher, although not statistically significant; oral clearance was lower and statistically significant ($p < 0.05$); and plasma half-life was longer, but not statistically significant. These findings suggested that metabolism of carbaryl was inhibited by cimetidine. Unexpectedly, the in vivo studies suggested that, in spite of the increased body exposure to carbaryl caused by cimetidine pretreatment, the inhibition of AChE by carbaryl was reduced in the pretreated individuals. Authors concluded with a tenable hypothesis that carbaryl is metabolized (by a cimetidine-inhibitable enzyme) to a more pharmacodynamically active metabolite.

The HSRB commented that one of the statements made in the EPA summary of the paper appeared to be incorrect, namely, “The results of the in vitro assays confirmed that, at a concentration of 10 or 100 $\mu\text{g/mL}$, cimetidine did not impact the RBC AChE inhibition caused by increasing concentrations of carbaryl.” In fact, at 100 $\mu\text{g/ml}$ both cimetidine and carbaryl

inhibit erythrocyte AChE, so the total observed inhibition is impacted. Furthermore, EPA's statement implies that the combined effect is additive, but the graphs at least raise the possibility that the effect may not be additive at 100ug/ml cimetidine. The graphs appear not to be completely additive, and in any case the data are not conclusive on this point. The Board also pointed out an inconsistency in the paper, in that the paper reports 4 human subjects, but reports n=6 for in vitro studies. This discrepancy is not explained. However, these issues do not cause the Board to be concerned about the internal validity of the study.

The Board opined that the fundamental study design seems sound, and the results suggest that there is a real possibility of a drug-drug interaction between carbaryl and cimetidine. The second element of the charge is related to provision of reliable data. The Board expressed confidence in the analytical/experimental reliability of the results. With regard to the human population-level reliability and generalizability of the results, the small number of subjects, the unclear process for selecting study participants, and the fact that the putative underlying processes uncovered in this study (activity of drug metabolizing enzymes) is subject to substantial individual variability in humans, all taken together reduce the Board's confidence in the population-level reliability of the data. In other words, it is not clear if the study participants, as a sample of the larger population, are a representative sample providing generalizable data. Nevertheless, the study has acceptable internal validity, in that reliable measurements were made and reasonable conclusions drawn regarding the metabolism of carbaryl. The Board asserts that the data are of acceptable scientific merit for EPA scientists to use for the stated purpose, namely, for PBPK modeling.

Comments on statistical aspects of the study

Cimetidine-carbaryl interaction in humans is investigated in study with four non-smoking, drug-free normal males. In vitro assays, cimetidine did not impact the outcome, whereas with vivo exposure, pre-treatment of cimetidine had an effect. The results support the hypothesis that the main site of metabolism for carbaryl is the liver by drug-metabolizing enzymes that can be inhibited by cimetidine.

The sample size of n=4 subjects seems small and it is unclear how the sample size was determined. However, this may be a pilot study, as the purpose appears to be to investigate the

pathway for these four individuals in depth and there is not as much concern about the population-level inference.

Statistical methods used for the data analysis include regression analysis, ANOVA, and repeated measures analysis. However, details are largely missing about the software package and procedures used. It appears that data and the code for analyzing the data are not available, making it a challenge to evaluate the quality of the data analysis. The statistical comments do not imply that the data are unreliable for EPA's intended uses, since generalizability to a larger population is not the main concern.

Ethics

Charge to the Board:

Does available information support a determination that the study was conducted in substantial compliance with subpart Q of 40 CFR part 26?

Board Response:

While the research was conducted before implementation of 40 CFR 26 subpart K and L, the information provided supports a determination that the studies were conducted in substantial compliance with subparts K and L of 40 CFR Part 26.

HSRB Detailed Recommendations and Rationale:

40 CFR 26 subpart Q applies to studies initiated prior to the implementation of the human subjects regulations at 40 CFR 26 subpart K and L; subpart Q requires that EPA not rely on data from studies if there is clear and convincing evidence that the conduct of the research was fundamentally unethical or deficient relative to prevailing ethical standards at the time the research was conducted. Subpart K requires that studies initiated on or after April 7, 2006 involving intentional exposure of human subjects to a pesticide be reviewed and approved by an institutional review board (IRB) that meets the membership and review criteria listed in that subpart. As this study was conducted prior to 2006, subpart Q is the applicable standard. Consistent with standards applicable at the time the research was conducted, the Vanderbilt Institutional Committee for the Protection of Human Subject reviewed and approved of this study. While we do not know if this Committee meets the criteria stated in 40 CFR 26 subpart K,

there was independent review at a reputable research university, the HSRB believes this 1991 study complied with the spirit of independent review.

40 CFR 26 subpart K mandates studies minimize risk to subjects, equitably select subjects, seek and appropriately document informed consent, make adequate provisions to ensure safety of subjects, and protect the privacy of subjects and confidentiality of data.

Minimize Risk: There is not a great deal of information provided about steps the investigators took to minimize participant risk. The dosages used in the study were below the level to cause symptoms and were “not expected to cause any additional short term adverse events.”

Equitable Selection: The investigator did not provide information about equitability of selecting the 4 male participants in this study. It appears to be a convenience sample of individuals affiliated with the University of Pittsburgh Center of Clinical Pharmacology, probably including the principal investigator. The Board did discuss the inclusion of only men in this study and noted the time period of the research (early 1990’s), prior to the widespread recognition of the importance of including women as well as men in biomedical research in order to obtain reliable information about the effects of treatments or exposures in females. The Board agreed that, given the time period of the study, inclusion of only male participants was consistent with typical research practice, particularly for non-beneficial exposure studies.

Informed Consent: As the research was done 26 years ago, neither the investigators nor the independent review board could not provide informed consent documentation, but the investigator told EPA that participants 1) were told of the science and risks of the study, 2) were sufficiently educated to understand the risks, and 3) signed informed consent documentation. The investigator does not provide information about provisions to ensure safety, protect privacy or confidentiality. While not ideal, the board does not feel the lack of this information violates the intention of 40 CFR 26 subpart K, and does not provide clear and convincing evidence of unethical conduct which would violate the substantive standard of subpart Q.

40 CFR 26 subpart Q prohibits the EPA from relying on third-party research involving intentional exposure to a pesticide of human subjects who are children or pregnant or nursing women. The 4 participants were male, so this does not violate 40 CFR 26 subpart Q.

HSRB review of an unpublished study: A randomized double blind study with malathion to determine the residues of malathion dicarboxylic acid (DCA), malathion monocarboxylic acid (MCA), dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), and dimethyl dithiophosphate (DMDTP) in human urine.

CHARGE TO THE BOARD AND BOARD RESPONSE

Charge to the Board:

Did the research on plasma levels of malathion and malaoxon, and urinary metabolites of malathion, as described in the study reports “A randomized double blind ascending single oral dose study with malathion to determine the No Effect Level on plasma and RBC cholinesterase activity” and “Determination of residues of malathion dicarboxylic acid (DCA), malathion monocarboxylic acid (MCA), dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), and dimethyl dithiophosphate (DMDTP) in human urine,” generate scientifically sound, reliable data?

Board Response:

The Board concludes that the research on plasma levels of malathion and malaoxon, and urinary metabolites of malathion, as described in the study reports “A randomized double blind ascending single oral dose study with malathion to determine the No Effect Level on plasma and RBC cholinesterase activity” and “Determination of residues of malathion dicarboxylic acid (DCA), malathion monocarboxylic acid (MCA), dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), and dimethyl dithiophosphate (DMDTP) in human urine,” generated scientifically sound data that are reliable for the uses proposed by the EPA.

HSRB Detailed Recommendations and Rationale:

The unpublished study report describes two studies. The first was a study in which 48 human participants were administered placebo or malathion at one of 5 different doses ranging from 0.5 mg/kg to 15 mg/kg. The study was entitled “A randomized double blind ascending single oral dose study with malathion to determine the no effect level on plasma and RBC cholinesterase activity” and it was conducted by Inveresk Research, Elphinstone Research Centre, Tranent, EH 32 2NE, Scotland.¹ In this study, the pharmacodynamic endpoints of plasma and erythrocyte AChE inhibition were assessed. The second study was entitled “Determination of residues of malathion dicarboxylic acid (DCA), malathion monocarboxylic acid (MCA), dimethyl phosphate (DMP), dimethyl thiophosphate (DMTP), and dimethyl dithiophosphate (DMDTP) in human urine,” and was conducted by Pacific Toxicology Laboratories, 6160 Variel Avenue, Woodland Hills, CA 91367.² In this second study, malathion and its metabolites from urine and plasma were analyzed.

As described above, the Agency plans to use PK data from the study for PBPK modeling purposes. Urine and plasma collection in the study was designed in a manner typical of pharmacokinetic studies, with pre-dosing and serial post-dosing samples collected. The design of the studies, the analytical methods used, and the data produced by the studies appear to be of a high scientific standard, and ideally suited to be used as inputs to aid the development and refinement of PB/PK models.

Comments on statistical aspects of the study

This is a randomized double blind ascending single oral dose study with a total of 34 subjects receiving treatments and 14 subjects in the control group. The sample size seems adequate for drawing population-level inference, although it is unclear from the protocol how the sample size was determined.

The statistical method used for the data analysis is a repeated measures analysis using SAS. It is

¹ The study and protocol were identified as ICR 013177. March 20, 2000. MRID 45125602.

² The Study and protocol were identified as PTL119801. October 11, 2000. MRID 45244601

unclear which SAS procedure was used and which covariance structure was specified. This information would be helpful to have.

In the repeated measures analysis, the main effects (dose level and time) and the interaction terms are specified. However, it is not clear why gender is not included as a main effect at the subject level. That is, it is unclear why were the data analyzed separately for male and female subjects. The model diagnostics are also unclear. More details and justifications for a particular choice (e.g., linear trend detection versus Bonferroni correction for multiple comparison) would be helpful to have. In addition, it is not clear how much the analysis can leverage the robustness of these methods against non-normality. Based on the description about the residuals, it seems that there is some heavy-tail (i.e., non-normal) distribution in the error term. It may be interesting to examine the robustness issue further.

Ethics

Charge to the Board:

Does available information support a determination that the study was conducted in substantial compliance with subpart Q of 40 CFR part 26?

Board Response:

While the research was conducted before implementation of 40 CFR 26 subparts K and L, the information provided supports a determination that the studies were conducted in substantial compliance with subpart Q of 40 CFR Part 26.

HSRB Detailed Recommendations and Rationale:

As mentioned above, 40 CFR 26 subpart Q applies to studies initiated prior to the implementation of the human subjects regulations at 40 CFR 26 subpart K and L; subpart Q requires that EPA not rely on data from studies if there is clear and convincing evidence that the conduct of the research was fundamentally unethical or deficient relative to prevailing ethical standards at the time the research was conducted. Subpart K requires that studies initiated on or after April 7, 2006 involving intentional exposure of human subjects to a pesticide be reviewed and approved by an institutional review board (IRB) that meets the membership and review

criteria listed in that subpart. As this study was conducted prior to 2006, subpart Q is the applicable standard.

The Inveresk Research Independent Ethics Review Committee reviewed and approved of this study. Based on information provided, the Committee meets the criteria stated in 40 CFR 26 subpart K and satisfies the standards of subpart Q, which is the applicable citation. The study was conducted in accordance with the guidelines set out in Declaration of Helsinki, 1964 and its 1975, 1989, 1996 amendments. The HSRB believes this study complied with the spirit of independent review. Amendments not reviewed by the Review Committee did not affect patient safety.

40 CFR 26 subpart K mandates studies minimize risk to subjects, equitably select subjects, seek and appropriately document informed consent, make adequate provisions to ensure safety of subjects, and protect the privacy of subjects and confidentiality of data.

Minimize Risk: The study listed above minimized risk to subjects by recruiting participants who were healthy (normal physical exam, laboratory data, and ECG), choosing doses not expected to cause adverse effects, and not escalating doses if there were any significant inhibition of cholinesterase. The study also contacted the participants' primary physician to determine if there was a concern with that individual participating in this study.

Equitable Selection: There are no specific information regarding the population from which participants were recruited, except that they were from the surrounding area and a generic advertisement was used. The use of randomization to different dosage or placebo allowed for equitable exposure to risk.

Informed Consent: The study physician explained the study and participants were allowed to ask questions. Written information regarding malathion. Additionally, this document contained most of the elements contained in a standard informed consent document (procedures, risks and benefits, withdrawing from the study, confidentiality, and physician contact information).

Safety: A physician provided oversight for the entire study. Participants resided in the study clinic for 2 days and were monitored at outpatient visits 3, 5, and 13 days after dosing.

Privacy and Confidentiality: Each participant was assigned a random individual identification number which protected privacy and confidentiality.

40 CFR 26 subpart Q prohibits the EPA from relying on third-party research involving intentional exposure to a pesticide of human subjects who are children or pregnant or nursing women. Materials submitted to EPA and HSRB indicate that no children or pregnant or nursing women enrolled in this study. Pregnancy testing occurred up to 21 days prior and again the day prior to the study. As a result, the study was conducted in substantial compliance with 40 CFR part 26, subpart Q.

HSRB review of a published study Methylisothiazolinone contact allergy and dose-response relationships, authored by Michael D. Lundov, Claus Zachariae, and Jeanne D. Johansen. Contact Dermatitis (2011) 64, 330-336.

CHARGE TO THE BOARD AND BOARD RESPONSE

Charge to the Board:

Is the research described in the published article “Methylisothiazolinone contact allergy and dose-response relationships” scientifically sound, providing reliable data?

Board response:

The board after discussion agreed that the research in the published article “Methylisothiazolinone Contact Allergy and Dose-Response Relationships” is scientifically sound, providing reliable data. In the use of this data for risk assessment to establish the elicitation threshold for methylisothiazolinone, the Board wishes to highlight some relevant exposure conditions or statistical concerns that should be considered when using this data.

HSRB detailed recommendations and rationale:

In this study authors wanted to 1) investigate the eliciting doses of MI in a patch test and in a ROAT for already sensitized subjects, 2) investigate whether phenoxyethanol influenced reactivity to MI, and 3) test a model for an exposure conversion between ROAT and patch test. The model will not be discussed here. For this study 11 test subjects (2 women and 9 men, average age of 49.7 years) with previous positive reactions to MCI/MI or MI were included. There were 14 controls (6 women and 8 men, average age 27.5) recruited into study. Subjects and controls were patch-tested first with 12 decreasing doses of MI (60 down to 0.0105 $\mu\text{g}/\text{cm}^2$) in 10% ethanol and 90% aqua, and then again with those same doses of MI in same mixture but with 0.4% phenoxyethanol (9.26 $\mu\text{g}/\text{cm}^2$). Blanks consisted of just the 0.4% phenoxyethanol in aqua and ethanol. Readings occurred on days 2, 3, 4 and 7 following occlusion for 2 days on the subject's backs. Placement of doses was double blinded. For the ROAT arm of study, patients applied 20 μl of 100ppm, 50 ppm and 5 ppm of MI mixture containing phenoxyethanol (using a fixed micropipette) twice a day on 3 x 3 cm on the volar part of forearm resulting in concentrations of 0.42, 0.21 and 0.021 $\mu\text{g}/\text{cm}^2/\text{day}$ (this amount is double the per application dose because the study involved two applications a day with no rinse off). Response were determined on day 2, 3, 4, 7, 14, and 21. In this study, an 8-point response scale from 0 (no reaction) to 8 (intensive erythema, infiltration and papules) was used for patch and ROAT. Responses greater than 5 on the response scale triggering stopping exposures in the ROAT arm.

Results: The study reported no statistical difference in reactions for test subjects in patch test with or without phenoxyethanol, or across threshold doses. Responses were seen at concentrations starting at 1.47 $\mu\text{g}/\text{cm}^2$ (55%) in the patch, while there was no response to 60 $\mu\text{g}/\text{cm}^2$ for controls in patch. In the ROAT study, for test subjects, 7/11 (64%) reacted to the 0.21 $\mu\text{g}/\text{cm}^2/\text{day}$, and 2/11 (18%) to the 0.021 $\mu\text{g}/\text{cm}^2/\text{day}$ (this dose is twice the per application dose due to twice daily exposure). Controls had no reaction in the ROAT study. Doses are described as low 1.47 $\mu\text{g}/\text{cm}^2$ for MI in patch test (55%, which would mean a concentration in cream of 49 ppm). The percentage of individuals who responded to 0.021 $\mu\text{g}/\text{cm}^2/\text{day}$ in the ROAT was 18%.

The HSRB expressed confidence that this study provides reliable and valid data. The Board noted that sufficient information was provided by the authors that the study has the potential to

be repeated and validated. The study objectives are stated; an acceptable control group is used; and the investigators used measures to reduce bias in the study by conducting a double blind study and by using an acceptable reading scale for responses. The investigators used methodologies to help assure adherence to study protocols (e.g., instructions to subjects, micropipette for application), and response rates related to application rates/concentrations were reported and related back to the objectives. These study characteristics provide support for the Board's conclusion that the data are scientifically sound.

The Board further recommended that EPA consider the following issues that may affect elicitation thresholds in the study:

- 1) Five sets of ROAT bottles were weighed to determine application conformance by subjects, however beyond stating adherence, no exact weights were reported and compared between control and test subjects.
- 2) Accumulated doses are mentioned in this study and compared to the patch results (again patch studies are occluded). This is done to build their model comparison between patch and ROAT. EPA may want to consider for risk assessment purposes the relationship of ROAT studies looking at elicitation threshold and potentially to relate back to induction threshold or even irritant thresholds that avoid induction and therefore elicitation points in participants. These relationships are only mentioned as a potential 'model' to explore elicitation thresholds, where induction of participants can be avoided or where human subjects for studies who have been induced are hard to find. Authors suggest repeated lower doses elicited quicker responses based on their comparison curves between patch and ROAT using equivalent ROAT doses, and an algorithmic relationship between the two is derived.
- 3) Although it is established that phenoxyethanol is not a sensitizer based on lack of response in subjects when phenoxyethanol was applied alone, this does not necessarily preclude that it could act synergistically or antagonistically with MI. For the ROAT arm, MI was not applied alone and therefore phenoxyethanol should not be entirely dismissed as having any vehicle effects when comparing to other studies.
- 4) MI-allergic subjects were previously tested at concentrations of up to 2000 ppm. Confirmatory patch testing was also conducted at this level. These are potentially high

induction threshold levels and the relationship between induction thresholds and elicitation thresholds are not well understood; thus the patch testing itself could have contributed to elicitation thresholds, although this is not known.

- 5) The average days to respond in the ROAT are not specifically reported and might be of interest. In addition, two of the 11 subjects in the ROAT did not follow the application scheme. It is not clear how this affected the statistical analysis.

Comments on statistical aspects of the study

Standard logistic regression analysis was used to estimate the dose–response relationship in the patch tests. The ED50 and 95% CI were estimated from the dose response curve. Comparison between the patch test reactions with or without phenoxyethanol was performed with Wilcoxon’s ranked sums test, and correlations between the individual threshold doses were investigated by Spearman’s ranked correlation. Differences in reactions to the same doses in the patch test and ROAT were investigated with McNemar’s test.

Based on the information related to statistical analysis provided in the article, the statistical validity of patch test results is questionable due to the following reasons: (1) because same subjects were used for with and the without treatments in the patch tests, the observed responses derived from treatments with and without phenoxyethanol were usually correlated, and this correlation was ignored in the dose-response models; and (2) the use of the statistically significant Spearman’s ranked correlation to conclude that treatments with and without phenoxyethanol produced similar rankings was inappropriate; (3) the Agency’s statistical validation confirmed the statistical results reported in the paper (ED50 and 95% CI were estimated from the dose response curve) was non-reproducible.

In summary, although there were flaws in the statistical analysis, the data presented in this article could be considered as scientifically sound and providing reliable data but limited to weight of evidence based applications when combined with other two reviewed papers.

Ethics

Charge to the Board:

Does available information support a determination that the study was conducted in substantial compliance with subparts K and L of 40 CFR part 26?

Board response:

The portions of the EPA regulations regarding the conduct of research with human subjects, 40 CFR part 26 subpart K and L, do not apply because the research was not conducted or supported by EPA, nor was it conducted with the intention to submit the results to EPA. EPA identified this study through a review of the public literature; the published article or any results of this research were not independently submitted to EPA.

The study was conducted in Denmark; therefore, the U.S. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) did not apply to the conduct of this research, but does for EPA use of data generated. The available information supports a determination that the study is in substantial compliance with subpart Q of 40 CFR Part 26 (Ethical Standards for Assessing Whether to Rely on the Results of Human Research in EPA Actions). Assuming scientific validity, the study may be used in actions under FIFRA (7 U.S.C. 136 et seq.) and section 408 of the Federal Food, Drug and Cosmetic Act (FFDCA).

HSRB Detailed Recommendations and Rationale:

In accordance with 40 CFR 26.1703, the research did not involve intentional exposure of any pregnant or nursing female subjects or any children. All of the subjects in this study were adults. Pregnancy and nursing were exclusion criteria for both test and control subjects. Female subjects were not tested for pregnancy before participation; they were asked if they were breastfeeding or could be pregnant. Given the minimal risk nature of the study (no subject had any severe or widespread reaction) and that patch testing during pregnancy or lactation is not known to be harmful, this seems adequate.

In accordance with 40 CFR 26.1704, the study was conducted under procedures at least as protective as those in subparts K and L of 40 CFR Part 26. The protocol and the published article state that the study was conducted according to the principles in the Declaration of Helsinki.

Documents provided to the EPA indicate that the study was reviewed and approved by the Capital Region of Denmark (the ethics committee with jurisdiction) on May 17, 2010. The ethics committee reviewed the study prior to initiation, amendments and reports with standards similar to those required by EPA (Danish Act on Research Ethics Review of Health Research Projects and the Ministerial Order No 806 of 12 July 2004 on Information and Consent).

The informed consent materials contained adequate information for the subjects (n=25) about the risks, discomforts and benefits of participation, and of their right not to participate or to withdraw (n=0). The risk-benefit ratio was determined to be acceptable and risks were minimized appropriately and were justified by the potential societal benefits associated with gathering data to determine the dose-response relationship in persons already sensitized to or allergic to the test article.

The selection of study participants appeared to be equitable and no children or pregnant women were enrolled. No conflicts of interest were declared. There is no evidence that the conduct of the research was fundamentally unethical or deficient relative to ethical standards and minimized the risk of harm to subjects.

HSRB review of a published study, Methylisothiazolinone in rinse-off products causes allergic contact dermatitis: a repeated open-application study, authored by K. Yazar, M. D. Lundov, A. Faurschou, M. Matura, A. Boman, J. D. Johansen, and C. Lidén. *British Journal of Dermatology* (2015) 173, 115-122.

CHARGE TO THE BOARD AND BOARD RESPONSE

Charge to the Board:

Is the research described in the published article “Methylisothiazolinone in rinse-off products causes allergic contact dermatitis: a repeated open-application study” scientifically sound, providing reliable data?

Board response:

The Board after discussion agreed that the research in the published article “Methylisothiazolinone in Rinse-Off Products Causes Allergic Contact Dermatitis: A Repeated Open-Application Study” is scientifically sound, providing reliable data.

HSRB Detailed Recommendations and Rationale:

In the use of this data for risk assessment to establish the elicitation threshold for methylisothiazolinone, the board wishes to highlight some relevant exposure conditions or statistical concerns that should be considered when using these data.

The objective of this study was to determine if currently allowed concentrations of MI in rinse-off products can cause allergic dermatitis. In this study consisting of 19 MI allergic subjects (mean age 40 years) and 19 control non-MI allergic subjects (mean age 37 years) participated. In one arm of the study 9 MI allergic subjects and all controls applied a 100 ppm MI soap five times a day to a 5 cm x 10 cm area of the ventral side of the forearm, resulting in a concentration of $0.48 \mu\text{g}/\text{cm}^2$ per application and a daily concentration of $2.4 \mu\text{g}/\text{cm}^2$. In the other arm of the study 10 MI allergic subjects and all controls applied a 50 ppm MI soap five times a day to a 5 cm x 10 cm area to the ventral side of the forearm resulting in a concentration of $0.24 \mu\text{g}/\text{cm}^2$ per application and a daily concentration of $1.2 \mu\text{g}/\text{cm}^2$. Controls used soaps with no MI.

Confirmatory patch test for MI subjects was 6 solutions of MI soap from 60 down to $0.48 \mu\text{g}/\text{cm}^2$, and vehicle control of aqua and 16% petrolatum. Control subjects only received the $60 \mu\text{g}/\text{cm}^2$ concentration soap. Patch tests were applied to back, occluded for 2 days, with readings on day 4 only. Readings for the ROAT were conducted once a week unless a reaction was experienced. Trained nurses and researchers conducted the reading for the patch, and researchers conducted readings for the ROAT. The study used an 8-point response scale from 0, (no reaction) to 8 (intensive erythema, infiltration and papules) for patch and ROAT. The investigators also used the Fisher scale (>25% area of application). Threshold concentration for a positive reaction in this study was a visible reaction of 1 on the scale on day 4 (if there was a continuous reaction). Subjects received instruction manuals and an instruction video on the application methods. The area of skin is moistened, soap applied and spread evenly and allowed to dry for 25s (some absorption into the skin is expected), area is rinsed and patted dry, and then a moisturizer (supplied by team) is applied once daily to area. Study participants were instructed not to use any of their own products during the study.

Results: 10/10 (100%) of participants who used the 100ppm ($0.48 \mu\text{g}/\text{cm}^2/\text{application}$) developed a reaction to MI soaps within 4-11 days (average 7.3 days); while 7/9 (78%) developed a reaction to the 50 ppm soaps within 5-21 days (average 8 days). Controls had no

reaction to MI soaps and neither subjects nor controls had reaction to the MI-free soap. There was statistical significance for the 100 ppm and 50 ppm soap compared to controls (Fisher test). In the patch test, some MI –allergic subjects (3/19) reacted to doses as low as 0.48 $\mu\text{g}/\text{cm}^2$. All MI-allergic subjects reacted to the 15 $\mu\text{g}/\text{cm}^2$ level.

The HSRB expressed confidence that this study provides reliable and valid data. The Board noted that sufficient information is provided by the authors that the study has the potential to be repeated and validated. The study objectives are stated; an acceptable control group is used; and the investigators used measures to reduce bias in the study by conducting a double blind study and by using an acceptable reading scale for responses. The investigators used methodologies to help assure adherence to study protocols (e.g., instructions to subjects, soap bottles with pump rates), and response rates related to application rates/concentrations are reported and related back to the objectives. These study characteristics provide support for the Board’s conclusion that the data are scientifically sound.

The Board further recommended that EPA consider the following issues that may affect elicitation thresholds in the study:

- 1) There is a need to clarify locations (anatomical) of application across studies.
Anatomical areas will differ in their sensitivity due to Stratum Corneum/Viable epidermis thickness and other structural differences resulting in varying local reactions. If cosmetics using MI are applied to various parts of the body, the most sensitive areas should be tested/considered in a risk assessment.
- 2) It is not clear why readings occurred on day 4 as opposed to day 2 and day 4 for the patch test.
- 3) Detailed information is provided in this study on where the MI and liquid soap preparation was obtained and chemical content of all applied test and control products. This will allow further analysis of the potential role of vehicle effects on MI elicitation thresholds.
- 4) Because this is a rinse-off study, the application, daily dose or accumulated may not be reflective of actual dose that remains on the skin, in the skin or absorbed into the skin (total dose compared to the ROAT). The actual LOAEL in this study may in fact be lower due to removal of the product following rinse activities, and pat and dry activities.

- 5) The control soap in the MI studies was preserved with methyl and ethyl paraben. Although there was no reaction to control soap in the ROAT, it might be important to note the presence of these preservatives.
- 6) MI for rinse off products can be found in liquid soaps and shampoos. Exposure rates will depend on customary uses of the products. This study tested liquid soap at 5 times a day and makes a statement that this may underestimate usage rate. There is no study reference for usage rates (volumes applied and areas of application), although a conservative approach (testing at higher doses and for higher usage rates) is appropriate in order to establish a safety factor. In the risk assessment, some questions arise in comparing MI studies with various application rates and therefore applied dose, or loading rates.
- 7) Vehicles effects for those who applied the optional provided lotion are not discussed.

Comments on statistical aspects of the study

The study goal was to determine if MI concentrations of 100 ppm and 50 ppm have the potential to elicit an allergic response in previously sensitized individuals. The treatment group consisted of 19 subjects with dermatitis and contact allergy to MI. Assignment to treatment group was determined by patch testing at 2000 ppm prior to start of the study. Ten subjects were assigned to 100 ppm dosages, and 9 subjects to 50 ppm. There was no indication of how the assignments were determined. The control group consisted of 19 subjects without contact allergy to MI as determined by no allergic reaction to patch testing prior to the start of the study. The investigators used Fisher's Exact Test to compare differences in the proportion of allergic responses for the two groups. This test would have been appropriate if underlying assumptions were met, however there was a problem related to study design, because there was no 50ppm control group. For MI of 100 ppm there was significant difference ($p = 5.0 \times 10^{-8}$) based on 10 allergic and 10 controls. The authors also claim there was a significant difference for an MI of 50 ppm ($p = 3.0 \times 10^{-5}$), but the control subjects were not tested at 50 ppm. The apparent assumption of the investigators is that the fact that subjects had no reaction at 100 ppm of MI for a control subject implies they would have no reaction at 50 ppm of MI. This might be a

reasonable guess, but for statistical purposes, the design of the study should have included a 50 ppm control group.

The investigators used McNemar's test for equality of proportions for comparison of pre-study patch test and ROAT on MI allergic subjects. For 10 allergic subjects at 100 ppm of MI there was a significant difference ($p = 0.00195$). None reacted to the patch test but all 10 reacted to the ROAT testing. The problem with the use of this test, however, is that the p-value is for two sided alternative (difference in either direction) but the authors' conclusion is that ROAT reactivity is higher (one sided conclusion). For all 19 allergic subjects,³ there was a significant difference ($p = 0.000122$) between the reaction to the patch test at 100 pm and the combined 100 ppm and 50 ppm reactions to the ROAT testing.

Finally, the investigators used Kendall's tau-B for an association for the allergic subjects between a positive MI patch test threshold and the threshold for ROAT. Two subjects with no ROAT reaction were not included. Kendall's tau-B is the difference between the number of concordant and discordant pairs of observations, which is not quite the same as the usual correlation coefficient, although the authors describe this as a test for correlation. The results show that Kendall's tau-B = 0.381 and $p = 0.062$; the p value from SAS is 0.0569 which indicates a computational difference, but does not change the overall finding that the test was not quite statistically significant.

In summary, the chosen statistical analyses are appropriate but some of the conclusions have what might be considered as minor flaws because the underlying assumptions are not met. Specifically, the lack of a 50 ppm control group, the one-sided conclusion from a two sided test, and the [problem with the Kendall's tau-B test] somewhat weaken the statistical analysis.

³ The Board noted a typographical error in the paper: the $n = 49$ for ROAT at 50 ppm should be $n = 19$.

Ethics

Charge to the Board:

Does the available information support a determination that the studies were conducted in substantial compliance with subparts K and L of 40 CFR Part 26?

Board response:

The portions of the EPA regulations regarding the conduct of research with human subjects, 40 CFR part 26 subpart K and L, do not apply because the research was not conducted or supported by EPA, nor was it conducted with the intention to submit the results to EPA. EPA identified this study through a review of the public literature; the published article or any results of this research were not independently submitted to EPA.

The study was conducted in Sweden and Denmark; therefore, the U.S. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) did not apply to the conduct of this research, but does for EPA use of data to set a human dermal sensitization endpoint/point of departure in its risk assessment for methylisothiazolinone.

The available information supports a determination that the study is in substantial compliance with subpart Q of 40 CFR Part 26 (Ethical Standards for Assessing Whether to Rely on the Results of Human Research in EPA Actions). Assuming scientific validity, the study may be used in actions under FIFRA (7 U.S.C. 136 et seq.) and section 408 of the Federal Food, Drug and Cosmetic Act (FFDCA).

In accordance with 40 CFR 26.1703, the research did not involve intentional exposure of any pregnant or nursing female subjects or any children. All of the subjects in this study were adults. Pregnancy and nursing were exclusion criteria for both test and control subjects. Female subjects were not tested for pregnancy before participation; they were asked if they were breastfeeding or could be pregnant. Given the minimal risk nature of the study (no subjects had any severe or widespread reaction) and that patch testing during pregnancy or lactation is not known to be harmful, this seems adequate.

In accordance with 40 CFR 26.1704, the study was conducted under procedures at least as protective as those in subparts K and L of 40 CFR Part 26. The protocol and the published article

state that the study was conducted according to the principles in the Declaration of Helsinki. Documents provided to the EPA indicate that the study was reviewed and approved by regional ethics review boards in Stockholm, Sweden and Capital Region, Denmark (the ethics committees with jurisdiction) in July 2013. The ethics committees reviewed the study prior to initiation with standards similar to those required by EPA (Swedish Act on the Ethical Review of Research Involving Humans; Danish Act on Research Ethics Review of Health Research Projects; and the Ministerial Order No 806 on Information and Consent).

The informed consent materials contained adequate information for the subjects (n=38) about the risks, discomforts and benefits of participation, and of their right not to participate or to withdraw (n=2). The risk-benefit ratio was determined to be acceptable and risks were minimized appropriately and were justified by the potential societal benefits associated with gathering data to determine if products preserved with allowed concentrations of the test article have the potential to elicit allergic contact dermatitis in previously sensitized individuals.

The selection of study participants appeared to be equitable and no children or pregnant women were enrolled. No conflicts of interest were declared. There is no evidence that the conduct of the research was fundamentally unethical or deficient relative to ethical standards and minimized the risk of harm to subjects.

HSRB review of a published study, An evaluation of dose/unit area and time as key factors influencing the elicitation capacity of methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) in MCI/MI-allergic patients, authored by Claus Zachariae, Anne Lerbaek, Pauline M. McNamee, John E. Gray, Mike Wooder, and Torkil Menné. Contact Dermatitis (2006) 55, 160-166.

CHARGE TO THE BOARD AND BOARD RESPONSE

Charge to the Board:

Is the research described in the published article “An evaluation of dose/unit area and time as key factors influencing the elicitation capacity of methylchloroisothiazolinone/methylisothiazolinone (MCI/MI) in MCI/MI-allergic patients” scientifically sound, providing reliable data?

Board Response:

The Board after discussion agreed that the research in the published article “An evaluation of dose/unit area and time as key factors influencing the elicitation capacity of methylchloroisothiazolinone/ methylisothiazolinone (MCI/MI) in MCI/MI allergic patients” is scientifically sound, providing reliable data.

In the use of this data for risk assessment to establish the elicitation threshold for methylisothiazolinone, the Board highlighted some relevant exposure conditions or statistical concerns that should be considered when using this data.

The authors evaluated the allergic response of MCI/MI patients to the application of a mixture of MCI/MI where authors wanted to determine the elicitation response to a mixture of MCI/MI and the influence of time and concentration (i.e., the exposure scenario) on elicitation thresholds. This study enrolled 29 eczema patients aged 18 and over who had a previous response to 100 ppm MCI/MI in a patch test, where response was confirmed for 25 with a diagnostic patch test to 100 ppm MCI/MI. There were healthy 10 volunteers enrolled with no response to 100 ppm MCI/MI in a patch test. The dose of MCI/MI for diagnostic patch was $3 \mu\text{g}/\text{cm}^2$ (determined from applying $15 \mu\text{l}$ of 100 ppm on a 0.5 cm^2 area). A controlled patch using deionized water with 10% ethanol was applied for two days with readings at days 2, 3, 4, and 7. The design of the double blind placebo ROAT was for subjects to apply 2 drops (estimate volume of $0.05668 \mu\text{l}$) of a MCI/MI mixture, twice a day for 4 weeks to 9 cm^2 area of the volar part of forearm, and allow to dry before putting on clothing. ROAT 1 was a total dose of $0.025 \mu\text{g}/\text{cm}^2$ per day (4 drops over the course of a day using a concentration of 2 ppm MCI/MI over a 9 cm^2 area). ROAT 2 was conducted 4 weeks after the end of ROAT 1, and also for 4 weeks. ROAT 2 was a total dose of $0.094 \mu\text{g}/\text{cm}^2$ per day (4 drops over the course of a day using a concentration of 7.5 ppm MCI/MI over a 9 cm^2 area). Response was graded on a five-point scale (negative, doubtful, weakly positive, moderately positive, or strongly positive) for outcomes of erythema, papules and vesicles. Test bottles were weighed before and after use, which helps to confirm overall dose and daily dose over the 4-week period. Average daily dose across subjects (those who had negative or positive responses and from ROAT 1 and ROAT 2) and controls was comparable according to the authors, although no significance testing was reported on that comparison.

Seven out of 25 (28%) subjects showed a positive response in ROAT 1 and the average number of days to response was 16.5 (reported as non-significant compared to vehicle control). Fourteen out of 25 (56%) subjects showed a positive response in ROAT 2 and average days to response was 12.1 days (reported as significant compared to vehicle). The difference between ROAT 1 and ROAT 2 responses was statistically significant. The majority of reactions were classified as weak or moderate; the 10 non-allergic controls did not show reactions in the ROAT arms of the study.

The HSRB expresses confidence that this study provides reliable and valid data. The Board noted that sufficient information is provided by the authors that the study has the potential to be repeated and validated. The study objectives were stated; an acceptable control group was used; and the investigators used measures to reduce bias in the study by conducting a double blind study and by using an acceptable reading scale for responses. The investigators used methodologies to help assure adherence to study protocols (e.g., instructions to subjects, bottles for application), and response rates related to application rates/concentrations are reported and related back to the objectives. These study characteristics provide support for the Board's conclusion that the data are scientifically sound.

The HSRB recommended that EPA consider the following scientific issues that may affect elicitation responses.

- 1) Application to same area, following a rinse out period might be expected to result in more rapid sensitivity for the ROAT 2 arm. However, EPA staff contacted the study team to determine whether the same area was used for ROAT 2; study team confirmed that a different area was used in the second application. Given the number of positive reactions and the days to reaction in ROAT 1 versus ROAT 2, this outcome is in fact reported. This may indicate a growing sensitivity to exposures of MCI/MI mixtures, for especially sensitive individuals. However, because a higher dose of MCI/MI was used for ROAT 2, it is not possible to determine whether repeated application, or higher dose, or both factors played a role. It is unclear what the outcome would have been if the same dose or even lower was used in ROAT 2 arm compared to ROAT 1. In this study, 5 of the 7 who showed positive responses to ROAT 1, showed positive responses also to ROAT 2, with reactions in first week.

The HSRB suggests that ROAT 2 may not offer any additional information on exposure conditions and threshold responses, given the higher dose used.

- 2) The mixture of MCI/MI is not confirmed although authors mention a typical mixture of 3:1. If this assumption is used to determine the concentration of MI, the ROAT 1 results is $(0.025 \mu\text{g (MCI/MI)/cm}^2 \text{ /day/ 4}) \times 1 = 0.00625 \mu\text{g (MI)/cm}^2 \text{ /day}$ and for ROAT 2 = $(0.094 \mu\text{g (MCI/MI)/cm}^2 \text{ /day /4}) \times 1 = 0.0235 \mu\text{g (MI)/cm}^2 \text{ /day}$. Although authors state that they believe that the elicitation threshold is in the proximity of $0.025 \mu\text{g (MCI/MI)/cm}^2 \text{ /day}$ (based on response in ROAT 1 to daily applications of this concentration), they found no significance for this level compared to control. In addition, this would indicate a dose of $0.00625 \mu\text{g (MI)/cm}^2 \text{ /day}$ of MI alone (28% responded), assuming all the response is attributed to the MI in the mixture. However, it may not be possible to disaggregate the response to the individual components in the mixture. In Lundov et al., 2011, page 334, MCI is mentioned as a more potent sensitizer than MI. This is not a study to determine the sensitivity of allergic patients to MI but to a mixture of MCI/MI. It is possible that MCI can act to increase or decrease the activity of MI (i.e., synergistic or antagonistic effects). The potential synergy or antagonism is not tested in this study but may be an important consideration depending on whether a determination is to be made of MI in specific mixtures, for specific application types. EPA may want to clarify if a distinction between MCI and MI is necessary in this risk assessment determination.
- 3) Authors have not confirmed what other ingredients are in the MCI/MI mixtures that might act as sensitizers or influence elicitation responses. Ethanol is mentioned an ingredient.
- 4) This study, compared to those by Lundov et al., 2011 and Yazar et al., 2015 used eczema patients as study subjects (although none with active eczema). This could result in higher response rates, or higher sensitivities for subjects, because these subjects might be more sensitive to lower concentrations of MI or MCI/MI mixtures. However, the various skin conditions (and their population prevalence) that influence elicitation thresholds are not well understood.
- 5) Age or sex of participants is not reported to determine demographic representation. Greater skin sensitivities might be expected for the very young and in the elderly.

- Sensitivity might also be greater on areas like the face or scrotum. In this study the forearm is used. In an elicitation response, greater sensitivity may be related to thickness of the stratum corneum and access to the viable epidermis. Thickness of layers of the skin vary over the body, and also by age and sex.
- 6) There is some suspicion that elicitation response (i.e., threshold) may be related to thresholds for induction. Full history of each participant is not known, and the study does not report whether these participants were already induced/sensitized and at what level, or if the confirmatory patch at 100 ppm is their threshold for induction. This is a lower level confirmatory patch than used in the other studies compared here.
 - 7) Response was based on a grading scale or weak, moderate or strong for outcomes of erythema, papules and vesicles. It is not clear if this is a slightly different reading scale than that used in the two other studies under review, in which an 8-point reading scale is mentioned.

Comments on statistical aspects of the study

The study goal was to determine the effect of concentration and time on elicitation capacity of MCI/MI in sensitized subjects using ROAT testing. The treatment group consisted of 29 eczema subjects; the number of males and females is not specified. Assignment to treatment group required at least one prior positive reaction to patch test with MCI/MI concentration of 100 ppm. The control group consisted of 10 healthy subjects with no allergic reaction to pre-study patch test at 100 ppm. In the study, a pre-study patch test followed by two 4 week stages of ROAT testing separated by a 4-week washout period. Stage 1 was ROAT with an MCI/MI concentration of 2 ppm ($0.025 \mu\text{g}/\text{cm}^2$); stage 2 was ROAT with an MCI/MI concentration of 7.5 ppm ($0.094 \mu\text{g}/\text{cm}^2$). The investigators used Fisher's Exact Test which was appropriate for comparing the proportion of subjects in the allergic group having a positive reaction to the control group proportion. A logistic regression was used for the allergic group subjects to compare the proportions of positive reactions in the stage 1 ROAT and stage 2 ROAT. A subject effect was included in the analysis. The problem with the logistic regression analysis is that apparently subjects were not considered as random effects. Also, with a random subject effect, significance of the results using SAS GLIMMIX depended on the method used to fit the logistic model ($p = 0.0407$ with Residual pseudo-likelihood; $p = 0.4936$ with Laplace). The investigators

also used Kaplan-Meier survival (no positive reaction) estimates over time to a positive reaction were calculated from the stage 1 and stage 2 data. The problem with this analysis is that the stage 1 and stage 2 are not independent because they come from the same set of subjects. In summary, the chosen statistical analyses might be appropriate but there are issues with whether or not the authors' underlying assumptions were reasonable.

Ethics

Charge to the Board:

Does available information support a determination that the study was conducted in substantial compliance with subpart Q of 40 CFR part 26?

Board Response:

The HSRB concludes that the available information supports a determination that the Zachariae et al. (2006) study was conducted in substantial compliance with subpart Q of 40 CFR part 26.

HSRB Detailed Recommendations and Rationale

The Agency's rules at 40 CFR part 26 subpart Q that are applicable to this review include the following:

§26.1703: Except as provided in §26.1706, EPA must not rely on data from any research subject to this subpart involving intentional exposure of any human subject who is a pregnant woman (and therefore her fetus), a nursing woman, or a child.

And,

§26.1704: EPA must not rely on data from any research subject to this section if there is clear and convincing evidence that: (1) The conduct of the research was fundamentally unethical (e.g., the research was intended to seriously harm participants or failed to obtain informed consent); or (2) The conduct of the research was 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 (based on knowledge available at the time the study was conducted) or impaired their informed consent.

§26.1703:

The manuscript indicates (p. 161-162) that subjects were at least 18 years of age and that pregnant or lactating women were excluded from study participation. EPA follow-up with the author noted that there was no pregnancy testing to confirm non-pregnant status.

§26.1704:

Ethics Committee Review: The manuscript (p. 162) indicates that a local ethics committee had reviewed and approved the study and that it was conducted according to Good Clinical Practice guidelines. The author states that he no longer has copies of the ethics committee approval since the study is more than a decade old. GCP standards (adopted June 1996) has clear standards for ethics committee membership and review processes which are consistent with Agency rules at 40 CFR part 26, Subpart K. GCP also requires compliance with the ethical standards of the Declaration of Helsinki, including risk minimization, informed consent of human subjects, and protection of vulnerable populations.

Informed Consent: The manuscript also indicates that informed consent from all subjects was obtained. Denmark's "Ministerial Order No 806 of July 2004 on Information and Consent at Inclusion of Trial Subjects in Biomedical Research Projects" provides robust guidelines for informed consent standards in this trial. There is no evidence to suggest that this study was in violation of these standards. As with ethics committee approval records, the author did not have a copy of the consent form available when requested by EPA.

Risk Minimization: Principal risk associated with this research involves allergic reaction to the MCI/MI material. This risk was minimized by providing subjects with instructions to visit the institution's dermatology center in the event of an allergic reaction outside of the study's scheduled monitoring visits. The author does not recall any adverse events that required medical treatment for subjects.

HSRB Review Of The ROAT Studies Considered Together

Charge to the Board:

When considered all together, do the three studies described in Lundov et al., Yazar et al., and Zachariae et al., provide a scientific weight of evidence in support of establishing a point of departure for the determination of an elicitation threshold for methylisothiazolinone (as identified by the Lundov et al., study) for use in risk assessments?⁴

Board Response:

The HSRB board agrees that when considered all together, the three studies described in Lundov et al., Yazar et al., and Zachariae et al., do provide a scientific weight of evidence in support of establishing a point of departure for the determination of an elicitation threshold for methylisothiazolinone (as potentially identified by the Lundov et al., study) for use in risk assessments.

⁴ For all three articles EPA has provided in English the following materials: **Zachariae et al., 2006:** Data Evaluation Record by two EPA reviewers, Ethics review by an EPA ethics Review officer, Ethics Questions completed by Dr. Zachariae, and the Ministerial Order 806 for Biomedical Research from the Danish Ministry of Interior and Health; **Lundov et al., 2011:** Data Evaluation Record by two EPA reviewers, Ethics Review by an EPA ethics review officer, Ethics Questions completed by Dr. Johansen, Ethical Application to the Danish National Bioethics Committee, the Ministerial Order 806 for Biomedical Research from the Danish Ministry of Interior and Health, and Danish Act on Research Ethics Review; **Yazar et al., 2011:** Data Evaluation Record by two EPA reviewers, Ethics Review by an EPA ethics review officer, Ethics Questions completed by Dr. Yazar, Ethical Application to the Danish National Bioethics Committee, Danish Approved Amendments, Swedish Ethical Application and Approval, Swedish Approved Amendments to the Ministerial Order 806 for Biomedical Research from the Danish Ministry of Interior and Health, and Danish Act on Research Ethics Review, Swedish Ethical Review Act

HSRB Detailed Recommendations and Rationale:

For all three studies EPA provides a summary of the study and findings and conclude that these studies do provide a weight of evidence to support the derivation of an elicitation point of departure for MI. For the Lundov study, the Lowest Observed Adverse Effect Level (LOAEL) is mentioned as $0.0105 \mu\text{g}/\text{cm}^2$ for MI, where for Zachariae et al., $0.025 \mu\text{g}/\text{cm}^2$ for MI (mixture of MCI/MI) is mentioned, and for Yazar et al., $0.24 \mu\text{g}/\text{cm}^2$ for MI (rinse-off product containing MI) is mentioned. EPA has evaluated all studies with respect to “Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment (USEPA, 2012)” and based on 16 screening criteria determined that all three studies are appropriate for quantitative use in establishing a point of departure for risk assessment. There was a finding for Zachariae et al., 2006 that for criteria #13: Adequate data provided on the chemical tested...that not enough information was provided. However, this lack of information does not prevent the study from being found to be scientifically sound and providing reliable data. There is a comparison of the Zachariae et al., and the Yazar et al., studies to the Lundov et al., et al., where Lundov et al., have established the (lowest) LOAEL of $0.0105 \mu\text{g}/\text{cm}^2$. These are all considered acceptable/non-guideline, non-registrant submitted studies.

EPA recognizes the small number of participants in each study, problems with demographic representation or reporting, lack of knowledge on the history of MI exposure for participants, and in some cases issues with adequate data for repeatable statistical analysis. In addition, EPA finds that the studies do not provide enough information to determine or construct a dose-response curve (Lundov et al with 3 applied concentration points is lacking in that regard). EPA also recognizes the potential influence of vehicles on the elicitation threshold for MI, and for some studies not enough information has been provided regarding the vehicles or their potential for influencing response. However, the intent is to establish weight of evidence in deriving an LOAEL for elicitation thresholds across the three studies. Because immune responses in individuals can vary so greatly, understanding or even repeating elicitation responses can be challenging. Elicitation thresholds are a relatively new area research for preservatives, and the influence of exposure conditions is also not well understood.

The HSRB recommended that the EPA take into account the following scientific issues when considering risks assessment based on these data. There is variability across the three studies in

types of applications and methods of applications. The objectives of the studies also vary. It recognized that sensitization to MI products might occur at lower product concentrations than currently allowed, however the limit or elicitation point is difficult to narrow down from these diverse studies, and for a particular product. Typical usage rates and application rates for the general public or even for the high-risk individual (high usage rate) is not discussed, making it more difficult to associate practical and likely dosage rate with a general and reasonable elicitation threshold. Care must be taken to compare appropriate doses/loadings across studies, where product type and application method must also be considered. In a risk assessment, appropriate/comparable doses might best be represented by daily doses. For the Lundov study, $0.021 \mu\text{g}/\text{cm}^2/\text{day}$ can be compared with $0.025 \mu\text{g}/\text{cm}^2/\text{day}$ MCI/MI in the Zachariae study (where MI is only $\frac{1}{4}$ of this), and for comparison with the $1.2 \mu\text{g}/\text{cm}^2/\text{day}$ (note rinse-off may greatly lower this level) in the Yazar study. The statistical significance reported across these doses, however, was not all strong, and the association of response to concentration in a product may be variable. Another option is to compare accumulated applied dose to an elicitation threshold across MI ROAT studies.

In establishing LOAEL and applying reasonable safety factors it is important to understand how consumers use these products in terms of number of applications, volume per application, and area of application. Determining an elicitation threshold and applied safety factors are dependent on understanding consumer behavior and the level of guidance or use recommendations that will or can be provided. For manufacturers, a $\mu\text{g}/\text{cm}^2/\text{day}$ must be translated to ppm in the product (or particular products) with consideration for vehicle effects and usage patterns.

There may be a rationale to further study the concentration levels of MI or MCI/MI to be allowed in a variety of consumer products, and to examine the extent to which individuals have become sensitized (induced) to MI. Worst-case tests should be performed taking into account sensitive areas, sensitive individuals, chemicals in the products that either promote absorption in the skin or retention in the skin.

Another potential risk assessment approach is to look at minimum levels of MI (or other products) needed for preservation. If it can be established that preservation of a product can be achieved at lower concentrations and with other established safer products, then this option needs to be explored. For example, Lundov et al mention previous studies showing that 5 ppm MI with

0.4% phenoxyethanol was adequate to preserve standard cosmetic creams (where phenoxyethanol rarely causes allergic response in subjects). Ultimately lower and safer levels of MI applied to protect MI allergic patients will need to satisfy those acceptable preservation levels.

In addition, in the risk assessment process, it would be of interest to relate elicitation thresholds back to effective concentration inducing a stimulation index of 3 (EC3 values). This is a measure used in the LLNA (local lymph node assay) to test whether a chemical is a sensitizer and potentially how potent.⁵ MI has an EC3 value of 0.4%⁶ (Yazar et al., 2015 pg. 116) and considered a strong sensitizer.⁷

In conclusion, there may not be full confidence that these three studies overall definitely establish *the* point of departure for an elicitation threshold in MI allergic patients, but that there may be *a* point of departure for an elicitation threshold in MI allergic patients. The studies do provide reliable data for the specific exposure conditions investigated and will assist in the risk assessment process for establishing appropriate and practical elicitation thresholds. The Board suggests that EPA carefully consider relationships among the studies and their findings, where vehicle effects, inductions thresholds, application methods, and average days to elicitation response all play a role.

In addition, the HSRB recommended that EPA consider the following scientific issues:

- 1) Different population subgroups may have different sensitivities. MI-sensitized individuals are the main focus for these ROAT elicitation studies. However, there may be other individuals affected by low levels of MI in products, such as those with eczema and sensitive skin problems. Data on eczema and contact dermatitis would be useful in a risk assessment and related to their influence on both induction and elicitation thresholds.

⁵ World Health Organization, 2008 “Skin Sensitization in Chemical Risk Assessment” Harmonization Project Document 5, found at <http://www.inchem.org/documents/harmproj/harmproj/harmproj5.pdf>

⁷ Leiva-Salinas and Silvestre (2013) “Update on Allergic Contact Dermatitis due to Methylchloroisothiazolinone/Methylisothiazolinone and Methylisothiazolinone” *Actas Dermosifilofr* 105(9): 840-846.

Cumulative effects from multiple products containing MI or mixtures of MCI/MI will also need to be considered.

- 2) In a risk assessment, longer follow up of MI allergic subjects may be needed to determine a safe level. Is it possible that a very low dose (below those tested in any of these studies) could produce a response in 3 or 4 months of continued use (beyond the times used in these ROAT studies)?
- 3) The HSRB also recommended considering warning labels on MI products and whether they adequately advise consumers about the risk of skin sensitization.
- 4) Finally, the HSRB recommended careful conversion of dosing calculations to determine how product concentration and usage patterns determine actual dosing applied in practice. In the Zachariae et al article, ppm must be converted to % (converted to $\mu\text{g/g}$) and combined with the volume applied and area of skin to determine a $\mu\text{g/cm}^2$, of MI. It is also necessary to know the $\text{g}/\mu\text{l}$ of the product (entire product) to make an assessment of $\mu\text{g/cm}^2$ of MI, where volume application of products is used. In the Lundov study, the weight of the product is not reported, but there was an application of $4.2 \text{ mg cream/cm}^2/\text{day}$ reported so this also corresponds to a total of $4.2 \text{ mg/cm}^2 \times 9 \text{ cm}^2 = 37.8 \text{ mg/day}$, so $40 \mu\text{l}$ of the product (two applications over the entire 9 cm^2 area), telling us the weight of the product $37.8 \text{ mg}/40 \mu\text{l}$ or $0.945 \text{ mg}/\mu\text{l}$ (i.e., weight of product per volume). Also, 100 ppm MI is equivalent to $100 \mu\text{g MI/g}$ in cream, therefore one application of this concentration would mean $(100 \mu\text{g MI/g cream} \times 0.945 \text{ mg}/\mu\text{l} \times 20 \mu\text{l} \times 1 \text{ g}/100 \text{ mg})/9 \text{ cm}^2 = 0.21 \mu\text{g MI/cm}^2$. There are other perhaps more direct ways to make this calculation, but this agrees with the $0.21 \mu\text{g MI/cm}^2$ reported for the highest dose of MI applied in the ROAT (Table 1), of the 100 ppm MI product. The Board recommended that EPA confirm how concentrations are determined and eliminate any confusion about concentrations applied in these studies.

References

- Paradis et al., 2014 “Developing a Framework for the Quantitative Risk Characterization of Dermal Sensitizers in the Workplace” 3M Company, St, Minnesota, found at https://schc.memberclicks.net/assets/meetings/spring2014/schc_poster_developing_framework.pdf
- EPA, 2004, FIFRA Scientific Advisory Panel Background Document: Proposed Hazard Identification Methodology for Assessment of Dermal Sensitization Risk, Washington, D.C.
- Anderson and Meade, 2014 “Potential Health Effects Associated with Dermal Exposure to Occupational Chemicals” *Environmental Health Insights*, 8(S1).
- World Health Organization, 2008 “Skin Sensitization in Chemical Risk Assessment” Harmonization Project Document 5, found at <http://www.inchem.org/documents/harmproj/harmproj/harmproj5.pdf>
- Ezendam, J., Braakhuis, H., and Vandebriel (2016) “State of the Art in Non-Animal Approaches for Skin Sensitization Testing: From Individuals Test Methods Towards Testing Strategies”, *Arch Toxicol.*, 90:2861-2883.
- Leiva-Salinas et al., (2014) “Update on Allergic Contact Dermatitis due to Methylchloroisothiazolinone/Methylisothiazolinone and Methylisothiazolinone” *Actas Dermosifiliogr*, 105(9): 840-846
- Zang et al., (2017) “Prediction of Skin Sensitization Potency Using Machine Learning Approaches”, *J. Appl. Toxicol.* Doi 10.1002/jat3424
- Griem et al., 2003 “Proposal for a Risk Assessment Methodology for Skin Sensitization Potency Data”, *Regulatory Toxicology and Pharmacology*, 38: 269-290
- Alves et al., 2015 “Predicting Chemically-Induced Skin Reactions. Part II: QSAR Models of Skin Permeability and the Relationship between Skin Permeability and Skin Sensitization”, *Toxicol Appl Pharmacol* 284(2):273-280.
- Steiling (2016) “Safety Evaluation of Cosmetic Ingredients Regarding their Skin Sensitization Potential”, *Cosmetics*, 3: doi 10.3390/cosmetics3020014.
- Kimber et al., (2002) “Allergic Contact Dermatitis”, *International Immunopharmacology*, 2: 201-211.
- Jaycock (1998) “Risk Assessment of Contact Allergens”, *American Journal of Contact Dermatitis*, 9(3): 155-161.

Dearman and Kimber (2003) “Factors Influencing the Induction Phase of Skin Sensitization”, American Journal of Contact Dermatitis, 14:188-194.

Gerberick and Robinson (2000) “A Skin Sensitization Risk Assessment Approach for Evaluation of New Ingredients and Products”, American Journal of Contact Dermatitis, 11:65-73.

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