UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON D.C., 20460

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

December 19, 2016

MEMORANDUM

SUBJECT: Ethics Review of Research Article by Michael Lundov, et al. on Methylisothiazolinone (2011)

FROM: Michelle Arling, Human Studies Ethics Review Officer (Acting)
Office of the Director
Office of Pesticide Programs

TO: Steven Weiss, Chief
Risk Assessment Science Support Branch
Antimicrobials Division
Office of Pesticide Programs

REF: Lundov, Michael et al. Methylisothiazolinone Contact Allergy and Dose-Response Relationships. *Contact Dermatitis.* Volume 68, January 2011. (MRID 50035303)

I have reviewed available information concerning the ethical conduct of the study referenced in the research article "Methylisothiazolinone Contact Allergy and Dose-Response Relationships" by Michael Lundov et al. If the research is determined to be scientifically acceptable, I find no barrier in regulation to the U.S. Environmental Protection Agency's reliance on this study in actions under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) or §408 of the Federal Food, Drug and Cosmetic Act (FFDCA). EPA will ask the Human Studies Review Board (HSRB) to comment on this study.

Summary Characteristics of the Research

This post-rule study used a patch test and a repeated open application test (ROAT) to investigate the eliciting doses of methylisothiazolinone with and without the addition of phenoxyethanol, and tested whether the previously developed model for conversion between patch test and ROAT data was also valid for methylisothiazolinone. The study included 11 test subjects (2 females, 9 males), aged 37-68 years, with methylisothiazolinone contact allergy, and 14 control subjects (6 females, 8 males), aged 20-44 years.

The patch test series for test subjects consisted of 12 decreasing doses of methylisothiazolinone, and the same 12 doses of methylisothiazolinone combined with phenoxyethanol. Control subjects were only tested with the highest dose of methylisothiazolinone and a patch without any test substance. A trained nurse from the allergy lab read the reactions on days 2, 3 or 4, and 7. Placement of the patches containing different doses was randomized.

For the ROAT, test subjects and controls applied three different concentrations of methylisothiazolinone with phenoxyethanol and one solution without any of the tested active ingredients from four different bottles, twice a day, on four different areas on the inner forearm. A trained nurse from the allergy lab read the reactions on days 2, 3 or 4, 7, 14 and 21, and noted if a reaction occurred between visits. For both the patch test series and the ROAT, readings were blinded.

The study design was reviewed and approved prior to implementation by an independent ethics committee of the Capital Region of Denmark. All participants received information about the study orally and in writing, and signed a written consent form prior to enrollment in the study.

To confirm that the study underwent an independent ethics review, EPA's Office of Pesticide Programs contacted the primary author on the article, Dr. Michael Lundov, by e-mail. Dr. Lundov's colleague and co-author on the article, Dr. Johansen, submitted the documentation provided to the ethics committee for review (including the informed consent form and information provided to test and control subjects), and correspondence from the independent ethics review committee. The study was conducted in Denmark, and the documentation provided to the ethics committee and correspondence from the independent ethics committee are in Danish. The original documents are included in Attachment 1. EPA contracted to translate the documents, the English versions of which are included in Attachment 2. EPA's questions to Dr. Johansen and her responses are included as Attachment 3.

1. Value of the Research to Society:

The objective of the study was to investigate the dose-response relationship in patients already sensitized to or allergic to methylisothiazolinone. As noted in the study, "Besides the few case reports with patients reacting to 10 and 30 ppm MI in patch testing (3, 4), there is no information available on the dose-response relationship in patients already sensitized to MI." (p. 331) In addition, the study "also tested whether the previously developed model for conversion between patch test and ROAT data was also valid for MI." (p. 331) The results were published in *Contact Dermatitis* in 2011. EPA is proposing to use the results of this study, in combination with results from other ROAT studies, to set a human dermal sensitization endpoint/point of departure in its risk assessment for methylisothiazolinone.

2. Subject Selection:

a. Demographics. A total of 25 subjects participated in the study. Eleven test subjects, aged 37-68 years (2 females, 9 males) participated in the study. Fourteen control subjects, aged 20-44 years (6 females, 8 males) participated in the study.

b. Inclusion/Exclusion Criteria. Based on the protocol reviewed by the independent ethics committee, for test subjects, the inclusion criteria were having had a positive reaction to methylisothiazolinone, being at least 18 years old, receiving written information about the study, and signing a written informed consent form. According to the article, "exclusion criteria were: age [less than] 18 years, eczema on the tested area, exposure to ultraviolet light within the last 3 weeks and during the study (e.g., sunbathing or solarium), systemic immunosuppressive therapy, pregnancy, breast feeding, and not being able to cooperate." (p. 331)

The document "Participant Information to Study Patients" provides more specific information about the conditions of study participation described in the article. The Participant Information form includes "not hav[ing] excessive active eczema, eczema on your arms or back" and "not being treated with immunosuppressive pills within 1 week of the start of the study." (Attachment 2, p. 16)

The inclusion criteria for control subjects were: being at least 18 years old, receiving written information about the study, and signing a written informed consent form. The article notes that healthy volunteers were recruited and that the exclusion criteria were the same for test and control subjects; the written information provided to control patients specifies that control subjects must "not have eczema or other skin diseases" and "not test positive for an allergy to methylchloroisothiazolinone/ methylisothiazolinone and methylisothaizolinone [sic]." (Attachment 2, p. 19) Dr. Johansen, an author of the study, noted that two other exclusion criteria were "no topical treatment with cortico-steroides [sic] 3 weeks prior to the experiment" and "no participation in other studies 3 weeks prior to the experiment."

c. Pregnancy and Nursing Status. According to the article and the information about the study provided to both test and control subjects, pregnant and nursing women were excluded as both test subjects and control subjects. Dr. Johansen confirmed that both test and control subjects "were asked if they were pregnant/or could be pregnant and/or breastfeeding."

d. Recruitment. Test subjects were recruited from a pool of 52 patients at the Gentofte Hospital who had a positive reaction to methylisothiazolinone from 2005 until the study's initiation. The researchers also contacted 50 patients with a confirmed allergy to methylchloroisothiazolinone/methylisothiazolinone and invited them to participate upon completion of a patch test to confirm an allergy to methylisothiazolinone. Potential test subjects received by mail written information about the study approved by the regional ethics committee (Attachment 2, pp. 16-18) and information from the National Bioethics Committee for Region Hovedstaden called "Before You Decide" (Attachment 2, p. 30). The written materials informed participants that Dr. Lundov would contact them by phone after about a week of receiving the materials. Dr. Lundov called potential test subjects, provided information about the study orally, answered questions, and invited patients to participate. If the potential test subject agreed to participate, Dr. Lundov verified that he or she satisfied the inclusion criteria and did not meet any of the exclusion criteria. Those who satisfied the criteria were invited to the clinic for an appointment, at which they received information about the study again

orally in an undisturbed environment and were provided and asked to sign the informed consent form.

Control subjects were recruited through an advertisement approved by the regional ethics committee and posted on a website (www.forogsperson.dk). (Attachment 2, p. 22) The advertisement invited interested persons to contact Dr. Lundov by phone or email. Dr. Lundov called individuals who responded with interest to the advertisement, provided information about the study orally, answered questions, and invited interested persons to participate. Potential control subjects received written information about the study approved by the regional ethics committee (Attachment 2, pp. 18-20) and information from the National Bioethics Committee for Region Hovedstaden called "Before You Decide" (Attachment 2, p. 30). If the interested person agreed to participate, Dr. Lundov verified that he or she satisfied the inclusion criteria and did not meet any of the exclusion criteria. Those who satisfied the control subject criteria were invited to the clinic for an appointment at which they received information about the study again orally in an undisturbed environment and were provided and asked to sign the informed consent form.

Potential test and control subjects had the option to have another person (e.g., friend or family member) present at the meeting at the clinic where information on the study was provided orally and the informed consent form was signed.

3. Risks and Benefits:

a. Risks. The risks and possible side effects are discussed in the informational materials provided to potential test and control subjects. As explained in the protocol, "the concentrations [used in the study] have been investigated previously in clinical studies and have not been associated with side effects. [Methylisothiazolinone] is a widely used preservative in cosmetics, and the risk of participating in the study will be similar to that of a short time use of cosmetic products, such as creams." (Appendix 2, p. 14) The risks to test subjects were eczema spots from the patch test and flare ups in eczema during the ROAT. The article states that "the following reading scale of reactions was used: 0, no reaction; 1. few papules with no erythema and no infiltration; 2. faint erythema with no infiltration or papules; 3. faint erythema with few papules and no homogeneous infiltration; 4. erythema and homogeneous infiltration; 5. erythema, infiltration, and a few papules; 6. erythema, infiltration, and papules; 7. erythema, infiltration and papules, and a few vesicles; and 8. intensive erythema, infiltration, and vesicles (12, 13)." (p. 331) The risks to control subjects were eczema spots from the patch test and during the ROAT, as well as developing an allergy to methylisothiazolinone. Risks were minimized through the inclusion and exclusion criteria, by selecting dose levels that would be unlikely to cause adverse effects (i.e., the concentration approved by the European Union for use in cosmetics), by limiting the exposure period to 4 weeks, and by closely monitoring the subjects throughout the study and inviting participants to visit the clinic outside the scheduled monitoring days if an adverse effect developed. If a participant developed an unexpected adverse effect, his or her participation would be stopped.

b. Benefits. There are no benefits to the subjects. Methylisothiazolinone is a known skin sensitizer and can cause contact allergy in persons who use products containing this ingredient. There is a potential benefit to society. This study evaluating concentrations of methylisothiazolinone that cause adverse effects in individuals with an allergy to methylisothiazolinone can be used to establish limits and protect both allergic and non-allergic individuals. Further, EPA plans to use these data, in combination with results from other ROAT studies, to set a human dermal sensitization endpoint/point of departure in its risk assessment for methylisothiazolinone.

c. Risk-Benefit Balance. The potential societal benefits from increased understanding of concentrations of methylisothiazolinone that cause adverse effects outweigh the small risks associated with the study.

4. Independent Ethics Review: The study was reviewed and approved by the Capital Region of Denmark on May 17, 2010. An amendment to the protocol to add mailing a second letter to potential test subjects who did not respond during the first recruitment letter was approved on June 3, 2010. In response to a request from EPA, Dr. Johansen provided a copy of the materials provided to and approved by the ethics committee, and the correspondence from the ethics committee approving the protocol and amendment. The original documents are included in Attachment 1, and the translated documents are included in Attachment 2.

5. Informed Consent: All subjects received information about the study in writing and orally, and were offered two opportunities to ask questions (in a telephone conversation with the study director, Dr. Lundov, and at the visit to the clinic, immediately prior to signing the informed consent form and enrolling in the study). All test and control subjects signed the informed consent form before participating. The consent form, in combination with the information provided to test and control subjects, appears to meet the requirements of 40 CFR 26.1116. The information provided to participants explains the research study, the purpose, expected duration of participation, and the procedures to be followed; adequately characterizes the risks and discomforts to subjects; and articulates the right to withdraw from the research at any time.

6. Respect for Subjects: Each participant received 500 kroner (approximately $71) per visit. Participants were expected to visit the hospital up to 6 times over the course of 3 weeks, and each visit was expected to take approximately 30 minutes. The information provided to test and control subjects stated that participation is voluntary and participants could withdraw from the study at any time. Further, in the information provided to test and control subjects, it was noted that subjects' participation would be discontinued if any unexpected side effects occurred. No subjects withdrew from the study and no subjects' participation was terminated based on the occurrence of unexpected side effects.

The information provided to test and control subjects also explained that their information is protected by confidentiality regulations. The subjects' identities were not revealed in the published article.

Applicable Standards

Standards Applicable to the Conduct of the Research

The portions of EPA's regulations regarding the conduct of research with human subjects, 40 CFR part 26 subpart A - L, do not apply since the research was neither conducted nor supported by EPA, nor was it conducted by a person with the intention to submit the results to EPA.

The protocol states that the study would be conducted according to the principles in the Declaration of Helsinki II (p. 331). In addition to the Declaration of Helsinki, ethical standards in place at the time the study was conducted included the Danish "Act on Research Ethics Review of Health Research Projects" (Attachment 4) and the "Ministerial Order No 806 of 12 July 2004 on Information and Consent at Inclusion of Trial Subjects in Biomedical Research Projects" (Attachment 5). The key ethical principles in the Declaration of Helsinki are respect for persons, beneficence and justice. The Danish Act establishes requirements for review of research protocols prior to implementation by an independent ethics committee, for providing information to and obtaining informed consent from study participants, and for adequate respect for study participants (e.g., confidentiality of data, adequate compensation, insurance coverage for study-related adverse effects). The Ministerial Order prohibits biomedical research unless informed consent has been obtained, and establishes the elements of informed consent, including that participation is voluntary and subjects are free to withdraw at anytime without negative effects. Potential subjects must receive information on the study orally and in a written document, both presented in a manner the potential subject can understand, prior to giving written consent to participate in the study.

Standards Applicable to the Documentation of the Research

EPA identified this study through a review of the public literature. No person has independently submitted the published article or any results of this research to EPA. Consequently, the requirements for the submission of information concerning the ethical conduct of completed human research contained in EPA regulations at 40 CFR part 26, subpart M do not apply.

Standards Applicable to EPA's Reliance on the Research

The Agency's rule (40 CFR part 26 subpart Q) defines standards for EPA to apply in deciding whether to rely on research—like this study—involving intentional exposure of human subjects. The applicable acceptance standards from 40 CFR part 26 subpart Q are these:

> **§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.
>
> **§26.1704(b).** 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.

FIFRA §12(a)(2)(P) also applied to this research. This provision 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.

EPA will submit this study for review by the Human Studies Review Board (HSRB) in conformance with 40 CFR §26.1604.

Compliance with Applicable Standards

All of the subjects in this study were adults. There is no evidence to indicate that any of the eight female subjects were pregnant or nursing. Pregnancy and nursing were exclusion criteria for both test and control subjects, and Dr. Johansen confirmed that prior to inclusion in the study participants were asked if they were or could be pregnant, or if they were nursing. Therefore, it is reasonable to conclude that the research did not involve intentional exposure of any pregnant or nursing female subjects or any children. EPA's reliance on the research is not prohibited by 40 CFR §26.1703.

The subjects provided written informed consent after receiving information in writing and orally about the study, the risks and benefits of their participation, and their ability to withdraw at any time. The protocol underwent independent ethics review and approval by the Capital Region ethics committee in Denmark. The study involved testing substances found in commercially available cosmetic products and at concentrations at or below the level permitted by the European Union. Based on these facts, and the absence of any information suggesting that the research was fundamentally unethical or intended to harm participants, I conclude that reliance on the research is not prohibited by 40 CFR §26.1704(b)(1).

Based on my evaluation of the research article and the information provided by Dr. Johansen (Attachments 1, 2, and 3), along with the Danish Act and Ministerial Order in effect at the time the study was conducted, I concluded that the conduct of the research was not 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. The study took adequate precautions to ensure participants' safety by limiting the exposure period, stopping participation in the event of a serious adverse reaction, and using a concentration of the test substance at or below levels approved by the European Union. The informed consent form (Attachment 2, pp. 28-29) in combination with the written information provided to test and control subjects, satisfy the requirements for informed consent under Danish law in place at the time the study was conducted. Therefore, reliance on this study is not prohibited by 40 CFR §26.1704(b)(2).

Consistent with the principle of respect for persons, the study purpose and potential risks and discomforts were explained to subjects orally and in writing, only subjects with the capacity

to understand the potential risks were allowed to participate, subjects were notified of insurance coverage paid for by the study sponsor for any incidents that occurred as a result of participation in the study, and all subjects provided written informed consent. Consistent with the principle of beneficence, the selected dose levels were unlikely to pose more than a minimal risk to subjects, subjects with medical conditions that could increase the likelihood of an adverse effect were excluded, and the research was conducted in a hospital by trained medical professionals.

Finally, there is no clear and convincing evidence to suggest undue influence or lack of fully informed, fully voluntary consent. The subjects received information about the study in writing and orally, and were permitted to bring another person to the meeting where they gave written consent to participate in the study. Test subjects were recruited from a pool of 52 patients at the Gentofte Hospital who had a positive reaction to methylisothiazolinone from 2005 until the study's initiation; there is no clear and convincing evidence to suggest that these subjects were vulnerable to undue influence by the medical staff at the hospital or the researchers regarding their decision about whether to participate in the research. The study design was reviewed and approved prior to implementation by an independent ethics committee of the Capital Region of Denmark.

Based on these facts, I conclude that the study was not deficient relative to the prevailing ethical standards in a way that placed participants at increased risk of harm or impaired their informed consent.

Conclusion

I find no barrier in law or regulation to reliance on this research (MRID 50035303) in EPA actions taken under FIFRA or §408 of FFDCA. I defer to others for a full review of the scientific validity of this study. If it were determined not to have scientific validity, it would also not be ethically acceptable.

cc: Steve Knizner
Tim McMahon
Tim Leighton

Attachments

Attachment 1: Ethical Application & Correspondence from Ethical Review Board (Danish)
Attachment 2: Ethical Application & Correspondence from Ethical Review Board (English)
Attachment 3: EPA Questions to and Responses from Dr. Johansen
Attachment 4: Act on Research Ethics Review of Health Research Projects (Denmark)
Attachment 5: Ministerial Order No 806 of 12 July 2004 on Information and Consent at Inclusion of Trial Subjects in Biomedical Research Projects (Denmark)