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

OFFICE OF CHEMICAL SAFETY AND
POLLUTION PREVENTION

*June 1, 2021*

**MEMORANDUM**

**SUBJECT:** Ethics Review of Research Article by Dwivedi et. al on Acute Effects of Acrolein in Human Subjects (2015)

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

**TO:** Dana Vogel, Director
Health Effects Division
Office of Pesticide Programs

**REF:** Dwivedi, A. et al. Acute effects of acrolein in human volunteers during controlled exposure. *Inhalation Toxicology*. Volume 27, Issue 14. pp. 810-821. December 4, 2015. MRID 51570802.

I have reviewed available information concerning the ethical conduct of the study referenced in the research article "Acute effects of acrolein in human volunteers during controlled exposure" by Aishwarya M. Dwivedi, Gunnar Johanson, Johnny C. Lorentzen, Lena Palmberg, Bengt Sjögren, and Lena Ernstgård. 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 research article in actions under the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) or §408 of the Federal Food, Drug and Cosmetic Act (FFDCA). The EPA will ask the Human Studies Review Board (HSRB) to comment on this study.

**Summary Characteristics of the Research**

The research article summarizes research "to estimate the threshold levels for acute irritation of acrolein" (p. 811) using human subjects. Irritation was measured through physical tests and observations (pulmonary function, nasal swelling, blink frequency, inflammatory markers) as well as subjective ratings by the human subjects. The study was conducted in two phases. For the pilot study, 8 individuals (4 female, 4 male) "were exposed to increasing concentrations of acrolein starting with 0.02, 0.02, 0.07, 0.1, 0.2, and 0.3 ppm, in an exposure chamber" (p. 811) in order to determine the levels of acrolein that should be used in the main study (0.05 ppm and 0.1 ppm). Following the pilot phase, the main study involved 18 individuals

(9 female, 9 male). Both acrolein and ethyl acetate were used in this phase; the ethyl acetate was used to mask the odor of acrolein. Each subject was exposed in six different scenarios: "clean air (control), 15 ppm ethyl acetate (EA), 0.05 ppm acrolein (ACR), 0.05 ppm ACR and 15 ppm EA (low ACR + EA), 0.1 ppm ACR (high ACR) and 0.1 ppm ACR, and 15 ppm EA (high ACR + EA)" (p. 811). Up to three subjects participated at a time, seated in the exposure chamber with a controlled climate. At least one week lapsed between each exposure session.

For each session, subjects rated their symptoms immediately before exposure, at three points during the exposure, and at 3 points after exposure. Subjects were asked to rate discomfort based on 10 indicators. Subjects' eye blink movement was measured with electromyography recorded starting two minutes before the exposure began for the duration of the exposure. Pulmonary function was measured before after and three and a half hours after exposure using a spirometer, and breathing frequency was measured for the duration of the exposure periods. Nasal swelling, inflammatory and coagulation markers in blood, and inflammatory markers in induced sputum were also measured at various intervals before and after the exposure periods.

To obtain more information and to confirm that the study underwent an independent ethics review, EPA's Office of Pesticide Programs contacted the author listed for correspondence related to the article, Dr. Lena Ernstgård. Failing to get a response, I reached out to the institute where the research was conducted, the Institute of Environment of Medicine and the Karolinska Institutet. Gunnar Johanson, Ph.D., indicated that the lead author was no longer with the institute and that Dr. Ernstgård had retired. Dr. Johanson responded to the EPA inquiry and provided as much information as possible given the age of the study and the relative inaccessibility of the office and records due to the COVID-19 pandemic restrictions in Sweden. Mr. Johanson responded to EPA's questions in English (Attachment 1) and provided the ethics application (Attachment 2) and supplemental materials (Attachment 3) in Swedish. I used Google Translate for relevant parts of the documents. Dr. Johanson is making efforts to obtain additional records documenting the ethical conduct of the study, and will provide them to EPA when it is possible to enter the office to retrieve them.

1. **Value of the Research to Society**: According to the article, "the basis for the [occupational exposure limit] is scanty" (p. 811). This study was conducted to measure the irritation to human subjects after exposure to acrolein in the eyes, nasal passage, and airway, and to evaluate markers of inflammation, and "to increase the tools to study low-level acrolein exposures" (p. 814). Because this study was measuring the sensory irritation potential in humans, non-human test methods could not be used to satisfy this need. Though previous research into the irritating effects of acrolein has been conducted, this research exposed subjects "to lower levels of acrolein and performed a wider spectrum of tests" (p. 811).

   EPA is proposing to use the results of this study to support a risk assessment for acrolein. The data will be used in a qualitative manner with another more recent study, as the threshold for eye irritation observed in this and the other recent study is similar to the point of departure (POD) EPA plans to use in an acrolein risk assessment.

2. **Subject Selection**:

*a.* ***Demographics.*** A total of 18 individuals (nine female, nine male) were enrolled in the study. Subjects ranged in age from 20 to 38 years old. Of these subjects, eight also participated in the pilot study (four female, four male).

*b.* ***Eligibility Criteria.*** According to the article, eligibility criteria included "20-50 years old, healthy, nonsmoker, and without chronic diseases" (p. 811). Additionally, Dr. Johanson noted that "[a]ny individual with any kind of dependency towards the investigators or unit staff was excluded" (Attachment 1). Pregnant women were excluded from participation and females' non-pregnant status was confirmed with a test on each day of exposure. Subjects also underwent a medical examination prior to enrollment and participation in an exposure session.

*c.* ***Recruitment.*** Participants were recruited through advertisements posted at the test location, Karolinska Institutet. The advertisement (in Swedish) is on page 1 of Attachment 3.

**3. Risks and Benefits**:

*a.* ***Risks.*** The article notes that "[a]crolein is considered to be highly irritating to eyes and respiratory tract" (p. 811). The risks to subjects were minimized through the exposure levels, subject screening, and research design. Subjects were informed of the risks associated with the participation prior to providing written consent. The levels of acrolein chosen for this experiment were at and half of the Swedish 8-hour exposure limits (0.1 ppm). No subjects wore contact lenses during the exposure periods.

*b.* ***Benefits.*** There were no directs benefits to the subjects participating in the study. The findings of this study may be used to inform risk assessments and acceptable exposure levels of acrolein. EPA will use the results of this study to support its risk assessment of acrolein.

*c.* ***Risk-Benefit Balance.*** Risks to subjects were effectively minimized. The potential societal benefits of greater understanding of the sensory irritation from exposure to acrolein outweigh the risks associated with the study.

**4. Independent Ethics Review**: According to the article, the study was reviewed and approved by the Regional Ethical Review Board in Stockholm. This is an independent ethics body that operates under the Declaration of Helsinki and the Swedish Ethical Review Act. Dr. Johanson confirmed that the ethics application was reviewed and approved prior to the initiation of the research. The application was submitted on January 12, 2012 and approved on February 2, 2012. The decision from the ethics committee is included on pages 15-17 of Attachment 3. The protocol was approved on 2-8-on the condition that researchers provide informed consent about the biobank used to store the study-related records. This was added, and is provided on page 8 of Attachment 3. The protocol was amended to expand the subject size for the main study from 16 to 18 subjects (Attachment 3, p. 14).

**5. Informed Consent**: All subjects provided informed consent after receiving oral and written information "about the design of the study, the possible hazards, and their right to

immediately and unconditionally interrupt the exposure" (p. 811). Dr. Johanson noted that consent meetings were held one-on-one between the prospective subject and the examining physician. After the information was presented and questions answered, individuals filled out the medical questionnaire and completed consent form. The overseeing doctor reviewed the responses and completed a basic medical exam, including analyzing a blood sample, then decided whether the subject was eligible to participate in the study. The information covered and the consent form (in Swedish) can be found in Attachment 3. At the request of the ethics committee, an additional consent related to the storage and use of tissue samples at a biobank at the Karolinska University Hospital was added to the consent documentation (Attachment 3, p. 8).

6. **Respect for Subjects**: Subjects were compensated for their participation. Subjects participating in the pilot study received 500 SEK (~$60). Subjects in the main study received 2,000 SEK per visit, for a total of 12,000 SEK (~$1,440). Dr. Johanson noted that subjects received compensation "after each exposure session so as not to pressure the subjects to complete all 6 sessions for economic reasons" (Attachment 1).

   Subjects were informed that they were free to withdraw at any time and without forfeiting benefits. No subjects withdrew from the study. Subjects would not be responsible for costs of treatment for study-related illnesses or injuries, or any accidents that occurred traveling to or from the study location for an exposure session. No subjects experienced adverse effects outside of what was expected as part of the study's investigation into sensory irritation and no medical treatment was necessary.

   Subjects' identities were protected. Only the physician and two researchers carrying out the exposures and subject monitoring were aware of the subjects' identities. All records were kept in a locked cabinet. Subjects were identified by number, and no subject's identity was 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 with the intention to submit the results to EPA.

The article notes that the study was conducted according to the principles in the Declaration of Helsinki (p. 811). In addition to the Declaration of Helsinki, the study was subject to the requirements of the Swedish "Act on Research Ethics Review of Health Research Projects" (Attachment 4). The key ethical principles in the Declaration of Helsinki are respect for persons, beneficence, and justice. The Swedish 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). It also 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. The Swedish Act also outlines the responsibilities of the ethics boards and the criteria for reviewing proposed human research.

***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.

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 18 female subjects were pregnant or nursing. Pregnancy was an exclusion criteria and female subjects' non-pregnant status was confirmed prior to each exposure session with a pregnancy test. There is no indication that nursing women were enrolled in the study. 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 Regional Ethics Review Board in Stockholm. The study involved testing acrolein at concentrations at or below the threshold levels set in Sweden. 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, the information provided by Dr. Johanson, and the Swedish Act 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 excluding subjects who would be at higher risk from participation (e.g., smokers), conducting a pilot study to establish doses to use in the main study, and using a concentration of the test substance at or below levels approved by the European Union. The supplemental materials provided, along with the affirmations from Dr. Johanson, satisfy the requirements for informed consent under Swedish 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, identity of the test substance, and potential risks and discomforts were explained to subjects orally and in writing. Only self-reported healthy adults were eligible to enroll, a physician conducted a medical exam prior to enrollment to confirm potential subjects' health and eligibility, subjects were notified of insurance coverage 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 result in anything more then temporary irritation, minimizing the risk to subjects. Additionally, subjects were monitored closely during and after the exposure sessions.

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. Dr. Johanson noted that while recruitment was conducted at the Karolinska Institutet and students were eligible to enroll, anyone associated with the study staff was excluded from participation. There is no clear and convincing evidence to suggest that any of the subjects were vulnerable to undue influence by 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.

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 51570802) 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: Dana Vogel
Donald Wilbur
Shalu Shelat

Attachments

Attachment 1: Responses to EPA questions on Dwivedi publication from Dr. Johanson (English)
Attachment 2: Ethics Review Application (Swedish)
Attachment 3: Supplemental Materials (Swedish)
Attachment 4: Act on Research Ethics Review of Health Research Projects (English translation)