15 April 2021

EPA Ethics Questions on Research Article by Aishwarya M. Dwivedi, Gunnar Johanson, Johnny C. Lorentzen, Lena Palmberg, Bengt Sjögren, and Lena Ernstgård

Research: *Acute effects of acrolein in human volunteers during controlled exposure* by Aishwarya M. Dwivedi, Gunnar Johanson, Johnny C. Lorentzen, Lena Palmberg, Bengt Sjogren, and Lena Ernstgard. Published in 2015 in Inhalation Toxicology, 27(14), 810-821, 2015.

Request: Please respond to the questions listed below regarding the ethical conduct of the study. Responses and documents can be sent to Michelle Arling of the U.S. Environmental Protection Agency (U.S. EPA) by May 15, 2021 at arling.michelle@epa.gov In some cases, I request copies of support documents; it would be very helpful to receive these copies in English if feasible. Thank you for your assistance.

Questions

1. Do you have any of the materials related to the study conduct, such as the protocol, informed consent, records of review by the ethics board? If so, can you share them?

The documents are attached. Unfortunately, all are in Swedish.

Test Subjects

2. On page 811, the article notes that "subjects were students recruited by advertisement at Karolinska Institutet." Was recruitment limited only to students? Were students targeted, or was participation open to anyone who fit the eligibility criteria?

The study was open to anyone that fit the criteria, however, in our experience it is easier to recruit students than working people and easier to recruit at Karolinska Institutet because of vicinity and interest in this kind of this research.

3. Were students of any of the investigators or study staff excluded from participating?

Yes, they were excluded. Any individual with any kind of dependency towards the investigators or unit staff was excluded.

4. What was the recruitment process for test subjects? If you used an advertisement to recruit subjects, can you share a copy with us?

Via advertisement (attached, bilaga/supplement 3) on billboards at Karolinska Institutet.

5. What were the inclusion criteria for test subjects? Please be explicit. Was there anything during the medical examination or clinical blood chemistry tests that disqualified subjects?

Inclusion criteria were: age 20-50 y, non-smoker, not pregnant, healthy as judged by our physician based on a short medical questionnaire (attached, bilaga 5:1) and a medical exam, no ongoing medication. Blood tests for pregnancy and, most likely, Hb, CRP, ASAT and ALAT. I could not retrieve the SOPs, nor the key linking to the subjects' journals, and can therefore not confirm the four latter four at this moment. A small number of potential participants (if any), for instance smokers, may have been excluded by the investigators before reaching the medical examination, these were never registered and cannot be tracked. Some may have been excluded by the physician for medical

reasons, this would be noted in the subject's journal. But as I don't have access to the key linking the acrolein study to the subjects, at the moment I cannot say if this is the case. If this issue is important to you, I can make more efforts to find out when the corona situation is better and people are back at work.

6. What were the circumstances and methods by which informed consent was obtained from the test subjects? Who conducted the consent meetings? Were they group sessions or individual meetings with each subject?

Written and oral consents were obtained on an individual basis by the examining physician (attached, bilaga 5:1).

7. Were subjects eligible to participate in both the pilot study and the main study? If so, how many subjects participated in both?

The 8 subjects that participated in the pilot study also participated in the main study.

8. Is it possible to obtain a copy of the consent form and any informational materials used for test subjects?

Attached (in Swedish).

9. If not, do you recall what the subjects were told during the consent process?

Attached

10. Were test subjects compensated for their participation? If so, what was the compensation?

Yes, 500 SEK (approx. 60 USD) in the pilot study, 2000 SEK (approx. 240 USD) per day in the main study (12000 SEK or approx. 1440 USD in total). These sums were paid after each exposure session so as not to pressure the subjects to complete all 6 sessions for economic reasons. The sums were considered and approved by the ethics committee.

11. Did any of the test subjects withdraw from the study? If so, how many?

None withdrew.

<u>Stopping Rules</u>

12. Were there stopping rules for the study? If so, please explain.

- Running eyes, cough, or other types of pronounced discomfort.
- Malfunctioning of the chamber (se Q18).
- The study subjects were informed that they were free to end the exposure at any time for whatever reason.

None of these happened.

<u>Medical Treatment</u>

13. a) Did any of the subjects require medical treatment as a result of their participation in the study?

No.

b) If so, please explain.
c) If so, who provided the medical treatment?

If it had happened, the physician on standby would take care of the treatment and remit as needed (the Karolinska Hospital is 1 min away).

d) Who paid for it?

If remitted to a hospital or other treatment, the treatment costs would be covered by the Swedish public health care system. The subjects pain, injury, disability, loss of income etc would be covered by the Swedish patient injury insurance. An extra insurance was added that would cover accidents when travelling to and from the Karolinska Institutet for the exposures.

Independent Ethics Review

14. The article states the research was approved by the Regional Ethical Review Board in Stockholm (p. 811). Can you please provide the following information?
    a) Is this an independent ethics review body?

Yes, it is a national authority (at the time divided in regions) that works independently from the research institutions. According to Swedish law, studies on humans have to undergo ethics review and approval prior to initiation. The authority is described at https://etikprovningsmyndigheten.se. The law is presented at https://www.riksdagen.se/sv/dokument-lagar/dokument/svensk-forfattningssamling/lag-2003460-om-etikprovning-av-forskning-som_sfs-2003-460 (both in Swedish only).

b) Is it possible to obtain a copy of the correspondence with, and approval from, the overseeing ethics committee?

Attached (in Swedish).

c) Was the proposed research reviewed and approved by this ethics committee prior to initiation of the research?

Yes (in accordance with Swedish law).

Identity of Test Substance

15. Were subjects fully informed of the identity, nature and function of the test substance(s) to which they would be exposed during the research?

Yes, in written and orally (written information attached, bilaga 4). However, they were not informed about which exposure condition in each of the six sessions.

Risk Mitigation Steps

16. On p. 813, the article notes that "We therefore decided, for ethical reasons, to use the Swedish 8 h OEL of 0.1 ppm as the high level and half of it as the low level." Can you explain the ethical reasons behind this decision?

We judged it to be ethically acceptable to expose for 2 hours at the OEL since this level is legally accepted for life-long exposure at work (8 h/d, 5 d/wk, 40 y). Our toxicological assessment (attached, bilaga 2, in Swedish) indicated that pronounced irritation such as tear-eyedness occur at significantly higher levels.

17. What were the risks associated with use of the test substances?

Unforeseen problems that would case higher than targeted levels in chamber air, e.g by failure of the chamber ventilation. Severe reaction, such as asthma.

18. Can you explain the steps taken during the study to reduce risks to participating subjects?

The acrolein level in chamber air, ventilation, under pressure, temperature and humidity were continuously monitored. The ventilation was equipped with an alarm. The exposure has large glass windows plus an intercom system. One of the investigators was always present outside the camber and had visual contact with the study subjects. A physician was standby in the same corridor.

Reports of Adverse Effects

19. Did you receive any reports of adverse effects after the study?

No.

Alternative Means of Obtaining this Information

20. Was there any other way of obtaining this information outside of the chamber studies conducted?

I am not sure what you mean. As described in the Introduction of our paper, there is insufficient data to identify the irritation threshold in humans, an important piece of information e.g. in setting health - based OELs. Alternative methods (in vitro studies, animal studies, field data) were judged insufficient.

Protecting Identity of Subjects

21. What steps did you take to protect the identity of the subjects in the raw data and study? (For example, subjects identities were not revealed in the research article.)

The identity of the subjects was only known to the responsible physician and the two researchers that carried out the actual exposures, blood sampling, spirometry etc. Subjects were given unique identity numbers which were used when entering analyzing and presenting results. The medical exams were filed and kept in a locked cabinet, as was the key linking names with identity numbers. Thus, identities were not revealed outside the inner circle of the three directly engaged in the experimental part.