Acrolein/PC 000701

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Risk Assessment Brand IV, Health Effects Division (7509P) **Date:** 05/20/2021

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Risk Assessment Branch IV, Health Effects Division (7509P) **Date:** 05/19/21

Template version 03/12

TXR# 0058181

DATA EVALUATION RECORD – Supplemental
See TXR # [insert number] for root DER

STUDY TYPE: Non-guideline Special Acute Study – Human Eye Irritation

PC CODE: 000701 **DP BARCODE: D458866**

TEST MATERIAL (PURITY): Acrolein (90% a.i. with 0.2 wt% hydroquinone as stabilizer)

SYNONYMS: 2-propenal

CITATION: Claeson, A. and Lind, N. (2016) Human exposure to acrolein: Time-dependence and individual variation in eye irritation. Department of Psychology, Umeå University, Umeå, Sweden. May 12, 2016. MRID 51570801. *Environmental Toxicology and Pharmacology* 45: 20-27. https://doi.org/10.1016/j.etap.2016.05.011

SPONSOR: Swedish Research Council FORMAS.

EXECUTIVE SUMMARY:

In a special acute toxicity study (MRID 51570801) Acrolein (90% a.i. diluted in heptane, batch/lot # no provided) was administered to 18 women and 8 men via eye-only exposure at concentrations of 0 (heptane only), 0.07, 0.16, or 0.36 mg/m^3 (0, 0.00007, 0.00016, or 0.0036 mg/L) for 30 minutes (control and acrolein treatments) or 15, 45, and 60 minutes (acrolein treatments only). Confidence level and magnitude of perception to acrolein exposure were recorded as a measurement of eye irritation every other minute during the 15-minute exposure or every 5 minutes during the three longer exposure periods. Two additional measurements of eye irritation included manual counting of eye-blinks from recorded video of participants during exposure and self-reported tear-film break-up times (BUTs) (i.e., ability to focus on a single point on a wall without closing of eyes).

When combining the responses from all participants, a large amount of variability was observed in confidence level of perceived acrolein exposure, with perceived intensity increasing only slightly with time. However, when participants were divided into groups of non-responders (low confidence of perceived exposure) and responders (high confidence of perceived exposure), the latter reported being able to detect acrolein at all exposure concentrations, with perceived intensity also generally increasing over time for all treatments (significant only for the 15 minute exposures). The shortest exposure time (15 minutes) and highest concentration (0.36 mg/m^3) also resulted in significantly more eye irritation both immediately after and 10 minutes after exposure. The highest concentration was able to be detected above chance after 6.8 minutes of

exposure. There was no effect of acrolein on blink frequency or self-reported tear-film BUTs, and while differences could be seen between responders and non-responders (29.9 ± 18 vs. 16.6 ± 8.3 and 18.2 ± 14.5 vs. 32.2 ± 18.8 for blink frequency and BUTs, respectively), they were not significant, likely due to the high variability in responders. A significantly higher false detection rate was also seen in responders compared to non-responders (0.35 vs. 0.08, respectively), indicating a possible subjectivity for detecting malodourous compounds (i.e., an inherent bias due to gender or general stress perception). The detection of acrolein in only 58% of subjects may also indicate a pre-existing trait of sensitivity towards acrolein exposure in the responders. This study confirms previous findings suggesting a lowest observed adverse effect level (LOAEL) of about 0.34 mg/m^3 is necessary for adverse eye irritation due to acrolein exposure.

This acute special study in humans is **acceptable/non-guideline**.

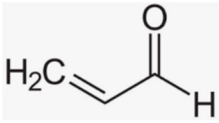
COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were not provided, as this is a literature study.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: Acrolein

Description: 0.2 wt% hydroquinone as stabilizer
Lot/batch #: Not reported; Sourced from Sigma Aldrich Co., St. Louis, MO
Purity: 90 % a.i.
Compound stability: Not reported
CAS # of TGAI: 107-02-8
Structure:



2. Vehicle and/or positive control: ≥99% heptane; Thermo Fisher Scientific, Waltham, MA

3. Subjects:

Species: Human: 8 male and 18 female volunteers
Strain: N/A
Age/weight at study initiation: 17-47 years of age; Body weights not reported
Source: Not reported; recruitment by billboard and local newspaper advertisements
Chamber temperature: 20-22°C
Chamber relative humidity: 15-21%
Chamber air exchange rate: 7.5 times/hour; 330 L/min

B. STUDY DESIGN:

1. In life dates: Not reported

2. Subject assignment and participant data collection: All subjects participated in the same dosing regimen (Table 1) that involved visiting the laboratory on four separate days (time between visits not reported) to undergo exposure conditions that differed in duration and concentration. Subjects were considered healthy non-smokers and non-pregnant (females), and two subjects normally wearing contact lenses were asked to not wear them during exposures. Subjects completed a chemical sensitivity scale (CSS) questionnaire that was used to assess behavioral changes and reactions to odorous and pungent substances, in addition to a perceived stress questionnaire (PSQ) to quantify the extent at which subjects perceived stress during the previous 4 weeks.

TABLE 1: Study design and range of mean sensory and confidence ratings in 18 women and 8 men

Test group	Concentration (C) (mg/m^3)	Duration (T) (min)	C X T[a] (mg/m^3-min)	Sensory rating (0-100)[b]	Confidence rating (1-4)[c]
Control	0	30	---	1.9-6.4	1.8-2.5
Low	0.07	60	0.4-4.2	0.0-11	1.5-2.7
Intermediate	0.16	45	0.8-7.2	2.3-6.9	1.7-2.7
High	0.36	15	0.7-5.0	0.3-13.8	1.9-3.3

[a] Range is the product of duration and acrolein concentration for ratings taken every other minute (15-minute exposure) or every five minutes (30-60 minute exposures)
[b] Borg CR-100 scale, which is a verbally anchored ratio scale used to measure sensory perception

[c] 1-2 indicates "no detection" and 3-4 indicates "yes detection"
Data from Table 2 on page 4 of MRID 51570801

3. **Dose selection rationale:** The dose levels were selected based on the results from previously reported sensory irritation thresholds[1-3] between 0.13 mg/m^3 and 1.2 mg/m^3. Additionally, low and high concentrations were approximately half the concentration of the Swedish occupational threshold limit for 15 minutes (0.7 mg/m^3) and 8 hours (0.2 mg/m^3), respectively. Heptane concentration in the control was 20.3 mg/m^3, and measured concentrations of heptane in the acrolein treatments did not differ appreciably from the control.

4. **Generation of the test atmosphere / chamber description:** Carbon-filtered air entered the chamber (1.5 X 0.9 X 2.0 m) through an inlet at floor level and exited in the chamber ceiling. Stimulus material (heptane or acrolein diluted in heptane) was continuously pumped via a syringe pump through a nebulizer, and aerosol from the nebulizer was mixed with air (4 L/min) in an evaporation chamber with a volume of approximately 1 L. The air mixture was further diluted and transported to the exposure chamber. Exposures were by eye only, with the subjects covering their nose and mouth with a fresh air mask.

   Concentrations of acrolein and heptane in the exposure chamber were monitored by direct injection into a coupled gas chromatography-flame ionizing detector (GC-FID) system. A syringe filled with 0.1 mL of air taken from the chamber was injected into the GC-FID, which was operated in splitless mode, with temperature starting at 35 °C and rising 2 °C/min until reaching 200 °C. Data were quantified using calibration curves from metered amounts of acrolein and heptane or, in the case of 0.07 mg/m^2 acrolein, derived by extrapolation from the standard curve.

C. METHODS:

1. **Measurements of eye irritation:** The influence of time on sensory irritation and detection was measured with judgements of confidence. Perceived intensity was measured using a magnitude estimation attached to a confidence level ranging from (1) not certain to (2) very certain. Ratings were then transformed into a scale of 1-4, where 1-2 represented an answer of "no" with certainty ranging from 1-2, and 3-4 represented an answer of "yes" with certainty ranging from 1-2. Perceived eye irritation was rated on the level-anchored category ratio (CR) Borg CR-100 scale, where descriptive adjectives correspond to specific numbers on the scale: 0 = nothing at all; 2 = minimal; 3 = extremely weak; 5 = very weak; 13 = weak; 25 = moderate; 37 = fairly strong; 50 = strong; 70 = very strong; and 90 = extremely strong.

   Two additional methods were used to assess eye irritation during exposure. The first involved filming of subjects and counting the number of eye-blinks manually with a hand

1 Gomes, R., Liteplo, R.G., Meek, M.E., 2001. Acrolein: hazard characterization and exposure-response analysis. J. Environ. Sci. Heal. Part C 19, 23–43.
2 Kuwabara, Y., Alexeeff, G.V., Broadwin, R., Salmon, A.G., 2007. Evaluation and application of the RD_{50} for determining acceptable exposure levels of airborne sensory irritants for the general public. Environ. Health Perspect. 115, 1609–1616.
3 Weber-Tschopp, A., Fischer, T., Gierer, R., Grandjean, E., 1977. Experimentally induced irritating effects of acrolein on men (author's transl). Int. Arch. Occup. Environ. Health 40, 117–130.

tally counter at three time points: 2 minutes after exposure, halfway through exposure, and 2 minutes prior to leaving the chamber. Blink frequency was calculated as the mean over 5 minutes, with blinks counted only when the majority of the eye was covered (i.e., ignoring twitches). The second method involved a self-reported tear-film break-up time (BUT), or the measurement of the length of time a subject was able to keep his/her eyes open while watching a fixed point on the wall, which was measured before exposure, immediately after exposure, and 10 minutes after the exposure. During exposure, the level of confidence and eye irritation were rated every other minute during the 15-minute exposure or every 5 minutes during the three other longer exposure periods. Ratings also took place before and immediately after each exposure session, together with BUT measurements.

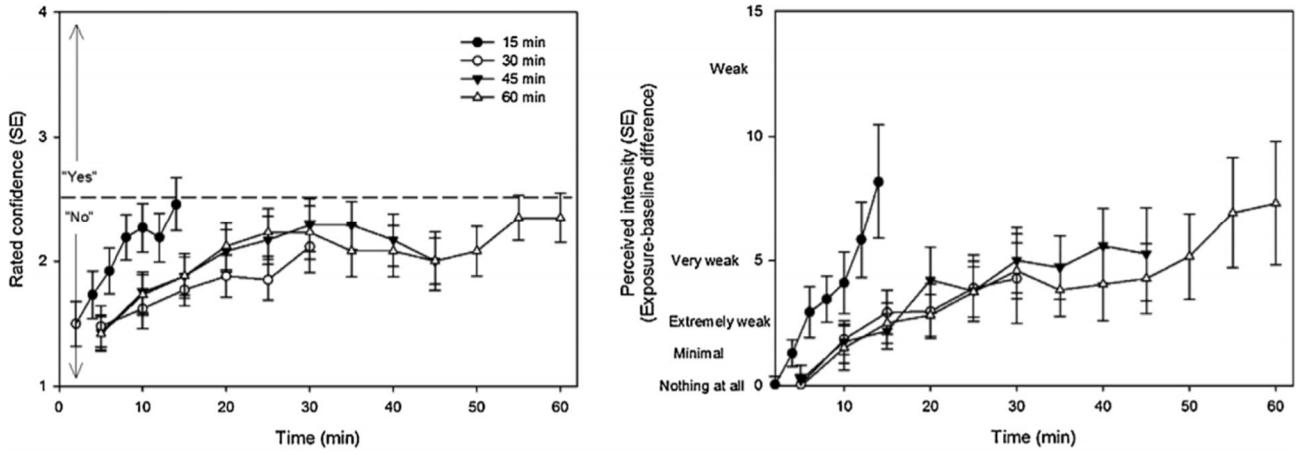
2. **Statistics:** Statistical analysis was conducted using a repeated-measures analysis of variance (ANOVA) and independent samples *t*-test, with an α-level set to 0.05. If the assumption of sphericity was violated, the significance level was adjusted with the Greenhouse-Geisser correction of the degrees of freedom to result in a stricter α-level.

   Several deficiencies were identified in the statistical methods stated, and the description of the methods lacked sufficient details (See Appendix).

II. RESULTS:

A. **Sensory perception:** Acrolein exposure increased detectability and perceived sensory irritation with time. No concentration elicited a reaction at the beginning of exposure. An initial response was a decrease in the level of confidence that a sensation had been experienced along with an increase in perceived sensitivity (Figure 1). Confidence rating increased with exposure time, but at no time did the average rating reach a confidence of "yes", despite an increase in perceived intensity over that same time from "nothing at all" to almost "weak" (See table 1 above).

Figure 1. Ratings of confidence (left) and magnitude (right) of eye irritation for all subjects during exposure to acrolein and heptane (15, 45, and 60 minutes) and heptane only (30 minutes)



There was a large difference among individuals regarding ratings of eye irritation, as 42% stated no detection at all while 58% rated it as clearly irritating. As a result, data were further divided into two groups based on confidence ratings for the 15-minute exposure. Responders

were considered those individuals with a confidence >2.5 (n=15), while non-responders did not reach this level of confidence (n=11). For all three acrolein exposures, confidence increased significantly with responders over time, but the perceived intensity as rated by the responders increased only with time for the 15-minute exposure (Figure 2). Additionally, the confidence level and perceived intensity was significantly different from the control exposure only during the 15-minute exposure (p<0.001), and the shortest exposure (15 minutes) with the highest concentration (0.36 mg/m^3) resulted in significantly more eye irritation immediately after and 10 minutes after the exposure had ended (Figure 3.)

Figure 2. Ratings of confidence (left) and magnitude (right) of eye irritation for responders only (n=15) during exposure to acrolein and heptane (15, 45, and 60 minutes) and heptane only (30 minutes). The dotted line (right) represents the highest rating for heptane-only conducted for 60 minutes at the same concentration as in the present study.

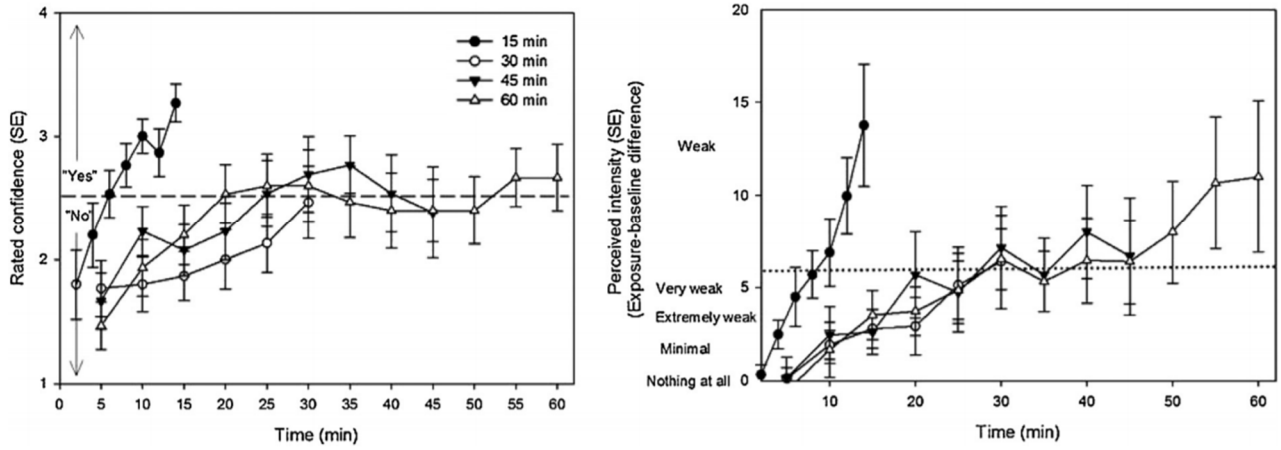
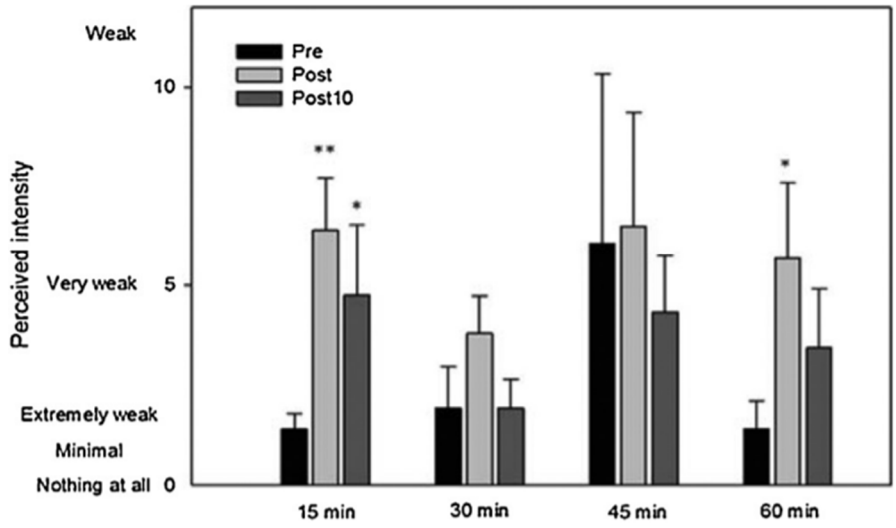


Figure 3. Ratings of magnitude of eye irritation for responders only (n=15) before, immediately after exposure, and 10 minutes after exposure to acrolein (15, 45, and 60 minutes) and heptane only (30 minutes).



To compare sensory irritation for responders only across exposure conditions and to distinguish from heptane exposure, the detection rate for each concentration corrected for false detections was calculated according to the equation:

$$P_C = (P_{Hit} - P_{FD} / 1 - P_{FD})$$

where P_C is the proportion of a correct response (confidence ≥ 3) corrected for bias, P_{Hit} is the proportion of a positive response to acrolein exposure, and P_{FD} is the positive response in the control exposure (the last P_{FD} for the 30-minute exposure was used as the false detection rate for exposures persisting beyond 30 minutes). Using this correction calculation as a function of the products of concentration and time (detection threshold calculated by fitting a least squares regression line), acrolein could be detected after 6.8 minutes of exposure at 0.36 mg/m^3. The mean false detection rate for the responders was 0.35, and for the non-responders was 0.08, with a significant difference between the two groups (p<0.05).

B. <u>Blink frequency and tear-film break-up time</u>: A two-way mixed model ANOVA showed no effects of exposure condition with or without acrolein on blink frequency or self-reported tear film BUTs, no significant interactions between time or concentration, and a significant effect of time only for blink frequency during the 60-minute exposure (p<0.05). Differences between responders and non-responders were also seen with blink frequency and self-reported tear-film BUTs, regardless of exposure condition. The number of counted eye-blinks during the four exposures was higher with responders compared to non-responders (29.9 ± 18 vs. 16.6 ± 8.3, respectively), with self-reported tear film BUTs showing a similar tendency (18.2 ± 14.5 vs. 32.2 ± 18.8). Neither difference was statistically significant, however.

III. DISCUSSION AND CONCLUSIONS:

A. <u>INVESTIGATORS' CONCLUSIONS</u> <u>(Pages 25-26 of MRID 51570801)</u>**:** Acrolein exposure below the threshold limit value for 15 minutes leads to sensory irritation in more than half of the participants. This irritation was time-dependent and took about 6.8 minutes of exposure to acrolein at 0.36 mg/m^3 to become detectable above chance. Confidence was generally low but increased with time for all exposures but the control. Exposure to the intermediate concentration indicates that exposure duration should be extended to 90 minutes in order to be detected above chance, while the lowest concentration was unable to generate any observable reaction. Concentration had a larger influence on sensory irritation than time. Large differences among individuals were seen as a general overall difference between the blink frequencies and self-reported tear film BUTs of responders compared to non-responders, as well as higher frequency of false detection rates. Acrolein resulted in both increased detectability and sensory irritation over time, with irritation still significant 10 minutes after exposure. Because acrolein was detected by only 58% of participants, a pre-existing trait of sensitivity toward exposure may be present in the responders, which should be taken into account for assessing occupational exposure limits that are set to avoid sensory irritation in the majority of people. Irritation to certain volatile organic compounds such as acrolein may depend on a combination of the compound, time of exposure, and the individual being exposed. This study confirms previous findings suggesting a lowest observed adverse effect level (LOAEL) of about 0.34 mg/m^3 is necessary for adverse eye

irritation due to acrolein exposure.

B. REVIEWER COMMENTS: When combining the responses from all participants, a good deal of uncertainty was seen in their confidence of perceived acrolein exposure, while perceived intensity increased slightly with time. However, when participants were divided into groups of non-responders (low confidence of perceived exposure) and responders (high confidence of perceived exposure), the latter reported being able to detect acrolein at all exposure concentrations, with perceived intensity also generally increasing over time for all treatments (significant only for the 15 minute exposures). The shortest exposure time (15 minutes) and highest concentration (0.36 mg/m^3) also resulted in significantly more eye irritation both immediately after and 10 minutes after exposure. The highest concentration was able to be detected above chance after 6.8 minutes of exposure. There was no effect of acrolein on blink frequency or self-reported tear-film BUTs, and while differences could be seen between responders and non-responders (29.9 ± 18 vs. 16.6 ± 8.3 and 18.2 ± 14.5 vs. 32.2 ± 18.8 for blink frequency and BUT, respectively), they were not significant, likely due to the high variability in responders. A significantly higher false detection rate was also seen in responders compared to non-responders (0.35 vs. 0.08, respectively), indicating a possible subjectivity for detecting malodourous compounds (i.e., an inherent bias due to gender or general stress perception). The detection of acrolein in only 58% of subjects may also indicate a pre-existing trait of sensitivity towards acrolein exposure in the responders. This study confirms previous findings[4-5] suggesting a lowest observed adverse effect level (LOAEL) of about 0.34 mg/m^3 is necessary for adverse eye irritation due to acrolein exposure.

C. STUDY DEFICIENCIES:

The following minor deficiencies were noted but do not alter the conclusions or interpretation of results for this study:

1) Heptane only (control) exposure lasted only 30 minutes rather than 60 minutes (longest acrolein exposure), and low relative humidity could have induced a sensory irritation over longer exposure times (i.e., through drier air).
2) The number of females was 2X that of males and may have biased responses towards a greater level of detection or false detection rates, as women naturally are more sensitive to sensory irritation[6-7].
3) Although methods were provided for measuring concentrations in exposure chambers, measured concentrations themselves were not provided.
4) Additional details regarding the clean air masks used to facilitate eye-only exposures were not provided, and it is unclear whether indirect inhalation exposure to the volatile compound may have remained possible in a manner influencing perceived sensitivity (e.g., slight odors).

4 Dwivedi, A.M., Johanson, G., Lorentzen, J.C., Palmberg, L., Sjögren, B., Ernstgård, L., 2015. Acute effects of acrolein in human volunteers during controlled exposure. Inhal. Toxicol. 8378, 1–12.
5 Trantallidi, M., Dimitroulopoulou, C., Wolkoff, P., Kephalopoulos, S., Carrer, P., 2015. EPHECT III: health risk assessment of exposure to household consumer products. Sci. Total Environ. 536, 903–913.
6 Claeson, A.-S., Nordin, S., 2011. Gender differences in nasal chemesthesis: a study of detection and perceived intensity. Chemosens. Percept. 4, 25–31.
7 Shusterman, D., Murphy, M.A., Balmes, J., 2003. Differences in nasal irritant sensitivity by age, gender, and allergic rhinitis status. Int. Arch. Occup. Environ. Health 76, 577–583.

5) The durations between visits of participants to the laboratory were not provided.
6) Several deficiencies were recognized for the statistical methods used by the investigators, and raw data was not available.

Appendix

Statistical Summary

The statistical analyses presented in the publication may not in all cases have been optimal or necessarily met some of the assumptions of the statistical tests used, and other alternative approaches may have been somewhat more appropriate. For example, this includes the investigators' use of repeated measures ANOVA, the use of "time" as a categorical variable, and the use of the independent samples t-test to evaluate repeated measures. More specifically:

- The repeated measures ANOVA used by the investigators assumes the variance-covariance matrix to be compound symmetric and was used to analyze the correlated data with the Greenhouse-Geisser correction when the assumption of sphericity1 was violated. While this approach is not unreasonable, a mixed-effects model is typically considered more appropriate as it has more options to better account for the actual structure of the associated variance-covariance matrices and represents the present state-of-the-science in statistics. These variance-covariance structure options that can be explicitly considered and modeled using mixed-effects models include compound symmetry, unstructured, or first-order autoregressive analyses, as well as using spatial power to correct for the inconsistent (non-uniform) intervals between measurements. A mixed effects model is also able to account for the random effects of subject or day implicit in the present study's design, and it is not clear how the repeated measures ANOVA used by the investigators was able to account for this in the analyses.

- It is unclear from the publication whether "Time" was set as a categorical variable in the repeated measures ANOVA model, and it is also unclear what comparisons were performed to conclude that detectability and perceived sensory irritation changed with time for each exposure group.

- The methods description in the study indicates that data were analyzed using the independent samples t-test. The independent samples t-test may have been used to compare the results of responders vs. non-responders for parameters such as blink frequency and self-reported tear-film breakup times. All data, however, seem to be repeated measures. The use of the independent samples t-test to analyze repeated measures data is in most instances not typically considered appropriate. Additionally, the high variability in the responders compared to the non-responders indicates that it may not have been normally distributed, and it is unclear what adjustments were conducted to achieve normality prior to analyses.

  The study investigators, upon request, kindly provided some of the raw data for the study and a subset of this raw data is undergoing further review in HED. It is important to note that that although HED will be using different statistical methods than used by the study authors in their publication, this does not necessarily mean that the conclusions reached regarding the outcomes will differ.

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1 Sphericity is the condition where that the variances of the differences between all possible pairs of within-subject conditions are equal. When violated, the Greenhouse-Geiser adjusts the degrees of freedom to better account for this violation. Typical present-day statistical practice, however, is to use mixed-effects models for which the variance-covariance structure can be explicitly modeled.