EPA Reviewer: Tim McMahon, PhD, Deborah Burgin, PhD, DABT **Signature**: _____
Risk Assessment Branch, Antimicrobials Division **Date:** _____
EPA Secondary Reviewer: Sarah Gallagher, PhD **Signature**: _____
Risk Assessment Branch 1, Existing Chemicals Risk Assessment Division (OPPT) **Date**:_____

DATA EVALUATION RECORD

STUDY TYPE: Controlled Human Exposure to Formaldehyde by Inhalation- non-guideline

PC CODE: 043001 **DP BARCODE:**
TXR#: N/A

TEST MATERIAL (PURITY): Formaldehyde test atmosphere (generated by vaporizing paraformaldehyde on a magnetic hot plate stirrer at 200°C).

SYNONYMS: HCHO, FA

CITATION: Joerg U. Mueller, Thomas Bruckner, Gerhard Triebig (2013): Exposure study to examine chemosensory effects of formaldehyde on hyposensitive and hypersensitive males. International Archives of Occupational and Environmental Health (2013) 86:107–117. DOI: 10.1007/s00420-012-0745-9

Mueller et al. references the following, conducted in the same facility: Isabelle Lang, Thomas Bruckner, Gerhard Triebig (2008). Formaldehyde and chemosensory irritation in humans: A controlled human exposure study. Regulatory Toxicology and Pharmacology 50 (1):23–36. DOI: 10.1016/j.yrtph.2007.08.012

Statistical review provided by: Eftim, S., Spruce, J., Rosenthal, C., Gan, R. (2023). Statistical Reanalysis of Data from Two Formaldehyde Inhalation Exposure Studies: Lang et al (2008) and Mueller et al (2013). Memorandum to Facey et al, EPA from S Eftim, ICF. February 21, 2023.

LABORATORIES: Institute and Out-patient Clinic of Occupational and Social Medicine, University of Heidelberg, Vossstrasse 2, 69115 Heidelberg, Germany; Institute of Medical Biometry and Informatics (IMBI), University Hospital of Heidelberg, Im Neuenheimer Feld 305, 69120 Heidelberg, Germany.

EXECUTIVE SUMMARY:

The study by Mueller et al (2013) was conducted with the objective of evaluating sensory irritant effects of formaldehyde on hypo- or hypersensitive individuals. Forty-one healthy, non-smoking male adults, average age 32 ± 9.9 years, were exposed for 5 days (4 hours per day) to formaldehyde (FA) vapor at

target concentrations of 0, 0.5, and 0.7 ppm, and to 0.3 ppm with peak exposures of 0.6 ppm (0.3/0.6 ppm), and 0.4 ppm with peak exposures of 0.8 ppm (0.4/0.8 ppm), in a repeated-measures crossover design. Concentrations in mg/m^3 equate to 0.0, 0.62, 0.86 mg/m^3 and 0.37 with peak of 0.74 (0.37/0.74 mg/m^3), and 0.49 with peak of 0.98 mg/m^3 (0.49/0.98 mg/m^3), respectively. The five exposure conditions were determined by blinded randomization. For exposure conditions that included peak exposures, they occurred four times for 15-min periods. Objective measurements included conjunctival redness, eye blinking frequency, tear film break-up time, and nasal flow rates. Subjective ratings on discomfort as well as the influence of personality factors on the subjective scoring were examined. These examinations were carried out approximately 1 hour prior to, during, and 1 hour after exposure, and were used to evaluate the irritating effects of formaldehyde.

For the assessment of subjective symptoms and complaints, the German version of the SPES (Swedish Performance Evaluation System) questionnaire was used. The questionnaire contained 31 questions about symptoms or states of irritation concerning different organ systems. The PANAS (Positive and Negative Affect Schedule) questionnaire served to determine subjects' positive and negative personality patterns and traits.

To assess hypo- and hyper-sensitivity, nasal sensitivity to CO_2 irritation was used as surrogate for individual chemosensory sensitivity. Conjunctival redness was measured by digital photography to document any dilatation of the conjunctival blood vessels indicative of irritant effects. Eye-blinking frequency was documented for approximately 5 min by means of a digital camcorder directly before FA exposure outside the exposure chamber and within the last 15 min of exposure inside the chamber. Self-reported tear film break-up time (sBUT) was measured by stopwatch. Nasal resistance and flow rates were assessed by anterior active rhinomanometry.

There were no exposure-related effects on conjunctival redness and blinking frequency. Tear film break-up time increased in the 0.4/0.8 ppm and 0.5 ppm exposure groups ($p < 0.05$). Nasal flow rates increased in hypersensitive subjects at 0.7 ppm ($p < 0.01$). ICF (2013) confirmed the accuracy of these findings.

The SPES survey sum score showed a statistically significant increase in hypersensitive subjects at 0.3/0.6 ppm ($p < 0.001$) and 0.4/0.8 ppm ($p < 0.01$); the perception of impure air increased in hypersensitive subjects at all exposure levels (including clean air, 0.01 ppm). Combined eye symptom survey scores were reported to be higher among hypersensitive subjects at all exposure concentrations except 0.7 ppm (0.86 mg/m^3). Changes in scores were not statistically significant and no exposure-response was observed. When controlled for "negative affectivity" these associations were not altered (indicating negative personality traits did not affect symptom reporting).

This study is classified as **acceptable/non-guideline.** It was not submitted for fulfillment of a guideline but was evaluated by the agency for determining a point of departure (POD) from acute inhalation exposure to formaldehyde. As such, this study can be used quantitatively in a weight-of-evidence in determining a POD for acute inhalation exposure to formaldehyde in the human population.

COMPLIANCE: This is a published study and as such, did not contain statements of compliance or confidentiality.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Materials:	
Description:	Paraformaldehyde, supplied by Merck, Darmstadt, Germany Formaldehyde vapor generated by vaporizing paraformaldehyde on a magnetic hot plate stirrer at 200°C. Peak exposures were generated by additional evaporation of formaldehyde until the desired peak concentration was reached. Real time monitoring of formaldehyde concentrations occurred in real time using an Asynco® Formaldehyde Monitor (Interscan, HCHO, model no. 4160-DSP, serial no. 821010, Karlsruhe, Germany).
Lot/Batch #:	Not provided
Purity:	Not provided
CAS # of TGAI:	50-00-0 (paraformaldehyde 30525-89-4)

2. Vehicle and/or positive control: Clean air (assumed). The publication noted (Table 1) that on days when the formaldehyde concentration should have been 0 ppm, the concentration was 0.01 ppm by real-time monitoring and 0.01 ± 0.01 ppm by HPLC. This background level is similar to the level reported in Lang et al. (2008) (testing was conducted at the same facility).

B. STUDY DESIGN and METHODS:

The objective of the study was, as stated in the publication, "to evaluate irritant formaldehyde effects on male volunteers, who were grouped as hypo- or hypersensitive against sensory irritation."

Exposure Facility

A description of the exposure chamber was not provided. As this study was performed in the same facility as the Lang et al. (2008) publication, it is assumed that the exposure chamber was the same. In the Lang study, exposures occurred in a 2.1m x 4.3 m x 2.5 m environmentally-controlled chamber with HEPA filters plus activated carbon filters. A ventilation rate of 7.0 m^3/minute produced one air change every 3.2 minutes. Temperature was maintained at 22.2 ±3.0 ºC and relative humidity at 50 ±10%. Exposure room air was vented to the building exhaust system without recirculation. An antechamber was used to prevent a sharp reduction in the exposure chamber concentration when the examiner or subjects entered or left the exposure chamber, as daily exposure concentration was not the same for each subject. No statements on

homogeneity of test atmospheres or temperature/relative humidity of the exposure chamber were provided.

The formaldehyde test atmosphere was generated by vaporizing paraformaldehyde on a magnetic hot plate stirrer at 200°C. Real-time monitoring of formaldehyde concentrations was carried out using an Asynco® Formaldehyde Monitor (Interscan, HCHO, model no. 4160-DSP, serial no. 821010, Karlsruhe, Germany). See Lang et al (2008) for more complete details.

To verify the results of the real-time monitoring, two air samples taken each exposure day were analyzed using dinitrophenylhydrazine and HPLC analysis.

Study Participants

A total of 41 males volunteered for participation in this study after written informed consent. The mean age of subjects was 32 ± 9.9 years. The following exclusion criteria applied to the subjects in this study:

(1) eye-blinking frequency of greater than 20/min
(2) allergy and/or skin diseases
(3) drug abuse or consumption of alcohol greater than 50 g/day
(4) exposure to FA at workplace or at home
(5) diseases of the respiratory tract, metabolism or heart
(6) inadequate vision without visual aids.

There was no information provided on inclusion criteria nor on screening of subjects prior to inclusion in the study regarding medical history or physical examination.

Approval and Informed Consent

As noted in the publication, “The trial was approved by the Ethics Committee of Heidelberg University and performed according to the Declaration of Helsinki.”

Inhalation Study Exposures

Formaldehyde exposures occurred on five consecutive days under five different exposure scenarios (Table 1). A maximum of four subjects were exposed per week, separated into two groups with exposures time-staggered by 1 h. During the 4-h daily exposure, subjects performed four cycle ergometer units (80 watts for 15 min) at predefined times to emulate occupational activity during a work shift. On days with exposure peaks, two of the four ergometric units were carried out during an exposure peak. Subjects were instructed not to eat shortly before an examination and during exposure, while drinking of non-sparkling mineral water was allowed.

Table 1 Results of the measured formaldehyde concentrations in the exposure chamber (mean ± SD); peak exposures were 15 min each; air samples for HPLC analysis were taken both during baseline and peak exposures

Concentration/ Exposure condition	FA concentration (ppm) (target)	FA concentration (ppm) (real-time monitoring)	FA concentration (ppm) (HPLC)
A	0.0	0.01 ± 0.00[a]	0.01 ± 0.01[a]
B	0.3 + 4 × 0.6 (peaks)	0.33 ± 0.02	0.32 ± 0.02
C	0.4 + 4 × 0.8 (peaks)	0.44 ± 0.03	0.39 ± 0.05
D	0.5	0.51 ± 0.01	0.49 ± 0.06
E	0.7	0.71 ± 0.02	0.70 ± 0.03

[a] Background level

The order of daily examination consisted of the following: 1) measurement of nasal flow rates and self-reported tear film break-up time (sBUT); 2) detection of CO_2 sensitivity as well as determination of conjunctival redness (photo documentation); 3) eye-blinking frequency (video recording); 4) recording of subjective symptoms and complaints using the SPES question aire (Swedish Performance Evaluation System). Subjects were examined approximately 1 hour before start and 1 hour after the end of exposure each day. The publication noted that "Apart from eye-blinking frequency recording and completion of the SPES questionnaire, which took place during the last 15 minutes of exposure inside the chamber, no examinations were carried out during the daily exposure phase."

Nasal resistance and flow rate were measured by anterior active rhinomanometry (AAR) in accordance with the International Committee on Standardization of Rhinometry (ICSR) and as described in Lang et al. (2008).

Tear film break up time (sBUT) was measured by instructing the subjects to hold their eyes open as long as possible and to keep them fixed on a mark on the opposite wall. The time elapsed until the first closure of the subject's eyelid was measured using a stopwatch.

Nasal sensitivity to CO_2 irritation was used as surrogate for individual chemosensory sensitivity to determine which subjects were hypersensitive and hyposensitive. CO_2 is known to irritate mucous membranes and cause concentration-dependent stinging and painful sensations with a very low inter-individual variability (Mueller et al, 2013). CO_2 sensitivity measurements were taken daily before and after exposure and during three follow-up tests at approximately one-week intervals after the end of exposure. Measurements recorded at the last follow-up

examination formed the basis for a breakdown of volunteers into different sensitivity groups based on their scores. According to the measurements, 20 hyposensitive and 21 hypersensitive subjects were divided by the median of the CO_2 sum score.

Conjunctival redness was measured using a digital camera (Fujifilm FinePix S1 Pro; Medical-Nikkor 120 mm f/4 IF Macro-lens with built-in ringflash) to "document any dilatation of the conjunctival blood vessels indicative of irritant effects of formaldehyde. A section of the participants' medial conjunctiva was photographed, and severity of conjunctival irritation was evaluated according to the publication of Lang (2008)."

Eye blinking frequency (EBF) was measured for approximately 5 minutes by means of a digital camcorder (JVC GR-D230E, serial no. 159P1566) outside the chamber immediately prior to formaldehyde exposure and within the last 15 minutes of exposure inside the chamber. Blinking frequency was counted by two investigators independently and blinded to the actual exposure concentrations.

Subjective symptoms and complaints were measured using the SPES questionnaire. Data were gathered twice a day, once prior to exposure and then shortly before the end of exposure. The SPES questionnaire contained 31 questions about symptoms or states of irritation concerning different organ systems (e.g., nose and eyes) as listed in Online Resource 1. Ratings of symptoms' strength or severity were also documented using a scale from 0 to 100 mm (endpoints ranged from 'not at all' to 'very strong').

Personality traits were assessed in the subjects before the first formaldehyde exposure, using the PANAS questionnaire. The PANAS questionnaire is a 20-item questionnaire in which a subject rates his/her emotional affectivity. Positive affectivity and negative affectivity traits were recorded as shown in the following table from supplementary material provided (Online Resource 2).

Table 2. Online Resource 2. PANAS questionnaire consists of two 10-item scales for positive and negative affectivity

Positive affectivity	Negative affectivity
Interested	Distressed
Excited	Upset
Strong	Guilty
Enthusiastic	Scared
Proud	Hostile
Alert	Irritable
Inspired	Ashamed

Determined	Nervous
Attentive	Jittery
Active	Afraid

Subjects had to document to which degree the respective feature generally applies to them (very slight/not at all; a little; moderately; quite a bit; extremely). Information from Mueller (2013) Online Resource 2.

Statistical Methods

The following information on statistics is taken from the publication.

"SAS version WIN 9.1 was used for statistical calculations. Categorical data were summarised by means of absolute and relative frequencies (counts and percentage). Quantitative data were summarised using the following summary statistics: number of observations, arithmetic mean, standard deviation (SD), minimum, median and maximum. To clearly demonstrate potential differences between effects, scaling was adapted and values outside the selected scale were omitted (marked by open squares). Possible differences between the five exposure conditions were verified by covariance analysis methods (ANCOVA), and potential divergences between categorical variables calculated based on the chi-square test. Differences in measurements before and after exposure were determined by one-sample t-test. The Spearman rank correlation coefficient and the corresponding p-values were calculated to demonstrate possible relations between the continuous variables or scores. The level of significance was set to 5%; alpha adjustment was not done."

Statistical re-analysis of the data was also performed by Dr. Sorina Eftim (Memorandum, S. Eftim et al, ICF, 2023). The Agency was unable to obtain the raw data from the study authors, so ICF was limited in what they were able to verify. Please refer to the report for more in-depth discussion of the statistical methods utilized. Where results could be verified by ICF, that is noted within each section.

RESULTS

Measured formaldehyde concentrations in this study were in good agreement with the target concentrations (Table 1).

Objective Symptom Results

Conjunctival Redness

The results on conjunctival redness (Online Resource 3) are reproduced below. There was no individual concentration-response relationship observed for conjunctival redness for any of the participants. According to the authors, for hypersensitive participants as a group, there appears to be an increase in conjunctival redness in the groups exposed to 0.5 and 0.7 ppm formaldehyde. However, ICF (2013) was unable to verify these results, in part because it is unclear how the authors analyzed this data.

Table 3 Online Resource 3 Results of conjunctival redness

		Changes in Conjunctival Redness (%)		
	Sensitivity	Decrease (-)	Constant (=)	Increase (+)
A (0 ppm)	•	0.00	85.00	15.00*
	+	23.81*	71.43	4.76
B (0.3 ppm/0.6 ppm)	•	10.00	90.00	0.00
	+	28.57	71.43	0.00
C (0.4 ppm/0.8 ppm)	•	20.00	70.00	10.00
	+	23.81	71.43	4.76
D (0.5 ppm)	•	21.05	78.95	0.00
	+	15.00	70.00	15.00
E (0.7 ppm)	•	25.00	70.00	5.00
	+	19.05	66.67	14.29

Table 3 contains percentage changes of conjunctival redness in hyposensitives (•) / hypersensitives (+) after exposure to the five FA concentrations A–E; * $p < 0.05$ compared to pre-exposure

Eye Blinking Frequency

Blinking frequency (EBF) was unaffected in any of the treatment groups, as shown in Figure 1. The lack of any meaningful change is likely due to the apparently large variability in blinking frequency measurements in the individuals in this study. ICF confirmed there were no consistent statistically significant changes.

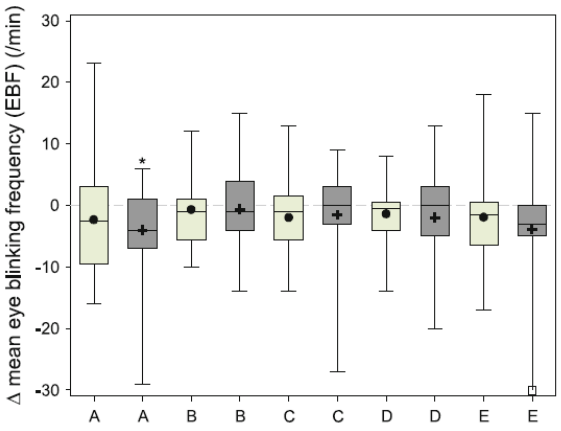

Fig. 1 Results of eye-blinking frequency (/min). Displayed are mean differences of EBF in hyposensitives (•)/hypersensitives (+) at the end of exposure compared to pre-exposure; FA concentration: A: 0 ppm; B: 0.3 ppm/0.6 ppm peaks; C: 0.4 ppm/0.8 ppm peaks; D: 0.5 ppm; E: 0.7 ppm; *p<0.05 compared to pre-exposure. For additional data see Online Resource 6 (source: Mueller et al., 2013)

Nasal Resistance and Flow

Data collected on nasal flow rate in this study is shown below (Table 4 Online Resource 8)

Table 4 Online Resource 8 Results of nasal flow measurement

FA concentration	sensitivity	mean	(±SD)	median	(range)
A (0 ppm; control condition)	●	-50.30	(± 314.09)	10.00	(-644.00 - 680.00)
	+	38.48	(± 213.51)	88.00	(-524.00 - 386.00)
B (0.3 ppm/0.6 ppm)	●	157.10#	(± 339.48)	45.00	(-388.00 - 1032.00)
	+	-10.67	(± 334.40)	- 4.00	(-784.00 - 692.00)
C (0.4 ppm/0.8 ppm)	●	-7.05	(± 303.20)	29.00	(-828.00 - 560.00)
	+	50.57	(± 346.11)	28.00	(-1148.00 - 648.00)
D (0.5 ppm)	●	-18.55	(± 406.98)	- 19.00	(-926.00 - 1236.00)
	+	-77.81	(± 306.18)	- 90.00	(-754.00 - 726.00)
E (0.7 ppm)	●	83.00	(± 266.55)	54.00	(-260.00 - 834.00)
	+	192.76**	(± 305.88)	136.00	(-184.00 - 764.00)

Table contains mean differences (± SD) and median (range) of nasal flow rates (ml/sec) in hyposensitives (●)/hypersensitives (+) after exposure to the five FA concentrations A–E;
** $p < 0.01$ compared to pre-exposure; # $p < 0.05$ compared to control condition (0 ppm)

There were no consistent increases or decreases in nasal flow rates. The authors noted that the hypo- and hypersensitive groups developed an increased mean nasal flow after exposure to 0.3/0.6 ppm and 0.7 ppm, respectively. However, "a consistent difference compared to the control condition was missing, no obvious FA-specific effect was detected." ICF confirmed

these results with a significant ($p < 0.01$) increase in nasal flow in hypersensitive males exposed to 0.7 ppm (0.86 mg/m^3) formaldehyde.

Subjective Symptoms Reporting

Results from the SPES 'sum score' and SPES subscores related to the objective parameters 'eyes', 'upper respiratory tract' and 'olfactory function' are shown in the results below (Table 5 Online Resource 9).

Table 5 Online Resource 9 Results of SPES 'sum score'

FA concentration	sensitivity	mean	(±SD)	median	(range)
A (0 ppm; control condition)	●	-0.20	(± 2.44)	0.00	(-8.00 - 3.06)
	+	0.97	(± 3.85)	0.55	(-4.65 - 16.23)
B (0.3 ppm/0.6 ppm)	●	0.57	(± 1.37)	0.21	(-3.13 - 3.03)
	+	2.20***	(± 2.24)	1.84	(-0.06 - 10.13)
C (0.4 ppm/0.8 ppm)	●	0.59	(± 2.63)	0.34	(-7.26 - 5.48)
	+	2.03**	(± 2.80)	1.81	(-4.19 - 8.52)
D (0.5 ppm)	●	0.63	(± 1.88)	0.31	(-2.00 - 7.13)
	+	0.84	(± 3.87)	0.77	(-10.48 - 8.61)
E (0.7 ppm)	●	0.68	(± 1.59)	0.29	(-1.94 - 5.26)
	+	1.63	(± 4.77)	1.58	(-15.42 - 10.06)

Table contains mean differences (± SD) and median (range) of SPES 'sum score' (mm) in hyposensitives (●)/hypersensitives (+) at the end of exposure to the five FA concentrations A–E;
** $p < 0.01$; *** $p < 0.001$ compared to pre-exposure

As shown above, the SPES symptom sum score was increased in all groups except hyposensitive males at 0 ppm and were significantly increased in hypersensitive males in the 0.3/0.6 ppm group and the 0.4/0.8 ppm group. ICF confirmed the hypersensitive males had a statistically significant increase compared to pre-exposure for the 0.3/0.6 ppm exposure ($p<0.001$) and the 0.4/0.8 ppm exposure group ($p<0.01$).

1) Eye Irritation

Results of the SPES subscore for eye irritation are reproduced below (Table 6 Online Resource 10). The authors reported no significant treatment-related changes in eye irritation scoring reported for either hyposensitive or hypersensitive males in this study at any of the formaldehyde concentrations tested. ICF confirmed the lack of significance for most groups, however, they

identified a significant increase in eye irritation in hypersensitive males exposed to 0.3 with peaks of 0.6 ppm ($p<0.05$) and borderline significance ($p = 0.05$) in hypersensitive males exposed to 0.5 ppm.

Table 6 Online Resource 10 Results of SPES subscore 'eye irritation'

FA concentration	sensitivity	mean	(±SD)	median	(range)
A (0 ppm; control condition)	●	- 0.17	(± 2.02)	0.00	(-4.86 - 4.00)
	+	1.96	(± 7.59)	0.00	(-3.57 - 34.00)
B (0.3 ppm/0.6 ppm)	●	0.23	(± 2.65)	0.00	(-5.86 - 4.71)
	+	2.13	(± 4.71)	0.86	(-1.43 - 21.14)
C (0.4 ppm/0.8 ppm)	●	0.62	(± 5.71)	0.14	(-15.71 - 12.43)
	+	1.43	(± 5.31)	0.14	(-13.00 - 12.43)
D (0.5 ppm)	●	- 0.09	(± 2.14)	0.00	(-6.29 - 2.43)
	+	1.24	(± 2.84)	0.86	(-4.43 - 11.00)
E (0.7 ppm)	●	0.94	(± 4.56)	0.00	(-8.57 - 13.29)
	+	0.52	(± 4.14)	0.14	(-12.71 - 6.57)

Table contains mean differences (± SD) and median (range) of 'eye irritation' (mm) in hyposensitives (●)/hypersensitives (+) at the end of exposure to the five FA concentrations A–E

It is interesting to note that the Lang et al. (2008) study, which used a similar design with similar concentrations, found a statistically significant increase over 0 ppm (A) in eye irritation scoring with exposures beginning at 0.3 ppm (C), shown below. Use of males alone, a greater number of volunteers, use of ethyl acetate in Lang et al. and CO_2 challenges in Mueller et al. may account for the differences seen between the two studies.

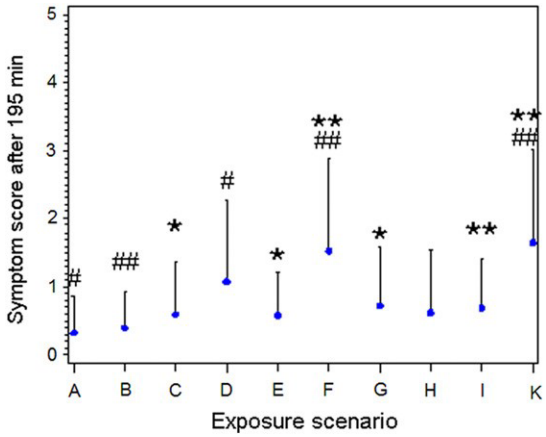

Fig. 7. Symptom score for eye irritation recorded during exposure (t = 195 min) to different concentrations of formaldehyde with or without ethyl acetate (EA). Results are expressed in means ± SD. A = 0 ppm, B = 0.15 ppm, C = 0.3 ppm, D = 0.3 ppm + 4 peaks of 0.6 ppm, E = 0.5 ppm, F = 0.5 ppm + 4 peaks of 1.0 ppm, G = 0 ppm + EA, H = 0.3 ppm + EA, I = 0.5 ppm + EA, K = 0.5 ppm + 4 peaks of 1.0 ppm + EA. Statistics: repeated measures ANOVA with contrasts; *p < 0.05 and **p < 0.01 compared to 0 ppm; #p < 0.05 and ##p < 0.01 compared to 0 ppm + EA. Source: Lang et al. (2008), p30

2) Nasal Irritation

Results of nasal irritation scoring are reproduced below from the supplementary material (Table 7 Online Resource 11). Note that for nasal irritation, the SPES questionnaire included the terms 'irritation of the nose', 'itchy nose', 'dry nose', 'running nose' and 'burning nose.'

Table 7 Online Resource 11 Results of SPES subscore 'nasal irritation'

FA concentration	sensitivity	mean	(±SD)	median	(range)
A (0 ppm; control condition)	●	-1.13	(± 4.12)	-0.10	(-14.20 - 5.80)
	+	-0.09	(± 1.60)	0.00	(-3.80 - 3.60)
B (0.3 ppm/0.6 ppm)	●	0.20	(± 1.84)	0.00	(-4.80 - 3.80)
	+	0.67	(± 2.62)	0.20	(-1.80 - 11.60)
C (0.4 ppm/0.8 ppm)	●	-0.56	(± 4.85)	0.00	(-11.40 - 8.80)
	+	-0.41	(± 3.64)	0.00	(-15.40 - 3.20)
D (0.5 ppm)	●	0.73	(± 5.29)	0.00	(-5.20 - 22.20)
	+	-0.74	(± 4.86)	0.86	(-19.60 - 5.60)
E (0.7 ppm)	●	-0.97	(± 4.56)	0.00	(-8.57 - 13.29)
	+	-0.71	(± 8.56)	0.00	(-34.40 - 14.60)

Table contains mean differences (± SD) and median (range) of 'nasal irritation' (mm) in hyposensitives (●)/hypersensitives (+) at the end of exposure to the five FA concentrations A–E

The data reported above show that there were no significant increases or decreases in nasal irritation at any of the formaldehyde concentrations tested, nor was there a concentration-response relationship observed. ICF confirmed the lack of significant changes. As with eye irritation, the nasal irritation results appear to contrast with the results of Lang et al. (2008), who reported increases in nasal irritation at similar concentrations of formaldehyde, shown in Figure 8 from the Lang paper.

As stated in Lang et al., "Nasal irritation was reported to be significantly higher in subjects exposed to 0.5 ppm with peaks of 1.0 ppm with or without ethyl acetate (EA) compared to either the 0 ppm condition or the 0 ppm + EA condition. In contrast, the exposure conditions of 0.3 ppm + EA or 0.5 ppm + EA, although resulting in significantly higher ratings than at the 0 ppm condition, did not result in significantly higher ratings than at 0 ppm + EA, indicating that the subjects did not differentiate between the irritation caused by formaldehyde and the perception of the EA odour. The nasal irritation rating ranged from about 0.5 to 2." ICF confirmed the findings in Lang, with an additional finding of a significantly higher mean score for 0.15 ppm (without EA) compared to 0 ppm + EA.

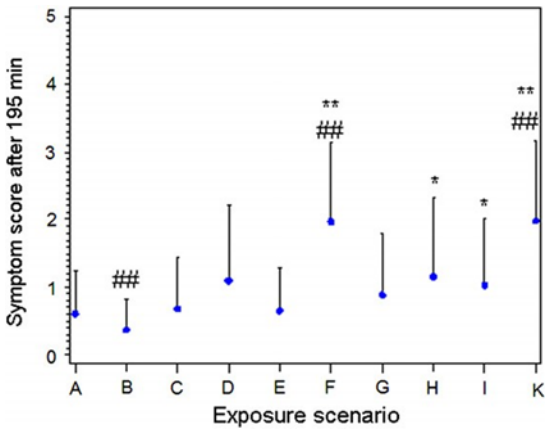

Fig. 8. Symptom score for nasal irritation recorded during exposure (t = 195 min) to different concentrations of formaldehyde with or without ethyl acetate (EA). Results are expressed in means ± SD. A = 0 ppm, B = 0.15 ppm, C = 0.3 ppm, D = 0.3 ppm + 4 peaks of 0.6 ppm, E = 0.5 ppm, F = 0.5 ppm + 4 peaks of 1.0 ppm, G = 0 ppm + EA, H = 0.3 ppm + EA, I = 0.5 ppm + EA, K = 0.5 ppm + 4 peaks of 1.0 ppm + EA. Statistics: repeated measures ANOVA with contrasts; *p < 0.05 and **p < 0.01 compared to 0 ppm; ##p < 0.01 compared to 0 ppm + EA. Source: Lang et al. (2008) p 31

3) Olfactory symptoms

In scoring the category of olfactory symptoms, the terms used in the SPES questionnaire were 'perception of impure air,' 'unpleasant smell', 'foul odor' and 'stench.' The data on olfactory symptoms from Online Resource 12 are reproduced below (Table 8).

Table 8 Online Resource 12 Results of SPES 'olfactory symptoms'

FA concentration	sensitivity	mean	(±SD)	median	(range)
A (0 ppm; control condition)	●	0.10	(± 4.05)	0.00	(-15.00 - 6.75)
	+	4.98	(± 11.11)	1.00	(-13.25 - 44.00)
B (0.3 ppm/0.6 ppm)	●	2.49	(± 5.84)	1.00	(-5.00 - 24.75)
	+	$8.43^{***\,\S}$	(± 8.28)	4.50	(-0.25 - 26.50)
C (0.4 ppm/0.8 ppm)	●	3.73^{*}	(± 7.29)	0.88	(-7.25 - 26.25)
	+	$11.60^{**\,\#\,\S\S}$	(± 14.25)	8.75	(0.00 - 58.25)
D (0.5 ppm)	●	2.04^{**}	(± 2.36)	1.38	(-0.25 - 9.00)
	+	6.23^{**}	(± 9.01)	4.00	(-11.75 - 22.25)
E (0.7 ppm)	●	2.71	(± 7.14)	0.50	(-1.00 - 31.50)
	+	7.31^{*}	(± 15.57)	6.75	(-40.25 - 38.50)

Table contains mean differences (± SD) and median (range) of 'olfactory symptoms' (mm) in hyposensitives (●)/hypersensitives (+) at the end of exposure to the five FA concentrations A–E; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ compared to pre-exposure; $^{\#}$ $p < 0.05$ compared to control condition (0 ppm); § $p < 0.05$; §§ $p < 0.01$ compared to hyposensitives

Increases in olfactory symptoms were observed in all formaldehyde exposure groups. The authors reported statistical significance in hypersensitive volunteers exposed to 0.3 ppm with 0.6 ppm peak exposures and in hyposensitive volunteers exposed to 0.4 ppm with 0.8 ppm peak exposures and 0.5 ppm compared to pre-exposure. Increases were also observed at the 0.5 ppm and 0.7 ppm groups compared to pre-exposure, but there was no concentration-response relationship across the concentration exposure groups. ICF confirmed significance for all exposure conditions above control for hypersensitive individuals and in hyposensitive individuals at the 0.4/0.8 ppm and 0.5 ppm exposures when compared to pre-exposure.

Hypersensitive participants also reported higher mean olfactory complaints than their hyposensitive counterparts. This difference between the two sensitivity groups was statistically significant in the 0.3 ppm/ 0.6 ppm peak exposure group (group B) and in the 0.4 ppm/ 0.8 ppm peak exposure group (group C). This finding was confirmed by ICF.

E. REVIEWER'S CONCLUSIONS:

As discussed in the IRIS Toxicological Review of Formaldehyde-Inhalation (USEPA, 2021), formaldehyde is known to be a sensory irritant of the eyes and respiratory tract, causing mild to severe symptoms, including itching, stinging, and watering eyes; sneezing and rhinitis; sore throat; coughing; and bronchial constriction. Symptoms of eye irritation are reported at lower concentrations than symptoms of the nose or throat. However, because of the wide variability in responses observed in some studies (including the present study), it is difficult to characterize the exposure-response relationship in the lower range of concentrations experienced by the general population and to derive a point of departure using the irritation response in the eye.

In the present study, forty-one male volunteers (categorized as hypersensitive and hyposensitive responders to irritation) were exposed for 5 days (4 h per day) in a randomized schedule to formaldehyde concentrations of 0, 0.5, and 0.7 ppm, and to 0.3 ppm with peak exposures of 0.6 ppm, and to 0.4 ppm with peak exposures of 0.8 ppm. Peak exposures were four times a day over a 15-min period for each peak exposure. It is not clear from the paper how the 15-minute peak exposures were spaced apart during the 4-hour exposure time.

Use of an exercise bike (ergometer) was also described in this paper, specifically, that "subjects had to perform four cycle ergometer units at 80 watts for 15 min at predefined times. On days with exposure peaks, two of the four ergometric units were carried out during an exposure peak." However, results of exercise on irritation responses were not described nor included in the publication.

Use of CO2 is appropriate because not only is it odorless, its effects are mediated by the trigeminal nerve which distinct from the activation of specialized chemical sensing like olfaction or taste. It acts on the same receptor, TRPV1, as formaldehyde and is thus suitable for evaluating individual sensitivity to chemical irritants like formaldehyde.

The authors of the paper concluded that "Formaldehyde concentrations of 0.7 ppm for 4 h and of 0.4 ppm for 4 h with peaks of 0.8 ppm for 15 min did not cause adverse effects related to irritation, and no differences between hypo- and hypersensitive subjects were observed." However, the results of the study appear to show that there were some significant responses to short-term inhalation exposure to formaldehyde. Specifically, 1) conjunctival redness in the hyposensitive group exposed to 0.5 ppm (0.62 mg/m^3) and 0.7 ppm (0.86 mg/m^3) formaldehyde appeared to be increased over control, although not labeled as statistically significant; 2) a significant ($p < 0.01$) increase in nasal flow in hypersensitive males exposed to 0.7 ppm (0.86 mg/m^3) formaldehyde; 3) total symptom scores (involving eyes, upper respiratory tract, and olfactory function) were significantly increased in hypersensitive males in the 0.3/0.6 ppm (0.37/0.74 mg/m^3) group and the 0.4/0.8 ppm group (0.49/0.98 mg/m^3); 4) increases in olfactory symptoms were observed in all of the formaldehyde exposure groups. Statistical significance was observed in hypersensitive volunteers at concentrations ≥ 0.3 ppm with 0.6 ppm (0.37/0.74 mg/m^3) peak exposures and in hyposensitive volunteers exposed to both 0.4 ppm with 0.8 ppm (0.49/0.98 mg/m^3) peak exposures. Increases were also observed at the 0.5 ppm and 0.7 ppm (0.62, 0.86 mg/m^3 respectively) groups compared to pre-exposure, but there was no concentration-response relationship across the concentration exposure groups.

Eye and nasal irritation responses were not reported to be increased in study participants at any concentration of formaldehyde tested in this study. These findings contrast with the results of the study of Lang et al. (2008), who reported significant eye irritation at concentrations as low as 0.3 ppm (0.37 mg/m3) compared to 0 ppm. Exposures to 0.5 ppm with peaks of 1.0 ppm with and without EA resulted in significantly higher nasal irritation compared to either 0 ppm or 0 ppm + EA. However, eye irritation scores averaged less than 2 ('somewhat') and nasal irritation rated from 0.5 to 2 for a maximum score of "somewhat."

Noted in IRIS: The measures of irritation evaluated by Lang et al. (2008) and Mueller et al. (2013) exhibited a large degree of variability, and it was difficult to define a meaningful magnitude of change in these measures that would be considered minimally adverse for the selection of a POD.

This study was not submitted for fulfillment of a guideline but was evaluated by the agency for determining whether a point of departure (POD) can be derived from acute inhalation exposure to formaldehyde using these data. As such, this study is part of a weight-of-evidence in determining a POD for acute inhalation exposure to formaldehyde in the human population. This study can be used quantitatively as part of a weight-of-evidence determination.

As this study was obtained from the peer reviewed open scientific literature, the OPP guidance document "Guidance for Considering and Using Open Literature Toxicity Studies to Support Human Health Risk Assessment (USEPA, 2012)" is also applicable when considering the use of open literature studies for risk assessment purposes. This guidance document presents criteria for

screening of studies, and criteria for whether the study is of sufficient quality to be used quantitatively. Screening criteria include the following:

1. The toxic effects are related to defined chemical exposure;
2. The toxic effects are on an appropriate test animal species;
3. The presence or absence of toxicological effects is observed;
4. A chemical concentration/dose or application rate is reported;
5. An explicit duration of exposure is included;
6. Toxicology information is reported for the chemical of interest or its structural analog;
7. The article is available in the English language;
8. The study results are presented as a full article (*i.e*., not an abstract);
9. The paper is a publicly available document;
10. The paper is the primary source of the data;
11. Treatment(s) are compared to acceptable controls;
12. The location of the study (*e.g*., laboratory vs. field) is reported;
13. Adequate data are provided on the chemical tested (*i.e*., test article characterization);
14. Adequate data are provided on the species tested;
15. The study results (findings) are adequately reported; and
16. The study findings are relevant to assessing human health risks.

From review of this study, it is concluded that the study can provide data for quantitative use as part of a weight of evidence determination in conjunction with other human exposure studies for deriving a point of departure for short-term inhalation exposures to formaldehyde. This is concluded based on the interpretation of the criteria as established in the guidance as follows:

• The point of departure from this study is lower *(i.e.,* more sensitive) than the PODs from cited inhalation animal toxicology studies in the formaldehyde final work plan that examined shorter-term inhalation exposures (EPA-HQ-OPP-2015-0739).
• The data in this study are reported in (or can be converted to) units that can be compared to other study results- results are reported in mg/cm^2, which can be compared to other studies.
• Sufficient information is provided to substantiate whether the study conclusions/endpoints/doses are accurate, reliable, and reasonable and a judgement can be made that the study findings could potentially be replicated. This study provided enough information to make a judgement on the reliability and reproducibility of the findings.