EPA Reviewer: Tim McMahon, PhD **Signature**: _____
Risk Assessment Branch, Antimicrobials Division **Date**: _____
EPA Secondary Reviewer: Deborah Burgin, PhD, DABT **Signature**: _____
Risk Assessment Branch, Antimicrobials Division **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 vapor (generated through thermal depolymerization of paraformaldehyde). Purity not stated, but concentrations continuously monitored in the exposure chambers.

SYNONYMS: HCHO

CITATION: Kulle, T.J.; Sauder, L.R.; Hebel, J.R.; Green, D.J.; Chatham, M.D. (1987): Formaldehyde Dose-Response in Healthy Non-Smokers. *JAPCA* 37: 919-924. DOI: 10.1080/08940630.1987.10466285

Kulle, T.J. (1993). Acute Odor and Irritation Response in Healthy Nonsmokers with Formaldehyde Exposure. Inhalation Toxicology 5(3): 323-332. DOI: 10.3109/08958379308998389 (statistical re-analysis of data from Kulle et al. 1987 paper)

LABORATORIES: University of Maryland, Baltimore.

EXECUTIVE SUMMARY:

This controlled human exposure study measured effects of formaldehyde vapor exposure on nasal airflow resistance, pulmonary function, eye irritation, and odor threshold. Nineteen healthy non-smoking human subjects (10 male, 9 female, average age 26 years; all non-smokers) participated in this study. Informed consent was obtained from all subjects, and subjects were financially compensated.

Each subject received 5 three-hour exposures to formaldehyde or control air with a week between exposures. The first group of 10 subjects (Group I) were exposed to 0.0, 0.5, 1.0, and 2.0 ppm formaldehyde (0.0, 0.62, 1.23, and 2.46 mg/m^3) at rest, and an additional 2.0 ppm exposure with exercise. The second group of 9 subjects (Group II) were exposed to 0.0, 1.0, 2.0,

or 3.0 ppm (0.0, 1.23, 2.46, or 3.69 mg/m^3) at rest, and an additional 2.0 ppm with exercise. Spirometric measurements (forced vital capacity, forced expiratory volume) were made prior to and during exposures at 0, 30, 60, 90, 120, 150, and 180 minutes. On the day incorporating exercise, the exercise was completed 2 minutes prior to spirometric measurements. Airway resistance and thoracic gas volume were measured prior to exposure, at the completion of the 3-hour exposure and at 24 hours post-exposure. Non-specific airway reactivity was assessed at the end of the 3-hour exposure period. Nasal resistance was measured before and immediately following exposures to 2.0 and 3.0 ppm formaldehyde. Symptoms were scored by each subject prior to exposure, immediately following exposure, and 24 hours post-exposure. Incidence and severity of odor, nose/throat irritation, eye irritation, chest discomfort/tightness, cough, headache, heart palpitations, and double vision were recorded.

At 0.5 ppm for three hours, no subjects reported eye irritation. At the 1.0 ppm formaldehyde exposure concentration, 3 of 19 subjects reported mild eye irritation and 1 reported moderate eye irritation. At the 2.0 ppm exposure concentration, 6 subjects reported mild irritation and 4 reported moderate eye irritation. Linear trends for increased odor and eye irritation ($p < 0.0001$) were observed from statistical analysis in Group II subjects exposed at rest. Nasal resistance was significantly increased at the 3.0 ppm formaldehyde concentration and was increased but not significant at 2.0 ppm. No significant decrements in pulmonary function were observed, and no increase in bronchial reactivity to methacholine (a standard substance used to assess bronchial airway reactivity) was observed at any concentration tested, at rest or after exercise. Exercise was observed to increase the incidence of nose/throat irritation but did not alter eye irritation or odor threshold response.

Because quantitative estimates for odor and irritant threshold concentrations could not be determined in the 1987 paper due to the statistical analysis used, Dr. Kulle re-analyzed the same population in 1993 using new methodologies. We include information and tables from the 1993 paper in this DER because similar data tables were not provided in the 1987 publication. A statistical analysis was also performed by Dr. Jonathan Cohen of ICF (Memorandum, J. Cohen, ICF, September 5, 2022).

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:

	Formaldehyde
Description:	Vapor generated from paraformaldehyde
Lot/Batch #:	Not provided
Purity:	not provided
CAS # of TGAI:	50-00-0

2. Vehicle and/or positive control: clean air. No other details provided.

B. STUDY DESIGN and METHODS:

The objective of the study was to determine effects of formaldehyde on human subjects from inhalation of formaldehyde vapor from acute exposures.

Exposure Facility

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±0.5ºC and relative humidity at 60±2%. Exposure room air was vented to the building exhaust system without recirculation.

Formaldehyde vapor was generated using the Balmat (1985) method and the concentration was continuously monitored by two CEA Model 555 Toxic Gas Monitors (CEA Instruments, Emerson, NJ) fitted with HCHO Reagent Modules. These colorimetric monitors employ the modified Schiff procedure, which is specific for formaldehyde. Exposure room samples were collected daily with midget impingers containing 1% sodium bisulfite and analyzed for HCHO using the NIOSH Chromotropic Acid technique: P & CAM #125.

Study Participants

Nineteen non-smoking participants were recruited for this study. As stated in the 1987 publication, “All subjects denied a history of allergy, asthma, hay fever, or upper respiratory infection in the 6 weeks prior to the study.” (p. ?)

In addition, as stated in the publication, “The subjects underwent a screening examination including medical history, physical exam, ECG, pulmonary function tests, and nonspecific airway reactivity by methacholine challenge. An exercise test was performed on the screening

day to determine the bicycle ergometer workload necessary to increase minute ventilation (VE) to 30-40 L/min."

For this study, *inclusion criteria* were not stated. *Exclusion criteria* were not stated.

IRB Approval and Informed Consent

As stated in the publication, "Approval was obtained from the Human Volunteers Research Committee at the University of Maryland. Informed consent was received from all volunteers, with financial compensation provided for study participation." (p.)

Inhalation Study Exposures

The intent of this study was to determine the effect of inhaled formaldehyde vapor on nasal mucociliary flow, nasal airflow resistance, forced expiratory vital capacity, and irritation threshold.

Nineteen healthy non-smoking human subjects (10 male, 9 female, average age 26 years; all non-smokers) participated in this study. Informed consent was obtained from all subjects, and subjects were financially compensated. All subjects underwent a screening examination, including medical history, physical exam, electrocardiogram, pulmonary function tests, and non-specific airway reactivity by methacholine challenge.

Each subject received 5 three-hour exposures to formaldehyde or control air with a week between exposures. Each subject served as their own control. The first group of 10 subjects (Group I) were exposed to 0.0, 0.5, 1.0, and 2.0 ppm formaldehyde (0.0, 0.62, 1.23, and 2.46 mg/m^3) at rest, with an additional 2.0 ppm exposure with exercise. The second group of 9 subjects (Group II) were exposed to 0.0, 1.0, 2.0 and 3.0 ppm (0.0, 1.23, 2.46 and 3.69 mg/m^3) at rest, and an additional 2.0 ppm with exercise. Spirometric measurements were made prior to and during exposures at 0, 30, 60, 90, 120, 150, and 180 minutes. On the day incorporating exercise, an 8-minute bicycle ride was performed every 30 minutes and minute ventilation was measured between the fourth and fifth minutes of each exercise period. Spirometric measurements were completed 2 minutes after each exercise period. Post-exposure measurements were obtained 24 hours after the 3.0 ppm at-rest and 2.0 ppm with exercise HCHO exposures.

Airway resistance and thoracic gas volume were measured prior to exposure, at the completion of the 3-hour exposure and at 24 hours post-exposure. Non-specific airway reactivity was assessed at the end of the 3-hour exposure period. Nasal resistance was measured before and immediately following at-rest exposures to 2.0 and 3.0 ppm formaldehyde. Symptoms were scored by each subject prior to exposure, immediately following exposure, and 24 hours post-

exposure. Incidence and severity of odor, nose/throat irritation, eye irritation, chest discomfort/tightness, cough, headache, heart palpitations, and double vision were recorded.

Statistical Methods

To assess the statistical significance of formaldehyde effects, the study authors used an analysis of variance (ANOVA) for randomized block design. Three ANOVAs were performed: for Group I, for Group II, and for the pooled groups of all 19 subjects using only those exposures common to both groups (0.0, 1.0, 2.0 ppm). When significant variations between exposures was detected by the F-test, further analyses were performed to identify the source of the variation. These included Tukey's test for pairwise comparisons of different exposures and an *F*-test for monotonic trend with increasing dose. The authors tested for a linear dose-response relationship for the nine subjects exposed to 0.0, 1.0, 2.0, and 3.0 ppm (Group 2) and all 19 subjects exposed to 0.0, 1.0, and 2.0 ppm. For Group 1, the authors tested for a log-linear dose response due to the dose spacing (0.0, 0.5, 1.0, 2.0 ppm). All analyses were conducted for each specific time point during exposure. The symptomatic responses were assigned severity levels of 0 = none, 1 = mild, 2 = moderated, and 3 = severe.

In 1993, Dr. Kulle reassessed the data with additional statistical methods to attempt to quantify threshold concentrations for the symptom responses. The new analysis compared individual subject response for each HCHO exposure. As stated in the publication, "The symptomatic response was determined as the difference between score at completion of exposure to that prior to exposure (t180-t0). Each symptom was scored across all HCHO concentrations, ranging from none to severe. Statistical significance was determined employing the McNemar chi-square test, a test for paired proportions that analyzes the number of symptomatic disagreements. Using a 2 x 2 table, the two noncongruent [discordant pairs] cells are analyzed for significance between the two HCHO concentrations. One cell contains the number of subjects reporting symptoms at the higher HCHO concentration but not at the lower HCHO concentration; the other cell contains the number of subjects reporting symptoms at the lower HCHO concentration but not at the higher HCHO concentration."

Statistical analysis of the data was also performed by Dr. Jonathan Cohen (Memorandum, J. Cohen, ICF, September 5, 2022). In this analysis, performed at the request of the agency, the available data were used to analyze the summarized symptom rates. Benchmark Dose (BMD) analyses were conducted for the odor sensation and sensory irritation rates and compared with the analyses presented in the IRIS report (EPA, 2022). Odor sensation and sensory irritation rates were examined using Fisher exact tests to analyze the differences in response rates at different dose levels. Cochran-Armitage tests were used for analysis of trends in the response rates. The analysis by Dr. Cohen noted that the tests assume statistical independence between results at different doses; this is an assumption which may not be valid due to the fact that the same subjects were tested at multiple doses, but cannot be evaluated in the absence of the raw data for

each subject. For odor sensation and eye irritation, statistically significant differences and trends were found in the response rates at the 5% level. For nose/throat irritation, no statistically significant differences were found in the response rates at the 10% level, but statistically significant trends in the response rates were observed at the 10% level. The current version of Benchmark Dose Software (BMDS Version 3.3rc10) was then used to fit and plot dose-response models, and to estimate the BMD as the dose at which there was a 10% extra risk above an assumed 0% risk for unexposed subjects. The BMDL was also estimated, defined as a one-sided 95% lower confidence limit for the BMD.

RESULTS

For all the subjects in this study, the pre-test pulmonary function and airway reactivity tests were determined to be within normal parameters.

Measured formaldehyde concentrations were reported as 0.51 ± 0.003, 1.01 ± 0.02, 2.00 ± 0.05, and 3.02 ± 0.03 (mean ± standard deviation) ppm, which correlated well with the desired inhalation concentrations for this study.

Symptom Reporting

Results of inhalation exposure to formaldehyde on reported symptoms were summarized in Table II of the 1987 publication. NB: this table only reports numerical values for Group II participants. Figure 1 (from the 1987 publication) shows the concentration-response for pooled Group I and Group II participants. Data from Group I participants were not reported in tabular format but were discussed within the paper. Summary tables for all dose levels for odor sensation (Table 2), eye irritation (Table 3), and nose/throat irritation (Table 4) are available in tabular format from Kulle (1993) and presented below.

Table II. Mean Symptom Differences ($t_{180} - t_0$) ± SE with formaldehyde exposure in Group II ($n = 9$).[a]

	HCHO concentration, ppm				Linear[b] dose significance
	0.0	1.0	2.0	3.0	
Odor sensation	0.00 ± 0.00	0.22 ± 0.15	0.44 ± 0.18	1.00 ± 0.29	$p < 0.0001$
Nose/throat irritation	0.00 ± 0.00	0.11 ± 0.11	0.33 ± 0.17	0.22 ± 0.15	$p = 0.054$
Eye irritation	0.00 ± 0.00	0.44 ± 0.24	0.89 ± 0.26	1.44 ± 0.18	$p < 0.0001$
Chest discomfort	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.11	0.00 ± 0.00	$p = 0.62$
Cough	0.00 ± 0.00	0.11 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	$p = 0.11$
Headache	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.11	$p = 0.33$

[a] Presence and severity of symptoms were scored as: 0 = none; 1 = mild (present, but not annoying); 2 = moderate (annoying); 3 = severe (debilitating).
[b] No significant nonlinear trends were detected.

Table II from page 921 of Kulle *et al* (1987)

TABLE 2. Odor Sensation Reported by Subjects for Each Formaldehyde Concentration

HCHO (ppm)	Subjects (*n*)	Percent of Subjects Reporting Symptom[a]			
		None	Mild	Moderate	Severe
0.0	19	95 (10/8)	5 (0/1)	0 (0/0)	0 (0/0)
0.5	10	60 (1/5)	40 (3/1)	0 (0/0)	0 (0/0)
1.0	19	74 (8/6)	26 (2/3)	0 (0/0)	0 (0/0)
2.0	19	42 (4/4)	42 (4/4)	16 (2/1)	0 (0/0)
3.0	9	22 (2/0)	67 (3/3)	0 (0/0)	11 (1/0)

[a]Numbers in parentheses represent numbers (M/F) of males and females reporting symptom.

Table 2 from page 327 of Kulle (1993)

TABLE 3. Eye Irritation Reported by Subjects for Each Formaldehyde Concentration

HCHO (ppm)	Subjects (*n*)	Percent of Subjects Reporting Symptom[a]		
		None	Mild	Moderate
0.0	19	95 (9/9)	5 (1/0)	0 (0/0)
0.5	10	100 (4/6)	0 (0/0)	0 (0/0)
1.0	19	74 (8/6)	21 (2/2)	5 (0/1)
2.0	19	47 (5/4)	32 (2/4)	21 (3/1)
3.0	9	0 (0/0)	56 (3/2)	44 (3/1)

[a]Numbers in parentheses represent numbers (M/F) of males and females reporting symptom.

Table 3 from page 328 of Kulle (1993)

TABLE 4. Nose/Throat Irritation Reported by Subjects for Each Formaldehyde Concentration

HCHO (ppm)	Subjects (*n*)	Percent Reporting Symptom[a]	
		None	Mild
0.0	19	84 (9/7)	16 (1/2)
0.5	10	90 (4/5)	10 (0/1)
1.0	19	95 (9/9)	5 (1/0)
2.0	19	63 (8/4)	37 (2/5)
3.0	9	78 (6/1)	22 (0/2)

[a]Numbers in parentheses represent numbers (M/F) of males and females reporting symptom.

Table 4 from page 329 of Kulle (1993)

As shown in the tables above, odor sensation (Table 2), eye irritation (Table 3), and nose/throat irritation (Table 4) were the most frequently reported symptoms from inhaled formaldehyde. Linear statistical significance was observed for odor sensation and eye irritation ($p < 0.0001$), while nose/throat irritation almost reached the 0.05 level of significance ($p = 0.054$). As shown in Figure 1 and Table 3 and as noted in the publication, "Eye irritation increased linearly from 0.5 to 3.0 ppm HCHO. At 2 ppm, 32 percent (6/19) of the subjects reported mild eye irritation and

21 percent (4/19) moderate eye irritation; at 3 ppm, all nine subjects experienced eye irritation, five at a mild and four at a moderate level." (Kulle et al, 1987).

As also noted in the 1987 publication, "The odor response paralleled that for eye irritation from 1 to 2 ppm HCHO, with a smaller rate of increase from 2 to 3 ppm. At 2 ppm HCHO, 42 percent (8/19) of the subjects reported a mild odorant response and 16 percent (3/19) a moderate response; at 3 ppm HCHO, 67 percent (6/9) of the subjects reported a mild odorant response with one subject experiencing a severe response (Table 2). For nose/throat irritation, a mild response was reported by 37 percent (7/19) of the subjects at 2 ppm and 22 percent (2/9) at 3 ppm HCHO." (Table 4).

For Group I participants exposed at rest, the 1987 paper stated that a "significant ($p < 0.05$ log-linear dose-response occurred with odor and eye irritation." Tests for non-linearity were not significant for Group I.

The 1987 paper noted that the reported threshold values for eye irritation from the open scientific literature at that time were reported in the range of 0.05-0.5 ppm HCHO. None of the [10] subjects in Group I reported eye irritation symptoms at the 0.5 ppm concentration. At the 1.0 ppm concentration, 3 of 19 subjects reported mild irritation symptoms and one reported moderate irritation. At 2.0 ppm, 6 subjects reported mild eye irritation and 4 reported moderate eye irritation. Odor was the second most predominant symptom reported and the only symptom reported to be increased at the 0.5 ppm concentration. The effect of exercise at the 2.0 ppm concentration did not have a significant effect on symptom reporting except for nose/throat irritation, which increased significantly at the 2.0 ppm concentration.

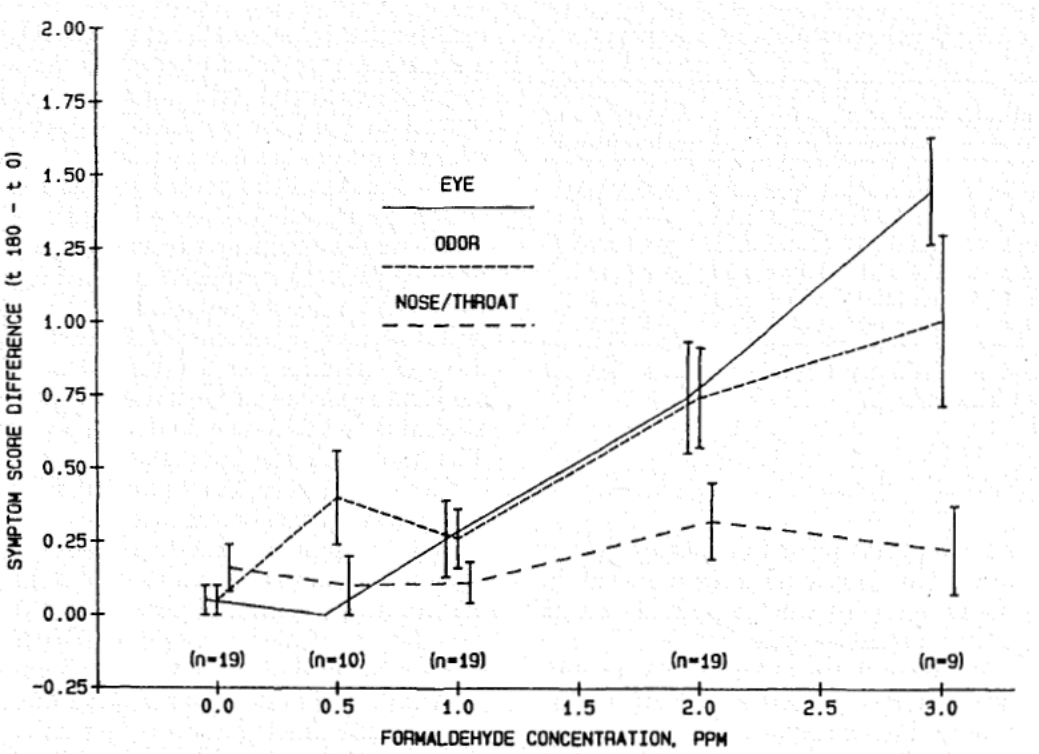

Figure 1. Mean sympton difference ±S.E. with formaldehyde exposure at rest.

Figure 1 from page 922 of Kulle (1987)

Nasal Resistance

As reported in the 1987 publication, "The mean increase in nasal resistance with at-rest exposure was not significant at 2 ppm HCHO (+10%, p =0.50), but was significant at 3.0 ppm (+27%, p <0.01)."

Pulmonary Function

As noted in the 1987 paper, "There were no significant decrements in pulmonary function (FVC, FEVi, FEF25-75%, SGaw) nor increases in bronchial reactivity to methacholine…as a result of acute 3-h exposures to 0.5-3.0 ppm HCHO at rest or with exercise (2.0 ppm HCHO), including 24-h postexposure." Numerical values were presented in Table III of the publication.

Point of Departure Estimates (Kulle, 1993)

As stated above, in 1993, Dr. Kulle evaluated the symptomatic data with additional statistical methods not previously used to estimate threshold values for odor and irritant responses to HCHO exposure. With the McNemar 2x2 test for paired proportions, the threshold for eye irritation was "estimated to be in the neighborhood of 0.5-1.0 ppm HCHO." The threshold for odor was estimated to be less than 0.5 ppm HCHO, and the threshold for nose-throat irritation was estimated to be around 1.0 ppm HCHO.

E. REVIEWER'S CONCLUSIONS:

The 1987 publication concluded that "This study demonstrates a symptomatic irritant response to inhalation of 0.5-3.0 ppm HCHO at concentrations representative of industrial, commercial, and domestic exposures." The publication also noted that "The dominant symptom reported in our study was eye irritation, with scoring increasing linearly from 0.5 to 3.0 ppm HCHO (Figure 1). The reported threshold for eye irritation is 0.05-0.5 ppm HCHO; however, as previously noted, in our study at 0.5 ppm HCHO none of our nine subjects had eye irritation, while at 1.0 ppm HCHO three of 19 reported mild eye irritation and one experienced moderate irritation."

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.