UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND

POLLUTION PREVENTION

MEMORANDUM

Date: August 5, 2021

SUBJECT: **Statistical Analysis of Data from Published Articles for the Human Studies Review Board Meeting of July 20-21, 2021**

PC Code: 000701
Decision No.: NA
Petition No.: NA
Risk Assessment Type: NA
TXR No.: 0058210
MRID No.: 51570802, 51570801

DP Barcode: NA
Registration Nos.: NA
Regulatory Action: Registration Review
Case No.: NA
CAS No.: 107-02-8
40 CFR: NA

FROM: David J. Miller, Branch Chief
Chemistry and Exposure Branch
Health Effects Division (7509P)

TO: Michelle Arling
OPP Immediate Office (7509P)

Jeremy Leonard, Ph.D., Toxicologist
Risk Assessment Branch IV
Health Effects Division (7509P)

Please find attached a copy of James Nguyen's write-up sent to you both by email (Wednesday, July 21, 2021 11:06 AM) before the start of Day #2 of the July 20-21 HSRB meeting covering human studies with acrolein. This addresses several of the clarification and other questions asked orally by the HSRB the previous day regarding the Dwivedi 2015 study.

For record-keeping purposes, you may wish to place this response in the official docket of the meeting.

Cc: Shalu Shelat (7509P)
James Nguyen (7509P)

Dwivedi 2015 study

HSRB question #1: Were the orders of exposure conditions randomized?

Short Answer: It is unclear if the orders of exposure conditions were randomized. The publication authors indicated that the exposure sequence followed a balanced design. As part of its re-analysis of the data, EPA did not attempt to verify this in the raw data we received from the authors. However, we note that the time intervals between any two consecutive exposures, i.e. the wash-out period, were 7 days or greater for almost of the study subjects and that more than half of the intervals were greater than 2 weeks. Given the long wash-out period between any two consecutive exposures and the fact that any exposure effects to the eye diminished rapidly over a very short period after the termination of exposure, we believe the carry-over effects were minimal, if they existed at all. Therefore, the effects of exposure order would similarly be minimal if they existed at all. We believe it is likely that an analysis to incorporate the order of exposure would complicate the model by adding another factor and not substantively alter the overall conclusion of the EPA re-analysis of the data whether or not the exposure sequence followed a balanced design.

Detailed Response: It is unclear if the orders of exposure conditions were randomized. In response to the HSRB question above, EPA statisticians reconstructed the sequences of exposure conditions of the subjects as below based on the dates of exposures in the raw data files recently provided to EPA by the study authors:

	Exposure Order					
Subject ID	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**
1	C	E	A	B	F	D
2	C	E	A	B	F	D
3	C	E	A	B	F	D
4	B	F	D	A	E	C
5	A	D	F	E	C	B
6	A	D	B	F	C	E
7	B	A	E	C	D	F
8	B	A	E	C	D	F
9	A[m]	D[m]	B	F	C	E
10	D	F	B	A	E	C
11	D	F	B	A	E	C
12	D	F	B	A	E	C
13	E	A	B	D	F	C
14	E	A	C	D	F	B
15	E	A	C	B	D	F
16	F	D	E	C	A	B
17	C	A	D	E	B	F
18	F	C	A	D	E	B

Summary exposures by orders:

1st exposure: 3A[m] + 3B + 4C + 3D + 3E + 2F = 18 subjects
2nd exposure: 6A + 0B + 1C + 4D[m] + 3E + 4F = 18 subjects
3rd exposure: 4A + 6B + 2C + 2D + 3E + 1F = 18 subjects
4th exposure: 4A + 4B + 3C + 3D + 2E + 2F = 18 subjects
5th exposure: 1A + 1B + 3C + 3D + 5E + 5F = 18 subjects
6th exposure: 0A + 4B + 5C + 3D + 2E + 4F = 18 subjects

Note: [m] indicates one subject was missing from the exposure condition.

As shown above, the order of exposure conditions were not the same for all subjects and the numbers of exposure conditions were not evenly distributed or the same at each exposure order number. Thus – **and contrary to the statement in the article that the exposure sequence followed a balanced design** – is appears that the exposure condition order was not balanced among the subjects. Importantly, however, we note the number of days between two consecutive exposures (i.e., wash-out period) shown below:

Number of days between two consecutive exposures (i.e., wash-out period)

Days between exposures	Frequency
5	1
6	1
7	16
8	1
9	1
12	4
14	39
16	5
19	2
>=23	18

The time interval between any two consecutive exposures, i.e. the wash-out period, was 7 days or greater for virtually all (but two) of the times and more than half of the intervals were greater than 2 weeks. Given the long wash-out period between any two consecutive exposures and the fact that any exposure effects of exposure on the eye diminished rapidly over a very short period after the termination of exposure, we believe the carry-over effects was minimal, if it existed at all. Therefore, the effects of exposure order of exposure conditions would similar be minimal if they existed at all. We believe an analysis to incorporate the order of exposure would considerably complicate the model by adding another factor and not substantively alter the overall conclusion of the EPA re-analysis of the data whether or not the exposure sequence followed a balanced design. In retrospect, we could have paid more attention to verifying with the raw data supplied the statement of the authors that a balanced design was used, but we nevertheless believe based on the long wash-out periods and short effect times of the exposure that this would have made little substantive difference to our conclusions and would have made for a considerably more complicated model that might have convergence issues.

HSRB question #2: Subjects were grouped as 3 at a time in the exposure chamber. Was this information available in the data?

Short Answer: There was no chamber information in the data provided by the authors. Our data analysis did not incorporate/evaluate the exposure chamber as a random effect in the models. Nevertheless, we believe an analysis that incorporated this information as a random effect would not change the overall substantive conclusion of EPA re-analysis.

Detailed Response: Our data analysis did not incorporate/evaluate the exposure chamber as a random effect in the models because there was no chamber information in the data provided to us by the study authors. In response to the HSRB query, however, we relooked at the data and tried to reconstruct the chamber information by combining the Date and Exposure condition as a new variable "Date_Exp". There were 47 different combinations of "Date + exposure condition", and the frequencies of these combinations were equal or less than 3[1].

Given that the actual exposure chamber information was not present in the raw data we received from the study authors, it would be possible to include this Date_Exp variable as a random effect in the model as a surrogate for exposure chamber. Theoretically, the results of this analysis would not be expected to substantially affect the point estimates of the exposure effects, but the 95% CI of the estimates may be wider (due to adding an additional random effect in the data if there was indeed a substantive exposure chamber effect) than that in the SAS analysis provided by EPA. Given the very low p-value ($p < 0.001$) of the only significant effect found in the eye irritational rating of the high acrolein exposure, we do not believe a data analysis to incorporate the "Date_Exp" variable as a random effect as a surrogate for exposure chamber would substantially change the p-value < 0.001 of the high acrolein exposure to a p-value > 0.05. Therefore, we don't believe that an analysis to incorporate the Date_Exp as a random effect in an attempt to simulate the chamber effect would change the overall conclusion of the EPA re-analysis.

[1] 10 combinations occurred 1 time, 15 combination occurred 2 times, and 22 combinations occurred 3 times