Peer Review of Data for a Modified Risk Tobacco Product (THS 2.2, PMI Study Numbers 15015, 1722, 1743): Part 3 In Vivo and In Vitro Studies

Prepared for:

Philip Morris International

Prepared by:

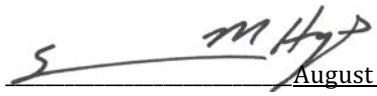


August 30, 2016

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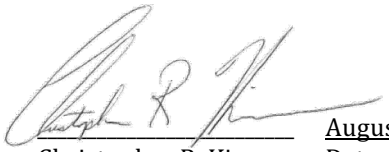
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The undersigned certify that the contents of the report provide an accurate description of the opinions of the peer review panel members.



August 30, 2016

Sean M. Hays Date
President, SciPinion



August 30, 2016

Christopher R. Kirman Date
Vice President, SciPinion

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1. Introduction

SciPinion LLC has been retained by Phillip Morris International (Sponsor) to conduct a scientific peer review of data related to a modified risk tobacco product. For this review, Scipinion was provided with several items from the Sponsor as summarized below.

(1) *Data Package* - The data package consisted of five published manuscripts:
   a. 8-month systems toxicology inhalation / cessation study in Apoe-/- mice (Phillips et al., 2016).
   b. Atherogenesis study in vitro - Adhesion assay (of monocytic cells to endothelial cells) (Poussin et al., 2016).
   c. Atherogenesis study in vitro - Transendothelial migration assay (van der Toorn et al., 2015).
   d. Systems Toxicology Assessment of Acute THS2.2 Aerosol Exposure in in vitro Human Nasal Epithelial Cultures (Iskandar et al., 2016).
   e. Systems toxicology assessment of the biological impact of a candidate Modified Risk Tobacco Product, on human organotypic oral (Zanetti et al., 2016).

   In addition, the sponsor provided web portals to access the raw data. The data package was reviewed to help understand science issues of interest and the types of expertise needed for the panel.

(2) *Expertise and Diversity Criteria* - Minimum and desired levels of experience for panel members were provided by the Sponsor. In addition, the Sponsor desired panel diversity of its members with respect to demographic factors (e.g., geographic location, years of experience, gender, sector of employment). This information was taken into consideration for the panel selection process (**Table 1**).

(3) *Draft Questions* – Draft questions for the expert panel members were provided by the Sponsor, and were reviewed and revised by SciPinion, and by an independent editor (see **Appendix A**).

The methods and results for the peer review of the data package are summarized below.

2. Methods

2.1 Process Overview

Peer reviews performed by SciPinion are conducted using the process summarized in **Figure 1**. The peer review is implemented through the use of a SciPi™ (pronounced sī-pī), a series of questions soliciting the experts' ***Sci***entific o***Pi***nions. The peer review process includes the following defined roles:

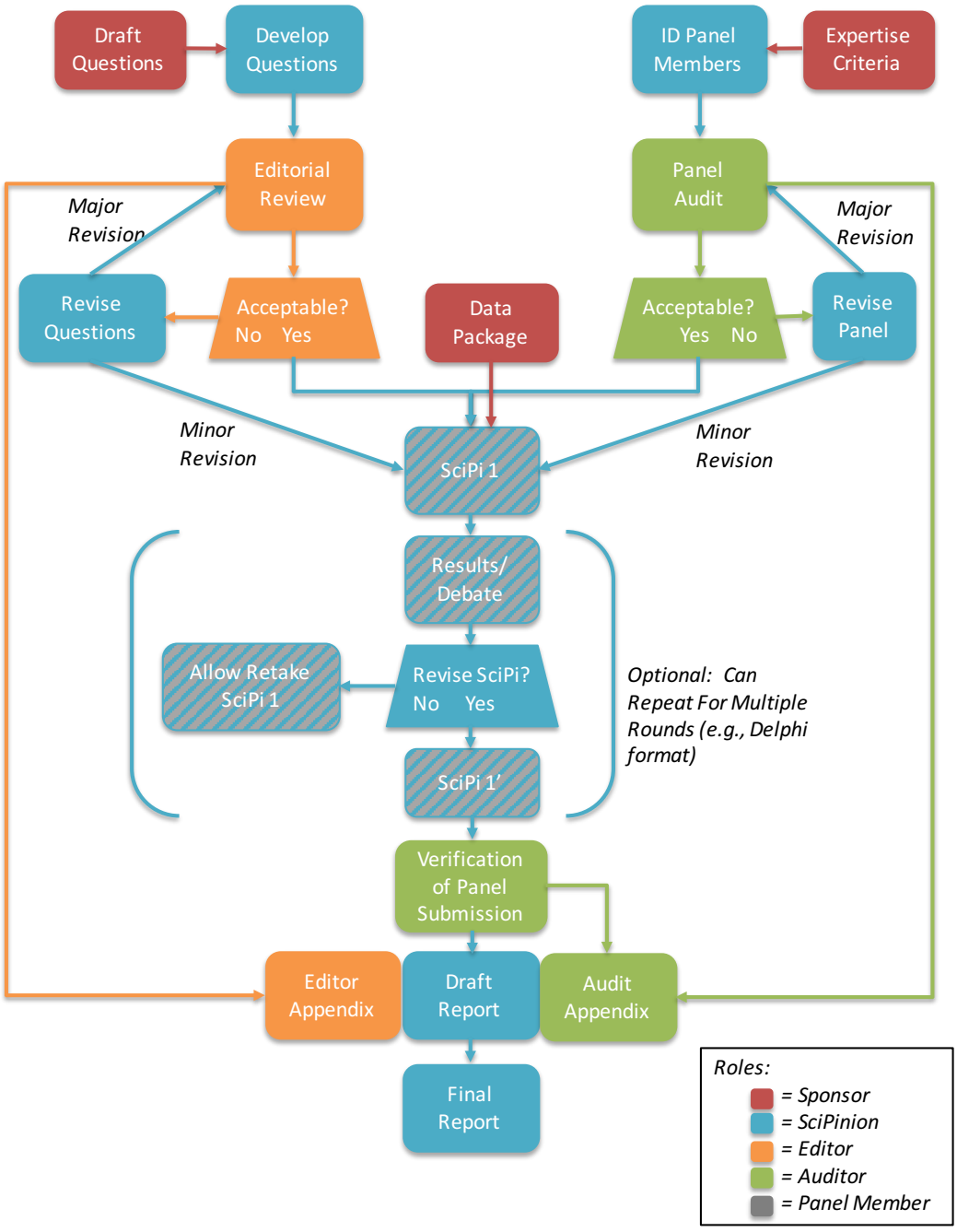
Table 1. Candidate Experts for Peer Review Panel

Expertise Criteria (souce of information):									
				Gender	**Highest Degree**	**Year of Highest Degree (or earlier)**	**Geographic Location (CV)**	**# Papers/ Reports (CV or online)**	**# First/Last Author (CV or online)**
				NA	*PhD/DVM /MD*	*2011*	NA	10	5
Candidate Values:									
Candidate ID #	**Name**	**Email**	**Title/Position**	**Gender**	***PhD/DVM /MD?***	**Year of Highest Degree**	**Geographic Location (CV)**	**# Papers/ Reports (CV or online)**	**# First/Last Author (CV or online)**
571	*Redacted*				PhD	1982	U.S.	46	21
1003					PhD	2009	U.S.	23	12
1019					PhD	1987	U.S.	66	60
1039					PhD	1987	Canada	53	43
1032					PhD	2009	Sweden	15	4
531					DVM	1966	U.S.	109	47
1291					PhD	1978	U.S.	115	55
669					PhD	2000	Singapore	93	51
601					PhD	1992	Denmark	98	44
1292					PhD	2002	Singapore	41	8
831					PhD	2007	Ireland	12	3
547					PhD	2002	Denmark	94	53
818					PhD	2010	France	16	6
1280					PhD	1991	U.S.	28	17
1297					PhD	2006	Sweden	18	8
1304					PhD	2003	Canada	32	13
1309					PhD	1998	U.S.	49	22
1312					PhD	2010	U.S.	58	39
1319					PhD	1996	U.S.	30	15
48					PhD	1986	U.S.	53	10
518					PhD	2001	U.S.	36	18
539					PhD	2006	Spain	26	6

- ***Sponsor*** – The role of the Sponsor in the peer review is depicted in red in **Figure 1**. Specifically, the Sponsor provides the following items: (1) data package (e.g., report, manuscript, data summary, raw data) to be reviewed; (2) minimum and desired expertise criteria for the Peer Reviewers; and (3) draft questions for the peer reviewers that form the SciPi™. The Sponsor may be the author of the data package, a public or private entity seeking to use the data package to support a decision, or a combination of multiple stakeholders or other entities who seek to

use the data package to support a decision. The Sponsor may choose to remain anonymous, known only to SciPinion.

Figure 1. SciPinion Review Process



- ***SciPinion*** – The role of SciPinion in the peer review is depicted in blue in **Figure 1**. As manager of the peer review process, the role of SciPinion is defined by the following tasks: (1) coordinate all other roles; (2) finalize questions included in the SciPi to be asked of the Peer Reviewers; (3) identify and assemble the panel of Peer Reviewers; (4) implement the SciPi™; (4) prepare and finalize a report that summarizes the methods and results of the peer review; and (5) as an option and at the Sponsor's discretion, SciPinion may post the results of public SciPis to their website (www.scipinion.com).
- ***Peer Reviewers*** – The role of the Peer Reviewer is depicted in grey in **Figure 1**. Peer reviewers are tasked with: (1) reviewing the data package; and (2) participating in the SciPi™ by providing their expert opinions in the answers to the questions. An important aspect of the peer review process at the heart of the SciPi™ is that Peer Reviewers work independently. The one exception to this independence occurs in SciPis with multiple rounds in which peer reviewers interact in later rounds (e.g., Delphi format). The other equally important aspect is the anonymity of the Peer Reviewers—to ensure they are free to offer their unbiased opinion, Peer Reviewers remain anonymous to the Sponsor and to any recipients of the report. Only SciPinion and the Auditor know the identities of the Peer Reviewers.
- ***Auditor*** – The role of the Auditor in the peer review is depicted in green in **Figure 1**. An Auditor is retained by SciPinion to independently verify that: (1) Peer Reviewers meet the minimum expertise requirements as defined for the SciPi™; and (2) the submitted responses can be attributed to the SciPinion user accounts belonging to the Peer Reviewers. The Auditor conducts the verification review through an independent audit process not under control of SciPinion or the Sponsor. The Auditor prepares a brief report containing the conclusions of their review. The identity of the Auditor(s) is included in this report.
- ***Editor*** – An independent Editor is retained by SciPinion to ensure that the questions in the SciPi™: (1) focus on the science issues associated with the data package; (2) are clearly written; and (3) are not leading or biased. The Editor prepares a brief report containing the conclusions of his/her review. The identity of the Editor is included in this report.

Importance of Anonymity

SciPinion advocates for the use of a double-blind format for its peer reviews, in which the identities of the Sponsor and the Peer Reviewers are withheld. The benefits of anonymity are three-fold: (1) scientists tend to be thoughtful and often introverted and by their very nature may not be willing participants to controversy and conflict; (2) scientists may be reluctant to offer opinions about controversial topics that might make them targets of public and private attacks; and, (3) increased participation by scientists to inform the most important societal decisions that involve influential and often controversial scientific information used to support regulatory decisions. By withholding the Sponsor's identity from the Peer Reviewers, any potential bias towards the Sponsor can be minimized. By providing anonymity to the Peer Reviewers, the Sponsor can expect to receive a minimally

biased opinion from the individual Peer Reviewers. If needed, experts who are willing to be identified can be recruited.

Importance of Independence

SciPinion advocates for the collection of opinions from its Expert Panel Members using a process that ensures their independence. While it is recognized there are many benefits to allowing experts to deliberate in face-to-face settings, this also introduces some potential pitfalls. Specifically, it introduces social influences that can result in conformity rather than consensus of its members (e.g., groupthink). Some examples of these types of influences include:

- domination of deliberations by an outspoken member;
- magnification of bias (e.g., deliberating panels can adopt more extreme views when influenced by like-minded members);
- reluctance of members to confront or contradict an expert perceived as having higher status;
- domination of deliberations of information commonly shared by members regardless of its importance, at the expense of important information known by few or one member; and
- undue influence of order by which opinions are expressed (e.g., views expressed first are more likely to be repeated and confirmed).

By ensuring the independence of its Expert Panel Members, SciPinion seeks to minimize these influences so that focus can be appropriately placed on the science issues associated with the data package. The goal of the peer review is to improve confidence in the validity of the input received from the Expert Panel Members to support decision-making by the Sponsor. Methods are available to permit some interaction between panel members, while minimizing these pitfalls (see description of multiple round SciPi format below).

Compensation

It is recognized that the majority of scientific journals rely upon a peer review process in which peer reviewers are not compensated. Instead, peer reviewers volunteer their time (typically a few hours) for this important service. This is the review model with which most scientists are familiar. However, as scientific manuscripts and their underlying data become increasingly large and complex, a few hours may not be sufficient to provide a robust review, and very few reviewers can be found who are qualified, available, and willing to donate a larger amount of their time to the review process. In these situations, the robustness of the peer review can suffer under a volunteer peer review model. For some peer reviews, SciPinion uses a compensated peer review model, in recognition that: (1) large and complex data sets can require a considerable amount of time to review; and (2) the peer reviewer's time is valuable, and is worthy of compensation. SciPinion adopts a compensated peer review model to add value to the peer review process, increasing its robustness both in terms of the number of scientists participating and the time

spent per peer reviewer, so that the Sponsor can have confidence that they will receive a high quality peer review to support decision-making. For this peer review, compensation of $7,000 USD/reviewer was offered for an estimate of 56 hours of the reviewers' time.

Confidentiality

Due to the sensitive nature of the data under review, all participants in the peer review were required to sign a non-disclosure agreement.

2.2 Peer Review Panel

This panel was tasked specifically to focus on reviewing the systems biology, bioinformatics, and toxicology components of the data package (additional panels were tasked with reviewing other components of the data package). For the purposes of this peer review, an expert is defined here as an individual who meets the minimum requirements for the experience criteria (**Table 1**). Ten peer review panel members were identified using the following steps: (1) Panel Recruitment; (2) Panel Expertise Verification Audit; (3) Panel Selection; (4) Panel Engagement; and (5) Panel Participation Verification. Each of these steps is summarized below.

Panel Recruitment

The goal of the panel recruitment is to cast as wide a net to reach out to as many potential candidates as is feasible. A total of 2,417 potential candidates were identified as having relevant experience in systems biology, bioinformatics, and toxicology using a variety of sources, including: (1) SciPinion's internal database; (2) searches for authors of recent publications on the topic of interest in online databases (e.g., Pubmed; Google Scholar); (3) searches of profiles on social media databases (e.g., LinkedIn); (4) general internet searches; and (5) referrals. Email addresses were obtained for as many potential candidates as possible with the exception of those who were considered conflicted by either working in the industry of the sponsor or the regulatory agency that may receive the reviewed materials in a regulatory review. An email invitation was sent to all 2,417 potential candidates, requesting interested candidates to volunteer on www.scipinion and upload a copy of their CV.

Panel Expertise Verification

Based on the recruitment effort, 28 candidates volunteered, and after an initial screening of expertise, 22 were retained. Because the panel members for this peer review were anonymous to the sponsor and to the readers of this report, candidate expertise and their CVs were reviewed by a team of two independent auditors (**Appendix C**). Each auditor scored the candidates as either 0 (does not meet minimum requirements), 1 (meets minimum requirements), or 2 (exceed minimum requirements) based on their ability to meet the defined expertise criteria. Three candidates receiving a combined score less than 2 (out of maximum combined score

of 4) were excluded from consideration. Expert panel members were selected from the remaining 22 candidates.

Panel Selection

Information relevant to panel selection criteria was extracted from CVs from the 22 candidates (**Table 1**). The source of panel candidate recruitment had no bearing on panel selection (i.e., candidates from all five sources listed above were treated equally). Panel members were selected based upon a consideration of two factors: (1) their expertise (based upon a proprietary SP-score calculated for each candidate that considers objective metrics for expertise); and (2) panel diversity (based upon use of Shannon's diversity index). To ensure that the panel was not comprised of a narrow demographic segment, diversity with respect to geographic location (weighted as equally important as expertise) and years of experience (weighted as 80% as important as expertise) were considered per request from the sponsor. Other demographic factors (e.g., gender, employment sector) were not considered. Ten experts were then selected by maximizing an expertise-diversity score. When compared to the panel with the most expertise (i.e., highest expertise score, but ignoring diversity), the selected panel yielded a slightly lower expertise score (-4%), this decrease was offset by a large increase in the diversity score (+8%) for the panel. Three members dropped out (2 due to change in work load; 1 after learning of the subject matter and sponsor). Experts who dropped out were replaced by alternates that yielded the next highest expertise-diversity score until ten panel members were obtained.

Panel Engagement

Ten panel members were placed under contract, and the email addresses corresponding to their SciPinion accounts were verified as belonging to the experts. Nine panel members participated in the peer review on schedule, and one member requested an extension to attend to a personal matter. The peer review period was started on May 3, 2016 and completed on June 28, 2016, during which panel members reviewed the data package and answered a series of questions in the form of a single round SciPi.

Panel Participation Verification

Upon completion of the SciPi by the expert panel, an independent Auditor verified that the SciPis were successfully completed by SciPinion user accounts corresponding to the selected expert panel members.

2.3 SciPi Questions

A set of draft questions to be included in the SciPi for the peer reviewers was provided by the sponsor. These questions were reviewed and edited by SciPinion, as well as by an independent editor, to ensure questions are not leading or biased. Editor comments from the independent reviewer were generally minor in nature (**Appendix D**), and revisions were made to the questions to address these

comments prior to panel engagement. Final questions for the SciPi are provided in **Appendix A**.

3. Results

Peer reviewer responses to the SciPi questions are provided in **Appendix B** and the raw data are available for download on www.scipinion.com and in the attached file survey_68_results.csv.

Evaluation of Potential Outliers

For question responses that can be readily codified to a numeric value (e.g., ratings of 1S5, confidence of high/medium/low), the results from all panel members were compared to assess whether any member(s) stands apart from the group. Based on a total of 351 responses (note S value is higher than the nominal number of questions, since each cell in the gridSformatted questions is treated as a separate response), panel members were flagged if: (1) their frequency responses in general agreement with other panel members is unusually low (i.e., less than 30% of responses between 0.5S and 1.5Sfold of the mean response for other members); (2) if the frequency of responses where the panel member is less confident than other members is unusually low (i.e., less than 5% of responses less than 0.5Sfold of the mean for other members); and (3) if the frequency of responses where the panel member is more confident than other members is unusually low (i.e., fewer than 5% of responses are greater than 1.5Sfold of the mean for other members). The results of this evaluation are provided in **Table 2**. Based on this evaluation one of the panel member (Expert 905) was flagged based on criteria 3 (only 4.3% of their responses were less confident than the other panel members). Upon inspection of the frequencies for the other response bins (59% similar, 37% more confident), this expert's responses were generally within the range of other panel members. For this reason, this small deviation was not considered sufficient to exclude this panel member from the group.

Demographics

The expert panel consisted of 10 scientists. In addition to the demographics information presented in **Appendix B**, the following information was obtained from panel member CVs:

- Gender: Male (6), Female (4)
- Countries of origin (unique): Five
- Total personSyears of experience: 206
- Years of experience: 21±15 years
- Number of publications: 50±31
- Number of first or last author publications: 25±18

On average, the expertise of the peer reviewers engaged in this review compare reasonable well with those typically involved in FDA advisory committees with

respect to years of experience (27±13 years; ERG, 2007[1]) and number of publications (170±146; ERG, 2007). The values for this panel are slightly (but not significantly) lower, which likely reflects the fact that this panel was selected specifically to be diverse with respect to years of experience (to help characterize potential correlations between this variable and the results collected).

Table 2. Outlier Evaluation

	Response Frequency		
Panel Member	**Expert is less confident than the other Experts (response<mean-0.5)**	**Expert confidence is similar to other experts (response within mean+/-0.5)**	**Expert is more confident than the other experts (response>mean+0.5)**
Expert 904	7.4%	60.1%	32.5%
Expert 905	36.8%	59.0%	***4.3%****
Expert 922	36.5%	49.0%	14.5%
Expert 908	19.1%	64.4%	16.5%
Expert 906	21.9%	58.1%	19.9%
Expert 917	7.1%	31.3%	61.5%
Expert 913	20.8%	51.3%	27.9%
Expert 912	37.6%	57.3%	5.1%
Expert 911	17.4%	74.4%	8.3%
Expert 903	28.2%	45.6%	26.2%
*Flagged as a potential outlier			
Flag	<5%	<30%	<5%
Number flags	0	0	1

Potential Conflicts of Interest

Some reviewers have previous experience with tobacco-related research. 2 reviewers have previously received funding in the past from PMI (experts 908 and 903), and 2 reviewers have received funding from another tobacco company (experts 913 and 903). However, reviewers have indicated that funds from tobacco companies represent only a small percentage of the current funding (<10%). 2 reviewers have served as an expert witness or non-testifying consultant in a tobacco-related case (experts 908 and 913). None of these experts were flagged in the evaluation of potential outliers. All ten reviewers have stated that they believe they can remain impartial with respect to this peer review.

Opinions on Science Issues

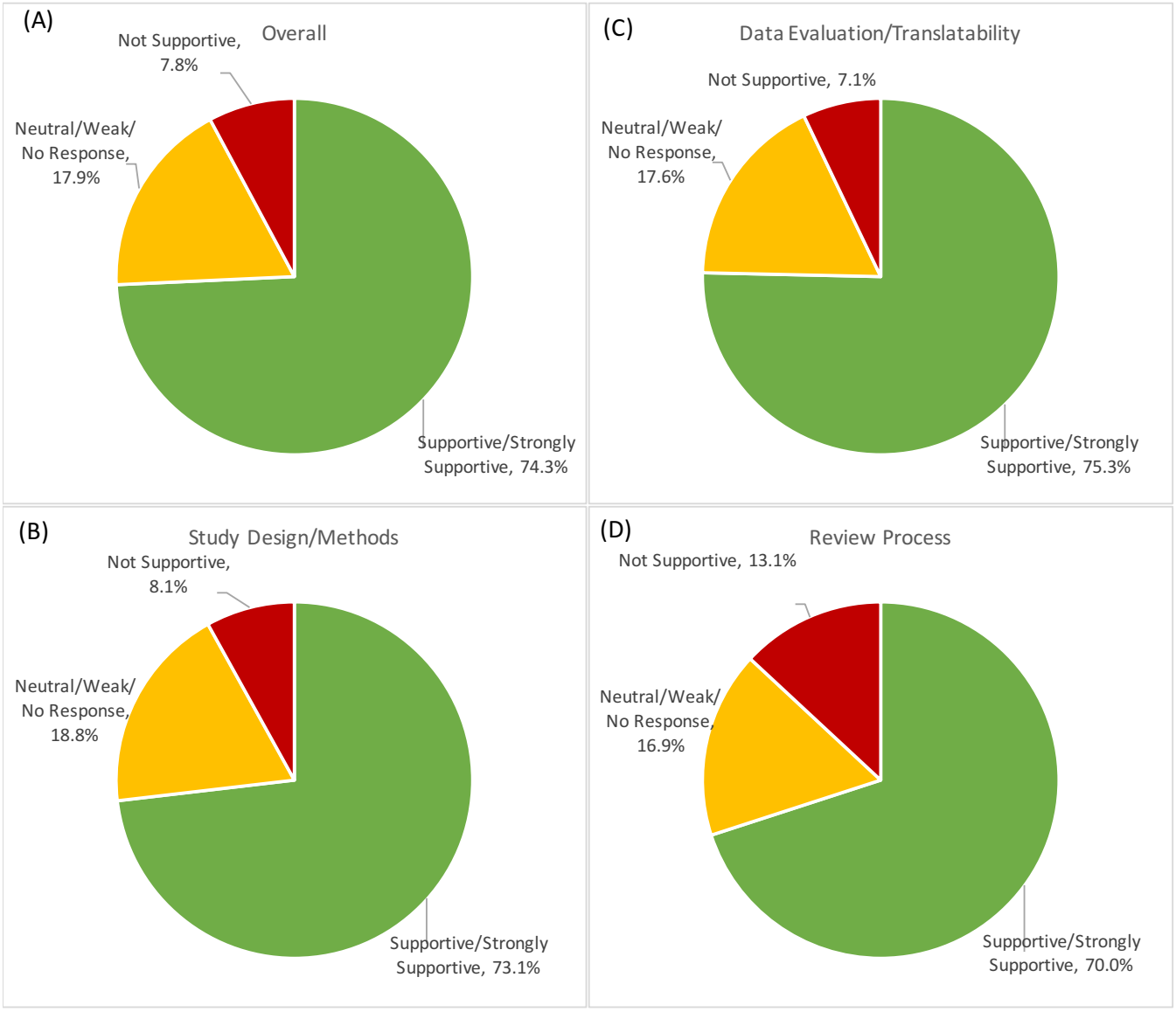
Science questions that can be readily codified as either "supportive/very supportive" (e.g., "yes", "confident" to "very confident") or critical (e.g., "no", "not

[1] ERG. 2007. Measuring Conflict Of Interest And Expertise On FDA Advisory Committees. Eastern Research Group. Task Order No. 14 Contract No. 223-03-8500.

confident") of the study authors methods and results, as well as for the peer review process, are summarized in **Figure 2**.

- *Overall* - the results were very positive, with 74.3% of responses being either supportive or very supportive, 17.9% of responses being neutral/weak, and 7.8% of responses being not supportive (**Figure 2A**).
- *Study design/methods* - 73.1% of responses being either supportive or very supportive, 18.8% of responses being neutral/weak, and 8.1% of responses being not supportive (**Figure 2B**).
- *Data evaluation/translatability* - 75.3% of responses being either supportive or very supportive, 17.6% of responses being neutral/weak, and 7.1% of responses being not supportive (**Figure 2C**).

Figure 2. Summary of Codified Results



The experts' opinions on specific science issues are provided in **Appendix B**. A few notable items are provided below:

- 9/10 experts considered the approach of matching aerosol exposures on the basis of nicotine to be reasonable.
- 70-90% of the experts considered the quality, annotation, and presentation of various endpoints from Phillips et al. to be sufficient.
- 60-90% of the experts considered the quality, annotation, and presentation of various endpoints from Poussin et al. and Van der Toorn et al. to be sufficient.
- 60-90% of the experts considered the quality, annotation, and presentation of various endpoints from Iskandar et al. and Zanetti et al. to be sufficient.
- 60-90% of the experts considered the interpretations and conclusions of the publications to be reasonable and well supported by the data.
- 9/10 experts considered that both switching and cessation lead to similar biological impacts based upon the evidence reviewed.

Opinions on Review Process

For results that can be readily codified for the review process, the results again are positive with 70.0% of responses being either supportive or very supportive, 16.9% of responses being neutral/weak, and 13.1% of responses being not supportive (**Figure 2D**). Reviewers opinions of the various tools (Aperio, sbv Improver, SDPplus, Garuda gadget, Tableau) were mixed. Reviewers were generally supportive of the inclusion of anonymity and compensation as part of the review process, but a majority (9/10) expressed interest in having anonymous interaction with the other panel members.

Appendix A: Survey Questions

SURVEY PREVIEW

Toxicologists, Systems Biologists, Cell Biologists, and Bioinformatics Scientists Wanted

Paid Peer Review Opportunity #68 $7,000 for ~56 hrs

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URL: https://scipinion.com/survey_preview/68

Appendix A: Survey Questions

INTRODUCTION

A series of studies has been conducted on the relative toxicity of a prototypic modified risk tobacco product (pMRTP) compared to a reference tobacco smoke. The data set for this peer review consists of the following items:

1. 8-month systems toxicology inhalation / cessation study in Apoe-/- mice (Phillips et al., 2016).
2. Atherogenesis study in vitro - Adhesion assay (of monocytic cells to endothelial cells) (Poussin et al., 2016).
3. Atherogenesis study in vitro - Transendothelial migration assay (van der Toorn et al., 2015).
4. Systems Toxicology Assessment of Acute THS2.2 Aerosol Exposure in in vitro Human Nasal Epithelial Cultures (Iskandar et al., 2016)
5. Systems toxicology assessment of the biological impact of a candidate Modified Risk Tobacco Product, on human organotypic oral (Zanetti et al., 2016)

Questions should enable the panelists to address key points in the study and verify relevance of the study design, methods, and interpretation of the results presented in the following publications (particularly the first one):

For some questions, you will be asked about the papers listed above with consideration of:

1. Potteaux et al. 2011 J Clin Invest 121(5):2025-36. PMID: 21505265
2. Konakahara et al. 2011 A neuronal transmembrane protein LRFN4 induces monocyte/macrophage migration via actin cytoskeleton reorganization. FEBS Lett 585(14):2377-84. PMID: 21704618

Peer reviewers are expected to accomplish the following:

- read in detail the above-mentioned references;
- access the systox webportal to review additional information;
- download some of the data from the systox webportal and review the quality of data annotation;
- provide commentary and appropriate rationale for all answers, including answers to multiple choice questions and feedback on why a reviewer cannot answer a particular question;
- review the results in light of existing publications in the same field and cite appropriate references when justifying their answers; and
- read additional publications/documents as necessary to answer some of the questions

If you have any questions regarding this peer review, please email them to: peerreview@scipinion.com

Thank you for your participation!

Appendix A: Survey Questions

SECTION 1: DEMOGRAPHICS

ID #1957

1) What is your experience (in years) in the respective fields?

	0 years	1-3 years	3-10 years	>10 years
General toxicology	○	○	○	○
Molecular toxicology	○	○	○	○
Systems toxicology	○	○	○	○
Risk assessment	○	○	○	○
Public health research	○	○	○	○
The conduct and/or interpretation of tobacco-related studies	○	○	○	○
Histopathology	○	○	○	○
Other	○	○	○	○

Please explain your answer(s)

ID #1958

2) Have you been involved in inhalation toxicology studies?

- ○ Yes, as an author
- ○ Yes, as a reviewer
- ○ No

Please explain your answer(s)

ID #1959

3) Are you familiar with 21st Century Toxicology concepts?

- ◯ Yes
- ◯ No

Please explain your answer(s)

ID #1960

4) Are you familiar with in vitro test methods for respiratory toxicology?

- ◯ Yes
- ◯ No

Please explain your answer(s)

ID #1961

5) Have you been involved in regulatory toxicological review, read-across panels, or other activity sponsored by a regulatory agency?

- ◯ Yes
- ◯ No

Please explain your answer(s)

ID #1962

6) How would you rate your confidence in leveraging or interpreting results obtained from omics data to compare the toxicological effects of different treatments or substances?

- ◯ Very high
- ◯ High
- ◯ Medium
- ◯ Low
- ◯ It depends on the type of omics data being generated

Please explain your answer(s)

SECTION 2: POTENTIAL CONFLICT OF INTEREST OR BIAS

ID #1963

1) Currently, what percentage of your funding comes from either grant or contract work from?

	<10%	10-50%	50-75%	>75%
Governments	○	○	○	○
Industry	○	○	○	○
Non-profit organizations	○	○	○	○
Tobacco companies	○	○	○	○
Other	○	○	○	○

Please explain your answer(s)

ID #2039

2) Either in the past or currently, have you received funding from (select all that apply)?

- ☐ The tobacco company Philip Morris International
- ☐ Another tobacco company
- ☐ US FDA
- ☐ US EPA
- ☐ NIEHS
- ☐ NSF
- ☐ European Commission
- ☐ Anti-tobacco non-profit organizations
- ☐ Animal rights organizations

Please explain your answer(s)

ID #1965

3) Have you ever been an employee of:

- ◯ A tobacco company
- ◯ The US FDA
- ◯ Any regulatory agency that had jurisdiction over tobacco

Please explain your answer(s)

ID #1966

4) Have you ever served as an expert witness, including deposition and testimony, or served as a consulting (non-testifying) expert in a tobacco related case?

- ◯ Yes
- ◯ No

Please explain your answer(s)

ID #1967

5) Do you know of any reason that you might be unable to provide impartial advice on this or any reason that your impartiality in the matter might be questioned?

ID #1968

6) Have you had any previous involvement with the document(s) under consideration including authorship, collaboration with the authors, or previous peer review functions? If so, please identify and describe that involvement.

- ◯ Yes
- ◯ No

Please explain your answer(s)

ID #1969

7) Have you served on previous advisory panels, committees or subcommittees that have addressed the topic under consideration? If so, please identify those activities.

- ◯ Yes
- ◯ No

Please explain your answer(s)

ID #1970

8) Do you own stock in a tobacco company?

- ◯ Yes
- ◯ No

Please explain your answer(s)

ID #1971

9) Is anyone in your immediate family an employee of:

	Yes	No
A tobacco company	○	○
FDA	○	○
An anti-smoking non-profit organization	○	○

Please explain your answer(s)

ID #1972

10) What is your smoking status?

- ○ Nonsmoker
- ○ Former smoker
- ○ Current smoker

Please explain your answer(s)

SECTION 3: STUDY DESIGN, ENDPOINTS, AND DATA QUALITY

ID #2028

1) For the evaluation of inhalation-related respiratory disease endpoints reported by Phillips et al. (2016) in an in vivo mouse model, how confident are you that the following criteria are met (1=low; 5=high)?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	1	2	3	4	5
Appropriate choice of model, including species, strain, and gender	○	○	○	○	○
Appropriate choice of controls	○	○	○	○	○
Appropriate number of replicates in respective groups	○	○	○	○	○
Appropriate choice of exposure modality	○	○	○	○	○
Appropriate duration of the exposure	○	○	○	○	○
Appropriate choice of endpoints	○	○	○	○	○
Appropriate choice of analytical methods	○	○	○	○	○

Please explain your answer(s)

ID #2029

2) For the evaluation of aerosol exposure-related effect on the nasal epithelium in an in vitro human model reported by Iskandar et al. (2016), how confident are you that the following criteria are met (1=low; 5=high)?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	1	2	3	4	5
Appropriate choice of model	○	○	○	○	○
Appropriate choice of controls	○	○	○	○	○
Appropriate number of biological and technical replicates in respective groups	○	○	○	○	○
Appropriate choice of exposure modality, including exposure equipment	○	○	○	○	○
Appropriate duration of the exposure	○	○	○	○	○
Appropriate choice of endpoints	○	○	○	○	○
Appropriate choice of analytical methods	○	○	○	○	○

Please explain your answer(s)

ID #2030

3) For the evaluation of aerosol exposure-related effect on the oral mucosa in an in vitro human model reported by Zanetti et al. (2016), how confident are you that the following criteria are met (1=low; 5=high)?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	1	2	3	4	5
Appropriate choice of model	○	○	○	○	○
Appropriate choice of controls	○	○	○	○	○
Appropriate number of biological and technical replicates in respective groups	○	○	○	○	○
Appropriate choice of exposure modality, including exposure equipment	○	○	○	○	○
Appropriate duration of the exposure	○	○	○	○	○
Appropriate choice of endpoints	○	○	○	○	○
Appropriate choice of analytical methods	○	○	○	○	○

Please explain your answer(s)

ID #2031

4) For the evaluation of inhalation-related cardiovascular disease endpoints in an in vivo mouse model reported by Phillips et al. (2016), how confident are you that these criteria are met (1=low; 5=high)?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	1	2	3	4	5
Appropriate choice of model, including species, strain, and gender	○	○	○	○	○
Appropriate choice of controls	○	○	○	○	○
Appropriate number of replicates in respective groups	○	○	○	○	○
Appropriate choice of exposure modality	○	○	○	○	○
Appropriate duration of the exposure	○	○	○	○	○
Appropriate choice of endpoints	○	○	○	○	○
Appropriate choice of analytical methods	○	○	○	○	○

Please explain your answer(s)

ID #2032

5) For the evaluation of aerosol exposure-related effect on the adhesion of monocytes on endothelial cells, an atherosclerosis-relevant mechanism, in a human in vitro model reported by Poussin et al. (2016), how confident are you that the following criteria are met (1=low; 5=high)?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	1	2	3	4	5
Appropriate choice of cell types	○	○	○	○	○
Appropriate choice of controls	○	○	○	○	○
Appropriate number of replicates in respective groups	○	○	○	○	○
Appropriate choice of exposure modality	○	○	○	○	○
Appropriate choice of endpoints	○	○	○	○	○
Appropriate choice of analytical methods	○	○	○	○	○

Please explain your answer(s)

ID #2033

6) For the evaluation of aerosol exposure-related effect on monocyte migration, a key atherosclerosis-relevant mechanism, in a human in vitro model reported by Van der Toorn et al. (2016), how confident are you that the following criteria are met (1=low; 5=high)?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	1	2	3	4	5
Appropriate choice of cell types	○	○	○	○	○
Appropriate choice of controls	○	○	○	○	○
Appropriate number of replicates in respective groups	○	○	○	○	○
Appropriate choice of exposure modality	○	○	○	○	○
Appropriate choice of endpoints	○	○	○	○	○
Appropriate choice of analytical methods	○	○	○	○	○

Please explain your answer(s)

ID #1979

7) In your opinion what is the utility domain of the Vitrocell Exposure system?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ○ It is useful for particulate, aerosol, and gaseous exposure
- ○ It is adaptable to different smoking regimes
- ○ It is suitable of different types of aerosols
- ○ Other (please specify in the comment box)
- ○ I cannot answer (please explain in the comment box)

Please explain your answer(s)

ID #1980

8) Given the scientific question and the available data generated in the studies, do you see any important gap(s) not covered by the authors? Please provide comments below.

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ◯ In the 8-month inhalation study reported in Phillips et al. 2016?
- ◯ In the in vitro assessment of adhesion reported in Poussin et al. 2016?
- ◯ In the in vitro assessment of transendothelial migration reported in Van der Toorn et al. 2016?
- ◯ In the in vitro assessment of effect on respiratory tissue reported in Iskandar et al?
- ◯ In the in vitro assessment of effect on oral tissue reported in Zanetti et al?

Please explain your answer(s)

ID #1981

9) How confident are you that the following criteria related to smoke/aerosol generation and characterization in terms of reproducibility were met in the publications? Please comment in the box below whether more details should be given in every single manuscript.

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

Answer Options: *Excellent* *Sufficient* *Poor* *I cannot answer*

	Sufficient level of details and explanation regarding smoke/aerosol generation	Sufficient level of details and explanation regarding smoke/aerosol characterization
Phillips et al.		
Poussin et al.		
van der Toorn et al.		
Iskandar et al.		
Zanetti et al.		

Please explain your answer(s)

ID #1982

10) Is the approach in the organotypic in vitro studies and in vivo study to match aerosol exposure levels on the basis of nicotine, instead of TPM concentration reasonable?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ◯ Yes (Please specify)
- ◯ No (Please specify)
- ◯ I cannot answer (please explain in the comment box)

Please explain your answer(s)

ID #1983

11) Is the approach in the in vitro studies by Poussin et al and Van der Toorn et al. to match the aerosol extract exposure levels on the basis of puffs/mL, instead of nicotine or other constituent concentrations, reasonable?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ◯ Yes (Please specify)
- ◯ No (Please specify)
- ◯ I cannot answer (please explain in the comment box)

Please explain your answer(s)

ID #1984

12) In your opinion, which tissue characteristics, if any, are missing from the 3D reconstructed buccal mucosa to support an assessment that sufficiently reflects the human response to exposure (e.g. cigarette smoke, aerosols)?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1985

13) In your opinion, which tissue characteristics, if any, are missing from the 3D reconstructed nasal epithelium to support an assessment that sufficiently reflects the human response to exposure (e.g. cigarette smoke, aerosols)?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1986

14) On a scale from 1 to 5 (5 being the highest), how relevant, informative, and intuitive are the various types of computational analyses (related to transcriptomics, lipidomics, proteomics) reported in the respective manuscripts?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

Answer Options: *1* *2* *3* *4* *5*

	Relevant	Informative	Intuitive
Transcriptomics: computational network modeling and NPA calculation			
Transcriptomics: gene set enrichment analysis			
miRNA analysis			
Proteomics: functional clustering			
Other (please specify)			

Please explain your answer(s)

ID #1987

15) Having inspected the study data in the various repositories provided, as well as in the Supplementary materials provided with the Phillips et al publication, and supplementary material provided on the systox portal, how do you rate the quality of the data, its annotation, and its presentation. Please comment if rated "insufficient" or "partially sufficient".

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	Insufficient	Partially sufficient	Sufficient
In-life observations, mortality, body weight, gross pathology	○	○	○
Biomarkers of exposure	○	○	○
Hematology and clinical chemistry (biomarkers of effect & lipoproteins)	○	○	○
Respiratory physiology	○	○	○
BAL fluid analysis (free lung cells and inflammatory mediators)	○	○	○
Histopathology and histomorphometry	○	○	○
Plaque size measurements (planimetry and microCT)	○	○	○
Transcriptomics analysis	○	○	○
Proteomics analysis	○	○	○
Lipidomics analysis	○	○	○

Please explain your answer(s)

ID #1988

16) Having inspected the study data in the various repositories provided, as well as in the Supplementary materials provided with the Poussin et al and Van der Toorn et al. publications reporting in vitro mechanistic assays, how do you rate the quality of the data, its annotation, and its presentation. Please comment if rated "insufficient" or "partially sufficient".

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	Insufficient	Partially sufficient	Sufficient
Poussin et al. Functional adhesion assay	○	○	○
Poussin et al. Inflammatory mediators	○	○	○
Poussin et al. Transcriptomics analysis	○	○	○
Van der Toorn et al. Transendothelial migration assay	○	○	○
Van der Toorn et al. Endothelial barrier integrity	○	○	○
Van der Toorn et al. Cytotoxicity and inflammatory behavior of THP-1 cells	○	○	○

Please explain your answer(s)

ID #1989

17) Having inspected the study data in the various repositories provided, as well as in the Supplementary materials provided with the Iskandar et al. and Zanetti et al. manuscripts reporting assessment of test items on organotypic tissue models, how do you rate the quality of the data, its annotation, and its presentation. Please comment if rated "insufficient" or "partially sufficient".

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	Insufficient	Partially sufficient	Sufficient
Iskandar et al. Histopathological evaluation	○	○	○
Iskandar et al. Cytotoxicity (AK assay)	○	○	○
Iskandar et al. CYP1A1/B1 activity assay	○	○	○
Iskandar et al. Characterization of pro-inflammatory mediators	○	○	○
Iskandar et al. Transcriptomics analysis	○	○	○
Iskandar et al. miRNA analysis	○	○	○
Zanetti et al. Histopathological evaluation	○	○	○
Zanetti et al. Cytotoxicity (AK assay)	○	○	○
Zanetti et al. CYP1A1/B1 activity assay	○	○	○
Zanetti et al. Characterization of pro-inflammatory mediators	○	○	○
Zanetti et al. Transcriptomics analysis	○	○	○
Zanetti et al. miRNA analysis	○	○	○
Zanetti et al. Endpoint reproducibility/variability analysis	○	○	○

Please explain your answer(s)

ID #1990

18) The standards for presenting omics data are still evolving. Regarding the publication of results from omics data in the studies evaluated, please rate the importance of each of the following criteria in determining the suitability of these data for their intended purposes and your confidence (scale of 1-5, 5 being highest confidence) that these criteria were met in the study (please download some files to judge the quality of the annotations and format)? Please also discuss in the text box any other criteria you deem useful or appropriate.

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

Answer Options: *1* *2* *3* *4* *5*

	Importance and fitness for purpose	Confidence that criteria were met
Availability of raw data		
Annotation of the metadata		
Sufficient detail in the narrative description of the methods		
Availability of the source code and/or software		
Possibility to run the code and/or software on own datasets to judge the quality of the tool		
Other (please specify)		

Please explain your answer(s)

SECTION 4: DATA EVALUATION AND INTERPRETATION

ID #1991

1) Are the results in the publication reporting the in vivo study (Phillips et al.) presented with sufficient detail, and were they clearly presented?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

Answer Options: *Insufficient* *Partially sufficient* *Sufficient*

	Sufficient detail	Clarity of presentation
In-life observations, mortality, body weight, gross pathology		
Biomarkers of exposure		
Hematology and clinical chemistry (biomarkers of effect & lipoproteins)		
Respiratory physiology		
BAL fluid analysis (free lung cells and inflammatory mediators)		
Histopathology and histomorphometry		
Plaque size measurements (planimetry and microCT)		
Transcriptomics analysis		
Proteomics analysis		
Lipidomics analysis		

Please explain your answer(s)

ID #1992

2) Are the results in the publications reporting the in vitro mechanistic assays (Poussin et al., Van der Toorn et al.) presented with sufficient detail, and were they clearly presented?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

Answer Options: *Insufficient* *Partially sufficient* *Sufficient*

	Sufficient detail	Clarity of presentation
Poussin et al. Functional adhesion assay		
Poussin et al. Inflammatory mediators		
Poussin et al. Transcriptomics analysis		
Van der Toorn et al. Transendothelial migration assay		
Van der Toorn et al. Endothelial barrier integrity		
Van der Toorn et al. Cytotoxicity and inflammatory behavior of THP-1 cells		

Please explain your answer(s)

ID #1993

3) Are the results in the publications reporting the in vitro assessment with the organotypic systems (Iskandar et al., Zanetti et al.) presented with sufficient detail, and were they clearly presented?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

Answer Options: *Insufficient* *Partially sufficient* *Sufficient*

	Sufficient detail	Clarity of presentation
Iskandar et al. Histopathological evaluation		
Iskandar et al. Cytotoxicity (AK assay)		
Iskandar et al. CYP1A1/B1 activity assay		
Iskandar et al. Ciliary beating frequency assay		
Iskandar et al. Characterization of pro-inflammatory mediators		
Iskandar et al. Transcriptomics analysis		
Iskandar et al. miRNA analysis		
Iskandar et al. Endpoint reproducibility/variability analysis		
Zanetti et al. Histopathological evaluation		
Zanetti et al. Cytotoxicity (AK assay)		
Zanetti et al. CYP1A1/B1 activity assay		
Zanetti et al. Characterization of pro-inflammatory mediators		
Zanetti et al. Transcriptomics analysis		
Zanetti et al. miRNA analysis		
Zanetti et al. Endpoint reproducibility/variability analysis		

Please explain your answer(s)

ID #1994

4) Are the interpretations and conclusions in the publications regarding the effects of exposure eto 3R4F cigarette and THS2.2 aerosol in the different models reasonably supported by the results?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	Reasonable and well supported by the data	Interpretation goes beyond what the data will support	Unsupported assertions or gaps in the interpretation
Lung / respiratory effects in Phillips et al. 2016	○	○	○
Vascular / atherosclerotic effects in Phillips et al. 2016	○	○	○
Clinical chemistry and systemic effects in Phillips et al. 2016	○	○	○
Monocyte-endothelial cell adhesion / events relevant to atherogenesis in Poussin et al. 2016	○	○	○
Monocyte chemotaxis and migration / events relevant to atherosclerotic changes in Van der Toorn et al. 2016	○	○	○
Respiratory effects in Iskandar et al. 2016	○	○	○
Oral effects in Zanetti et al. 2016	○	○	○

Please explain your answer(s)

ID #1995

5) In the transendothelial migration assay, how would you explain the unexpected decrease in monocyte transmigration upon smoke/aerosol extract exposure? Please specify evidence in the comment box below.

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ○ This response is likely a consequence of the leukemic origin of the THP-1 cells and can be expected to be opposite when primary monocytes are employed
- ○ The model design is only suitable to address reverse intravasation of monocytes/macrophages from the subendothelial space back into the vascular lumen as described by Potteaux et al., 2011 (PMID: 21505265)
- ○ Apparently a general cytotoxic or a more specific anti-locomotory effect (e.g., on the organization of the actin cytoskeleton- cf. Konakahara et al., 2011 (PMID: 21704618) of the exposure antagonized the expected increase in transendothelial migration
- ○ The exposure modality (pre-treatment of monocytic and endothelial cells or common exposure during the transmigration phase determines whether CSE exposure is stimulatory or inhibitory
- ○ Transmigration assays mimic in vivo mechanisms only under flow conditions and results from static assays are not representative for the initiation of atherosclerosis
- ○ Another mechanism not mentioned above (please explain in the comment box)
- ○ I cannot answer (please explain in the comment box)

Please explain your answer(s)

ID #1996

6) Considering the results derived from the repetition of the assessment in the nasal organotypic model across multiple phases, how do you rate the reproducibility of this model and consequently its usefulness in product assessment?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	strongly agree	agree	disagree	strongly disagree
The model is useful to get an overall idea of relative effects	○	○	○	○
The model allows quantitative analyses and assessment	○	○	○	○
The model is appropriate for mechanistic understanding, but not for product assessment	○	○	○	○
The model is not appropriate for assessment of inhaled toxicants	○	○	○	○
I cannot answer (please explain in the comment box)	○	○	○	○

Please explain your answer(s)

ID #1997

7) Causal biological networks have been developed for understanding and interpreting omics data using a systems toxicology approach (http://causalbionet.com). These networks are developed based on a priori biological knowledge about causal signaling pathways. How confident are you that the specific application of the causal network model by Phillips et al. and Iskandar et al. provides an appropriate high-level evaluation of the biological impact (direct or indirect) of an exposure on the transcriptome of tissues investigated?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ◯ Extremely confident
- ◯ Very confident
- ◯ Confident
- ◯ Somewhat confident
- ◯ Not confident
- ◯ I cannot answer (please explain in the comment box)

Please explain your answer(s)

ID #1998

8) How critical and informative do you consider the following endpoints for assessing the relative impact of various tobacco products on the in vivo model system considered?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

Answer Options: *low* *medium* *high* *cannot answer*

	Critical	Informative
In-life observations, mortality, body weight, gross pathology		
Biomarkers of exposure		
Hematology and clinical chemistry (biomarkers of effect & lipoproteins)		
Respiratory physiology		
BAL fluid analysis (free lung cells and inflammatory mediators)		
Histopathology and histomorphometry		
Plaque size measurements (planimetry and microCT)		
Transcriptomics analysis		
Proteomics analysis		
Lipidomics analysis		
The totality of the data, collectively		

Please explain your answer(s)

ID #1999

9) How critical and informative do you consider the following endpoints for assessing the relative impact of various tobacco products on early steps of atherogenesis?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

Answer Options: *low* *medium* *high* *cannot answer*

	Critical	Informative
Poussin et al. Functional adhesion assay		
Poussin et al. Inflammatory mediators		
Poussin et al. Transcriptomics analysis		
Van der Toorn et al. Transendothelial migration assay		
Van der Toorn et al. Endothelial barrier integrity		
Van der Toorn et al. Cytotoxicity and inflammatory behavior of THP-1 cells		
The totality of the data, collectively		

Please explain your answer(s)

ID #2000

10) How critical and informative do you consider the following endpoints for assessing the relative impact of various tobacco products on the respiratory tract?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

Answer Options: *low* *medium* *high* *cannot answer*

	Critical	Informative
Iskandar et al. Histopathological evaluation		
Iskandar et al. Cytotoxicity (AK assay)		
Iskandar et al. CYP1A1/B1 activity assay		
Iskandar et al. Ciliary beating frequency assay		
Iskandar et al. Characterization of pro-inflammatory mediators		
Iskandar et al. Transcriptomics analysis		
Iskandar et al. miRNA analysis		
Zanetti et al. Histopathological evaluation		
Zanetti et al. Cytotoxicity (AK assay)		
Zanetti et al. CYP1A1/B1 activity assay		
Zanetti et al. Characterization of pro-inflammatory mediators		
Zanetti et al. Transcriptomics analysis		
Zanetti et al. miRNA analysis		
Zanetti et al. Endpoint reproducibility/variability analysis		
The totality of the data, collectively		

Please explain your answer(s)

ID #2001

11) Do the study designs and models allow a mechanism-based evaluation for mechanisms such as oxidative stress and inflammation across endpoints and between studies?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ○ Yes, the systems approach applied in each study enhances the understanding of pathomechanisms across the various endpoints and between the various studies
- ○ Yes, a systems approach can potentially enhance the understanding of pathomechanisms across endpoints and between different studies, but considering the current studies, additional aspects would be required in order to make the evaluation of pathomechanisms more conclusive: (please indicate missing aspects in the text box)
- ○ No, comparisons across endpoints or different model systems are not reliable; the only reliable approach to understand pathomechanisms is to use separate, mechanism-specific investigations in each of that various dedicated model systems
- ○ I cannot answer (please explain in the comment box)

Please explain your answer(s)

ID #2002

12) The analysis of lung transcriptomics data using the causal network model (Philips et al. 2016) predicted a robust CS-induced increase in extracellular matrix (ECM) degradation that was not observed in the cessation group. For each of the statements please rate your confidence level (scale of 1-5, 5 being highest confidence).

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	1	2	3	4	5
The quantified CS exposure-related histopathological changes in the lung tissue are consistent with ECM degradation throughout CS exposure	○	○	○	○	○
The inflammatory mediators in BALF are reflective of an observed increase in ECM degradation during CS exposure	○	○	○	○	○
Some of the lung functional parameters are likely reflective of ECM/tissue degradation (please specify which in the comment box)	○	○	○	○	○
There is another explanation for the predicted increase of the network that is not related to ECM degradation per se (please specify in the comment box)	○	○	○	○	○
I cannot answer (please explain in the comment box)	○	○	○	○	○

Please explain your answer(s)

ID #2003

13) In your opinion, how useful is the assessment of CYP1A1/CYP1B1 activity levels of the in vitro nasal and oral epithelial models following cigarette smoke/aerosol exposure in deducing the potential harmful and deleterious effects for cells/tissues of cigarette smoke/aerosol exposure? Do you believe other CYP enzymes should be monitored as well?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #2004

14) To demonstrate a reduced level of potentially toxic effects of MRTPs compared with conventional cigarettes, please rate the weight of the following evidence on a 1-5 scale (5 representing strong weight). Please insert any relevant comment in the box below.

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	1	2	3	4	5
Integration of diverse data modalities within a single study for one biological system (e.g., respiratory)	○	○	○	○	○
Analysis of the effects of the products on multiple systems (respiratory, cardiovascular) in a single model (Apoe-/- mice)	○	○	○	○	○
Reproducibility of results across full experiment repetitions (see Iskandar et al.)	○	○	○	○	○
Concordance of results for specific mechanisms (e.g. oxidative stress or inflammation) across diverse studies and model systems (Apoe-/- mouse and in vitro assays)	○	○	○	○	○

Please explain your answer(s)

ID #2005

15) Can any of the inflammatory networks from the BIF analysis or secreted inflammatory mediators in BALF serve to explain the type of inflammatory cell infiltration observed during the histopathological evaluation in the cessation and switch group in the Apoe-/- THS switching study reported in Phillips et al, 2016?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #2006

16) Based on your evaluation of the data, is there any additional observation regarding THS2.2 or switch groups that is relevant for the interpretation of the results, but has not been discussed in the manuscripts?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

SECTION 5: TRANSLATABILITY AND OUTLOOK

ID #2007

1) Do the exposure conditions (dose/regimen/duration) and the biomarkers of exposure allow for extrapolation to the human situation, i.e. either substituting cigarette smoking by tobacco aerosol inhalation or quitting tobacco use altogether? Please comment on the following studies and cite any appropriate reference.

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

Answer Options: *Not appropriate* *Moderately appropriate* *Very appropriate*

	Dose	Regimen	Duration	Biomarkers of exposure
Phillips et al. 2016 - Apoe-/- THS switching study, respiratory effects				
Phillips et al. 2016 - Apoe-/- THS switching study, vascular effects				
Phillips et al. 2016 - Apoe-/- THS switching study, systemic effects				
Poussin et al. 2016 - In vitro adhesion assay: indirect condition				
Poussin et al. 2016 - In vitro adhesion assay: direct condition				
Poussin et al. 2016 - In vitro adhesion assay: fresh direct condition				
Van der Toorn et al. 2016 - In vitro transendothelial migration assay				
Iskandar et al. 2016 – Organotypic nasal tissue THS2.2 assessment				
Zanetti et al. 2016 – Organotypic oral tissue THS2.2 assessment				

Please explain your answer(s)

ID #2008

2) For future studies, which change(s) and/or additional experiment(s) would you suggest to investigate further the effects of both 3R4F and THS2.2 / other MRTP aerosol exposure on respiratory and/or oral tissues?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #2009

3) For future studies, which change(s) and/or additional experiment(s) would you suggest to investigate further the effects of both 3R4F and THS2.2 / other MRTP aerosol exposure on cardiovascular tissues?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #2010

4) For future studies, which change(s) and/or additional experiment(s) would you suggest to investigate further systemic effects of both 3R4F and THS2.2 / other MRTP aerosol exposure?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #2011

5) Considering the in vivo study and the underlying evidence you have reviewed, how does the biological impact of switching to THS2.2 compare with the biological impact from smoking cessation?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ◯ Switching leads to a greater biological impact than cessation
- ◯ Switching leads to a smaller biological impact than cessation
- ◯ Both switching and cessation lead to similar biological impacts.
- ◯ Those two groups cannot be compared (please explain the comment box)
- ◯ I cannot answer (please explain in the comment box)

Please explain your answer(s)

ID #2012

6) Can the approaches reported for the assessment of cigarette smoke or THS2.2 aerosols be applied for a risk assessment of other inhaled toxicants, e.g. environmental air pollution, workplace exposure, etc.?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #2013

7) What are the most important biological effects of cigarette smoke exposure that are relevant to human disease (COPD and CVD) and considering the 5 publications under review, how strong (1=weakest; 5=strongest) is the evidence supporting a reduction in effects from THS2.2 exposure compared with 3R4F exposure?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

Answer Options: 1 2 3 4 5

	Importance	Evidence strength for reduction
Inflammation		
Oxidative stress		
Extracellular matrix (ECM) synthesis and degradation		
Metabolic alterations		
Other (please specify)		

Please explain your answer(s)

ID #2014

8) The authors of the studies reported in the 5 publications and manuscripts under review aimed at reducing and refining the exposure of animals in vivo by assessing two human disease models in the same inhalation toxicity study and to maximize the use of various tissues from the same animals within an array of classical toxicity end points plus transcriptomics, proteomics, and lipidomics. Moreover, they have developed in vitro models to enable detailed comparative assessment and mechanistic evaluations. Do you think this approach was efficient and worthwhile, and how could it be further improved?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ◯ The approach was indeed efficient and should be further considered in future studies
- ◯ Some compromises in design (please identify these and describe in the comment box) are not weighed out by the benefits, and hence separate models would be more efficient
- ◯ Further reduction of animals could be envisioned by the following accomplishments: (please specify)
- ◯ I cannot answer (please explain in the comment box)

Please explain your answer(s)

ID #2015

9) Considering the mechanistic in vitro assays and the assessment using in vitro organotypic models, how would you characterize each measurement regarding its usefulness for comparison to human clinical exposure-response studies?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	very useful	useful	not useful	cannot answer
Poussin et al. Functional adhesion assay	○	○	○	○
Poussin et al. Inflammatory mediators	○	○	○	○
Poussin et al. Transcriptomics analysis	○	○	○	○
Van der Toorn et al. Transendothelial migration assay	○	○	○	○
Van der Toorn et al. Endothelial barrier integrity	○	○	○	○
Van der Toorn et al. Cytotoxicity and inflammatory behavior of THP-1 cells	○	○	○	○

Please explain your answer(s)

ID #2016

10) Considering the assessment using in vitro organotypic models, how would you characterize each measurement regarding its usefulness for comparison to human clinical exposure response studies?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	Very useful	Useful	Not useful	Cannot answer
Iskandar et al. Histopathological evaluation	○	○	○	○
Iskandar et al. Cytotoxicity (AK assay)	○	○	○	○
Iskandar et al. CYP1A1/B1 activity assay	○	○	○	○
Iskandar et al. Ciliary beating frequency assay	○	○	○	○
Iskandar et al. Characterization of pro-inflammatory mediators	○	○	○	○
Iskandar et al. Transcriptomics analysis	○	○	○	○
Iskandar et al. miRNA analysis	○	○	○	○
Zanetti et al. Histopathological evaluation	○	○	○	○
Zanetti et al. Cytotoxicity (AK assay)	○	○	○	○
Zanetti et al. CYP1A1/B1 activity assay	○	○	○	○
Zanetti et al. Characterization of pro-inflammatory mediators	○	○	○	○
Zanetti et al. Transcriptomics analysis	○	○	○	○
Zanetti et al. miRNA analysis	○	○	○	○
Zanetti et al. Endpoint reproducibility/variability analysis	○	○	○	○

Please explain your answer(s)

SECTION 6: TOOLS AND REVIEW PROCESS

ID #2017

1) Was the access to the digitalized slides in the Aperio platform useful for your review?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ◯ Yes
- ◯ No
- ◯ I did not visit the portal

Please explain your answer(s)

ID #2018

2) How would you qualify the annotation (e.g. use of ontologies, richness of annotation), and the structure (isa-tab format) of the data available in the underlying database (via the sbv IMPROVER systems toxicology Web Portal (1=low; 5=high)?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

Answer Options: *1* *2* *3* *4* *5*

	Sufficient Detail	Intuitive	Informative / Helpful
Data annotation			
Description of protocols (M&M)			
Use of ontologies			
Raw data format			
Processed data format			
ISA tab format			
Illustrations in M&M			

Please explain your answer(s)

ID #2040

3) In collaboration with Selventa, the SDPplus platform has been made available for this review. The SDPplus platform allows users to evaluate transcriptomics data in reverse causal analyses, including the network perturbation amplitude analyses. Algorithms built into this platform use measurable biology to infer casual mechanisms or biological impact on causal network models. Based on your experience with the SDPplus platform, please select all that apply:

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- [ ] SDPplus allows users to assess which key processes or pathways are most impacted by a given treatment
- [ ] SDPplus allows users to explore large-scale omics study datasets in a comprehensive manner
- [ ] The implementation of the network perturbation amplitude (NPA) and Biological Impact Factor (BIF) in SDPplus allows users to assess and compare transcriptomics profiles in an insightful manner
- [ ] SDPplus is intuitive and easy to use and benefit from
- [ ] A platform such as SDPplus is very useful and should be made available to the whole scientific community
- [ ] I did not understand the intended purpose of the platform and/or the presentation of the results, and therefore did not benefit from it
- [ ] I cannot comment on SDPplus (please explain in the comment box)
- [ ] None of the above

Please explain your answer(s)

ID #2041

4) In collaboration with the Systems Biology Institute in Japan, custom-built software called Garuda gadget, have been made available for this review. Follow the instructions in the details section below to be able to install and use the gadgets. Based on your experience with the gadgets, please select all that apply:

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- [ ] Garuda gadgets allows users to assess which key processes or pathways are enriched for biological functions for a given set of genes under a specific condition of study
- [ ] Garuda gadgets allows users to explore large-scale omics study datasets and perform enrichment for genes and proteins
- [ ] Garuda gadgets allows users to assess and compare the transcriptomics profiles for a specific gene or set of genes between two study conditions
- [ ] Garuda gadgets allow the visualization of pathways associated with the genes in transcriptomics profile data
- [ ] Garuda gadgets are very useful and should be made available to the whole scientific community
- [ ] I cannot comment on Garuda (please explain in the comment box)
- [ ] None of the above

Please explain your answer(s)

ID #2021

5) Tableau is a software for visualization and data analytics (http://www.tableau.com). Usually, authors of scientific publications choose specific data visualization (figure) to convey a particular message. Reviewers and readers may want to view the data in other ways to fully grasp the relevance of it. To allow for interactive browsing of study results, a Tableau workbook was prepared (see question-specific link in the Introduction). In your opinion, this workbook (select all that apply):

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ○ Is superfluous given the images that are already provided in the publication
- ○ Could be useful but is not easy to understand
- ○ Is useful and should be included as supplementary material for publication of large studies whenever possible

Please explain your answer(s)

ID #2022

6) What additional material/information would you wish to have to better assess the relevance of the studies and their results?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #2042

7) Regarding the information provided on the sbv IMPROVER systems toxicology Web Portal (select all that apply)

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- [ ] The navigation on the website was intuitive and I found the information I needed easily
- [ ] The content was not easily accessible
- [ ] Background information provided in the question pages was misleading
- [ ] Both enough and appropriate information were provided in the question pages to answer to the questions
- [ ] The tutorial videos were useful to efficiently review the material
- [ ] The tutorial videos and tools were distracting for the review purposes
- [ ] I was lacking information and had to go back to the article reprint on the publisher's website
- [ ] I was missing key functionalities (please specify below)
- [ ] I did not visit the portal

Please explain your answer(s)

ID #2024

8) How could this peer review process be improved?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #2025

9) Your participation in this peer review is completely anonymous. For future peer reviews, how important was anonymity to your participation in this peer review?

- ◯ Very important: I don't want anyone to know that I took part in this peer review
- ◯ Somewhat important: I would be comfortable having my name disclosed, but want my individual responses to the questions to remain undisclosed.
- ◯ Not important: I would be comfortable having my name and individual responses to the questions disclosed

Please explain your answer(s)

ID #2026

10) Your participation in this peer review was completely independent. For future peer reviews, would you prefer to have anonymous interaction with other panel members (e.g., online comment/debate)? Please indicate the reasons for your answer in the text box.

- ◯ Yes
- ◯ No

Please explain your answer(s)

ID #2027

11) Your participation included an offer of compensation. For future peer reviews, how important is compensation for your participation?

- ◯ Not important: I would be willing to participate for free
- ◯ Somewhat important: I prefer some level of compensation for my participation, but this could be less than received for this effort
- ◯ Important: I require compensation for my participation, and the level of compensation is appropriate
- ◯ Very Important: I require compensation for my participation, but the level of compensation would need to be increased

Please explain your answer(s)

Appendix B.

SURVEY RESULTS

Toxicologists, Systems Biologists, Cell Biologists, and Bioinformatics Scientists Wanted

Paid Peer Review Opportunity #68 $7,000 for ~56 hrs

Generated: 2016-06-30 14:52:15 +0000
URL: https://scipinion.com/surveys/68/report.pdf?debug=true

Appendix B: Survey Results

SECTION: DEMOGRAPHICS

ID #1957 (https://scipinion.com/surveys/68/results/1957)

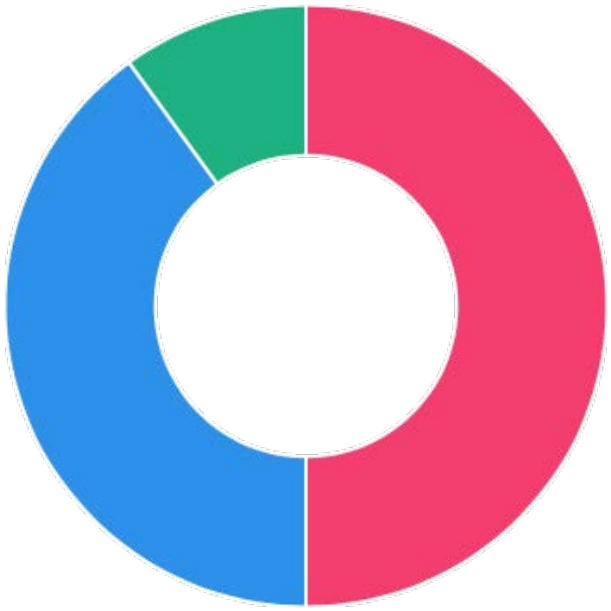
What is your experience (in years) in the respective fields?

	0 years	1-3 years	3-10 years	>10 years	Total
General toxicology	0.00% 0	10.00% 1	20.00% 2	70.00% 7	10
Molecular toxicology	0.00% 0	22.22% 2	55.56% 5	22.22% 2	9
Systems toxicology	0.00% 0	37.50% 3	37.50% 3	25.00% 2	8
Risk assessment	0.00% 0	0.00% 0	50.00% 5	50.00% 5	10
Public health research	0.00% 0	0.00% 0	40.00% 4	60.00% 6	10
The conduct and/or interpretation of tobacco-related studies	30.00% 3	40.00% 4	20.00% 2	10.00% 1	10
Histopathology	10.00% 1	20.00% 2	20.00% 2	50.00% 5	10
Other	20.00% 1	0.00% 0	0.00% 0	80.00% 4	5

Appendix B: Survey Results

ID #1958 (https://scipinion.com/surveys/68/results/1958)

Have you been involved in inhalation toxicology studies?



Legend

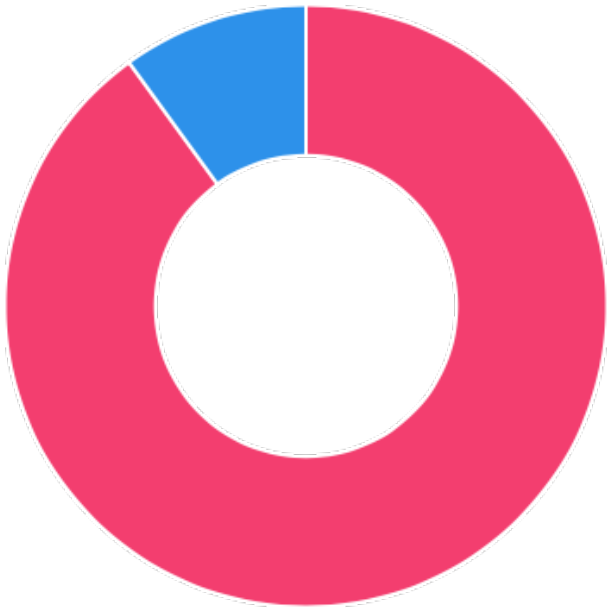
- Yes, as an author: 5 *(50%)*
- Yes, as a reviewer: 4 *(40%)*
- No: 1 *(10%)*

answers: 10
skips: 0

Appendix B: Survey Results

ID #1959 (https://scipinion.com/surveys/68/results/1959)

Are you familiar with 21st Century Toxicology concepts?



Legend

Yes: 9 *(90%)*

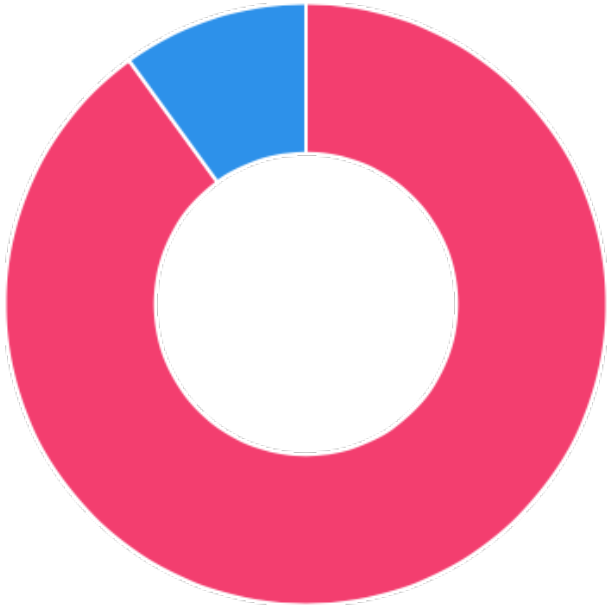
No: 1 *(10%)*

answers: 10

skips: 0

ID #1960 (https://scipinion.com/surveys/68/results/1960)

Are you familiar with in vitro test methods for respiratory toxicology?



Legend

Yes: 9 *(90%)*

No: 1 *(10%)*

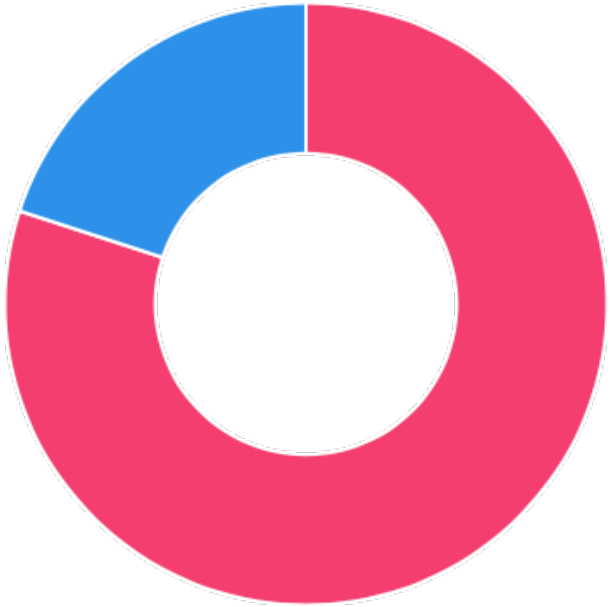
answers: 10

skips: 0

Appendix B: Survey Results

ID #1961 (https://scipinion.com/surveys/68/results/1961)

Have you been involved in regulatory toxicological review, read-across panels, or other activity sponsored by a regulatory agency?



Legend

Yes: 8 *(80%)*

No: 2 *(20%)*

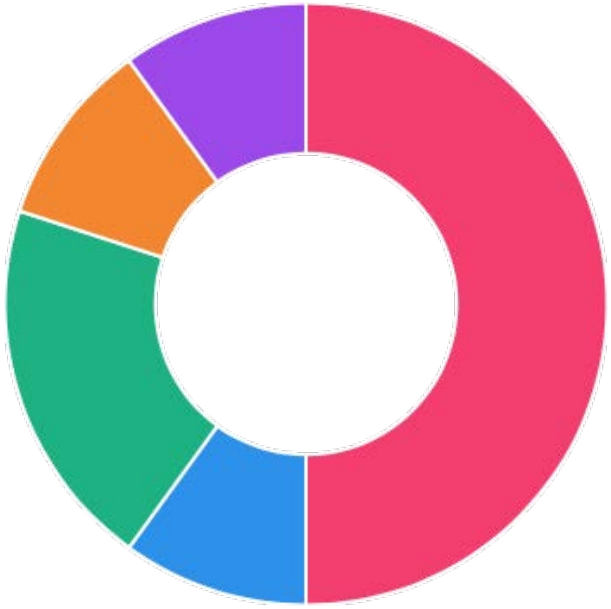
answers: 10

skips: 0

Appendix B: Survey Results

ID #1962 (https://scipinion.com/surveys/68/results/1962)

How would you rate your confidence in leveraging or interpreting results obtained from omics data to compare the toxicological effects of different treatments or substances?



Legend

- Very high: 5 *(50%)*
- High: 1 *(10%)*
- Medium: 2 *(20%)*
- Low: 1 *(10%)*
- It depends on the type of omics data being generated: 1 *(10%)*

answers: 10

skips: 0

SECTION: POTENTIAL CONFLICT OF INTEREST OR BIAS

ID #1963 (https://scipinion.com/surveys/68/results/1963)

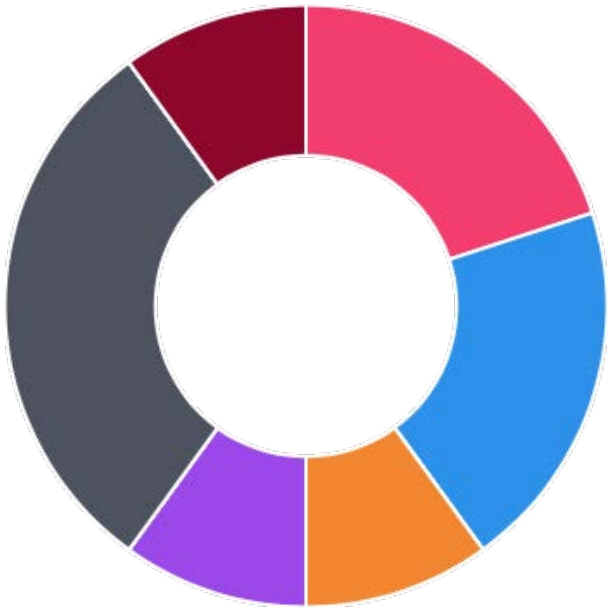
Currently, what percentage of your funding comes from either grant or contract work from?

	<10%	10-50%	50-75%	>75%	Total
Governments	22.22% 2	11.11% 1	22.22% 2	44.44% 4	9
Industry	37.50% 3	50.00% 4	0.00% 0	12.50% 1	8
Non-profit organizations	60.00% 3	40.00% 2	0.00% 0	0.00% 0	5
Tobacco companies	100.00% 7	0.00% 0	0.00% 0	0.00% 0	7
Other	80.00% 4	0.00% 0	20.00% 1	0.00% 0	5

Appendix B: Survey Results

ID #2039 (https://scipinion.com/surveys/68/results/2039)

Either in the past or currently, have you received funding from (select all that apply)?



Legend

- The tobacco company Philip Morris International: 2 *(20%)*
- Another tobacco company: 2 *(20%)*
- US FDA: 0 *(0%)*
- US EPA: 1 *(10%)*
- NIEHS: 1 *(10%)*
- NSF: 0 *(0%)*
- European Commission: 3 *(30%)*
- Anti-tobacco non-profit organizations: 0 *(0%)*
- Animal rights organizations: 1 *(10%)*

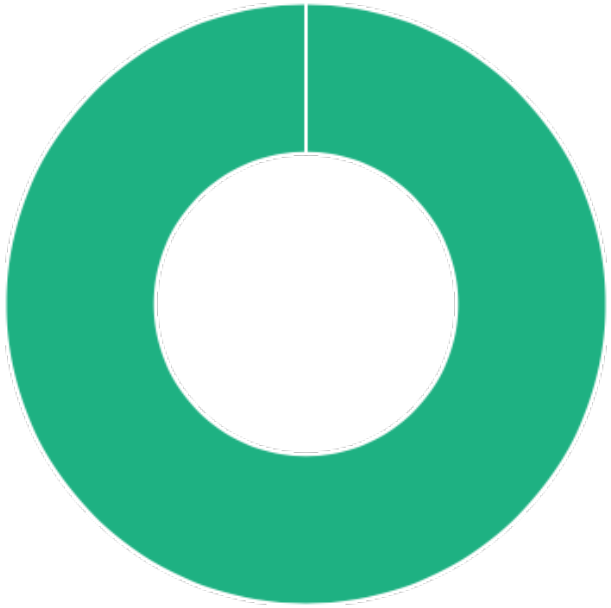
answers: 5

skips: 5

Appendix B: Survey Results

ID #1965 (https://scipinion.com/surveys/68/results/1965)

Have you ever been an employee of:



Legend

- A tobacco company: 0 *(0%)*
- The US FDA: 0 *(0%)*
- Any regulatory agency that had jurisdiction over tobacco: 1 *(100%)*

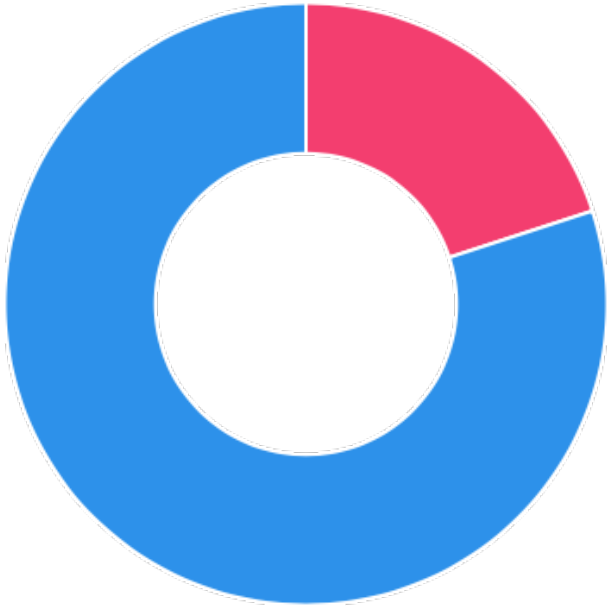
answers: 1

skips: 9

Appendix B: Survey Results

ID #1966 (https://scipinion.com/surveys/68/results/1966)

Have you ever served as an expert witness, including deposition and testimony, or served as a consulting (non-testifying) expert in a tobacco related case?



Legend

Yes: 2 *(20%)*

No: 8 *(80%)*

answers: 10

skips: 0

Appendix B: Survey Results

ID #1967 (https://scipinion.com/surveys/68/results/1967)

Do you know of any reason that you might be unable to provide impartial advice on this or any reason that your impartiality in the matter might be questioned?

None

No.

No, I don't have any conflict of interest relevant to the subject matter of this study.

No. That's what CROs and private consultants do. My former employer, a CRO, bases its role in toxicology and health research as an unbiased source of information; I do the same as a retired employee working as a private consultant.

No

No. I am an ex smoker (quit completely and permanently in 1991)

No - I look at the data as a scientist and I have no bias in this matter although it is laudable that efforts are being made to provide a much less harmful product in THS2.2. I don't like cigarette smoking but I don't extend this prejudice into judging scientific data.

I have no reasons to regard that I can be unable to provide impartial advice. I will be in a position to provide the impartial advice on the matter in question.

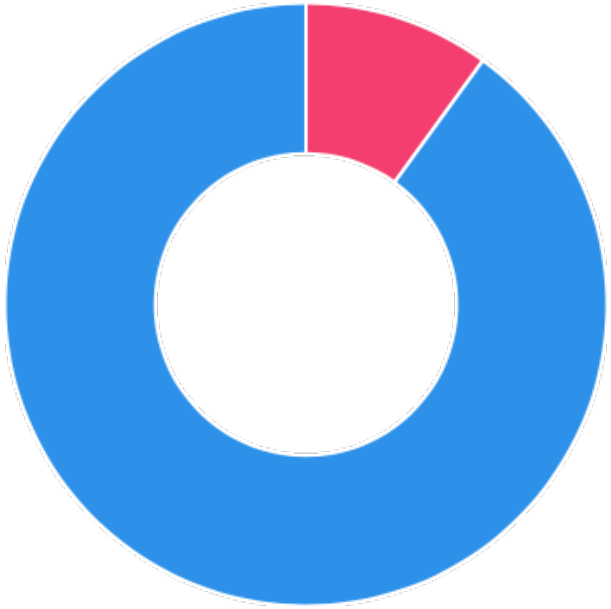
no

Not Applicable

Appendix B: Survey Results

ID #1968 (https://scipinion.com/surveys/68/results/1968)

Have you had any previous involvement with the document(s) under consideration including authorship, collaboration with the authors, or previous peer review functions? If so, please identify and describe that involvement.



Legend

Yes: 1 *(10%)*

No: 9 *(90%)*

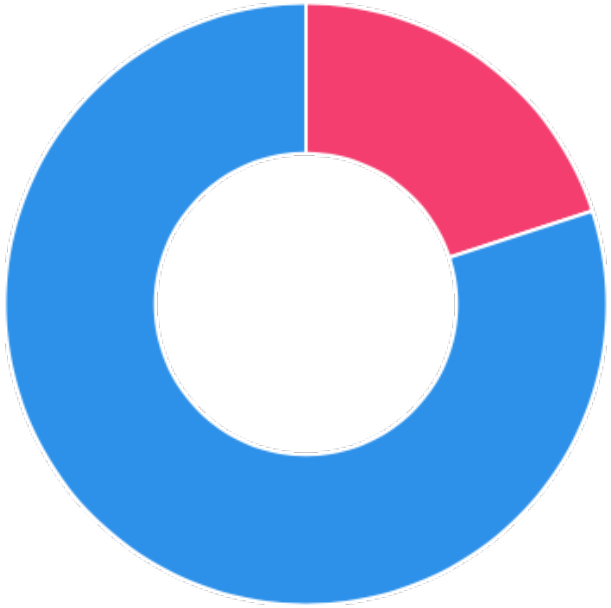
answers: 10

skips: 0

Appendix B: Survey Results

ID #1969 (https://scipinion.com/surveys/68/results/1969)

Have you served on previous advisory panels, committees or subcommittees that have addressed the topic under consideration? If so, please identify those activities.



Legend

Yes: 2 *(20%)*

No: 8 *(80%)*

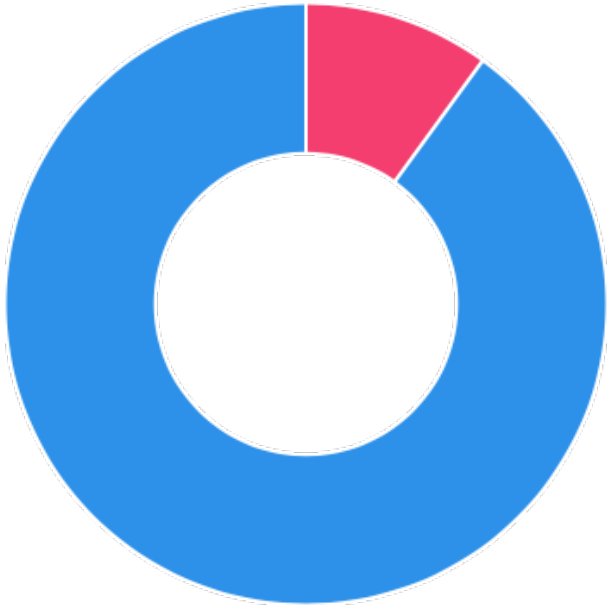
answers: 10

skips: 0

Appendix B: Survey Results

ID #1970 (https://scipinion.com/surveys/68/results/1970)

Do you own stock in a tobacco company?



Legend

Yes: 1 *(10%)*

No: 9 *(90%)*

answers: 10

skips: 0

Appendix B: Survey Results

ID #1971 (https://scipinion.com/surveys/68/results/1971)

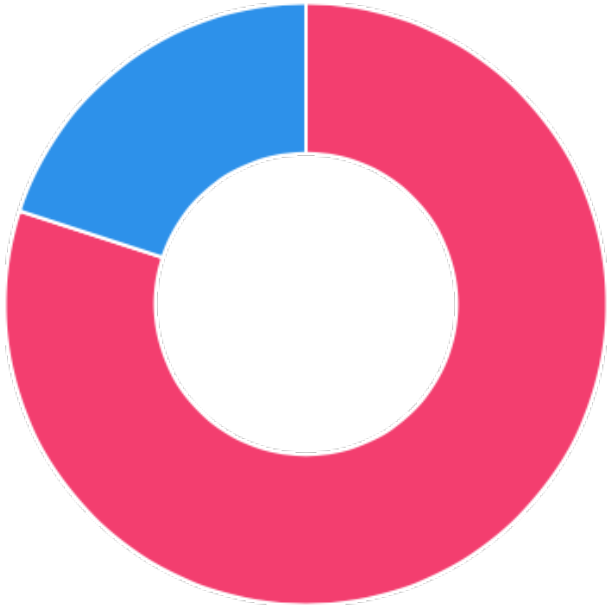
Is anyone in your immediate family an employee of:

	Yes	No	Total
A tobacco company	0.00% 0	100.00% 10	10
FDA	0.00% 0	100.00% 10	10
An anti-smoking non-profit organization	0.00% 0	100.00% 10	10

Appendix B: Survey Results

ID #1972 (https://scipinion.com/surveys/68/results/1972)

What is your smoking status?



Legend

- Nonsmoker: 8 *(80%)*
- Former smoker: 2 *(20%)*
- Current smoker: 0 *(0%)*

answers: 10
skips: 0

Appendix B: Survey Results

SECTION: STUDY DESIGN, ENDPOINTS, AND DATA QUALITY

ID #2028 (https://scipinion.com/surveys/68/results/2028)

For the evaluation of inhalation-related respiratory disease endpoints reported by Phillips et al. (2016) in an in vivo mouse model, how confident are you that the following criteria are met (1=low; 5=high)?

	1	2	3	4	5	Total
Appropriate choice of model, including species, strain, and gender	0.00% 0	10.00% 1	30.00% 3	20.00% 2	40.00% 4	10
Appropriate choice of controls	0.00% 0	0.00% 0	10.00% 1	40.00% 4	50.00% 5	10
Appropriate number of replicates in respective groups	0.00% 0	0.00% 0	0.00% 0	60.00% 6	40.00% 4	10
Appropriate choice of exposure modality	0.00% 0	10.00% 1	30.00% 3	40.00% 4	20.00% 2	10
Appropriate duration of the exposure	0.00% 0	0.00% 0	20.00% 2	70.00% 7	10.00% 1	10
Appropriate choice of endpoints	0.00% 0	0.00% 0	30.00% 3	20.00% 2	50.00% 5	10
Appropriate choice of analytical methods	0.00% 0	0.00% 0	10.00% 1	40.00% 4	50.00% 5	10

Appendix B: Survey Results

ID #2029 (https://scipinion.com/surveys/68/results/2029)

For the evaluation of aerosol exposure-related effect on the nasal epithelium in an in vitro human model reported by Iskandar et al. (2016), how confident are you that the following criteria are met (1=low; 5=high)?

	1	2	3	4	5	Total
Appropriate choice of model	0.00% 0	0.00% 0	10.00% 1	60.00% 6	30.00% 3	10
Appropriate choice of controls	0.00% 0	10.00% 1	0.00% 0	50.00% 5	40.00% 4	10
Appropriate number of biological and technical replicates in respective groups	0.00% 0	0.00% 0	30.00% 3	30.00% 3	40.00% 4	10
Appropriate choice of exposure modality, including exposure equipment	0.00% 0	0.00% 0	10.00% 1	40.00% 4	50.00% 5	10
Appropriate duration of the exposure	0.00% 0	0.00% 0	20.00% 2	50.00% 5	30.00% 3	10
Appropriate choice of endpoints	0.00% 0	0.00% 0	0.00% 0	60.00% 6	40.00% 4	10
Appropriate choice of analytical methods	0.00% 0	0.00% 0	10.00% 1	60.00% 6	30.00% 3	10

ID #2030 (https://scipinion.com/surveys/68/results/2030)

For the evaluation of aerosol exposure-related effect on the oral mucosa in an in vitro human model reported by Zanetti et al. (2016), how confident are you that the following criteria are met (1=low; 5=high)?

	1	2	3	4	5	Total
Appropriate choice of model	0.00% 0	0.00% 0	10.00% 1	50.00% 5	40.00% 4	10
Appropriate choice of controls	0.00% 0	10.00% 1	0.00% 0	70.00% 7	20.00% 2	10
Appropriate number of biological and technical replicates in respective groups	0.00% 0	10.00% 1	10.00% 1	60.00% 6	20.00% 2	10
Appropriate choice of exposure modality, including exposure equipment	0.00% 0	0.00% 0	0.00% 0	60.00% 6	40.00% 4	10
Appropriate duration of the exposure	0.00% 0	0.00% 0	30.00% 3	40.00% 4	30.00% 3	10
Appropriate choice of endpoints	0.00% 0	0.00% 0	20.00% 2	40.00% 4	40.00% 4	10
Appropriate choice of analytical methods	0.00% 0	0.00% 0	10.00% 1	40.00% 4	50.00% 5	10

Appendix B: Survey Results

ID #2031 (https://scipinion.com/surveys/68/results/2031)

For the evaluation of inhalation-related cardiovascular disease endpoints in an in vivo mouse model reported by Phillips et al. (2016), how confident are you that these criteria are met (1=low; 5=high)?

	1	2	3	4	5	Total
Appropriate choice of model, including species, strain, and gender	0.00% 0	10.00% 1	30.00% 3	20.00% 2	40.00% 4	10
Appropriate choice of controls	0.00% 0	0.00% 0	10.00% 1	30.00% 3	60.00% 6	10
Appropriate number of replicates in respective groups	0.00% 0	0.00% 0	0.00% 0	50.00% 5	50.00% 5	10
Appropriate choice of exposure modality	0.00% 0	20.00% 2	20.00% 2	30.00% 3	30.00% 3	10
Appropriate duration of the exposure	0.00% 0	0.00% 0	20.00% 2	60.00% 6	20.00% 2	10
Appropriate choice of endpoints	0.00% 0	10.00% 1	0.00% 0	50.00% 5	40.00% 4	10
Appropriate choice of analytical methods	0.00% 0	0.00% 0	20.00% 2	30.00% 3	50.00% 5	10

Appendix B: Survey Results

ID #2032 (https://scipinion.com/surveys/68/results/2032)

For the evaluation of aerosol exposure-related effect on the adhesion of monocytes on endothelial cells, an atherosclerosis-relevant mechanism, in a human in vitro model reported by Poussin et al. (2016), how confident are you that the following criteria are met (1=low; 5=high)?

	1	2	3	4	5	Total
Appropriate choice of cell types	0.00% 0	0.00% 0	10.00% 1	50.00% 5	40.00% 4	10
Appropriate choice of controls	0.00% 0	0.00% 0	10.00% 1	60.00% 6	30.00% 3	10
Appropriate number of replicates in respective groups	0.00% 0	10.00% 1	20.00% 2	40.00% 4	30.00% 3	10
Appropriate choice of exposure modality	10.00% 1	10.00% 1	20.00% 2	40.00% 4	20.00% 2	10
Appropriate choice of endpoints	0.00% 0	10.00% 1	0.00% 0	40.00% 4	50.00% 5	10
Appropriate choice of analytical methods	0.00% 0	20.00% 2	20.00% 2	20.00% 2	40.00% 4	10

Appendix B: Survey Results

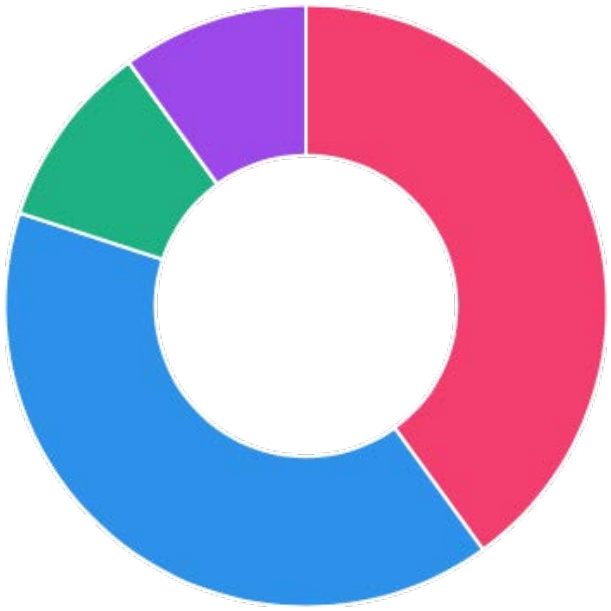
ID #2033 (https://scipinion.com/surveys/68/results/2033)

For the evaluation of aerosol exposure-related effect on monocyte migration, a key atherosclerosis-relevant mechanism, in a human in vitro model reported by Van der Toorn et al. (2016), how confident are you that the following criteria are met (1=low; 5=high)?

	1	2	3	4	5	Total
Appropriate choice of cell types	0.00% 0	0.00% 0	0.00% 0	60.00% 6	40.00% 4	10
Appropriate choice of controls	0.00% 0	0.00% 0	30.00% 3	30.00% 3	40.00% 4	10
Appropriate number of replicates in respective groups	10.00% 1	0.00% 0	10.00% 1	50.00% 5	30.00% 3	10
Appropriate choice of exposure modality	0.00% 0	11.11% 1	33.33% 3	44.44% 4	11.11% 1	9
Appropriate choice of endpoints	0.00% 0	0.00% 0	20.00% 2	40.00% 4	40.00% 4	10
Appropriate choice of analytical methods	0.00% 0	0.00% 0	20.00% 2	30.00% 3	50.00% 5	10

ID #1979 (https://scipinion.com/surveys/68/results/1979)

In your opinion what is the utility domain of the Vitrocell Exposure system?



Legend

- It is useful for particulate, aerosol, and gaseous exposure: 4 *(40%)*
- It is adaptable to different smoking regimes: 4 *(40%)*
- It is suitable of different types of aerosols: 1 *(10%)*
- Other (please specify in the comment box): 0 *(0%)*
- I cannot answer (please explain in the comment box): 1 *(10%)*

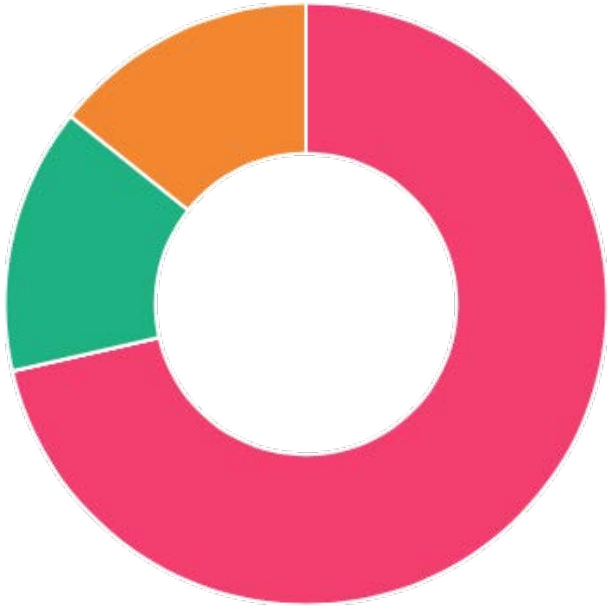
answers: 10

skips: 0

Appendix B: Survey Results

ID #1980 (https://scipinion.com/surveys/68/results/1980)

Given the scientific question and the available data generated in the studies, do you see any important gap(s) not covered by the authors? Please provide comments below.



Legend

- In the 8-month inhalation study reported in Phillips et al. 2016?: 5 *(71%)*
- In the in vitro assessment of adhesion reported in Poussin et al. 2016?: 0 *(0%)*
- In the in vitro assessment of transendothelial migration reported in Van der Toorn et al. 2016?: 1 *(14%)*
- In the in vitro assessment of effect on respiratory tissue reported in Iskandar et al?: 1 *(14%)*
- In the in vitro assessment of effect on oral tissue reported in Zanetti et al?: 0 *(0%)*

answers: 7

skips: 3

Appendix B: Survey Results

ID #1981 (https://scipinion.com/surveys/68/results/1981)

How confident are you that the following criteria related to smoke/aerosol generation and characterization in terms of reproducibility were met in the publications? Please comment in the box below whether more details should be given in every single manuscript.

	Sufficient level of details and explanation regarding smoke/aerosol generation	Sufficient level of details and explanation regarding smoke/aerosol characterization	Total
Phillips et al.	*Excellent* **50.00%** 5 *Poor* **10.00%** 1 *Sufficient* **40.00%** 4	*Excellent* **55.56%** 5 *Poor* **11.11%** 1 *Sufficient* **33.33%** 3	10
Poussin et al.	*Poor* **20.00%** 2 *Sufficient* **80.00%** 8	*Poor* **22.22%** 2 *Sufficient* **77.78%** 7	10
van der Toorn et al.	*Poor* **10.00%** 1 *Sufficient* **90.00%** 9	*Excellent* **11.11%** 1 *Poor* **22.22%** 2 *Sufficient* **66.67%** 6	10
Iskandar et al.	*Excellent* **20.00%** 2 *Sufficient*	*Excellent* **11.11%** 1 *Poor* **11.11%**	10

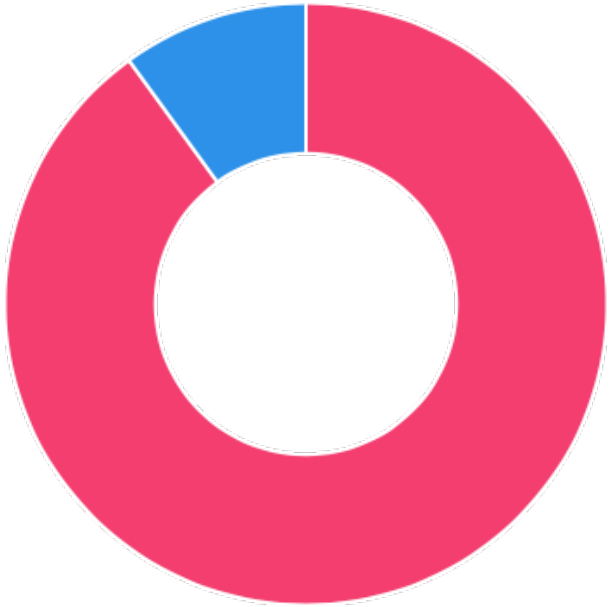
Appendix B: Survey Results

	80.00% 8	1 *Sufficient* **77.78%** 7	
Zanetti et al.	*Excellent* **20.00%** 2 *Poor* **20.00%** 2 *Sufficient* **60.00%** 6	*Excellent* **11.11%** 1 *Poor* **11.11%** 1 *Sufficient* **77.78%** 7	10

Appendix B: Survey Results

ID #1982 (https://scipinion.com/surveys/68/results/1982)

Is the approach in the organotypic in vitro studies and in vivo study to match aerosol exposure levels on the basis of nicotine, instead of TPM concentration reasonable?



Legend

- Yes (Please specify): 9 *(90%)*
- No (Please specify): 1 *(10%)*
- I cannot answer (please explain in the comment box): 0 *(0%)*

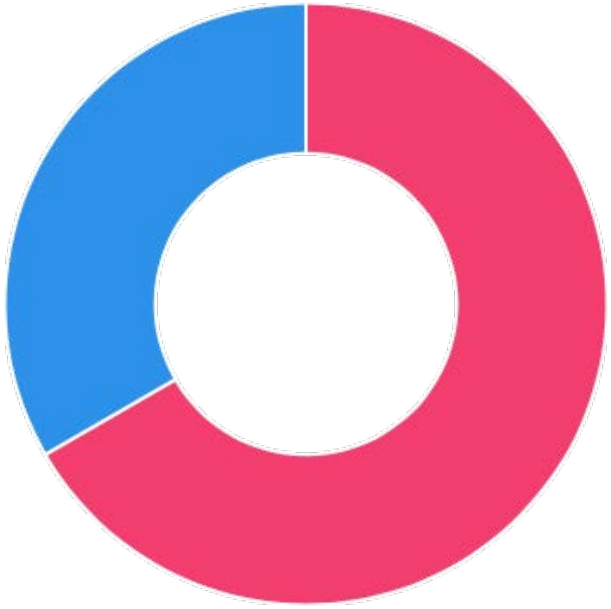
answers: 10

skips: 0

Appendix B: Survey Results

ID #1983 (https://scipinion.com/surveys/68/results/1983)

Is the approach in the in vitro studies by Poussin et al and Van der Toorn et al. to match the aerosol extract exposure levels on the basis of puffs/mL, instead of nicotine or other constituent concentrations, reasonable?



Legend

- Yes (Please specify): 6 *(67%)*
- No (Please specify): 3 *(33%)*
- I cannot answer (please explain in the comment box): 0 *(0%)*

answers: 9

skips: 1

ID #1984 (https://scipinion.com/surveys/68/results/1984)

In your opinion, which tissue characteristics, if any, are missing from the 3D reconstructed buccal mucosa to support an assessment that sufficiently reflects the human response to exposure (e.g. cigarette smoke, aerosols)?

Although, the overall approach is reasonable, absence of natural microenviroment such as adjacent connective tissue, inflammatory cells, immunosurveillance often makes it less ideal model to assess the overall adverse effects of tobacco products.

1. Recreating the influence of saliva in altering exposure conditions that were attempted with the vitrocell method. That is, how do we know the vitrocell system creates the exposure conditions that occur in vivo when the dilution/swallowing influence of saliva is not accounted for? Does this affect interpreting the comparison of the 3F4R and THS2.2 results? 2. Immune cell recruitment to the area could be of interest if this is believed to be operating in contributing to tissue injury and the response to CS. Can this be recreated by adding immune cells to the sub-basal cell media compartment? 3. Do we know if the in-vitro mucosal layer retains the same level of metabolic competency of the mucosa in an intact human? Maybe the in-vitro mucosa loses some of its metabolic capacity. This could conceivably lessen the response, or reduce the response to either CS and/or THS2.2 This is an uncertainty that could be addressed either in the discussion or by comparing freshly harvested, explanted mucosa to the mucosa in the vitrocel system with respect to the standard CYPS, ALDH(s) and conjugating enzymes that are likely present in the mucosa.

I think their organotypic model is functional but would be complete with having coat of fibroblasts overlaid with buccal epithelial striatum to fully represent buccal mucosa.

In this study authors have shown that air liquid interface (ALI) - 3D model of bucal mucosa contain structure and functional polarization typical of native epithelium, with an organized stratified epithelium, technically which is sufficient and comparable with in vivo tissue. However it would have been ideal to mention the thickness of the stratified epithelium in different time points in ALI condition. Additionally the morphological/structural changes of basal cells with alteration of nucleus numbers are also important for ALI-3D model of buccal mucosa.

Probably deeper tissue layer lamina propria is missing from the 3D reconstructed buccal mucosa. Although the authors have discussed this point in discussion section of the paper, it is still a missing characteristic in the proposed 3D model.

1) Amount/length of exposure 2) Depth of exposure may not mimic the human system 3) It is not clear whether the temperature of the CS in the system is identical to that seen in natural exposure to CS. 4) Cell-cell and cross-tissue interactions may not be captured in such systems.

Model seems appropriate, but is of course just a model.

One criticism of the manuscripts by Iskander et al and Zanetti et al is the use of a single individual tissue donor. The authors point out that this reduces the impact of one source of variability. However, future studies should include multiple tissue donors from variable age groups, sexes, and other potential sources of variation in response to exposure. A very useful interim step in development of this tool would be use of organotypic tissues from rodents and/or nonhuman primates to develop the method in species most frequently used as models. The biggest gap in this and any other in vitro toxicology study is the lack of any systemic response system available in vivo. Another obvious criticism is the lack

of a fibroblast layer beneath the epithelium, as is the case in most living mucosal surfaces, and is doable as described in the paper by Schlage et al.

Detail of cell integrity and tight junctions; cell communication and diffusionary properties. Detail on specialized cells for this application.

I think this in vitro assay is very convincing, although it may be speculated that some aspects of absorption, distribution, metabolism and excretion may not be completely resembled in the in vitro assay

Appendix B: Survey Results

ID #1985 (https://scipinion.com/surveys/68/results/1985)

In your opinion, which tissue characteristics, if any, are missing from the 3D reconstructed nasal epithelium to support an assessment that sufficiently reflects the human response to exposure (e.g. cigarette smoke, aerosols)?

As stated above for question #12	None.
None...no tissue characteristics are missing in the respective studies.	Possibly all the tissue characteristics are met in 3D reconstructed nasal epithelium that could reflect human response to cigarette smoke and aerosols exposures.
1) Amount/length of exposure 2) Depth of exposure may not mimic the human system 3) It is not clear whether the temperature of the CS in the system is identical to that seen in natural exposure to CS. 4) Cell-cell and cross-tissue interactions may not be captured in such systems.	Some of the methods used by Iskander et al are either repeated in the Results or included only as part of the Results. This may have been done to more clearly describe what was done and the resulting observations. To quote Talikka et al (2014)," In the MucilAir model, the fibroblasts are cultured as a monolayer on a porous membrane on the opposite side of the epithelial cells, whereas in the EpiAirway model, the fibroblasts are placed just below the epithelial cell in a collagen matrix." I am not able from the text of the Iskander et al paper to determine if the MuciAir model used in their paper included the cultured fibroblast layer decribed in Talikka et al. If it was included, this should be described in the Iskander paper in a manner similar to the description in the Talikka paper. If it was not, an explanation should be provided. To answer the immediate question, the intact human nasal mucosa is more complicated than the in vitro systems described; however, the data from the paper indicate an in vitro response that simulates the human nasal mucosa response to CS. I am not able to open/view the Tissue Provider webpage.
Model seems appropriate, but is of course just a model.	verification of confluence and communicability (cell junctions) as well as viability at the outset.
1. It is a great in-vitro system that may be more than adequate to ask the questions being asked. It is a good model for investigating the human response. However, and obviously, it cannot recreate the complex flow characteristics through the nose but this may not be necessary unless one is attempting to map lesions with location of nasal tumors (if known) and to compare CS to THS2.2. There are no neuronal cells but these cannot be added to the olfactory mucosa. Could T or B cells, or macrophages, be added to the media to see if their is	same as above

> recruitment to the mucosal layer? 2. The same comment about metabolic capacity loss in-vitro and how does it compare to fresh explants? Loss of metabolic capacity in-vitro (e.g., ALDH) could be an important factor in comparing the relative lack of response to THS2.2 to 3R4F. One could speculate that the in-vitro nasal epithelium is more at risk to CS due to low of aldehyde dehydrogenases.

Appendix B: Survey Results

ID #1986 (https://scipinion.com/surveys/68/results/1986)

On a scale from 1 to 5 (5 being the highest), how relevant, informative, and intuitive are the various types of computational analyses (related to transcriptomics, lipidomics, proteomics) reported in the respective manuscripts?

	Relevant	Informative	Intuitive	Total
Transcriptomics: computational network modeling and NPA calculation	*3* **33.33%** 3 *4* **44.44%** 4 *5* **22.22%** 2	*1* **10.00%** 1 *2* **10.00%** 1 *3* **10.00%** 1 *4* **50.00%** 5 *5* **20.00%** 2	*1* **33.33%** 3 *2* **11.11%** 1 *4* **33.33%** 3 *5* **22.22%** 2	10
Transcriptomics: gene set enrichment analysis	*3* **33.33%** 3 *4* **22.22%** 2 *5* **44.44%** 4	*1* **10.00%** 1 *2* **10.00%** 1 *3* **10.00%** 1 *4* **50.00%** 5 *5* **20.00%** 2	*1* **11.11%** 1 *2* **11.11%** 1 *3* **11.11%** 1 *4* **44.44%** 4 *5* **22.22%** 2	10

Appendix B: Survey Results

miRNA analysis	2 22.22% 2 3 44.44% 4 4 22.22% 2 5 11.11% 1	1 20.00% 2 2 20.00% 2 3 30.00% 3 4 20.00% 2 5 10.00% 1	1 11.11% 1 2 22.22% 2 3 22.22% 2 4 22.22% 2 5 22.22% 2	10
Proteomics: functional clustering	3 44.44% 4 4 33.33% 3 5 22.22% 2	1 10.00% 1 3 40.00% 4 4 50.00% 5	1 11.11% 1 3 33.33% 3 4 55.56% 5	10
Other (please specify)	3 33.33% 1 4 66.67% 2	1 33.33% 1 4 66.67% 2	1 33.33% 1 4 66.67% 2	3

Appendix B: Survey Results

ID #1987 (https://scipinion.com/surveys/68/results/1987)

Having inspected the study data in the various repositories provided, as well as in the Supplementary materials provided with the Phillips et al publication, and supplementary material provided on the systox portal, how do you rate the quality of the data, its annotation, and its presentation. Please comment if rated "insufficient" or "partially sufficient".

	Insufficient	Partially sufficient	Sufficient	Total
In-life observations, mortality, body weight, gross pathology	0.00% 0	10.00% 1	90.00% 9	10
Biomarkers of exposure	0.00% 0	10.00% 1	90.00% 9	10
Hematology and clinical chemistry (biomarkers of effect & lipoproteins)	0.00% 0	10.00% 1	90.00% 9	10
Respiratory physiology	0.00% 0	10.00% 1	90.00% 9	10
BAL fluid analysis (free lung cells and inflammatory mediators)	0.00% 0	10.00% 1	90.00% 9	10
Histopathology and histomorphometry	0.00% 0	30.00% 3	70.00% 7	10
Plaque size measurements (planimetry and microCT)	10.00% 1	0.00% 0	90.00% 9	10
Transcriptomics analysis	10.00% 1	20.00% 2	70.00% 7	10
Proteomics analysis	10.00% 1	10.00% 1	80.00% 8	10
Lipidomics analysis	0.00% 0	10.00% 1	90.00% 9	10

Appendix B: Survey Results

ID #1988 (https://scipinion.com/surveys/68/results/1988)

Having inspected the study data in the various repositories provided, as well as in the Supplementary materials provided with the Poussin et al and Van der Toorn et al. publications reporting in vitro mechanistic assays, how do you rate the quality of the data, its annotation, and its presentation. Please comment if rated "insufficient" or "partially sufficient".

	Insufficient	Partially sufficient	Sufficient	Total
Poussin et al. Functional adhesion assay	0.00% 0	10.00% 1	90.00% 9	10
Poussin et al. Inflammatory mediators	10.00% 1	0.00% 0	90.00% 9	10
Poussin et al. Transcriptomics analysis	20.00% 2	0.00% 0	80.00% 8	10
Van der Toorn et al. Transendothelial migration assay	0.00% 0	40.00% 4	60.00% 6	10
Van der Toorn et al. Endothelial barrier integrity	0.00% 0	40.00% 4	60.00% 6	10
Van der Toorn et al. Cytotoxicity and inflammatory behavior of THP-1 cells	0.00% 0	40.00% 4	60.00% 6	10

Appendix B: Survey Results

ID #1989 (https://scipinion.com/surveys/68/results/1989)

Having inspected the study data in the various repositories provided, as well as in the Supplementary materials provided with the Iskandar et al. and Zanetti et al. manuscripts reporting assessment of test items on organotypic tissue models, how do you rate the quality of the data, its annotation, and its presentation. Please comment if rated "insufficient" or "partially sufficient".

	Insufficient	Partially sufficient	Sufficient	Total
Iskandar et al. Histopathological evaluation	0.00% 0	10.00% 1	90.00% 9	10
Iskandar et al. Cytotoxicity (AK assay)	0.00% 0	10.00% 1	90.00% 9	10
Iskandar et al. CYP1A1/B1 activity assay	0.00% 0	22.22% 2	77.78% 7	9
Iskandar et al. Characterization of pro-inflammatory mediators	0.00% 0	10.00% 1	90.00% 9	10
Iskandar et al. Transcriptomics analysis	10.00% 1	0.00% 0	90.00% 9	10
Iskandar et al. miRNA analysis	10.00% 1	10.00% 1	80.00% 8	10
Zanetti et al. Histopathological evaluation	0.00% 0	10.00% 1	90.00% 9	10
Zanetti et al. Cytotoxicity (AK assay)	0.00% 0	20.00% 2	80.00% 8	10
Zanetti et al. CYP1A1/B1 activity assay	10.00% 1	20.00% 2	70.00% 7	10
Zanetti et al. Characterization of pro-inflammatory mediators	0.00% 0	30.00% 3	70.00% 7	10
Zanetti et al. Transcriptomics analysis	10.00% 1	20.00% 2	70.00% 7	10
Zanetti et al. miRNA analysis	20.00% 2	20.00% 2	60.00% 6	10
	20.00%	0.00%	80.00%	

Appendix B: Survey Results

Zanetti et al. Endpoint reproducibility/variability analysis	2	0	8	10

Appendix B: Survey Results

ID #1990 (https://scipinion.com/surveys/68/results/1990)

The standards for presenting omics data are still evolving. Regarding the publication of results from omics data in the studies evaluated, please rate the importance of each of the following criteria in determining the suitability of these data for their intended purposes and your confidence (scale of 1-5, 5 being highest confidence) that these criteria were met in the study (please download some files to judge the quality of the annotations and format)? Please also discuss in the text box any other criteria you deem useful or appropriate.

	Importance and fitness for purpose	Confidence that criteria were met	Total
Availability of raw data	*3* **20.00%** 2 *4* **10.00%** 1 *5* **70.00%** 7	*2* **11.11%** 1 *3* **11.11%** 1 *4* **33.33%** 3 *5* **44.44%** 4	10
Annotation of the metadata	*2* **10.00%** 1 *4* **50.00%** 5 *5* **40.00%** 4	*2* **22.22%** 2 *4* **44.44%** 4 *5* **33.33%** 3	10
Sufficient detail in the narrative description of the methods	*3* **20.00%** 2 *4* **30.00%**	*1* **11.11%** 1 *3* **11.11%** 1 *4*	10

	3 *5* **50.00%** 5	**33.33%** 3 *5* **44.44%** 4	
Availability of the source code and/or software	*1* **10.00%** 1 *2* **10.00%** 1 *3* **20.00%** 2 *4* **20.00%** 2 *5* **40.00%** 4	*1* **33.33%** 3 *3* **11.11%** 1 *4* **33.33%** 3 *5* **22.22%** 2	10
Possibility to run the code and/or software on own datasets to judge the quality of the tool	*1* **20.00%** 2 *3* **40.00%** 4 *4* **20.00%** 2 *5* **20.00%** 2	*1* **33.33%** 3 *3* **22.22%** 2 *4* **33.33%** 3 *5* **11.11%** 1	10
Other (please specify)	*4* **50.00%** 1 *5* **50.00%**	*4* **100.00%** 2	2

	1		

SECTION: DATA EVALUATION AND INTERPRETATION

ID #1991 (https://scipinion.com/surveys/68/results/1991)

Are the results in the publication reporting the in vivo study (Phillips et al.) presented with sufficient detail, and were they clearly presented?

	Sufficient detail	Clarity of presentation	Total
In-life observations, mortality, body weight, gross pathology	*Partially sufficient* **10.00%** 1 *Sufficient* **90.00%** 9	*Partially sufficient* **20.00%** 2 *Sufficient* **80.00%** 8	10
Biomarkers of exposure	*Insufficient* **10.00%** 1 *Partially sufficient* **10.00%** 1 *Sufficient* **80.00%** 8	*Insufficient* **10.00%** 1 *Partially sufficient* **10.00%** 1 *Sufficient* **80.00%** 8	10
Hematology and clinical chemistry (biomarkers of effect & lipoproteins)	*Insufficient* **10.00%** 1 *Sufficient* **90.00%** 9	*Partially sufficient* **10.00%** 1 *Sufficient* **90.00%** 9	10
Respiratory physiology	*Insufficient* **10.00%** 1 *Sufficient* **90.00%** 9	*Partially sufficient* **10.00%** 1 *Sufficient* **90.00%** 9	10
	Insufficient **10.00%**	*Partially sufficient*	

BAL fluid analysis (free lung cells and inflammatory mediators)	1 *Partially sufficient* **10.00%** 1 *Sufficient* **80.00%** 8	**10.00%** 1 *Sufficient* **90.00%** 9	10
Histopathology and histomorphometry	*Insufficient* **10.00%** 1 *Sufficient* **90.00%** 9	*Partially sufficient* **10.00%** 1 *Sufficient* **90.00%** 9	10
Plaque size measurements (planimetry and microCT)	*Insufficient* **10.00%** 1 *Partially sufficient* **10.00%** 1 *Sufficient* **80.00%** 8	*Sufficient* **100.00%** 10	10
Transcriptomics analysis	*Insufficient* **10.00%** 1 *Partially sufficient* **10.00%** 1 *Sufficient* **80.00%** 8	*Insufficient* **10.00%** 1 *Sufficient* **90.00%** 9	10
Proteomics analysis	*Insufficient* **10.00%** 1 *Sufficient* **90.00%** 9	*Insufficient* **10.00%** 1 *Sufficient* **90.00%** 9	10

Lipidomics analysis	*Insufficient* **10.00%** 1 *Sufficient* **90.00%** 9	*Insufficient* **10.00%** 1 *Sufficient* **90.00%** 9	10

Appendix B: Survey Results

ID #1992 (https://scipinion.com/surveys/68/results/1992)

Are the results in the publications reporting the in vitro mechanistic assays (Poussin et al., Van der Toorn et al.) presented with sufficient detail, and were they clearly presented?

	Sufficient detail	Clarity of presentation	Total
Poussin et al. Functional adhesion assay	*Partially sufficient* **10.00%** 1 *Sufficient* **90.00%** 9	*Partially sufficient* **22.22%** 2 *Sufficient* **77.78%** 7	10
Poussin et al. Inflammatory mediators	*Partially sufficient* **10.00%** 1 *Sufficient* **90.00%** 9	*Partially sufficient* **11.11%** 1 *Sufficient* **88.89%** 8	10
Poussin et al. Transcriptomics analysis	*Insufficient* **10.00%** 1 *Partially sufficient* **10.00%** 1 *Sufficient* **80.00%** 8	*Insufficient* **11.11%** 1 *Partially sufficient* **22.22%** 2 *Sufficient* **66.67%** 6	10
Van der Toorn et al. Transendothelial migration assay	*Insufficient* **10.00%** 1 *Partially sufficient* **10.00%** 1 *Sufficient* **80.00%** 8	*Partially sufficient* **22.22%** 2 *Sufficient* **77.78%** 7	10
	Partially sufficient	*Partially sufficient*	

Van der Toorn et al. Endothelial barrier integrity	**10.00%** 1 *Sufficient* **90.00%** 9	**11.11%** 1 *Sufficient* **88.89%** 8	10
Van der Toorn et al. Cytotoxicity and inflammatory behavior of THP-1 cells	*Partially sufficient* **20.00%** 2 *Sufficient* **80.00%** 8	*Partially sufficient* **22.22%** 2 *Sufficient* **77.78%** 7	10

Appendix B: Survey Results

ID #1993 (https://scipinion.com/surveys/68/results/1993)

Are the results in the publications reporting the in vitro assessment with the organotypic systems (Iskandar et al., Zanetti et al.) presented with sufficient detail, and were they clearly presented?

	Sufficient detail	Clarity of presentation	Total
Iskandar et al. Histopathological evaluation	*Insufficient* **10.00%** 1 *Partially sufficient* **10.00%** 1 *Sufficient* **80.00%** 8	*Insufficient* **10.00%** 1 *Partially sufficient* **10.00%** 1 *Sufficient* **80.00%** 8	10
Iskandar et al. Cytotoxicity (AK assay)	*Sufficient* **100.00%** 10	*Sufficient* **100.00%** 10	10
Iskandar et al. CYP1A1/B1 activity assay	*Partially sufficient* **20.00%** 2 *Sufficient* **80.00%** 8	*Partially sufficient* **20.00%** 2 *Sufficient* **80.00%** 8	10
Iskandar et al. Ciliary beating frequency assay	*Partially sufficient* **10.00%** 1 *Sufficient* **90.00%** 9	*Sufficient* **100.00%** 10	10
Iskandar et al. Characterization of pro-inflammatory mediators	*Partially sufficient* **10.00%** 1 *Sufficient* **90.00%** 9	*Partially sufficient* **10.00%** 1 *Sufficient* **90.00%** 9	10

Iskandar et al. Transcriptomics analysis	*Partially sufficient* **20.00%** 2 *Sufficient* **80.00%** 8	*Insufficient* **10.00%** 1 *Partially sufficient* **10.00%** 1 *Sufficient* **80.00%** 8	10
Iskandar et al. miRNA analysis	*Partially sufficient* **10.00%** 1 *Sufficient* **90.00%** 9	*Insufficient* **10.00%** 1 *Sufficient* **90.00%** 9	10
Iskandar et al. Endpoint reproducibility/variability analysis	*Insufficient* **10.00%** 1 *Partially sufficient* **10.00%** 1 *Sufficient* **80.00%** 8	*Insufficient* **10.00%** 1 *Partially sufficient* **10.00%** 1 *Sufficient* **80.00%** 8	10
Zanetti et al. Histopathological evaluation	*Partially sufficient* **20.00%** 2 *Sufficient* **80.00%** 8	*Partially sufficient* **20.00%** 2 *Sufficient* **80.00%** 8	10
Zanetti et al. Cytotoxicity (AK assay)	*Partially sufficient* **20.00%** 2 *Sufficient* **80.00%** 8	*Partially sufficient* **20.00%** 2 *Sufficient* **80.00%** 8	10

Zanetti et al. CYP1A1/B1 activity assay	*Partially sufficient* **30.00%** 3 *Sufficient* **70.00%** 7	*Partially sufficient* **30.00%** 3 *Sufficient* **70.00%** 7	10
Zanetti et al. Characterization of pro-inflammatory mediators	*Insufficient* **10.00%** 1 *Partially sufficient* **20.00%** 2 *Sufficient* **70.00%** 7	*Insufficient* **10.00%** 1 *Partially sufficient* **20.00%** 2 *Sufficient* **70.00%** 7	10
Zanetti et al. Transcriptomics analysis	*Insufficient* **10.00%** 1 *Partially sufficient* **10.00%** 1 *Sufficient* **80.00%** 8	*Insufficient* **20.00%** 2 *Sufficient* **80.00%** 8	10
Zanetti et al. miRNA analysis	*Partially sufficient* **30.00%** 3 *Sufficient* **70.00%** 7	*Insufficient* **10.00%** 1 *Partially sufficient* **20.00%** 2 *Sufficient* **70.00%** 7	10
Zanetti et al. Endpoint reproducibility/variability analysis	*Partially sufficient* **20.00%** 2 *Sufficient*	*Partially sufficient* **20.00%** 2 *Sufficient*	10

Appendix B: Survey Results

	80.00% 8	80.00% 8	

Appendix B: Survey Results

ID #1994 (https://scipinion.com/surveys/68/results/1994)

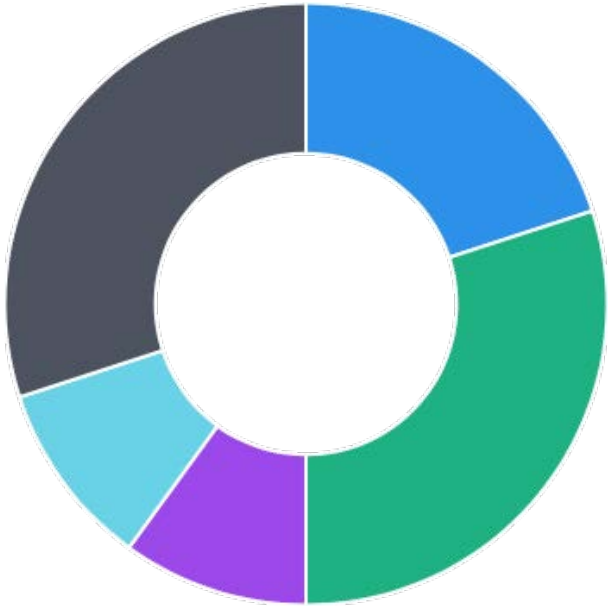
Are the interpretations and conclusions in the publications regarding the effects of exposure eto 3R4F cigarette and THS2.2 aerosol in the different models reasonably supported by the results?

	Reasonable and well supported by the data	Interpretation goes beyond what the data will support	Unsupported assertions or gaps in the interpretation	Total
Lung / respiratory effects in Phillips et al. 2016	**90.00%** 9	**0.00%** 0	**10.00%** 1	10
Vascular / atherosclerotic effects in Phillips et al. 2016	**90.00%** 9	**0.00%** 0	**10.00%** 1	10
Clinical chemistry and systemic effects in Phillips et al. 2016	**90.00%** 9	**0.00%** 0	**10.00%** 1	10
Monocyte-endothelial cell adhesion / events relevant to atherogenesis in Poussin et al. 2016	**80.00%** 8	**10.00%** 1	**10.00%** 1	10
Monocyte chemotaxis and migration / events relevant to atherosclerotic changes in Van der Toorn et al. 2016	**70.00%** 7	**10.00%** 1	**20.00%** 2	10
Respiratory effects in Iskandar et al. 2016	**60.00%** 6	**20.00%** 2	**20.00%** 2	10
Oral effects in Zanetti et al. 2016	**60.00%** 6	**20.00%** 2	**20.00%** 2	10

Appendix B: Survey Results

ID #1995 (https://scipinion.com/surveys/68/results/1995)

In the transendothelial migration assay, how would you explain the unexpected decrease in monocyte transmigration upon smoke/aerosol extract exposure? Please specify evidence in the comment box below.



Legend

- This response is likely a consequence of the leukemic origin of the THP-1 cells and can be expected to be opposite when primary monocytes are employed : 0 *(0%)*
- The model design is only suitable to address reverse intravasation of monocytes/macrophages from the subendothelial space back into the vascular lumen as described by Potteaux et al., 2011 (PMID: 21505265): 2 *(20%)*
- Apparently a general cytotoxic or a more specific anti-locomotory effect (e.g., on the organization of the actin cytoskeleton- cf. Konakahara et al., 2011 (PMID: 21704618) of the exposure antagonized the expected increase in transendothelial migration: 3 *(30%)*
- The exposure modality (pre-treatment of monocytic and endothelial cells or common exposure during the transmigration phase determines whether CSE exposure is stimulatory or inhibitory : 0 *(0%)*
- Transmigration assays mimic in vivo mechanisms only under flow conditions and results from static assays are not representative for the initiation of atherosclerosis : 1 *(10%)*
- Another mechanism not mentioned above (please explain in the comment box): 1 *(10%)*
- I cannot answer (please explain in the comment box): 3 *(30%)*

answers: 10
skips: 0

ID #1996 (https://scipinion.com/surveys/68/results/1996)

Considering the results derived from the repetition of the assessment in the nasal organotypic model across multiple phases, how do you rate the reproducibility of this model and consequently its usefulness in product assessment?

	strongly agree	agree	disagree	strongly disagree	Total
The model is useful to get an overall idea of relative effects	60.00% 6	40.00% 4	0.00% 0	0.00% 0	10
The model allows quantitative analyses and assessment	10.00% 1	80.00% 8	10.00% 1	0.00% 0	10
The model is appropriate for mechanistic understanding, but not for product assessment	0.00% 0	40.00% 4	60.00% 6	0.00% 0	10
The model is not appropriate for assessment of inhaled toxicants	0.00% 0	10.00% 1	70.00% 7	20.00% 2	10
I cannot answer (please explain in the comment box)	0.00% 0	0.00% 0	0.00% 0	100.00% 1	1

Appendix B: Survey Results

ID #1997 (https://scipinion.com/surveys/68/results/1997)

Causal biological networks have been developed for understanding and interpreting omics data using a systems toxicology approach (http://causalbionet.com). These networks are developed based on a priori biological knowledge about causal signaling pathways. How confident are you that the specific application of the causal network model by Phillips et al. and Iskandar et al. provides an appropriate high-level evaluation of the biological impact (direct or indirect) of an exposure on the transcriptome of tissues investigated?



Legend

- Extremely confident: 0 *(0%)*
- Very confident: 3 *(30%)*
- Confident: 4 *(40%)*
- Somewhat confident: 3 *(30%)*
- Not confident: 0 *(0%)*
- I cannot answer (please explain in the comment box): 0 *(0%)*

answers: 10

skips: 0

Appendix B: Survey Results

ID #1998 (https://scipinion.com/surveys/68/results/1998)

How critical and informative do you consider the following endpoints for assessing the relative impact of various tobacco products on the in vivo model system considered?

	Critical	Informative	Total
In-life observations, mortality, body weight, gross pathology	*high* **70.00%** 7 *low* **10.00%** 1 *medium* **20.00%** 2	*high* **70.00%** 7 *medium* **30.00%** 3	10
Biomarkers of exposure	*high* **70.00%** 7 *medium* **30.00%** 3	*high* **80.00%** 8 *medium* **20.00%** 2	10
Hematology and clinical chemistry (biomarkers of effect & lipoproteins)	*high* **80.00%** 8 *low* **10.00%** 1 *medium* **10.00%** 1	*high* **80.00%** 8 *medium* **20.00%** 2	10
Respiratory physiology	*high* **80.00%** 8 *medium* **20.00%** 2	*high* **90.00%** 9 *medium* **10.00%** 1	10
	high	*high*	

Appendix B: Survey Results

BAL fluid analysis (free lung cells and inflammatory mediators)	**60.00%** 6 *medium* **40.00%** 4	**60.00%** 6 *medium* **40.00%** 4	10
Histopathology and histomorphometry	*high* **80.00%** 8 *medium* **20.00%** 2	*high* **80.00%** 8 *medium* **20.00%** 2	10
Plaque size measurements (planimetry and microCT)	*high* **90.00%** 9 *medium* **10.00%** 1	*high* **90.00%** 9 *medium* **10.00%** 1	10
Transcriptomics analysis	*high* **40.00%** 4 *low* **10.00%** 1 *medium* **50.00%** 5	*high* **40.00%** 4 *low* **10.00%** 1 *medium* **50.00%** 5	10
Proteomics analysis	*high* **50.00%** 5 *medium* **50.00%** 5	*high* **50.00%** 5 *medium* **50.00%** 5	10
Lipidomics analysis	*high* **60.00%** 6 *medium*	*high* **60.00%** 6 *medium*	10

	40.00% 4	40.00% 4	
The totality of the data, collectively	*high* 100.00% 10	*high* 100.00% 10	10

Appendix B: Survey Results

ID #1999 (https://scipinion.com/surveys/68/results/1999)

How critical and informative do you consider the following endpoints for assessing the relative impact of various tobacco products on early steps of atherogenesis?

	Critical	Informative	Total
Poussin et al. Functional adhesion assay	*high* **80.00%** 8 *medium* **20.00%** 2	*high* **80.00%** 8 *medium* **20.00%** 2	10
Poussin et al. Inflammatory mediators	*high* **80.00%** 8 *medium* **20.00%** 2	*high* **70.00%** 7 *medium* **30.00%** 3	10
Poussin et al. Transcriptomics analysis	*high* **30.00%** 3 *low* **10.00%** 1 *medium* **60.00%** 6	*high* **40.00%** 4 *low* **10.00%** 1 *medium* **50.00%** 5	10
Van der Toorn et al. Transendothelial migration assay	*high* **60.00%** 6 *low* **10.00%** 1 *medium* **30.00%** 3	*high* **40.00%** 4 *low* **10.00%** 1 *medium* **50.00%** 5	10
		high	

Appendix B: Survey Results

Van der Toorn et al. Endothelial barrier integrity	*high* **50.00%** 5 *medium* **50.00%** 5	**40.00%** 4 *low* **20.00%** 2 *medium* **40.00%** 4	10
Van der Toorn et al. Cytotoxicity and inflammatory behavior of THP-1 cells	*high* **60.00%** 6 *low* **10.00%** 1 *medium* **30.00%** 3	*high* **60.00%** 6 *low* **20.00%** 2 *medium* **20.00%** 2	10
The totality of the data, collectively	*high* **90.00%** 9 *medium* **10.00%** 1	*high* **80.00%** 8 *medium* **20.00%** 2	10

Appendix B: Survey Results

ID #2000 (https://scipinion.com/surveys/68/results/2000)

How critical and informative do you consider the following endpoints for assessing the relative impact of various tobacco products on the respiratory tract?

	Critical	Informative	Total
Iskandar et al. Histopathological evaluation	*high* **77.78%** 7 *medium* **22.22%** 2	*high* **66.67%** 6 *medium* **33.33%** 3	9
Iskandar et al. Cytotoxicity (AK assay)	*high* **66.67%** 6 *medium* **33.33%** 3	*high* **66.67%** 6 *medium* **33.33%** 3	9
Iskandar et al. CYP1A1/B1 activity assay	*high* **44.44%** 4 *low* **22.22%** 2 *medium* **33.33%** 3	*high* **33.33%** 3 *low* **44.44%** 4 *medium* **22.22%** 2	9
Iskandar et al. Ciliary beating frequency assay	*high* **77.78%** 7 *medium* **22.22%** 2	*high* **66.67%** 6 *medium* **33.33%** 3	9
Iskandar et al. Characterization of pro-inflammatory mediators	*high* **55.56%** 5 *medium*	*high* **66.67%** 6 *medium*	9

	44.44% 4	33.33% 3	
Iskandar et al. Transcriptomics analysis	*high* 22.22% 2 *low* 11.11% 1 *medium* 66.67% 6	*high* 33.33% 3 *low* 22.22% 2 *medium* 44.44% 4	9
Iskandar et al. miRNA analysis	*high* 33.33% 3 *low* 33.33% 3 *medium* 33.33% 3	*high* 22.22% 2 *low* 44.44% 4 *medium* 33.33% 3	9
Zanetti et al. Histopathological evaluation	*high* 55.56% 5 *low* 11.11% 1 *medium* 33.33% 3	*high* 44.44% 4 *low* 11.11% 1 *medium* 44.44% 4	9
Zanetti et al. Cytotoxicity (AK assay)	*high* 55.56% 5 *low* 22.22% 2 *medium*	*high* 44.44% 4 *low* 22.22% 2 *medium*	9

	22.22% 2	**33.33%** 3	
Zanetti et al. CYP1A1/B1 activity assay	*high* **55.56%** 5 *low* **33.33%** 3 *medium* **11.11%** 1	*high* **55.56%** 5 *low* **44.44%** 4	9
Zanetti et al. Characterization of pro-inflammatory mediators	*high* **55.56%** 5 *low* **11.11%** 1 *medium* **33.33%** 3	*high* **55.56%** 5 *medium* **44.44%** 4	9
Zanetti et al. Transcriptomics analysis	*high* **22.22%** 2 *low* **22.22%** 2 *medium* **55.56%** 5	*high* **33.33%** 3 *low* **33.33%** 3 *medium* **33.33%** 3	9
Zanetti et al. miRNA analysis	*high* **22.22%** 2 *low* **33.33%** 3 *medium* **44.44%**	*high* **22.22%** 2 *low* **44.44%** 4 *medium* **33.33%**	9

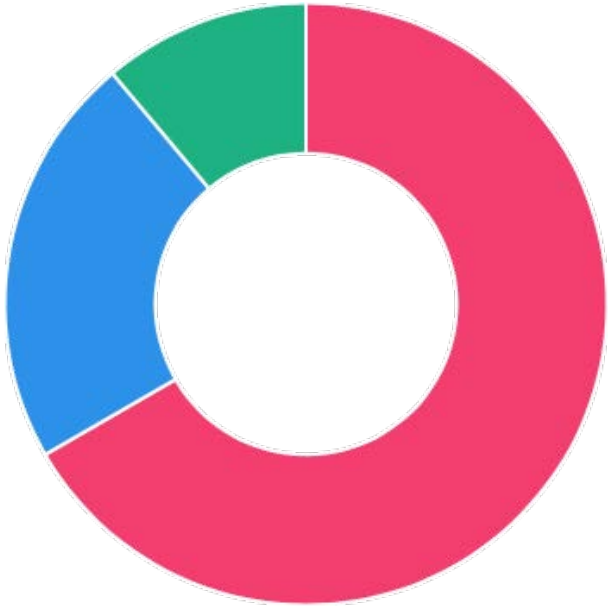
Appendix B: Survey Results

	4	3	
Zanetti et al. Endpoint reproducibility/variability analysis	*high* **66.67%** 6 *low* **22.22%** 2 *medium* **11.11%** 1	*high* **77.78%** 7 *low* **22.22%** 2	9
The totality of the data, collectively	*high* **66.67%** 6 *low* **11.11%** 1 *medium* **22.22%** 2	*high* **55.56%** 5 *medium* **44.44%** 4	9

Appendix B: Survey Results

ID #2001 (https://scipinion.com/surveys/68/results/2001)

Do the study designs and models allow a mechanism-based evaluation for mechanisms such as oxidative stress and inflammation across endpoints and between studies?



Legend

Yes, the systems approach applied in each study enhances the understanding of pathomechanisms across the various endpoints and between the various studies: 6 *(67%)*

Yes, a systems approach can potentially enhance the understanding of pathomechanisms across endpoints and between different studies, but considering the current studies, additional aspects would be required in order to make the evaluation of pathomechanisms more conclusive: (please indicate missing aspects in the text box): 2 *(22%)*

No, comparisons across endpoints or different model systems are not reliable; the only reliable approach to understand pathomechanisms is to use separate, mechanism-specific investigations in each of that various dedicated model systems: 1 *(11%)*

I cannot answer (please explain in the comment box): 0 *(0%)*

answers: 9

skips: 1

Appendix B: Survey Results

ID #2002 (https://scipinion.com/surveys/68/results/2002)

The analysis of lung transcriptomics data using the causal network model (Philips et al. 2016) predicted a robust CS-induced increase in extracellular matrix (ECM) degradation that was not observed in the cessation group. For each of the statements please rate your confidence level (scale of 1-5, 5 being highest confidence).

	1	2	3	4	5	Total
The quantified CS exposure-related histopathological changes in the lung tissue are consistent with ECM degradation throughout CS exposure	11.11% 1	0.00% 0	0.00% 0	44.44% 4	44.44% 4	9
The inflammatory mediators in BALF are reflective of an observed increase in ECM degradation during CS exposure	22.22% 2	0.00% 0	11.11% 1	44.44% 4	22.22% 2	9
Some of the lung functional parameters are likely reflective of ECM/tissue degradation (please specify which in the comment box)	11.11% 1	0.00% 0	11.11% 1	44.44% 4	33.33% 3	9
There is another explanation for the predicted increase of the network that is not related to ECM degradation per se (please specify in the comment box)	33.33% 3	11.11% 1	11.11% 1	33.33% 3	11.11% 1	9
I cannot answer (please explain in the comment box)	33.33% 1	0.00% 0	0.00% 0	33.33% 1	33.33% 1	3

ID #2003 (https://scipinion.com/surveys/68/results/2003)

In your opinion, how useful is the assessment of CYP1A1/CYP1B1 activity levels of the in vitro nasal and oral epithelial models following cigarette smoke/aerosol exposure in deducing the potential harmful and deleterious effects for cells/tissues of cigarette smoke/aerosol exposure? Do you believe other CYP enzymes should be monitored as well?

The metabolism of xenobiotics includes oxidative reactions by phase I enzymes that convert lipophilic chemical compounds into their hydrophilic forms, followed by phase II conjugation enzymes, and finally the phase III membrane transporters. The most prominent phase I enzymes are cytochrome P450(also known as CYPs) that detoxify or activate xenobiotic compounds. This phase I enzymes are also known to be responsible for the metabolism of compounds present in tobacco ,such as nicotine, benzene, polycyclic aromatic hydrocarbons(PAHs). Expression CYPs are highly tissue specific. Although the liver is known to be the main organ responsible for the metabolism of xenobiotics, but in case of CS/Tobacco products respiratory tract/lung served as an primary target organ. Hence it is highly relevant of valuable to measure the CYP1A1 activity form ALI-3D model of human nasal and oral epithelial cells following CS/tobacco exposure to screen the first level of cytotoxicity. determiantion of CYP1B1 enzyme activity following CS expsoure might be also useful.

I strongly believe that other CYP enzymes should be monitored. More importantly, at least in the mouse model multiple CYPs have to be measured in various tissues especially liver and lung to generate testable hypothesis on the impact of THS 2.2. It appears that CYP induction due to increased/prolonged dose of THS 2.2 could lead to extensive inflammatory responses (perhaps via induction of CYPs) which is poorly studied here. In general, mechanistic aspects of THS 2.2 at high doses are very weakly studied/reported here.

These reflect AHR activation (maybe PAHs or maybe combustion product-formed AHR ligands). How these changes explain bioactivation to active intermediates, or detoxification, is a very difficult question to answer in view of all the CS and THS2.2 mixture components (that could be metabolized by CYP1A1/1B1. Unless you tie these induced enzymes to their products, e.g,. DNA adducts, metabolites of PAHs, genotoxicity, they don't serve much purpose. And, in one of the studies (Zanetti) the activity levels versus mRNA results were contradictory. Overall, I don't find these data very helpful for describing toxicity (more biomarkers of exposure than causal factors). Furthermore, the method Iskandar used is not the conventional way I would have measured 1A1 and 1B1 (e.g., mRNA, protein, EROD). You could add additional CYPs but I'm not familiar with what CYPs, beyond 1A1/1B1 activating PAHs, are involved with tobacco smoke mixtures. And, the Iskandar CYP1A1 data are not consistent with the fast activation by the AHR and they do not show concentration-dependancy. Maybe add ALDH information in the nasal mucosa studies for removing aldehydes from the organotypic nasal system?

Iskander et al: High dose of CS induced toxicity in the epithelium and altered its capability to metabolize nitrosamines and PAHs. No real conclusions reached, thus limited usefulness using the system in this paper. Iskander et al need to make this manuscript look as organized as Iskander et al paper on bronchial in vitro study. Zanetti et al: The figure... shows that, at both timepoints, there were no substantial changes in CYP activity in 3R4F CS-exposed inserts, compared with air-exposed controls. In contrast, a dose-dependent increase in CYP1A1/CYP1B1

It is informative when proper system controls and number of assessments are in place

Primarily, this study is quite useful to understand the metabolizing or xenobiotics of cigarette smoke/aerosol exposure by CYP1A1/CYP1B1 activity. It is plausible that the cytochrome P450 system plays an important role in neutralizing the deleterious effects of CS exposure toxicants. The study appropriately used the

activity, up to 15%, was observed in cultures exposed to THS2.2 aerosol, at both post exposure timepoints. In addition, CYP activity was significantly higher in cultures exposed to the low concentration of THS2.2 than in those receiving comparable exposures to 3R4F CS at 24 h. At 48 h post-exposure, this was only the case for one concentration of THS2.2 (0.46). An AK release cytotoxicity assay was performed, as a control, testing basolateral medium of the same culture inserts also used to measure CYP activity. The results were consistent with those illustrated in the figure cytotoxicity assay results . Discussion: THS2.2 had a lower impact than CS, except for a transiently higher CYP1A1/1B1 activity. In my opinion there are better tools to study P450 in CS and other chemicals; skin painting, oral exposures in vivo. I believe it is possible to study in in vitro cell systems from rodents already exposed to PAHs to see the effect of metabolic changes (enzyme induction) This is important in rodent nasal olfactory epithelium, exemplified by naphthalene toxicity studies.

important CYP1A1/CYP1B1 enzymes' activity of cytochrome P450 system.

yes, because, it may infer the levels of PAHs, nitrosamines, and arylamines in the exposed smoke/aerosol

Measurements of CYP levels may be a good marker of exposure and physiological adaptation to CS, whereas CYP levels may be less useful as direct markers of harmful or deleterious effects.

Not used only to deduce potential harmful effects. Also a marker of exposure.

Appendix B: Survey Results

ID #2004 (https://scipinion.com/surveys/68/results/2004)

To demonstrate a reduced level of potentially toxic effects of MRTPs compared with conventional cigarettes, please rate the weight of the following evidence on a 1-5 scale (5 representing strong weight). Please insert any relevant comment in the box below.

	1	2	3	4	5	Total
Integration of diverse data modalities within a single study for one biological system (e.g., respiratory)	0.00% 0	11.11% 1	0.00% 0	44.44% 4	44.44% 4	9
Analysis of the effects of the products on multiple systems (respiratory, cardiovascular) in a single model (Apoe-/- mice)	0.00% 0	0.00% 0	22.22% 2	33.33% 3	44.44% 4	9
Reproducibility of results across full experiment repetitions (see Iskandar et al.)	0.00% 0	0.00% 0	22.22% 2	44.44% 4	33.33% 3	9
Concordance of results for specific mechanisms (e.g. oxidative stress or inflammation) across diverse studies and model systems (Apoe-/- mouse and in vitro assays)	0.00% 0	0.00% 0	11.11% 1	33.33% 3	55.56% 5	9

ID #2005 (https://scipinion.com/surveys/68/results/2005)

Can any of the inflammatory networks from the BIF analysis or secreted inflammatory mediators in BALF serve to explain the type of inflammatory cell infiltration observed during the histopathological evaluation in the cessation and switch group in the Apoe-/- THS switching study reported in Phillips et al, 2016?

BIF analysis by Philips et aldemonstarted that the most perturbed biological mechanisms following 3R4F CS exposure is the inflammatory processes (accounting for almost half of the RBIF), while tissue repair and angiogenesis, cell fate, and cell stress network perturbations were other major contributors. Author have also reported that slight or no perturbation was seen for THS2.2 aerosol exposure except for month 3, with a RBIF around 7% and a composition very similar to that of the 3R4F group. Strongly reduced perturbation patterns were also seen after cessation and switching, indicating an almost complete resolution of CS related perturbations after 6 months of cessation or switching to THS2.2 aerosol

It is not clear that they would explain.

I have to say I am not sure if the question refers to the inflammatory cell infiltration observed during the first two months of exposure of the cessation/switching groups to CS, or to the inflammatory cell infiltration in the six months following cessation or switching to the THS2.2 product. I am going to assume you meant the latter time frames (months 3-8). The text of the paper describes the most obvious results of the MAP analysis of the cessation and switch groups: ". Both switching and cessation resulted in the return of protein levels toward sham/THS2.2 aerosolexposedgroup levels, reaching sham levels in most but not all cases; exceptions included immunoglobulin A, eotaxin, interleukin-18, macrophage inflammatory protein (MIP)-2, tissueinhibitor of metalloproteinase (TIMP)-1. Those analytes showing exposure-related changes were mainly associated with inflammation,including many proteins previously observed to respond to CS, such as myeloperoxidase, MIP-1, -2, and -3,monocyte chemoattractant protein-1, -3, and -5, interleukins, aswell as several tissue remodeling enzymes and growth factors, vascular cell adhesion molecule, epidermal growth factor, vascular endothelial growth factor, MMP-9, and TIMP-1). The proteolytic activity of the BALF largely paralleled the protein abundance of MMP-9 (Supplementary Figure 12). Taken together, these results are well aligned with the influx of inflammatory cells observed in the BALF ." I concur with the authors conclusions, noting that the only proteins in the cessation group that had significant changes at all 3 post-CS exposure measurements were IgA and IL-18. The continuing increases in igA in these groups likely reflects the continuing presence of increased numbers of lymphocytes/plasma cells noted in the BALF data (Figure 8) and at histopathologic examination (Figure 9) Regarding the BIF heatmap in Figure 14B, the only perturbations in the Inflammatory Processes network NOT starred in 2 of 3 months analyses of the Cessation group are Mast Cell Activation and Cytotoxic T-cell signaling. All the other starred perturbations listed are potential participants in the inflammatory cell infiltrates described for this group in the two months of CS exposure. Mast cell response was a prominent perturbation in Phillips et al 2015. Further comparing the two papers, the heatmap for 2015 paper has similar perturbations in the switch and cessation groups. There were many fewer perturbations in the Switch group compared to

the Cessation group. The only point at which significant perturbations in switch group matched those of the cessation group was at 6 months, which was also the peak month for relative biologic impact for the CS-exposed group. The authors of Phillips et al 2016 had no speculation on this diference nor do I, but it is a possible subject for further computational analysis and speculation on differences.

Does this question refer to the increased above baseline for the dendritic cells at 3 months? The Systox Portal Improv was too small to see.

Partially??? Isn't the point that inflammation, although the most significant and consistent finding (in my opinion), could be acting in concert with other changes, e.g, aldehyde-induced clastogenicity/cytotoxicity, increased cell proliferation, to produce the pathology of CS? In my opinion, the multi-pronged approach taken in Phillips is really convincing. You would have to knockout individual inflammatory pathways to see how these contributed to the secretion of inflammatory mediators triggered by CS, e.g. SiRNA, pharmacological inhibitors. These will be tedious studies.

Yes, macrophages and neutrophils signalling, epithelial mucus hypersecretion of IPN and secreted inflammatory mediators in BALF (chemo attractants and cytokines) such as MCP1, IL18, IL1 alpha, beta, TNF alpha, etc could help to understand the inflammatory cell infiltration observed during the histopathological evaluation in the cessation and switch group in the Apoe-/- THS 2.2 switching study

some what but not perfect correlation

Yes, at least the inflammatory mediators in BAL fluid are useful for explaining the cell infiltration observed. The BIF analysis may still be at a more exploratory level but has huge potential for future toxicological studies.

Unable to answer.

ID #2006 (https://scipinion.com/surveys/68/results/2006)

Based on your evaluation of the data, is there any additional observation regarding THS2.2 or switch groups that is relevant for the interpretation of the results, but has not been discussed in the manuscripts?

No additional observation regrading THS2.2 or switch group required further for the interpretation of that data provided in the manuscripts.

The results are very self explanatory and well presented. Philips et. al study is very well organized and provides robust evidence for the claims made. I don't have any thing else to add- mostly because the data is well exploited in this study.

biomarkers for cytotoxicity potential such as alterations of free cytosolic Ca2+ homeostasis are indicators of cell fate as are cell necrosis, fibrosis and regeneration Without comprehensive genetic characterization "omics" analysis is unreliable and in these cases (intentionally) confounding.

See above. I suggest adding a discussion of the different suspected reasons for the lesser number of perturbations in the switch group compared to the cessation group. Laryngeal and heart sections from the switching and cessation groups would provide a more precise definition of the difference in effects of CS at these different time points. One obvious point of comparison is with the Phillips 2015 paper with a similar protocol using a different strain of mouse.

1. Need to vary the THS2.2 exposure - have you created the maximum use/exposure profile that may occur in humans? 2. Begin to measure in-vivo the difference in THS2.2 and CS components that are systemically absorbed/metabolized, espeicallly for systemic toxicity such as atherosclerosis. 3. Take this study out to a 2-year cancer bioassay if you feel the mouse model is adequate for predicting human lung cancer risk (or other cancers like esophageal).

An observation that how well the THS2.2 or switch groups address the already or highly likely or in process COPD subjects addicted to CS considering it an irreversible phenomenon. It will also be interesting to see how this will reflect in human clinical trails. Secondly, it seems there is no clear interpretation of how the TSH2.2 at highest nicotine levels could have a comparable effects with 3R4F with lower nicotine content (for example in Zanetti et al.) Considering there are insignificant carbonyls levels in TSH2.2 treatments, whether the observed effects could be attributed to highest nicotine levels. One can relate the effect of 3R4F with lower nicotine compared THS2.2 highest nicotine levels to carbonyls.

None

No, I definitely believe that the overwhelming amount of data covers all relevant aspects of classical and modern toxicology.

I did not see anything that wasn't discussed, and discussed fairly.

SECTION: TRANSLATABILITY AND OUTLOOK

ID #2007 (https://scipinion.com/surveys/68/results/2007)

Do the exposure conditions (dose/regimen/duration) and the biomarkers of exposure allow for extrapolation to the human situation, i.e. either substituting cigarette smoking by tobacco aerosol inhalation or quitting tobacco use altogether? Please comment on the following studies and cite any appropriate reference.

	Dose	Regimen	Duration	Biomarkers of exposure	Total
Phillips et al. 2016 - Apoe-/- THS switching study, respiratory effects	*Moderately appropriate* **40.00%** 4 *Very appropriate* **60.00%** 6	*Moderately appropriate* **60.00%** 6 *Very appropriate* **40.00%** 4	*Moderately appropriate* **50.00%** 5 *Very appropriate* **50.00%** 5	*Moderately appropriate* **40.00%** 4 *Very appropriate* **60.00%** 6	10
Phillips et al. 2016 - Apoe-/- THS switching study, vascular effects	*Moderately appropriate* **40.00%** 4 *Very appropriate* **60.00%** 6	*Moderately appropriate* **50.00%** 5 *Very appropriate* **50.00%** 5	*Moderately appropriate* **50.00%** 5 *Very appropriate* **50.00%** 5	*Moderately appropriate* **40.00%** 4 *Very appropriate* **60.00%** 6	10
Phillips et al. 2016 - Apoe-/- THS switching study, systemic effects	*Moderately appropriate* **40.00%** 4 *Very appropriate* **60.00%** 6	*Moderately appropriate* **30.00%** 3 *Very appropriate* **70.00%** 7	*Moderately appropriate* **50.00%** 5 *Very appropriate* **50.00%** 5	*Moderately appropriate* **40.00%** 4 *Very appropriate* **60.00%** 6	10
Poussin et al. 2016 - In vitro adhesion assay: indirect condition	*Moderately appropriate* **50.00%** 5 *Not appropriate* **10.00%** 1 *Very appropriate* **40.00%** 4	*Moderately appropriate* **50.00%** 5 *Not appropriate* **20.00%** 2 *Very appropriate* **30.00%** 3	*Moderately appropriate* **50.00%** 5 *Not appropriate* **20.00%** 2 *Very appropriate* **30.00%** 3	*Moderately appropriate* **40.00%** 4 *Not appropriate* **20.00%** 2 *Very appropriate* **40.00%** 4	10
		Moderately appropriate	*Moderately appropriate*	*Moderately appropriate*	

Appendix B: Survey Results

Poussin et al. 2016 - In vitro adhesion assay: direct condition	*Moderately appropriate* **60.00%** 6 *Very appropriate* **40.00%** 4	**60.00%** 6 *Not appropriate* **10.00%** 1 *Very appropriate* **30.00%** 3	**50.00%** 5 *Not appropriate* **10.00%** 1 *Very appropriate* **40.00%** 4	**50.00%** 5 *Not appropriate* **10.00%** 1 *Very appropriate* **40.00%** 4	10
Poussin et al. 2016 - In vitro adhesion assay: fresh direct condition	*Moderately appropriate* **70.00%** 7 *Very appropriate* **30.00%** 3	*Moderately appropriate* **70.00%** 7 *Not appropriate* **10.00%** 1 *Very appropriate* **20.00%** 2	*Moderately appropriate* **60.00%** 6 *Not appropriate* **10.00%** 1 *Very appropriate* **30.00%** 3	*Moderately appropriate* **50.00%** 5 *Not appropriate* **10.00%** 1 *Very appropriate* **40.00%** 4	10
Van der Toorn et al. 2016 - In vitro transendothelial migration assay	*Moderately appropriate* **70.00%** 7 *Very appropriate* **30.00%** 3	*Moderately appropriate* **70.00%** 7 *Very appropriate* **30.00%** 3	*Moderately appropriate* **60.00%** 6 *Not appropriate* **10.00%** 1 *Very appropriate* **30.00%** 3	*Moderately appropriate* **60.00%** 6 *Very appropriate* **40.00%** 4	10
Iskandar et al. 2016 – Organotypic nasal tissue THS2.2 assessment	*Moderately appropriate* **40.00%** 4 *Very appropriate* **60.00%** 6	*Moderately appropriate* **40.00%** 4 *Very appropriate* **60.00%** 6	*Moderately appropriate* **30.00%** 3 *Not appropriate* **10.00%** 1 *Very appropriate* **60.00%** 6	*Moderately appropriate* **40.00%** 4 *Very appropriate* **60.00%** 6	10
	Moderately appropriate	*Moderately appropriate*	*Moderately appropriate* **40.00%**	*Moderately appropriate*	

Appendix B: Survey Results

Zanetti et al. 2016 – Organotypic oral tissue THS2.2 assessment	**40.00%** 4 *Very appropriate* **60.00%** 6	**40.00%** 4 *Very appropriate* **60.00%** 6	4 *Not appropriate* **10.00%** 1 *Very appropriate* **50.00%** 5	**50.00%** 5 *Very appropriate* **50.00%** 5	10

ID #2008 (https://scipinion.com/surveys/68/results/2008)

For future studies, which change(s) and/or additional experiment(s) would you suggest to investigate further the effects of both 3R4F and THS2.2 / other MRTP aerosol exposure on respiratory and/or oral tissues?

This reviewer would suggest to do additional experiments like immunohistochemistry for important players (total collagen, MMPs, elastin, alpha SMA, mucins etc) in COPD development.

As all these studies were single exposures , multiple exposures studies with time between exposures revealing an adaptability needs to be studied in detail.

Please see suggestions for respective individual studies. Employing RNA-seq might be more appropriate for several of the transcriptomics studies. To address human tissue-specific effects, organ-on-chip kind of experiments might be more informative.

I suggest addition of microscopic examination and morphometry of laryngeal tissue sections, as described in numerous publications of rodent inhalation studies on tobacco smoke/modified tobacco products. I encourage further modification of the in vitro assays utilizing respiratory tract epithelium to add submucosal tissues containing fibroblasts and other mesenchymal cells to the mucosa used in the tests. I encourage the use of longer durations of exposure in both individual exposure times total days of exposure. Acute exposures to in vitro oral, nasal , vascular, or pulmonary cell systems do not provide information on the longer term effects of exposure, which are the keys to learning more about the pathogenesis of atherogenesis, COPD, or cancer in these tissue systems. As stated above a number of times, the key to dissecting the pathogenesis of changes induced in chronic exposures to particulate aerosols is to study the inflammatory component. Chronic irritation induces chronic inflammation, metaplasia and hyperplasia of respiratory tract epithelium. Study this process in vitro and in vivo.

1. Good analytical on blood levels of key aldehydes, PAHs, or other CS and THS2.2. components, that are believed driving the atherogenic response, would be very informative. You need to determine how CS compares to THS2.2 in terms of potential bad actors (e.g., aldehydes, PAHs, etc.) in systemic circulation. For the direct, site-of-contact effects in on mucosal surfaces, this is not an issue. This should be done for both the mouse and humans to see how the mouse model replicates (or doesn't replicate) the human situation of smoking cigarettes or inhaling the heated THS2.2 products. If you can isolate the major components of cigarette smoke (and THS2.2), you might want to consider non-combustion/heated mixtures of these components and conduct inhalation studies to confirm which components are triggering the respiratory and endothelial/monocyte response. Whereas, it is likely the complex mixture components that work together, you may find that is is only key components of the CS mixture (that are not present to any extent in THS2.2) that are causing the omics, biochemical, cellular and histopath changes. This may key you in on additional technologies to go specifically after reduction of these substances. 2. Add genotoxicity endpoints (adducts, crosslinks, micronuclei, comet assays) or any other nuclear change that could be helpful. 4. Begin teasing out nuclear receptor pathways that are predominantly altering the omics signatures being seen with the THS2.2 mixtures. Could you add agonists/antagonists to THS2.2 to counter these nuclear receptor pathways should THS2.2 cause pathology (in the event that THS2.2 is not totally lacking adverse effects on lung pathology and atherogenesis). 5. Since chewing tobacco still carries risk of oral injury/cancer, how can you distinguish THS2.2. from chewing tobacco? Is this something of any convern, e..g., people pointing out that THS2.2, even though it does not deliver combustion products, would compare to chewing tobacco use?

> For future studies to investigate the effects of both 3R4F and THS2.2 / other MRTP aerosol exposure on respiratory tissue, it would be good to explore further molecular mechanism for the onset of chronic lung disease using ALI-3D model with either primary lung cells or human lung cell line (e.g. 16HBE) in co-culture with macrophages. This will provide further in sight of cell-cell cross talk as well as the host-defence mechanism. Establishment of Co-culture of bronchial epithelial cells and endothelia cells would be also good to study the role pulmonary barrier function in the progression of emphysema following exposure to cigarettes smoke.

> Consider longer exposures. Considering exposure group that switches from THS to CS

> Suggest to repeat for better control for all parameters to include rationale for animal model and limited targetted biomarkers without reliance on a previous study of the same group. Determination of particulate size, quantity and distribution.

> Long term in vivo studies to assess both non-neoplastic and neoplastic end points

> Although not a directly toxic effects, the effects of CS of health status of teeth and gums may be relevant to investigate.

Appendix B: Survey Results

ID #2009 (https://scipinion.com/surveys/68/results/2009)

For future studies, which change(s) and/or additional experiment(s) would you suggest to investigate further the effects of both 3R4F and THS2.2 / other MRTP aerosol exposure on cardiovascular tissues?

Hemodynamics could be an additional study that could be considered for investigating further effects of both 3R4F and THS2.2 / other MRTP aerosol exposure on cardiovascular system.

As all these studies were single exposures , multiple exposures studies with time between exposures revealing an adaptability needs to be studied in detail.

1. (see answers in #2). Can you develop an in-vivo model that replicates oral inhalation that occurs when human smoke whereas an obligate nasal breather likely does not represent the site-of-contact response for comparing CS to THS2.2. or the systemic absorption that occurs across the lung (atherosclerotic endpoint). 2. Toxicokinetic studies of blood level-time courses comparing CS with THS2.2 components in blood over time. Maybe these can be done in humans (or primates)? 3. What uncertainties exist when trying to extrapolate what may occur chronically? Does the stark difference between CS and THS2.2 hold in-vitro when the in-vitro systems are taken out to a longer time point. Maybe the only way to deal with this is to continue conducting animal studies??? Can the van der Torn or Poussin methods be extended out further in time? 4. Add pharmaceutical modulators of the pathways to see if you can prevent CS effects and convert the CS signatures (omics, cellular, biochemical and histopath) to a THS2.2 signature (this is along the lines of trying to identify key nuclear receptor pathways but this time, instead of attempting to rule-out any adverse effects for THS2.2, see how CS's pathways are soley responsible for toxicity.

Most of the results shown are qualitative in nature when dealing with imaging data. So it is hard to assess the statistical significance except when specific assays have been conducted. So the authors need to devise approaches to address this.

I suggest addition of sections of heart and associated major vascular structures to tissues fixed and examined microscopically and used for morphometry. I encourage the use of longer durations of exposure in both individual exposure times total days of exposure. Acute exposures to in vitro oral, nasal , vascular, or pulmonary cell systems do not provide information on the longer term effects of exposure, which are the keys to learning more about the pathogenesis of atherogenesis, COPD, or cancer in these tissue systems.

For future studies to investigate the effects of both 3R4F and THS2.2 / other MRTP aerosol exposure on cardiovascular tissue, it would be good to explore further molecular mechanism for the onset of cardiovascular impairments using cardiac co-culture system like 3-D co-culture of fibroblast with cardiomyocytes or co-culture system of endothelial cells with vascular smooth muscle cells (VSMC). This will provide further in sight of cell-cell cross talk as well as the

Might suggest a comparison of mouse strains for baseline levels (genetic differences), particularly for lipid levels

Long term in vivo studies to assess both non-neoplastic and neoplastic end points

> cardiac cell–cell junction density-dependent connectivity. Additionally It will be also good to measure cardiovascular parameters (heart rate, blood pressure, activity and body temperature) using radiotelemetry system in rodents model to study the effects of both 3R4F and THS2.2 / other MRTP aerosol exposure on cardiovascular impairments.

> I have no specific proposals

> Consider longer exposures. Considering exposure group that switches from THS to CS

ID #2010 (https://scipinion.com/surveys/68/results/2010)

For future studies, which change(s) and/or additional experiment(s) would you suggest to investigate further systemic effects of both 3R4F and THS2.2 / other MRTP aerosol exposure?

As all these studies were single exposures , multiple exposures studies with time between exposures revealing an adaptability needs to be studied in detail.

I encourage the use of longer durations of exposure in both individual exposure times total days of exposure. Acute exposures to in vitro oral, nasal , vascular, or pulmonary cell systems do not provide information on the longer term effects of exposure, which are the keys to learning more about the pathogenesis of atherogenesis, COPD, or cancer in these tissue systems. I suggest addition of microscopic examination and morphometry of laryngeal tissue sections, as described in numerous publications of rodent inhalation studies on tobacco smoke/modified tobacco products.

2-Year studies to the point of cancer (lung, esophageal) and infarcts (if mice develop infarcts) maybe coupled to measures of ischemia. It would be interesting to prove that THS2.2 at the maximum exposure level did not increase lung tumors in experimental animals. 3. If smoking is linked to hypertension could you add an animal model prone to hypertension?

I have no specific proposals

None

Consider longer exposures. Considering exposure group that switches from THS to CS

For future studies to investigate further the systemic effect of both 3R4F and THS2.2 / other MRTP aerosol exposure it would have been good to measure the role/ changes of intracellular calcium concentration at systemic (blood) level using calcium imaging technique. As intracellular Ca2+ level plays and important role in the regulation of VSMCs and endothelial cell proliferation for the onset of cardiovascular diseases or atherosclerosis plaque formation.

Strict Determination of particulate size, quantity and distribution; avoid whole animal exposure (use nose only) for 7 days/week

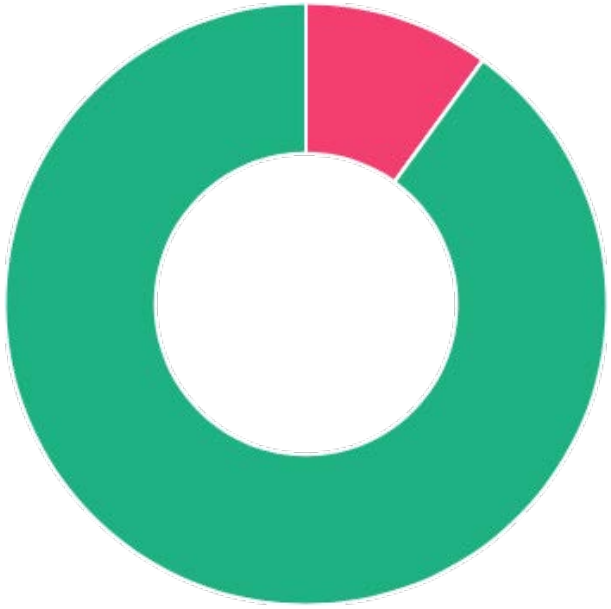
Histology and proteomics in heart could add value for systemic effects of both 3R4F and THS2.2 / other MRTP aerosol exposure.

Long term in vivo studies to assess both non-neoplastic and neoplastic end points both in respiratory and cardiovascular systems

Appendix B: Survey Results

ID #2011 (https://scipinion.com/surveys/68/results/2011)

Considering the in vivo study and the underlying evidence you have reviewed, how does the biological impact of switching to THS2.2 compare with the biological impact from smoking cessation?



Legend

- Switching leads to a greater biological impact than cessation: 1 *(10%)*
- Switching leads to a smaller biological impact than cessation: 0 *(0%)*
- Both switching and cessation lead to similar biological impacts.: 9 *(90%)*
- Those two groups cannot be compared (please explain the comment box): 0 *(0%)*
- I cannot answer (please explain in the comment box): 0 *(0%)*

answers: 10

skips: 0

ID #2012 (https://scipinion.com/surveys/68/results/2012)

Can the approaches reported for the assessment of cigarette smoke or THS2.2 aerosols be applied for a risk assessment of other inhaled toxicants, e.g. environmental air pollution, workplace exposure, etc.?

Yes the assessment of cigarette smoke or THS2.2 aerosols can be applied for a risk assessment of environmental air pollutants and workplace exposure. The approach could possibly represent a realistic exposure conditions and could be emulated to evaluate the risk of occupational and environmental pollutants particularly airborne.

Yes.

In principle yes although specific use cases depend on the toxicants under study.

Yes, as pointed out in the publications you refer to under additional details. Future inhalation or oral exposure studies on many potential inhaled or orally exposed compounds and environmental or occupational contaminants could and should utilize the in vivo and in vitro methods and the data presented in these papers.

Potentially. Depends on the toxicant - for example, whether it is a gas, aerosol or adsorbed on particles.

Possibly but the true assessment lies in the set up and control of the study. The present study seemed designed to culminate in a certain result (which was achieved) but its control and technical methodology were not sufficiently rationalized, particularly where method differences exist for the 3R4F vs THS2.2 processes.

Yes. You're paving the way for methodological advances fortesting respiratory toxicology (e.g. 3Rs for respiratory sensitization, irritation and general toxicology). You are doing very good work but keep in mind the limitation of bioinformatics with multiple comparisons and all of the skepticism that us older toxicology types hold towards some of these black box methods and the conclusions one can draw from bioinformatics.

Yes, the approaches reported for the assessment of cigarette smoke or THS2.2 aerosols can be surely use for the risk assessment of other inhaled toxicants. Apoe-/- mice have already been extensively used to study the molecular mechanism for the onset of cardio-pulmonary impairments following exposure to cigarette smoke and environmental pollutants (ultrafine aprticles). Similarly the systems (in vitro) used by Poussin et al and Van der Toorn et al publications are the very well established and standard approaches to explore the effect of inhaled toxicants on monocyte adhesion to endothelial cells and transendothelial migration, which plays an important role in the progression of atherogenesis. Although application of toxicants (aerosol/particles) in the submerged in vitro model does not represent the in vivo situation and other disadvantage of submerged cell exposure to particles is that the motion of particles/toxicants in liquids is mainly driven by random motion (diffusion). Consequently, under submerged conditions a substantial fraction of the particles will either remain in the liquid or be lost to the lateral walls of the cell culture vessel, which alters the actual cellular dose. However, both the authors have measured a wide variety of exposure mediated endpoints to explore the details patho-mechanism of atherogenesis , which will provide the next level of knowledge for evaluation of

inhaled toxicants and risk assessments. Correspondingly, the relevancy and applicability of both in vitro human 3-D nasal and oral epithelial culture models as test systems for toxicity assessment of inhalation exposure, by Iskandar et al. and Zanetti et al. are more close to in vivo model as this ALI-3D are considered as the physiologically relevant in vitro model and becoming a realistic alternative of in vivo model for toxicity screening. Additionally this ALI-3D system will provide an ideal platform to study cell to cell interaction and risk assessment of several chronic diseases (e.g. pulmonary and cardiac diseases) following exposure to occupational and environmental toxicants.

YES

I definitely think so. A broad range of e.g. urban air pollutants (e.g. diesel exhaust particles, combustion particles) and workplace exposure (e.g. insoluble dust) cold be assessed in a similar manner.

Appendix B: Survey Results

ID #2013 (https://scipinion.com/surveys/68/results/2013)

What are the most important biological effects of cigarette smoke exposure that are relevant to human disease (COPD and CVD) and considering the 5 publications under review, how strong (1=weakest; 5=strongest) is the evidence supporting a reduction in effects from THS2.2 exposure compared with 3R4F exposure?

	Importance	Evidence strength for reduction	Total
Inflammation	*3* **10.00%** 1 *4* **10.00%** 1 *5* **80.00%** 8	*3* **10.00%** 1 *4* **50.00%** 5 *5* **40.00%** 4	10
Oxidative stress	*3* **30.00%** 3 *4* **20.00%** 2 *5* **50.00%** 5	*2* **20.00%** 2 *3* **10.00%** 1 *4* **50.00%** 5 *5* **20.00%** 2	10
Extracellular matrix (ECM) synthesis and degradation	*3* **30.00%** 3 *4* **60.00%** 6 *5* **10.00%** 1	*2* **10.00%** 1 *3* **20.00%** 2 *4* **70.00%** 7	10

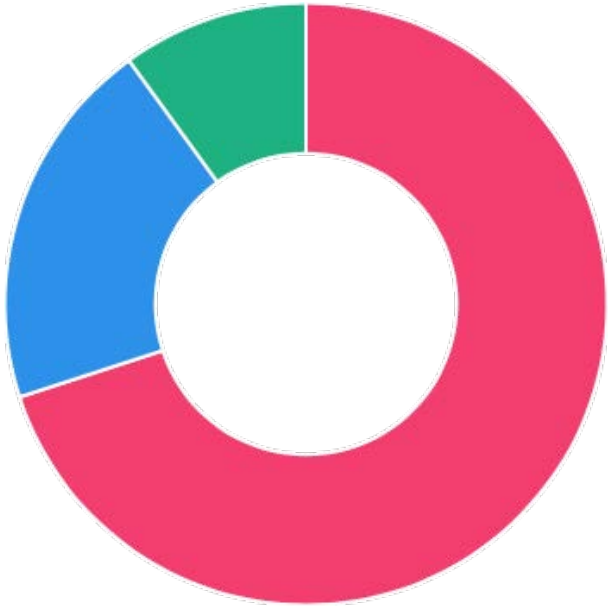
Appendix B: Survey Results

Metabolic alterations	*3* **70.00%** 7 *4* **30.00%** 3	*2* **10.00%** 1 *3* **30.00%** 3 *4* **40.00%** 4 *5* **20.00%** 2	10
Other (please specify)	*4* **50.00%** 1 *5* **50.00%** 1	*4* **100.00%** 2	2

Appendix B: Survey Results

ID #2014 (https://scipinion.com/surveys/68/results/2014)

The authors of the studies reported in the 5 publications and manuscripts under review aimed at reducing and refining the exposure of animals in vivo by assessing two human disease models in the same inhalation toxicity study and to maximize the use of various tissues from the same animals within an array of classical toxicity end points plus transcriptomics, proteomics, and lipidomics. Moreover, they have developed in vitro models to enable detailed comparative assessment and mechanistic evaluations. Do you think this approach was efficient and worthwhile, and how could it be further improved?



Legend

- The approach was indeed efficient and should be further considered in future studies: 7 *(70%)*
- Some compromises in design (please identify these and describe in the comment box) are not weighed out by the benefits, and hence separate models would be more efficient: 2 *(20%)*
- Further reduction of animals could be envisioned by the following accomplishments: (please specify): 1 *(10%)*
- I cannot answer (please explain in the comment box): 0 *(0%)*

answers: 10

skips: 0

Appendix B: Survey Results

ID #2015 (https://scipinion.com/surveys/68/results/2015)

Considering the mechanistic in vitro assays and the assessment using in vitro organotypic models, how would you characterize each measurement regarding its usefulness for comparison to human clinical exposure-response studies?

	very useful	useful	not useful	cannot answer	Total
Poussin et al. Functional adhesion assay	40.00% 4	50.00% 5	10.00% 1	0.00% 0	10
Poussin et al. Inflammatory mediators	30.00% 3	70.00% 7	0.00% 0	0.00% 0	10
Poussin et al. Transcriptomics analysis	20.00% 2	50.00% 5	30.00% 3	0.00% 0	10
Van der Toorn et al. Transendothelial migration assay	20.00% 2	70.00% 7	10.00% 1	0.00% 0	10
Van der Toorn et al. Endothelial barrier integrity	30.00% 3	50.00% 5	10.00% 1	10.00% 1	10
Van der Toorn et al. Cytotoxicity and inflammatory behavior of THP-1 cells	30.00% 3	50.00% 5	10.00% 1	10.00% 1	10

ID #2016 (https://scipinion.com/surveys/68/results/2016)

Considering the assessment using in vitro organotypic models, how would you characterize each measurement regarding its usefulness for comparison to human clinical exposure response studies?

	Very useful	Useful	Not useful	Cannot answer	Total
Iskandar et al. Histopathological evaluation	30.00% 3	60.00% 6	10.00% 1	0.00% 0	10
Iskandar et al. Cytotoxicity (AK assay)	20.00% 2	70.00% 7	10.00% 1	0.00% 0	10
Iskandar et al. CYP1A1/B1 activity assay	10.00% 1	70.00% 7	20.00% 2	0.00% 0	10
Iskandar et al. Ciliary beating frequency assay	40.00% 4	60.00% 6	0.00% 0	0.00% 0	10
Iskandar et al. Characterization of pro-inflammatory mediators	10.00% 1	90.00% 9	0.00% 0	0.00% 0	10
Iskandar et al. Transcriptomics analysis	0.00% 0	100.00% 10	0.00% 0	0.00% 0	10
Iskandar et al. miRNA analysis	0.00% 0	70.00% 7	30.00% 3	0.00% 0	10
Zanetti et al. Histopathological evaluation	30.00% 3	70.00% 7	0.00% 0	0.00% 0	10
Zanetti et al. Cytotoxicity (AK assay)	20.00% 2	80.00% 8	0.00% 0	0.00% 0	10
Zanetti et al. CYP1A1/B1 activity assay	20.00% 2	60.00% 6	10.00% 1	10.00% 1	10
Zanetti et al. Characterization of pro-inflammatory mediators	30.00% 3	70.00% 7	0.00% 0	0.00% 0	10
Zanetti et al. Transcriptomics analysis	0.00% 0	90.00% 9	10.00% 1	0.00% 0	10
Zanetti et al. miRNA analysis	0.00% 0	70.00% 7	20.00% 2	10.00% 1	10
	10.00%	70.00%	10.00%	10.00%	

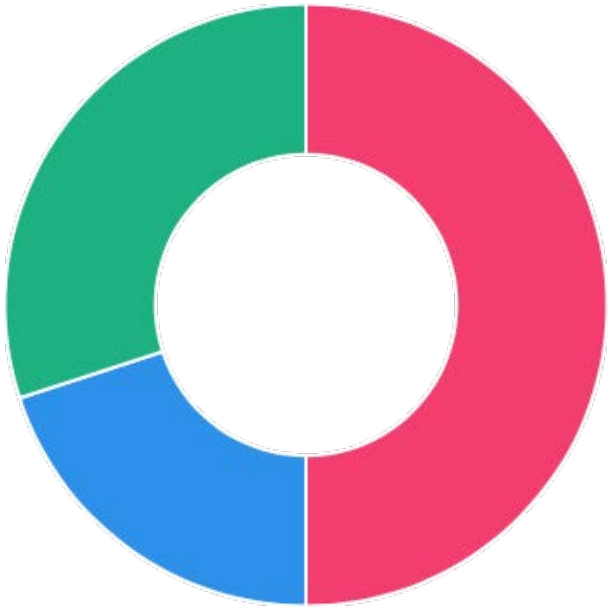
Appendix B: Survey Results

Zanetti et al. Endpoint reproducibility/variability analysis	1	7	1	1	10

SECTION: TOOLS AND REVIEW PROCESS

ID #2017 (https://scipinion.com/surveys/68/results/2017)

Was the access to the digitalized slides in the Aperio platform useful for your review?



Legend

- Yes: 5 *(50%)*
- No: 2 *(20%)*
- I did not visit the portal: 3 *(30%)*

answers: 10
skips: 0

Appendix B: Survey Results

ID #2018 (https://scipinion.com/surveys/68/results/2018)

How would you qualify the annotation (e.g. use of ontologies, richness of annotation), and the structure (isa-tab format) of the data available in the underlying database (via the sbv IMPROVER systems toxicology Web Portal (1=low; 5=high)?

	Sufficient Detail	Intuitive	Informative / Helpful	Total
Data annotation	*3* **11.11%** 1 *4* **66.67%** 6 *5* **22.22%** 2	*1* **10.00%** 1 *2* **10.00%** 1 *3* **10.00%** 1 *4* **60.00%** 6 *5* **10.00%** 1	*3* **22.22%** 2 *4* **66.67%** 6 *5* **11.11%** 1	10
Description of protocols (M&M)	*2* **11.11%** 1 *4* **44.44%** 4 *5* **44.44%** 4	*2* **10.00%** 1 *3* **10.00%** 1 *4* **60.00%** 6 *5* **20.00%** 2	*3* **22.22%** 2 *4* **44.44%** 4 *5* **33.33%** 3	10
	3 **11.11%**	*2* **10.00%** 1	*3* **22.22%**	

Appendix B: Survey Results

Use of ontologies	1 *4* **55.56%** 5 *5* **33.33%** 3	*3* **10.00%** 1 *4* **60.00%** 6 *5* **20.00%** 2	2 *4* **55.56%** 5 *5* **22.22%** 2	10
Raw data format	*1* **11.11%** 1 *2* **11.11%** 1 *3* **11.11%** 1 *4* **33.33%** 3 *5* **33.33%** 3	*1* **30.00%** 3 *2* **10.00%** 1 *3* **10.00%** 1 *4* **40.00%** 4 *5* **10.00%** 1	*1* **11.11%** 1 *2* **11.11%** 1 *3* **22.22%** 2 *4* **44.44%** 4 *5* **11.11%** 1	10
Processed data format	*3* **11.11%** 1 *4* **55.56%** 5 *5* **33.33%** 3	*1* **10.00%** 1 *2* **10.00%** 1 *4* **60.00%** 6 *5* **20.00%** 2	*2* **11.11%** 1 *4* **66.67%** 6 *5* **22.22%** 2	10
		1		

Appendix B: Survey Results

ISA tab format	*3* **12.50%** 1 *4* **50.00%** 4 *5* **37.50%** 3	**11.11%** 1 *2* **11.11%** 1 *4* **55.56%** 5 *5* **22.22%** 2	*2* **12.50%** 1 *4* **62.50%** 5 *5* **25.00%** 2	9
Illustrations in M&M	*4* **33.33%** 3 *5* **66.67%** 6	*2* **10.00%** 1 *4* **30.00%** 3 *5* **60.00%** 6	*4* **33.33%** 3 *5* **66.67%** 6	10

Appendix B: Survey Results

ID #2040 (https://scipinion.com/surveys/68/results/2040)

In collaboration with Selventa, the SDPplus platform has been made available for this review. The SDPplus platform allows users to evaluate transcriptomics data in reverse causal analyses, including the network perturbation amplitude analyses. Algorithms built into this platform use measurable biology to infer casual mechanisms or biological impact on causal network models. Based on your experience with the SDPplus platform, please select all that apply:



Legend

- SDPplus allows users to assess which key processes or pathways are most impacted by a given treatment: 2 *(11%)*
- SDPplus allows users to explore large-scale omics study datasets in a comprehensive manner: 2 *(11%)*
- The implementation of the network perturbation amplitude (NPA) and Biological Impact Factor (BIF) in SDPplus allows users to assess and compare transcriptomics profiles in an insightful manner: 4 *(22%)*
- SDPplus is intuitive and easy to use and benefit from: 2 *(11%)*
- A platform such as SDPplus is very useful and should be made available to the whole scientific community: 2 *(11%)*
- I did not understand the intended purpose of the platform and/or the presentation of the results, and therefore did not benefit from it: 3 *(17%)*
- I cannot comment on SDPplus (please explain in the comment box): 3 *(17%)*
- None of the above: 0 *(0%)*

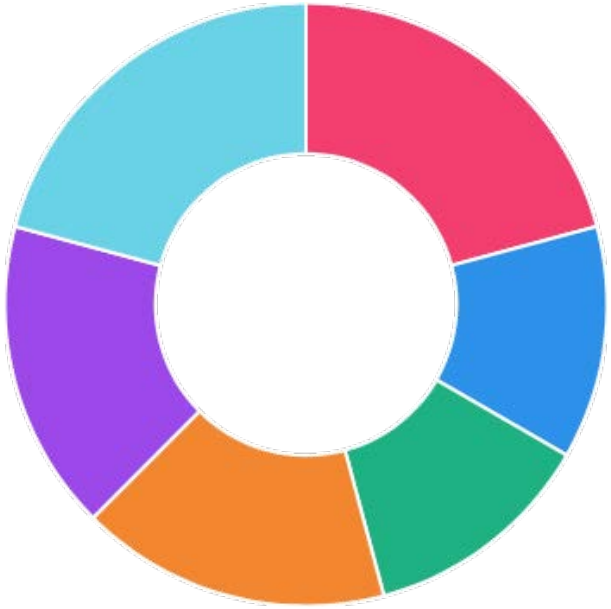
answers: 10

skips: 0

Appendix B: Survey Results

ID #2041 (https://scipinion.com/surveys/68/results/2041)

In collaboration with the Systems Biology Institute in Japan, custom-built software called Garuda gadget, have been made available for this review. Follow the instructions in the details section below to be able to install and use the gadgets. Based on your experience with the gadgets, please select all that apply:



Legend

- Garuda gadgets allows users to assess which key processes or pathways are enriched for biological functions for a given set of genes under a specific condition of study: 5 *(21%)*
- Garuda gadgets allows users to explore large-scale omics study datasets and perform enrichment for genes and proteins: 3 *(13%)*
- Garuda gadgets allows users to assess and compare the transcriptomics profiles for a specific gene or set of genes between two study conditions: 3 *(13%)*
- Garuda gadgets allow the visualization of pathways associated with the genes in transcriptomics profile data: 4 *(17%)*
- Garuda gadgets are very useful and should be made available to the whole scientific community: 4 *(17%)*
- I cannot comment on Garuda (please explain in the comment box): 5 *(21%)*
- None of the above: 0 *(0%)*

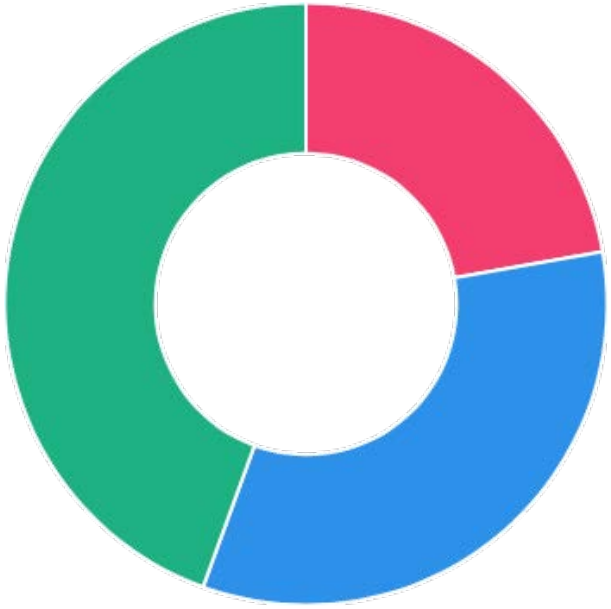
answers: 10

skips: 0

Appendix B: Survey Results

ID #2021 (https://scipinion.com/surveys/68/results/2021)

Tableau is a software for visualization and data analytics (http://www.tableau.com). Usually, authors of scientific publications choose specific data visualization (figure) to convey a particular message. Reviewers and readers may want to view the data in other ways to fully grasp the relevance of it. To allow for interactive browsing of study results, a Tableau workbook was prepared (see question-specific link in the Introduction). In your opinion, this workbook (select all that apply):



Legend

- Is superfluous given the images that are already provided in the publication: 2 *(22%)*
- Could be useful but is not easy to understand: 3 *(33%)*
- Is useful and should be included as supplementary material for publication of large studies whenever possible: 4 *(44%)*

answers: 9

skips: 1

ID #2022 (https://scipinion.com/surveys/68/results/2022)

What additional material/information would you wish to have to better assess the relevance of the studies and their results?

Sufficient material.

Authors have extensively provided the data or information for reviewing and it is sufficient to assess the relevance of the studies and their results.

Would like to know who is sponsoring this survey and how the results are being used; Information on how the platform of causal biological network models was developed and by whom.

More specific information correlating omics data with morphologic data.

The materials/information provided here are sufficient and details to review the studies.

I have no concerns with the availability of the datasets.

For the next time - provide pdfs of paper copies with tables, graphics, etc. compiled so you don't have to piecemeal looking at the data. I'd prefer printing off hard copies to study and easily refer back to information with simultaneous comparisons being made possible, as opposed to a single computer screen. For each reviewer, they should receive a packet of materials as well as access to the website materials. I do not review complicated and dense materials/data on computer screens as a typical course of action.

There was sufficient material with greater details from these manuscripts

None.

Appendix B: Survey Results

ID #2042 (https://scipinion.com/surveys/68/results/2042)

Regarding the information provided on the sbv IMPROVER systems toxicology Web Portal (select all that apply)



Legend

- The navigation on the website was intuitive and I found the information I needed easily: 6 *(26%)*
- The content was not easily accessible: 4 *(17%)*
- Background information provided in the question pages was misleading: 0 *(0%)*
- Both enough and appropriate information were provided in the question pages to answer to the questions: 4 *(17%)*
- The tutorial videos were useful to efficiently review the material: 5 *(22%)*
- The tutorial videos and tools were distracting for the review purposes: 1 *(4%)*
- I was lacking information and had to go back to the article reprint on the publisher's website: 1 *(4%)*
- I was missing key functionalities (please specify below): 2 *(9%)*
- I did not visit the portal: 0 *(0%)*

answers: 10

skips: 0

Appendix B: Survey Results

ID #2024 (https://scipinion.com/surveys/68/results/2024)

How could this peer review process be improved?

Better communication between reviewers and staff.

Some of the manuscripts/drafts (perhaps they are under review) had poor descriptions and representations of the study designs although the methods/results were comprehensive making it very time consuming to understand the study design and its rational. If this can be fixed it would significantly help the reviewers.

1. Hard Copies. (not of the raw data but of the multiple data tabs provided for each study. 2. Place the question surveys directly in the methods/data areas for each study. Do not force the reviewer to quit their review, go to the general SciPinion location, and then wade through the extensive series of questions. This was not efficient.

This is an excellent work platform; all the information regarding each manuscript are nicely available for reviewers.

The peer review process is of good standards according to this reviewer. It is well designed and executed. However, it may be improved by providing similarity index of the manuscripts with that of related published articles.

There is a question on how these papers are singled out for review. Is it at the request of the Sponsor or by SciPinion? Is it because of the potential impact of the subject to public health? This should be stated in the Introductory page. How is this information being used? By whom? More information provided to the survey experts is strongly suggested. Lastly, How is SciPinion associated with Phillip Morris since Boue et al, 2015 indicates a collaboration? Please cite any conflicts of interest of the SciPinion forum. With this, the process seems reasonable.

It would be really helpful to allow more time for peer-reveiw process.

I think everything work fine.

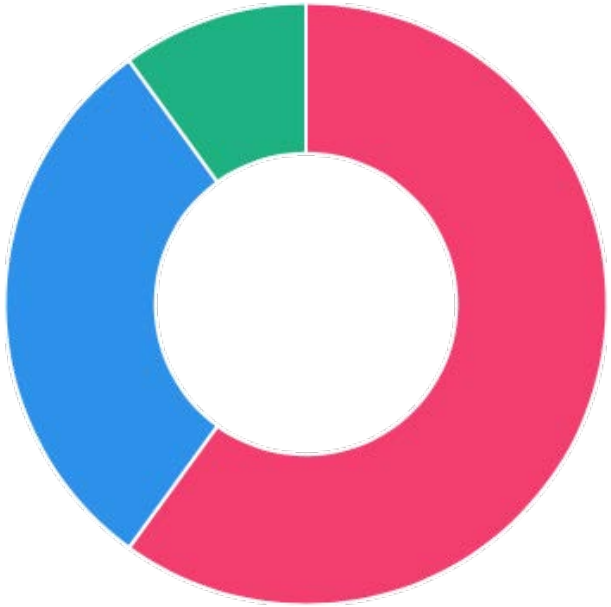
Overall, I found the process easy to follow and information was available when I needed it and where I expected to find it.

See above; more access to the systems i listed above. The Zanetti et al paper was very disorganized and long and should not be reviewed until it is reorganized and shortened. Please provide a full page width box for reviewers to type in their answers and comments.

Appendix B: Survey Results

ID #2025 (https://scipinion.com/surveys/68/results/2025)

Your participation in this peer review is completely anonymous. For future peer reviews, how important was anonymity to your participation in this peer review?



Legend

Very important: I don't want anyone to know that I took part in this peer review: 6 *(60%)*

Somewhat important: I would be comfortable having my name disclosed, but want my individual responses to the questions to remain undisclosed.: 3 *(30%)*

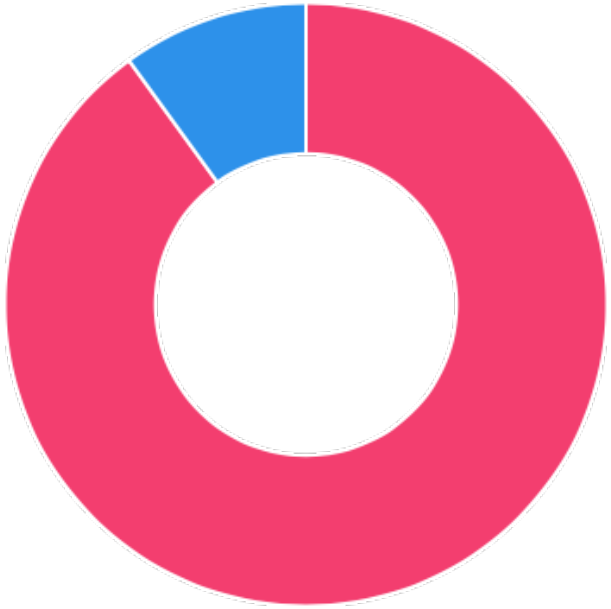
Not important: I would be comfortable having my name and individual responses to the questions disclosed: 1 *(10%)*

answers: 10

skips: 0

ID #2026 (https://scipinion.com/surveys/68/results/2026)

Your participation in this peer review was completely independent. For future peer reviews, would you prefer to have anonymous interaction with other panel members (e.g., online comment/debate)? Please indicate the reasons for your answer in the text box.



Legend

Yes: 9 *(90%)*

No: 1 *(10%)*

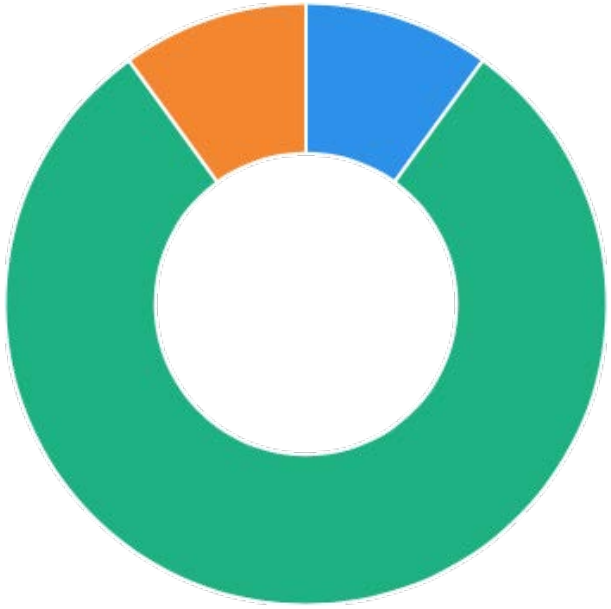
answers: 10

skips: 0

Appendix B: Survey Results

ID #2027 (https://scipinion.com/surveys/68/results/2027)

Your participation included an offer of compensation. For future peer reviews, how important is compensation for your participation?



Legend

- Not important: I would be willing to participate for free: 0 *(0%)*
- Somewhat important: I prefer some level of compensation for my participation, but this could be less than received for this effort: 1 *(10%)*
- Important: I require compensation for my participation, and the level of compensation is appropriate: 8 *(80%)*
- Very Important: I require compensation for my participation, but the level of compensation would need to be increased: 1 *(10%)*

answers: 10
skips: 0

Appendix B: Survey Results

Appendix C: Auditor's Report
NeutralScience Audit 3 (NSA3) for SciPinion Expert Panel

April 27, 2016

Documents received for review

Audit package documents were received from SciPinion on April 14, 2016 with update addition of 2 candidates on April 18. The complete package was confirmed as received by the Auditors on April 18, 2106. The complete package as received from SciPinion is included with the email transmittal for this report, including the Table 1 Excel file as referenced in the Auditor reports. Table 1 is also printed in Appendix 1. SciPinion requested audit of only the 22 candidates at the top of Table 1. These candidates are listed in the summary table below. The Auditors also reviewed the general topic of the panel as presented in the Audit package.

Auditors selected (CV's provided in Appendix 2)

Jim Willis

Babasaheb (Bob) Sonawane, PhD

Audit findings

The individual audits are provided in Appendix 2 with comments on each candidate review. A ranking system was used by the Auditors where 0 indicates that the candidate did not meet the minimum expertise criteria, 1 indicates that the candidate met the criteria, and 2 indicates that in addition to meeting the criteria the candidate was well qualified for the described task. A summary of findings is presented below.

Summary table

Sonawane	Willis	Composite	Candidate ID #	Name
2	1	3	571	[redacted]
2	2	4	1003	[redacted]
1	1	2	1019	[redacted]
2	0	2	1039	[redacted]
1	0	1	1032	[redacted]
2	2	4	531	[redacted]
1	2	3	1291	[redacted]
2	0	2	669	[redacted]
2	1	3	601	[redacted]
1	1	2	1292	[redacted]
1	0	1	831	[redacted]
2	2	4	547	[redacted]
1	1	2	818	[redacted]
2	1	3	1280	[redacted]
2	2	4	1297	[redacted]
0	0	0	1304	[redacted]
1	0	1	1309	[redacted]
2	0	2	1312	[redacted]
2	1	3	1319	[redacted]
2	0	2	48	[redacted]

1	1	2	518	[redacted]
1	0	1	539	[redacted]

Eight candidates received a score of 0 (does not meet minimum criteria) from one of the Auditors.



Richard Canady, PhD
NeutralScience L3C
rcanady@neutralscience.org

Appendix 1 – "Table 1" as received from SciPinion April 18, 2016 and referenced in the Auditor reports.

[To maintain anonymity, Table 1 has been deleted]

Appendix 2 – Individual Auditor Reports including CV's for Auditors and with reference to associated files in email transmittal to SciPinion.

Auditor Report: SciPinion Scientific Review Package NSA3

Independence of the Auditor
The identity of the Sponsor of this peer review was not conveyed by SciPinion or by NeutralScience to this Auditor.

Panel Expertise Verification
I have reviewed the expertise of the 20 candidates identified by Candidate ID # in the **Audit Summary Table** below. My review has included CV's, publications, indicators of experience available through independent sources, web pages of home institutions, selected manuscripts and the descriptions, data and minimum experience criteria for the peer review panel as described by SciPinion through NeutralScience on April 15, 2016. Based on my review and comparison to minimum expertise criteria and the peer review needed, I have provided a score for each candidate of **0** (does not meet minimum criteria), **1** (meets minimum criteria) or **2** (meets minimum criteria and is also well qualified for this peer review).

Audit Summary Table

Candidate ID #	Name	**Review outcome***
571	[redacted]	2
1003		2
1019		1
1039		2
1032		1
531		2
1291		1
669		2
601		2
1292		1
831		1
547		2
818		1
1280		2
1297		2
1304		0
1309		1
1312		2
1319		2
48		2
518		1
539		1

Notes on each candidate appear in Appendix 1 to this report.

Auditor: Babasaheb (Bob) Sonawane, PhD

Signature: B P Sonawane Date: ~~April 23,216~~ April 24, 2016

Appendix 1

Auditor comments

Candidate ID #	Name	Review outcome*	Auditor comments
571	[redacted]	2	Extensive and broad experience in toxicology and regulatory aspects of pharmaceuticals and food contaminants safety(preclinical toxicology
1003		2	Excellent experience in toxicological pathology and molecular biology
1019		1	A very good experience mostly limited to site-specific risk assessments. Good knowledge and experience of some regulatory aspects toxic pollutants
1039		2	A very good wide experience in toxicology and risk assessment of drinking water contaminants
1032		1	Relevant experience in cardiovascular research using genetics and statistical software packages. Her contributations to or/role in as being one of the several authors of publications is questionable.
531		2	A very well recognized expertise in inhalation toxicology, toxicological pathology of several chemical agents exposure on respiratory tract. Excellent record of publications. Well qualified.
1291		1	Research focus on health effects of Red Tide Toxins and some other agents. Limited knowledge /experience in PK/TK and safety assessment of of environmental agents, A fairly good record of publications
669		2	Excellent demonstrated international experience and collaboration with several institutions/ organizations on environmental and occupational exposure and health effects of nanomaterials and airborne pollutants. A very good record of publications as well as risk communication.
601		2	Expertise in chemical food safety, computational methods and endocrine disrupting chemicals (phthalates) and fungicides etc; A very good record of publications in repro/ developmental toxicology, biochemistry and endocrinology. Well qualified.

1292	[redacted]	1	Good research experience in cardiovascular biology, thrombosis, hematology etc; A very good record publications mostly as a co-author.
831		1	Limited focus on nanomaterials research. several presentations to European organizations. publication record is scant to adequate
547		2	A very well recognized expertise in cardiovascular and pulmonary toxicology. Reviewer of manuscripts for several scientific journals. Excellent record of pulications on health effects of variety of chemicals. Highly qualified as reviewer toxicological tasks. A highly qualified as a reviewer.
818		1	Expertise in metals(Zn, Al, Ni,Vanadium,etc;) and genotoxicty. Limited experience in inmmunotoxicology. Publication record is adequate.
1280		2	Excellent knowledge and experience in health risk assessment of environmental agents, some PBPK modeling, quality assurance of tox. data, and recent involvement in NextGen risk assessment of NCEA/ORD/EPA. A fairly good record of publications and presentations to professional society meetings.
1297		2	A very good research experience in pulmonary, and cardiovascular biology and immunology effects on exposure to fine particles, and nanoparticles at molecular level, however, limited number of publications.
1304		0	Research publications in immunochemistry, mouse mammary gland and liver cancer. Participation in several activities but seems to be unfocused and the demonstrated record of publications is very scant.
1309		1	A very good record of academic research experience in developmental biology, specifically on Sea Urchin. Excellent record of mentoring and training graduate and post-doctoral fellows. A good record as a reviewer of

	[redacted]		scientific manuscripts of developmental biology types of journals.
1312		2	Excellent expertise in computational biology, and bioinformatics, regulatory networks of transcriptional and post-transcriptional, levels and genomics data. Peer reviewer of several journals and very good record of presentations and publications. Highly qualified for review of systems biology and bioinformatics data.
1319		2	A very good record of research experience in cancer systems biology, gamma radiation immunology and MOA information. A good record of publications and presentations at national and international level meetings.
48		2	A very well recognized scientist with a good record of publications in pharmacology and toxicology. A very well qualified to review scientific issues and reports on variety of subjects from in vitro/in vivo toxiclogical data to molecular mechanisms of toxicity as well as NextGen risk evaluation.
518		1	A fairly good record of publications in lung cell biology, immunology and genetics.
539		1	Expertise in mechanisms of lung toxicity, specifically tobacco/ cigarette smoke in mice. Limited experience as a reviewer.

* Score of 0 indicates that the candidate did not meet the minimum expertise criteria, 1 indicates that the candidate met the criteria, and 2 indicates that in addition to meeting the criteria the candidate was well qualified for the described task.

CURRICULUM VITAE

Babasaheb (Bob) Sonawane, Ph.D.

Address: 13204 Moran Drive
North Potomac, MD 20878
Email: bobrsonawane@yahoo.com
Tel: 301-869-7757 (home); 301-801-8938 (cell)

EDUCATION:

1971 **Ph. D.**, *in Entomology-Toxicology,* Univ. of Missouri, Columbia, Missouri, USA
1965 **M.S. with Honors** *in Entomology***,** University of Pune, Pune, India
1962 **B.S. with Honors,** *in Agricultural Sciences,* University of Pune, Pune, India

PROFESSIONAL EXPERIENCE:

1995 – 2015 *Supervisory Interdisciplinary Toxicologist*
Effects Identification and Characterization Group
National Center for Environmental Assessment-Washington Division,
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

1990 – 1995 *Chief, Reproductive and Developmental Toxicology Branch*
Human Health Assessment Group
Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

1985 – 1990 *Biologist*
Reproductive Effects Assessment Group
Office of Health and Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

1983 – 1985 *Senior Staff Fellow*
Division of Toxicology

Center for Veterinary Medicine
Food and Drug Administration
5600 Fishers Lane
Rockville, MD

1976 – 1983 *Research Assistant Professor of Pediatrics and Animal Biology*
School of Medicine and School of Veterinary Medicine
University of Pennsylvania
Philadelphia, PA

1975 – 1976 *Senior Research Associate*
Depart of Pediatrics
Children's Hospital of Philadelphia
Philadelphia, PA

1972 – 1975 *NIH Post-Doctoral Fellow*
National Institute of Environmental Health Sciences
Research Triangle Park, NC

1971 – 1972 *Research Specialist in Toxicology*
School of Medicine
University of Missouri
Columbia, MO

DETAIL ASSIGNMENTS

Oct. 1999 – Feb. 2000 *Acting Director*
National Center for Environmental Assessment-Washington Division
NCEA/ORD/US EPA
Washington, DC

April – July 1990 *Acting Deputy Director*
Environmental Criteria and Assessment Office
Office of Health and Environmental Assessment
Cincinnati, OH

Sep. – Dec., 1989 *Acting Branch Chief*
Reproductive and Developmental Toxicology Branch

Human Health Assessment Group
Office of Health and Environmental Assessment
ORD/US EPA
Washington, DC

LEADERSHIP PROVIDED TO THE SCIENTIFIC COMMUNITY (Selected examples):

- Member Interagency Committee on National Children Study (2006-2007).
- Member Indo-US Sub-Commission for Science and Technology (1987 – 2002).
- EPA liaison to the Environmental Health Committee of the American Academy of Pediatrics (1993 – 2001).
- Member of the Office of Toxic Substance Testing/Research Priorities Task Force SARA section 110 (1999).
- Represented ORD to the Agency's Science and Policy Committee for Reinvention of Federal Government- Vice President Gore Initiative (1993) and served on the National Performance Review Committee (1993).
- EPA's Developmental Toxicity Risk Assessment Guidelines (1991).
- Invited Participant to the FDA's Dental Products Review Panel (1991).
- Member of Office of International Activities, United Nations Advisory Working Group (1991).
- Member of the Committee Advising Office of Mobile Sources –Methanol as a Fuel in the 1990s.
- Member, Risk Assessment Forum for "Cholinesterase Inhibition as An Indication of Adverse Toxicological Effect" (1987-1989).
- Member of the Toxicity Matrix Work group, SARA Section 313 (1988).
- EPA's Reproductive Toxicity Risk Assessment Guidelines (1987).
- Manuscripts reviewer for the scientific journals: Carcinogenesis, Cytokine, Science, Critical Reviews in Toxicology, Journal of Toxicology and Environmental Health, Environmental Health Perspectives, Reproductive Toxicology, Neurotoxicology, Teratology, Environmental Research, Toxicological Sciences, Regulatory Toxicology and Pharmacology, Toxicology and Applied Pharmacology, Risk Analysis, Environment International, etc.
- Member AAALAC Board representing-Teratology Society.
- Organizer/Participant/Sponsor of over 50 scientific conferences, symposia/workshops at National and International levels related to children's environmental health, toxicology, risk assessment of chemicals, benefit- cost analysis etc,
- Member ORD/EPA Aging Research Plan Team.
- Member of several inter- and intra-agency committees, workgroups and taskforces related to toxicology, children's environmental health and risk assessment of environmental agents.
- Member of the Chemical Emergency Preparedness Program of EPA.

- Member of the Review Board on the California Proposition 65.

TECHNICAL ASSISTANCE/LEADERSHIP PROVIDED TO THE AGENCY (Examples):

- Member of the ORD/EPA-wide Committee on Honorary Awards (2008 to 2014).
- EPA Representative and Reviewer of 12th Report on Carcinogens (ROC) to Congress (2011) prepared by the National Toxicology Program of NIEHS/HHS (Aristolochic acids, Captafol, Certain Glass Wool Fibers, Cobalt-tungsten Carbide-powders and Hard Metals, Formaldehyde, ortho –Nitrotoluene, Riddelline, and Styrene).
- Provided leadership, reviewer and/or provided technical assistance in developing, coordinating and managing several health risk assessment of chemicals for the Agency programs. Recent examples include but not limited to: Libby Amphibole Asbestos, Ethylene oxide, Phthalate Esters, Chromium III and VI, Benzene, Trichloroethylene, Tetrachloroethylene, Phosgene, Vinyl Chloride, 1,3- Butadiene, Formaldehyde, n-butanol, Vanadium Pentoxide, Tetrahydrofuran, Nitrobenzene, MTBE, MMT, PCBs non-cancer, Dioxins,1,2 Dichloroethane, Methyl Mercury, Methanol, Antimony, Diesel Exhaust, PAH's, Dioxins, Cadmium, Lead, Strontium an Strontium Compounds, Glass Wool Fibers, etc.
- Provided leadership and/or assisted in developing NCEA/ORD annual operating plans and participated in the development of ORD's multi-year research and research strategies.
- Directed, and/or conducted research on developing methods, models, risk assessment guidelines, and databases to improve and better characterize uncertainties in risk assessment. Some of the recent examples include, but not limited to:
  - *Report: Lymphohematopoietic Cancers Induced by Chemicals and Other Agents:*
  - *Overview and Implications for Risk Assessment, Enzyme ontogeny database,*
  - *Use of Transgenic strains of mice and rats in MOA of cancer and non-cancer health risk evaluation,*
  - *A Framework for Assessing Risk to Children, Approaches for Incorporating Pharmacokinetics in Children's Risk Assessment,*
  - *Dose-metrics Adjustment and Scaling Factors Across Life stages,*
  - *Bisphenol-A Common Mode of Action—A Case Study,*
  - *Environmental Risk Factors and Prostate Cancer, Early Origins of Neurodegenerative Disease,*
  - *Biomarkers of Exposure and Effects in Children, Structure-Activity Application to Developmental Toxicity,*
  - *Aging and Toxic Response,*
  - *Application of PBPK Models in Risk Assessment,*
  - *Biomarkers of Leukemia in Children,*
  - *Genetic Polymorphisms and Metabolism of Xenobiotics*
  - *Excretion of Chemicals in Breast Milk, etc.*

Planned, organized, sponsored and/or participated in several National and International Scientific Workshops/Meetings and Conferences promoting children's environmental health and risk assessment issues. Examples include:

- Symposium on Aging Population as a Sensitive Subgroup - California Environmental Protection Agency held in Pacific Grove , CA (2006)
- Seven International Neuro-toxicology Conferences (1995 -2001)
- DOD/EPA Annual TriServices Conferences (1996-2001)
- WHO Conference on Environmental Threat to Children's Health, Manila, Philippines (2000)
- International Workshops on Indoor Molds and Children's Health in Alexandria VA (1998)
- Children Environmental Health Network (CEHN) – Research Needs Conferences (1995, 1997)
- Eight Indo-US Workshops, Conferences and Symposia on lead and other heavy metals
- Indian National Congress in Lucknow, India
- Indian Pharmacology Society Meeting in Nagpur, India
- Environment and Genetic Susceptibility in New Delhi, India
- Pediatric Pharmacology, in Ahmadabad, India
- Three Joint Conferences with Industrial Toxicology Research Center in Lucknow, India on toxicology and risk assessment issues related to health effects heavy metals, pharmaceuticals, pesticides and children's environmental health,
- International Symposium on Chelating Agents in Pharmacology and Therapeutics held in Pilsner (Czech Republic)
- NATO Conference on Environmental Health held in L'viv, Ukraine
- Other Conferences related to Women and Children's Environmental Health Risk Assessment in Ukraine, Thailand, Belgium, Italy, France, Canada, Argentina, Australia etc.

INVITED PRESENTATIONS (Examples):

- Organizer of Roundtable Discussion and Co-Chair: Trichloroethylene Exposure and Development of Fetal cardiac malformations: What the Date Tells Us about Inhalation exposure Resulting from Vapor Intrusion and Potential health Risks to pregnant Women. 55th Annual meeting of the Society of toxicology, New Orleans, LA; March, 2016.
- Organizer and Chairperson, Symposium 12- Environmental Epigenomics and Disease Susceptibility; 46th Annual Meeting of Environmental Mutagen and Genomic Society. New Orleans, LA; September, 2015.
- Primary Sponsor, Symposium 7 on The Carcinogenicity of Outdoor Air Pollution; 46th Annual Meeting of Environmental Mutagen and Genomic Society. New Orleans, LA; September, 2015.

- Chairperson, Perspectives on the Evaluation and Interpretation of Mutagenicity and Genotoxicity Data, 42nd Annual Environmental Mutagen Society meeting, Montreal, Quebec; October 15–19, 2011.
- Co-Chair, Mode of Action and Risk Assessment, Benzene-2009, Health Effects and Mechanisms of Bone Marrow Toxicity; Technische Universität München, Munich, Germany; September 7-11, 2009.
- Co-chair; Formaldehyde and Leukemia: Epidemiology, Potential Mechanisms, and Implications for Risk Assessment; EMS Annual Meeting, San Juan, Puerto Rico; October 18-21, 2008.
- Member, WHO/IPCS/CICAD Final Review Board; meeting held in Helsinki, Finland; Strontium and Strontium Compounds; Cyclic Acid Anhydrides, Inorganic Chromium III, Chromium VI and Compounds, March, 2007.
- Member, Steering Committee; Hershey Medical Center Technical Workshop: Optimizing the Design and Interpretation of Epidemiologic Studies for Assessing Neurodevelopment Effects from *in utero* Chemical Exposure, September, 2005.
- Member, WHO/IPCS/Concise International Chemical Assessment Document 68; Tetrachloroethene; Cobalt and inorganic cobalt compounds, 13th CICAD Final Review Board; Nagpur, India; November, 2005.
- Organizer and Co-chair, WHO/UNEP/ILO International Programme on Chemical Safety - Workshop on Advances in the Use of Biomarkers in Children; Buenos Aires, Argentina; November, 2005.
- Organizer and Co-chair; Workshop: How do neurotoxicants exploit genetic susceptibility? Twenty-First International Neurotoxicology Conference, Honolulu, Hawaii; February 10 -13, 2004.
- Member, Organizing Committee; Report of the International Conference on Environmental Threats to the Health of Children: Hazards and Vulnerability, Bangkok, Thailand; March, 2002.
- Invited to participate in the "Sixth International Symposium on Chelating Agents in Pharmacology, Toxicology and Therapeutics", Pilsen, Czech Republic, September, 2000.
- Invited to plan a Conference on "Chemical Excretion in Human Milk" with March of Dimes and to participate in the National Advisory Council's meeting for the Children's Health and the Environment (New York, NY); November, 2000.
- Member, Organizing Committee of Toxicology and Risk Assessment Conferences with DOD and other federal agencies at Wright Patterson US Air Force Base in Dayton, OH, for five years.
- Children's Health and the Environment 2000, ed: JM Cranmer, CF Bearer, CF Chaisson, DA Cory-Slechta, LR Goldman, DC Rice, W Slikker, Jr., BR Sonawane, HA Tilson, B Weiss, RT Zoeller. NeuroToxicology 2001; 22(5):555-722.

- Children's Health and the Environment: Mechanisms and Consequences of Developmental Neurotoxicology, ed: JM Cranmer, MA Aschner, CF Bearer, LG Costa, CT DeRosa, LR Goldman, AG Kirshner, DC Rice, BR Sonawane, HA Tilson, and B Weiss, NeuroToxicology 2000; 21(1-2):229-238; and 21(6):923-1155. 18th International Neurotoxicology Conference, Colorado Springs, CO; September 23-26, 2000.
- Invited to participate in the World Health Organization (WHO) Workshop on "Environmental Threats to Children" held in association with the Pacific Basin Consortium Meeting; Manila, Philippines; April, 2000.
- Pesticides and Susceptible Populations: Who is at Risk and When? ed: JM Cranmer, W Slikker, V Moser, B Weiss, M Aschner, L Costa, D Ray, BR Sonawane, NeuroToxicology 2000; 1(1-2):9-227. 17th International Neurotoxicology Conference, Little Rock, AR; October 17-20, 1999.
- Invited as a speaker and serve as a discussant in three sessions at "Lead Poisoning: An International Conference on Prevention and Treatment', Bangalore, India; February, 1999.
- Participated as Member of Steering Committee, Planning Meeting on "Workshop on Benzene Leukemogenesis – State of the Sciences", Ottawa, Canada; July, 1998.
- Invited by the American Petroleum Institute (API) to participate "Benzene: State of the Sciences Workshop Planning Meeting. Chicago, IL; June, 1998.
- Invited to American Crop Protection Association (ACPA) 1998 Spring Conference on "Redefining the Crop Protection Industry"; Arlington, VA; April, 1998.
- Invited by the Great Lakes Association of Colleges of Medicine, Inc. to the 7th Annual Training Seminar in Heavy Metals Toxicology to give a lecture on Arsenic Toxicology. Orlando FL; March, 1998.
- 16th International Neurotoxicology Conference, Little Rock, AR. September 13-16, 1998.
- Invited Presentation at 5th Annual Convention on Human Embryology and Birth Defects Related to Environmental Factors held in Manila, Philippines; 1996.
- Invited presentation on Sono-embryology to the 8th Post-graduate Course on Genetics, Congenital Anomalies, Fetal Therapy; Pediatric Adolescent Gynecology and Updates on Ultrasound held at Philippine Children Hospital Medical Center, Manila; November, 1996.
- Human Embryology and Birth Defects Related to Environmental Factors and Database of Fetal Risk on Maternal Exposure to Infections, Pharmaceuticals and Environmental Toxic Agents, Fifth Annual Convention, Manila, Philippines; November, 1996.
- Neurotoxicity of Mercury: Indicators and Effects of Low-Level Exposure, ed: JM Cranmer, RW Amler, TW Clarkson, SE Gilbert, DC Rice, W Slikker Jr, B Sonawane, HA Tilson, MA Verity. NeuroToxicology 1996; 17(1):1-299.

PUBLICATIONS (Selected from over 120):

1. Segal D, Lin YS, Ginsberg G, Sonawane B. (2015) A conceptual framework for evaluating the interaction of a chemical and nonchemical stressor in human health risk assessments: a case study for lead and psychosocial stress. *Human and Ecological Risk Assessment.* 21:7, 1840-1868.
2. Thomas, R., Hubbard, A.E., McHale, C.M., Zhang, L., Rappaport, S.M., Lan, Q., Rothman, N., Vermeulen, R., Guyton, K.Z., Jinot, J., Sonawane, B.R. and Smith, M.T. (2014) Characterization of changes in gene expression and biochemical pathways at low levels of benzene exposure. *PLoS One*, 9, e91828.
3. Olden, K., Lin, Y.S., Gruber, D. and Sonawane, B. (2014) Epigenome: biosensor of cumulative exposure to chemical and nonchemical stressors related to environmental justice. *Am J Public Health*, 104, 1816-21.
4. Lin, Y.S., Ho, W.C., Caffrey, J.L. and Sonawane, B. (2014) Low serum zinc is associated with elevated risk of cadmium nephrotoxicity. *Environ Res*, 134, 33-8.
5. Lin, Y.S., Ginsberg, G., Lin, J.W. and Sonawane, B. (2014) Mercury exposure and omega-3 fatty acid intake in relation to renal function in the US population. *Int J Hyg Environ Health*, 217, 465-72.
6. Lin, Y.S., Ginsberg, G., Caffrey, J.L., Xue, J., Vulimiri, S.V., Nath, R.G. and Sonawane, B. (2014) Association of body burden of mercury with liver function test status in the U.S. population. *Environ Int*, 70, 88-94.
7. Ginsberg, G., Sonawane, B., Nath, R. and Lewandowski, P. (2014) Methylmercury-induced inhibition of paraoxonase-1 (PON1)-implications for cardiovascular risk. *J Toxicol Environ Health A*, 77, 1004-23.
8. Eastmond, D.A., Keshava, N. and Sonawane, B. (2014) Lymphohematopoietic cancers induced by chemicals and other agents and their implications for risk evaluation: An overview. *Mutat Res Rev Mutat Res*.
9. Lin, Y.S., Caffrey, J.L., Lin, J.W., Bayliss, D., Faramawi, M.F., Bateson, T.F. and Sonawane, B. (2013) Increased risk of cancer mortality associated with cadmium exposures in older Americans with low zinc intake. *J Toxicol Environ Health A*, 76, 1-15.
10. Eastmond, D.A., Vulimiri, S.V., French, J.E. and Sonawane, B. (2013) The use of genetically modified mice in cancer risk assessment: challenges and limitations. *Crit Rev Toxicol*, 43, 611-31.
11. Vulimiri, S.V., Berger, A. and Sonawane, B. (2011) The potential of metabolomic approaches for investigating mode(s) of action of xenobiotics: case study with carbon tetrachloride. *Mutat Res*, 722, 147-53.
12. Pachkowski, B.F., Guyton, K.Z. and Sonawane, B. (2011) DNA repair during in utero development: a review of the current state of knowledge, research needs, and potential application in risk assessment. *Mutat Res*, 728, 35-46.

13. Gwinn, M.R., DeVoney, D., Jarabek, A.M., Sonawane, B., Wheeler, J., Weissman, D.N., Masten, S. and Thompson, C. (2011) Meeting report: mode(s) of action of asbestos and related mineral fibers. *Environ Health Perspect*, 119, 1806-10.
14. Gwinn, M.R., Craig, J., Axelrad, D.A., Cook, R., Dockins, C., Fann, N., Fegley, R., Guinnup, D.E., Helfand, G., Hubbell, B., Mazur, S.L., Palma, T., Smith, R.L., Vandenberg, J. and Sonawane, B. (2011) Meeting report: Estimating the benefits of reducing hazardous air pollutants--summary of 2009 workshop and future considerations. *Environ Health Perspect*, 119, 125-30.
15. Ginsberg, G., Angle, K., Guyton, K. and Sonawane, B. (2011) Polymorphism in the DNA repair enzyme XRCC1: utility of current database and implications for human health risk assessment. *Mutat Res*, 727, 1-15.
16. Zhang, L., Freeman, L.E., Nakamura, J., Hecht, S.S., Vandenberg, J.J., Smith, M.T. and Sonawane, B.R. (2010) Formaldehyde and leukemia: epidemiology, potential mechanisms, and implications for risk assessment. *Environ Mol Mutagen*, 51, 181-91.
17. Ginsberg, G., Guyton, K., Johns, D., Schimek, J., Angle, K. and Sonawane, B. (2010) Genetic polymorphism in metabolism and host defense enzymes: implications for human health risk assessment. *Crit Rev Toxicol*, 40, 575-619.
18. Walker, K., Ginsberg, G., Hattis, D., Johns, D.O., Guyton, K.Z. and Sonawane, B. (2009) Genetic polymorphism in N-Acetyltransferase (NAT): Population distribution of NAT1 and NAT2 activity. *J Toxicol Environ Health B Crit Rev*, 12, 440-72.
19. Thompson, C.M., Sonawane, B. and Grafstrom, R.C. (2009) The ontogeny, distribution, and regulation of alcohol dehydrogenase 3: implications for pulmonary physiology. *Drug Metab Dispos*, 37, 1565-71.
20. Thompson, C.M., Johns, D.O., Sonawane, B., Barton, H.A., Hattis, D., Tardif, R. and Krishnan, K. (2009) Database for physiologically based pharmacokinetic (PBPK) modeling: physiological data for healthy and health-impaired elderly. *J Toxicol Environ Health B Crit Rev*, 12, 1-24.
21. Neafsey, P., Ginsberg, G., Hattis, D. and Sonawane, B. (2009) Genetic polymorphism in cytochrome P450 2D6 (CYP2D6): Population distribution of CYP2D6 activity. *J Toxicol Environ Health B Crit Rev*, 12, 334-61.
22. Neafsey, P., Ginsberg, G., Hattis, D., Johns, D.O., Guyton, K.Z. and Sonawane, B. (2009) Genetic polymorphism in CYP2E1: Population distribution of CYP2E1 activity. *J Toxicol Environ Health B Crit Rev*, 12, 362-88.
23. Keshava, N., Chen, C., White, P., Sonawane, B. and Bussard, D. (2009) Biomarkers in toxicology and risk assessment: informing critical dose-response relationships by Swenberg et al., 2008. *Chem Res Toxicol*, 22, 8-10; author reply 10-2.
24. Guyton, K.Z., Kyle, A.D., Aubrecht, J., Cogliano, V.J., Eastmond, D.A., Jackson, M., Keshava, N., Sandy, M.S., Sonawane, B., Zhang, L., Waters, M.D. and Smith, M.T. (2009) Improving

prediction of chemical carcinogenicity by considering multiple mechanisms and applying toxicogenomic approaches. *Mutat Res*, 681, 230-40.

25. Ginsberg, G., Smolenski, S., Neafsey, P., Hattis, D., Walker, K., Guyton, K.Z., Johns, D.O. and Sonawane, B. (2009) The influence of genetic polymorphisms on population variability in six xenobiotic-metabolizing enzymes. *J Toxicol Environ Health B Crit Rev*, 12, 307-33.
26. Ginsberg, G., Smolenski, S., Hattis, D., Guyton, K.Z., Johns, D.O. and Sonawane, B. (2009) Genetic Polymorphism in Glutathione Transferases (GST): Population distribution of GSTM1, T1, and P1 conjugating activity. *J Toxicol Environ Health B Crit Rev*, 12, 389-439.
27. Ginsberg, G., Neafsey, P., Hattis, D., Guyton, K.Z., Johns, D.O. and Sonawane, B. (2009) Genetic polymorphism in paraoxonase 1 (PON1): Population distribution of PON1 activity. *J Toxicol Environ Health B Crit Rev*, 12, 473-507.
28. Thompson, C.M., Sonawane, B., Barton, H.A., DeWoskin, R.S., Lipscomb, J.C., Schlosser, P., Chiu, W.A. and Krishnan, K. (2008) Approaches for applications of physiologically based pharmacokinetic models in risk assessment. *J Toxicol Environ Health B Crit Rev*, 11, 519-47.
29. Meek, M.E., Sonawane, B. and Becker, R.A. (2008) Foreword: Biomonitoring Equivalents special issue. *Regul Toxicol Pharmacol*, 51, S3.
30. Makris, S.L., Thompson, C.M., Euling, S.Y., Selevan, S.G. and Sonawane, B. (2008) A lifestage-specific approach to hazard and dose-response characterization for children's health risk assessment. *Birth Defects Res B Dev Reprod Toxicol*, 83, 530-46.
31. Gift, J.S., McGaughy, R., Singh, D.V. and Sonawane, B. (2008) Health assessment of phosgene: approaches for derivation of reference concentration. *Regul Toxicol Pharmacol*, 51, 98-107.
32. Foos, B. and Sonawane, B. (2008) Overview: Workshop on children's inhalation dosimetry and health effects for risk assessment. *J Toxicol Environ Health A*, 71, 147-8.
33. Firestone, M., Sonawane, B., Barone, S., Jr., Salmon, A.G., Brown, J.P., Hattis, D. and Woodruff, T. (2008) Potential new approaches for children's inhalation risk assessment. *J Toxicol Environ Health A*, 71, 208-17.
34. Walker, K., Hattis, D., Russ, A., Sonawane, B. and Ginsberg, G. (2007) Approaches to acrylamide physiologically based toxicokinetic modeling for exploring child-adult dosimetry differences. *J Toxicol Environ Health A*, 70, 2033-55.
35. Chiu, W.A., Barton, H.A., DeWoskin, R.S., Schlosser, P., Thompson, C.M., Sonawane, B., Lipscomb, J.C. and Krishnan, K. (2007) Evaluation of physiologically based pharmacokinetic models for use in risk assessment. *J Appl Toxicol*, 27, 218-37.
36. Barone, S., Jr., Brown, R.C., Euling, S., Cohen Hubal, E., Kimmel, C.A., Makris, S., Moya, J., Selevan, S.G., Sonawane, B., Thomas, T. and Thompson, C.M. (2006) Visión General De La Evaluación Del Rieso En alud Infantil Empleando Un Enfoque Por Etapas De Desarrollo [Overview of a Life Stage Approach to Children's Health Risk Assessment]. *Acta Toxicol. Argent.*, 14(Sup), 7-10.

37. Amler, R.W., Barone, S., Jr., Belger, A., Berlin, C.M., Jr., Cox, C., Frank, H., Goodman, M., Harry, J., Hooper, S.R., Ladda, R., LaKind, J.S., Lipkin, P.H., Lipsitt, L.P., Lorber, M.N., Myers, G., Mason, A.M., Needham, L.L., Sonawane, B., Wachs, T.D. and Yager, J.W. (2006) Hershey Medical Center Technical Workshop Report: optimizing the design and interpretation of epidemiologic studies for assessing neurodevelopmental effects from in utero chemical exposure. *Neurotoxicology*, 27, 861-74.
38. Landrigan, P.J., Sonawane, B., Butler, R.N., Trasande, L., Callan, R. and Droller, D. (2005) Early environmental origins of neurodegenerative disease in later life. *Environ Health Perspect*, 113, 1230-3.
39. LaKind, J.S., Brent, R.L., Dourson, M.L., Kacew, S., Koren, G., Sonawane, B., Tarzian, A.J. and Uhl, K. (2005) Human milk biomonitoring data: interpretation and risk assessment issues. *J Toxicol Environ Health A*, 68, 1713-69.
40. Ginsberg, G., Hattis, D., Russ, A. and Sonawane, B. (2005) Pharmacokinetic and pharmacodynamic factors that can affect sensitivity to neurotoxic sequelae in elderly individuals. *Environ Health Perspect*, 113, 1243-9.
41. Brown, R.C., Lockwood, A.H. and Sonawane, B.R. (2005) Neurodegenerative diseases: an overview of environmental risk factors. *Environ Health Perspect*, 113, 1250-6.
42. Berlin, C.M., LaKind, J.S., Fenton, S.E., Wang, R.Y., Bates, M.N., Brent, R.L., Condon, M., Crase, B.L., Dourson, M.L., Ettinger, A.S., Foos, B., Furst, P., Giacoia, G.P., Goldstein, D.A., Haynes, S.G., Hench, K.D., Kacew, S., Koren, G., Lawrence, R.A., Mason, A., McDiarmid, M.A., Moy, G., Needham, L.L., Paul, I.M., Pugh, L.C., Qian, Z., Salamone, L., Selevan, S.G., Sonawane, B., Tarzian, A.J., Rose Tully, M. and Uhl, K. (2005) Conclusions and recommendations of the expert panel: technical workshop on human milk surveillance and biomonitoring for environmental chemicals in the United States. *J Toxicol Environ Health A*, 68, 1825-31.
43. Ginsberg, G., Slikker, W., Jr., Bruckner, J. and Sonawane, B. (2004) Incorporating children's toxicokinetics into a risk framework. *Environ Health Perspect*, 112, 272-83.
44. Ginsberg, G., Hattis, D. and Sonawane, B. (2004) Incorporating pharmacokinetic differences between children and adults in assessing children's risks to environmental toxicants. *Toxicol Appl Pharmacol*, 198, 164-83.
45. Ginsberg, G., Hattis, D., Russ, A. and Sonawane, B. (2004) Physiologically based pharmacokinetic (PBPK) modeling of caffeine and theophylline in neonates and adults: implications for assessing children's risks from environmental agents. *J Toxicol Environ Health A*, 67, 297-329.
46. Ginsberg, G., Hattis, D., Miller, R. and Sonawane, B. (2004) Pediatric pharmacokinetic data: implications for environmental risk assessment for children. *Pediatrics*, 113, 973-83.

47. Daston, G., Faustman, E., Ginsberg, G., Fenner-Crisp, P., Olin, S., Sonawane, B., Bruckner, J., Breslin, W. and McLaughlin, T.J. (2004) A framework for assessing risks to children from exposure to environmental agents. *Environ Health Perspect*, 112, 238-56.
48. Bostwick, D.G., Burke, H.B., Djakiew, D., Euling, S., Ho, S.M., Landolph, J., Morrison, H., Sonawane, B., Shifflett, T., Waters, D.J. and Timms, B. (2004) Human prostate cancer risk factors. *Cancer*, 101, 2371-490.
49. Olin, S.S. and Sonawane, B.R. (2003) Workshop to develop a framework for assessing risks to children from exposure to environmental agents. *Environ Health Perspect*, 111, 1524-6.
50. Hattis, D., Ginsberg, G., Sonawane, B., Smolenski, S., Russ, A., Kozlak, M. and Goble, R. (2003) Differences in pharmacokinetics between children and adults--II. Children's variability in drug elimination half-lives and in some parameters needed for physiologically-based pharmacokinetic modeling. *Risk Anal*, 23, 117-42.
51. Landrigan, P.J., Sonawane, B., Mattison, D., McCally, M. and Garg, A. (2002) Chemical contaminants in breast milk and their impacts on children's health: an overview. *Environ Health Perspect*, 110, A313-5.
52. LaKind, J.S., Birnbach, N., Borgert, C.J., Sonawane, B.R., Tully, M.R. and Friedman, L. (2002) Human milk surveillance and research of environmental chemicals: concepts for consideration in interpreting and presenting study results. *J Toxicol Environ Health A*, 65, 1909-28.
53. Ginsberg, G., Smolenski, S., Hattis, D. and Sonawane, B. (2002) Population distribution of aldehyde dehydrogenase-2 genetic polymorphism: implications for risk assessment. *Regul Toxicol Pharmacol*, 36, 297-309.
54. Ginsberg, G., Hattis, D., Sonawane, B., Russ, A., Banati, P., Kozlak, M., Smolenski, S. and Goble, R. (2002) Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. *Toxicol Sci*, 66, 185-200.
55. Berlin, C.M., Jr., LaKind, J.S., Sonawane, B.R., Kacew, S., Borgert, C.J., Bates, M.N., Birnbach, N., Campbell, R., Dermer, A., Dewey, K.G., Ellerbee, S.M., Furst, P., Giacoia, G.P., Gartner, L., Groer, M., Haynes, S.G., Humerick, S.S., Lawrence, R.A., Lorber, M., Lovelady, C., Mason, A., Needham, L.L., Picciano, M.F., Plautz, J., Ryan, J.J., Selevan, S.G., Sumaya, C.V., Tully, M.R., Uhl, K., Vesell, E. and Wilson, J.T. (2002) Conclusions, research needs, and recommendations of the expert panel: technical workshop on human milk surveillance and research for environmental chemicals in the United States. *J Toxicol Environ Health A*, 65, 1929-35.
56. Ginsberg GL, Hattis DB, Smolenski S, Russ A, Kozlak M, Goble RL, Beck NB, Sonawane B. Assessing pharmacikinetic differences beween children and adults: implications for risk assessment. Presented at the 11th International Congress on Toxicology, Brisbale, Australia. July 2001. Abs # P1B11.

57. Beck NB and Sonawane B. Children's health and air toxics: how recent risk assessments are addressing the issues. Presented at the 11th International Congress on Toxicology, Brisbale, Australia. July 2001. Abs # P1B12.
58. Sonawane B, Ginsberg GL, Goldstein DA and Beck NB. A conceptual framework to evaluate the risks of environmental chamical exposures to children: issues for consideration. Presented at the 11th International Congress on Toxicology, Brisbale, Australia. July 2001. Abs # P1B13
59. Sonawane B and Beck NB. Cancer risk assessment of benzene, an international update. Presented at the 11th International Congress on Toxicology, Brisbale, Australia. July 2001. Abs # P1B27.
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65. Sonawane, B.R. (1995) Chemical contaminants in human milk: an overview. *Environ Health Perspect*, 103 Suppl 6, 197-205.
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BOOK CHAPTERS/ISSUE EDITOR/EDOTORIALS (Examples):

1. Vulimiri, S.V., Sonawane, B. and Szabo, D.T. (2014) Systems Biology Application in Toxicology. In Wexler, P. (ed.), *Encyclopedia of Toxicology*. Elsevier Inc. Academic Press, vol. 4, pp. 454-458.
2. Dzubow, R.C., Sonawane, B., Bullock, M.B. and Landrigan, P.J. (2011) Issues and challenges for determining environmental risk factors and cuases of disease among children. In Nriagu, J.O. (ed.), *Encyclopedia of Environmental Health, Five-Volume Set*. Elsevier, Waltham, MA, pp. 304-307.
3. Johns, D.O., Oesterling Owens, E., Thompson, C.M., Sonawane, B., Hattis, D. and Krishnan, K. (2010) Chapter 5. Physiological Parameters and Databases for PBPK Modeling. In Krishnan, K. and Andersen, M.E. (eds.), *Quantitative Modeling in Toxicology*. John Wiley & Sons, Ltd., Chichester, UK. .
4. Thompson, C., Nong, A., Sonawane, B. and Krishnan, K. (2006) Considerations for Applying Physiologically Based Pharmacokinetic Models in Risk Assessment. In Lipscomb, J.L. and Ohanian, E.V. (eds.), *Toxicokinetics and Risk Assessment*. Informa Healthcare USA, Inc., New York, NY, pp. 123-139.
5. Sonawane, B.R. (1990) Overview of risk factors during reproduction and pregnancy: Proceedings of the Symposium. In *Interaction of Environmental and Genetic Factors on Pregnancy Outcome*.

6. Sonawane, B.R. and Yaffe, S.J. (1987) Drug disposition in utero: Reproductive function in the offspring. In Kacew, S. and Lock, S. (eds.), *Toxicologic and Pharmacologic principles in Pediatrics*. Hemisphere Publishing Corp, New York, pp. 41-65.
7. Sonawane, B.R. (1987) Principles of drug disposition in the fetus and the newborn. In Vidyasagar (ed.), *Textbook of Neonatology*. Interprint, New Delhi, pp. 18-26.
8. Sonawane, B.R. and Yaffe, S.J. (1985) Physiological disposition of drugs in the fetus and newborn. In Fabro, S. and Scialli, A.R. (eds.), *Principles of Drug and Chemical Action in Pregnancy*. Marcel Dekker, New York.
9. Sonawane, B.R. and Yaffe, S.J. (1985) Nutritional aspects of pharmacology. In Watkins, J.B. and Walker, W.A. (eds.), *Nutrition in Pediatrics-Basic Science and Clinician Applications*. Little, Brown & Co, pp. 341-356.
10. Yaffe, S.J. and Sonawane, B. (1984) Clinical problems in perinatal neurobiology. In Caciagli, F. and Paoleppi, R. (eds.), *Developmental Neuroscience: Physiological and Pharmacological Aspects*. Elsevier Science Publications, Amsterdam, pp. 299-310.
11. Sonawane, B.R. and Catz, C. (1981) Nutritional status and drug metabolism during deelopment. In Soyka, L.F. and Redmond, G.P. (eds.), *Drug Metabolism and the Immature Human*. Raven Press, New York.
12. Sonawane, B. and Yaffe, S.J. (1981) Clinical implications of hepatic immaturity: Bilirubin glucuronidation and induction by phenobarbital. In Redmond, G.P. and Soyka, L.F. (eds.), *Drug Metabolism in the Immature Human*. Raven Press, New York, pp. 119-128.
13. Sonawane, B.R., et al. (1979) *Hepatic metabolsim of drugs in the liver and biliary system in infants and children*. Churchill Livingston, Edinburg and London.

PRESENTATIONS/ABSTRACTS (Selected from over 150):

1. Sonawane, BR, Vulimiri, SV, Adams, LS Nath, RG and Fritz, JM (2015) MicroRNA Expression in Lymphohematopoietic Malignancies and Following Formaldehyde Exposure. EMGS meeting, New Orleans, LA, September, 2015.
2. Nath RG, Sonawane B, Vulimiri SV, Lin YS. Mechanisms of Cadmium Carcinogenesis. American Association for Cancer Research (AACR) AACR Annual Meeting. Philadelphia, PA. April 18 - 22, 2015.
3. Vulimiri, SV,N. Keshava, J. M. Fritz and B. R. Sonawane.(2015). Understanding the Molecular Mechanism of Formaldehyde-Induced DNA-Protein Crosslink Repair. SOT Abstract # 549.
4. Lin, Y, Kadry A. M., Sams R., Cowden J. and Sonawane B. R. (2015) Arsenic Methylation Is Associated with Body Mass Index among US Adults. SOT Abstract # 1300.
5. Keshava, N, and B. R. Sonawane. (2015). Evaluation of Cancer and Noncancer Effects of Cumene. SOT Abstract #1870.

6. Segal, S, Y. Lin, G. Ginsberg and B. R. Sonawane. (2015). A Framework for Assessing Chemical/Nonchemical Interactions: A Case Study of Lead and Psychosocial Stress. SOT Abstract # 1878.
7. Sonawane, BR, Y. Lin, R. R. Dietert and G. L. Ginsberg. (2015). Increased Susceptibility to Chemical Toxicity Associated with Pre-Existing Diseases: Framework and Case Studies. SOT Abstract # 1884.
8. Vulimiri, SV, Y. Lin, J. Kancherla, G. L. Ginsberg, B. P. Foos and B. R. Sonawane. (2014). Scoping the Need for PBPK Modeling of Child-Adult Metabolism Differences: Case Studies Applying an Enzyme Ontogeny Database. SOT Abstract #892.
9. Olden, K, Y. Lin and B. R. Sonawane. (2014). Environmental Health Disparities in the Age of Epigenetics. SOT Abstract # 959.
10. Sonawane, BR, R. Nath, P. Lewandowski and G. L. Ginsberg. (2014). Methylmercury Inhibition of Serum Paraoxonase 1 Activity and the Risk of Cardiovascular Disease. SOT Abstract # 1812.
11. Birchfield, N, T. Berner, R. Nath, B. R. Sonawane, P. White, J. Ball, R. P. Subramaniam, C. J. Brinkerhoff, K. Hogan, J. Gift, B. Buckley, E. O. Owens, A. D. Kraft, B. S. Glenn, L. Flowers, J. Jinot and C. Scott. (2014). When Does Exogenous Exposure to Endogenously Produced Chemicals and Essential Elements Become a Problem? SOT Abstract # 1831c.
12. Nath RG, Sonawane B, Vulimiri SV, Lin YS. Epigenetic effects of cadmium. Environmental Mutagenesis and Genomics Society, Orlando. September 13–17, FL, 2014.
13. Gwinn, M.R., A. M. Jarabek and B. R. Sonawane. Office of Research & Development, US Environmental Protection Agency, Washington, DC. (2014). Potential Mode of Action for Respiratory Effects following Exposure to Libby Amphibole Asbestos. SOT Abstract # 1831k.
14. Kushman, ME, A. D. Kraft, K. Guyton, B. Sonawane, W. A. Chiu, S. Makris and I. Rusyn. (2013). A Systematic Approach for Identifying, Evaluating, and Presenting Mode-of-Action Evidence in IRIS Toxicological Reviews. SOT Abstract #710.
15. Makris, SL and Sonawane, BR (2014). Are standard testing guidelines adequate to screen chemicals for prenatal programming of toxicological response? PPTOX –IV meeting, Boston, MA Oct 26-29.
16. Sonawane, BR, Nath, RG, Makris, SL (2014). Epigenetic effects of di(2-ethylhexyl) phthalate. Environmental Mutagen and Genomics Society meeting Orlando, FL Sept 13-17.
17. Vulimiri, SV, D. A. Eastmond, J. E. French and B. Sonawane. (2013). The Use of Genetically Modified Mice in Cancer Risk Assessment: Challenges and Limitations. SOT Abstract #1957
18. Lin YS, Caffrey JL, Lin JW, Bayliss D, Ho WC, Sonawane BR. The role of dietary zinc in cadmium nephrotoxicity. Society of Risk Analysis. Baltimore, MD. Dec 8-12, 2013.
19. Lin YS, Ginsberg G, Sonawane BR. The relationship of mercury exposure, omega-3 intake, and risk of chronic kidney disease. Society of Risk Analysis. Baltimore, MD. Dec 8-12, 2013.

20. Thomas, R, A. E. Hubbard, C. M. McHale, L. Zhang, S. M. Rappaport, Q. Lan, N. Rothman, R. Vermeulen, K. Z. Guyton, J. Jinot, B. R. Sonawane and M. T. Smith. (2012). A systems biology analysis to inform human health risk assessment of benzene. SOT Abstract #145.
21. Eastmond, DA and B. Sonawane. (2012). Factors influencing mutagenic mechanism of action determinations for environmental chemicals. SOT Abstract #2651.
22. Eastmond, DA, S. V. Vulimiri and B. Sonawane. (2011). An evaluation of factors for classifying a chemical as inducing cancer in either rodents or humans via a mutagenic mode of action using a weight of evidence approach. SOT Abstract #1934.
23. Gwinn, MR, D DeVoney, AM Jarabek, B Sonawane, J Wheeler, DN Weissman, S Masten, C Thompson Meeting report: mode(s) of action of asbestos and related mineral fibers. Environmental Health Perspectives, Dec, 2011.
24. Gwinn, MR, J Craig, DA Axelrad, R Cook, C Dockins, N Fann, R Fegley, DE Guinnup, G Helfand, B Hubbell, SL Mazur, T Palma, RL Smith, J Vandenberg, B Sonawane. Meeting report: Estimating the benefits of reducing hazardous air pollutants-summary of 2009 workshop and future considerations. Environmental Health Perspectives. Jan, 2011.
25. Eastmond, D.A., Vulimiri, S.V., Sonawane, B. (2011) An evaluation of factors for classifying a chemical as inducing cancer either in rodents or humans via a mutagenic mode of action using a weight of evidence approach. The Toxicologist, Sciences, Vol. 120, Suppl. 2:414.
26. Gwinn, M, D. DeVoney, A. M. Jarabek, B. Sonawane, C. Bas, and H. Dawson. Mode(S) of Action of Asbestos and Related Mineral Fibers: A Meeting Summary Report, ASTM Johnson Conference; July 25-29, 2011, University of Vermont, Burlington, VT.
27. Johns, D. R. Dewoskin, C.M. Thompson, K. Krishnan, H.A. Barton, B. Sonawane. Development of a Physiological Parameters Database for Physiologically-Based Pharmacokinetic (PBPK) Modeling. Presented at the 49th Annual Meeting of Society of Toxicology. March 7-11, 2010. Salt Lake City, Utah.
28. DeVoney, D, J. Whalan and B. Sonawane. (2010). Evidence for formaldehyde-induced leukemia and lymphoma in long-term rodent bioassays. SOT Abstract #520.
29. Sonawane, B, T. Bateson, J. Whalan and D. DeVoney. (2010). Epidemiologic evidence for formaldehyde-induced lymphohematopoietic malignancies. SOT Abstract #665.
30. Johns, D, R. Dewoskin, C. Thompson, K. Krishnan, H. A. Barton and B. Sonawane. (2010). Development of a physiological parameters database for physiologically-based pharmacokinetic (PBPK) modeling. SOT Abstract #1564.
31. Ginsberg, G, J. Schimek, K. Z. Guyton, D. O. Johns and B. Sonawane. (2010). Polymorphism in NADPH:quinone oxidoreductase (NQO-1): population distribution and potential use in risk assessment. SOT Abstract # 1928.
32. Guyton, KZ, Amy D. Kyle, Jiri Aubrecht, Vincent J. Cogliano, David A. Eastmond, Marc Jackson, Nagalakshmi Keshava, Martha S. Sandy, Babasaheb Sonawane, Luoping Zhang, Michael D. Waters, Martyn T. Smith. Improving Prediction of Chemical Carcinogenicity by

Considering Multiple Mechanisms and Applying Toxicogenomic Approaches; Symposium of the Standing Committee on Risk Analysis Issues and Reviews; National Research council; Washington, DC May 11-13, 2009.

33. Sonawane, BR, W. Slikker, Jr., L.P. Zhang, E. Faustman, H. Mortensen, B. Lyn-Cook, G. Ginsberg, M. Smith. Genetic Polymorphisms in Metabolism of Xenobiotics: Implications for Health Risk Assessment of Drugs and Environmental Agents. Presented at the 40 Annual EMS Meeting, October 24 –28, 2009; St. Louis, Missouri.
34. Vulimiri SV, Sonawane B. Metabolomics in Risk Assessment; presented at the 40th Annual Meeting of the Environmental Mutagen Society; St. Louis, Missouri, U.S.A. October 24–28, 2009.
35. DeVoney, D, M. Gwinn, T. Bateson, B. Sonawane , K. Guyton, P. Sullivan, A Mechanistic View of the Determinants of Toxicity of Mineral Fibers for Quantitative Risk Assessment. ASTM Johnson Conference July 14- 18, 2008, University of Vermont, Burlington, Vermont.
36. Johns, D, K. Z. Guyton, B. Sonawane and G. Ginsberg. (2008). Exploring genetic polymorphisms in xenobiotic metabolizing enzymes that may affect response to environmental toxicants. SOT Abstract #421.
37. Hogan, K.A., S. Barone, R. C. Brown, W. Chiu, K. Z. Guyton, L. Kopylev, S. Makris, R. McGaughy, C. Siegel Scott, B. Sonawane, R. Subramaniam and P. White. (2008). Addressing uncertainty in the estimation of human risks from tetrachloroethylene exposure. SOT Abstract #914.
38. Sonawane, BR, D. Johns, C. Thompson, H. Barton, D. Hattis, R. Tardif and K. Krishnan. (2008). Evaluation of physiological parameters in adult rats and mice for populating an access database. SOT Abstract #997.
39. Gwinn, M.R., K. Z. Guyton, B. Sonawane and D. DeVoney. (2008). Determinants of asbestos fiber-induced genotoxicity. SOT Abstract #1685.
40. Sonawane, B. D. Johns, C.M. Thompson, H. Barton, D. Hattis, R. Tardif, and K. Krishnan. Evaluation of Physiological Parameters in Adult Rats and Mice for Populating an ACCESS Database. Presented at the Society of Toxicology Annual Meeting. March 16-20, 2008. Seattle, WA. (Blue Ribbon Award).
41. Singh, DV, J. Gift, R. McGaughy and B. Sonawane. Alternative approaches for noncancer risk assessment of phosgene. Presented at the Society of Toxicology Annual Meeting, Charlotte, North Carolina, March 25-29, 2007.
42. Thompson, CM. B. Sonawane and K. Krishnan. Physiologically Based Pharmacokinetic (PBPK) Modeling in the Elderly. Society of Toxicology. March 25-29, 2007. Charlotte, NC.
43. Sonawane, B. C.M. Thompson, D. Hattis, R. Tardif, and K. Krishnan Physiological Parameters in Healthy and Diseased Elderly. Society of Toxicology. March 25-29, 2007. Charlotte, NC.

44. Johns DO, Moya J, Ginsberg GL, Sonawane B. Characterization of factors affecting exposure to environmental agents in aging populations. Annual conference of the International Society of Exposure Analysis, Durham, NC, October 2007.
45. Moya J, Johns D, Barone RC, Thompson C and Sonawane B. Characterization of environmental risk factors to older adults. Presented at BOSC HHRA Subcommittee Meeting, Bethesda, MD November 2007.
46. Krishnan K, Thompson C, Johns D, Hattis D, Tardif R, Sonawane B; Changes in Physiological Parameters Across Lifestages. Society For Risk Analysis Annual Meeting 2007.
47. Sonawane, B., Future Directions, Implications for Research, Risk Assessments, and Public Policy: Presented at the Environmental Mutagen Society 38th Annual Meeting Agenda Atlanta, Georgia; October 20-24, 2007.
48. Sonawane, BR, C. Thompson, D. Hattis, R. Tardiff and K. Krishnan. (2007). Physiological parameters in healthy and diseased elderly. SOT Abstract #1529.
49. Singh, DV, J. Gift, R. McGaughy and B. Sonawane (2007). Alternative approaches for noncancer risk assessment of phosgene. SOT Abstract #1542.
50. Sonawane, B and Vincent J. Cogliano. Predicting chemical carcinogenicity: Where Have We Been and Where Do; We Want To Go: Motivations for Change. EMS Annual meeting, Hyatt Regency; Atlanta, Georgia, U.S.A. October 20–24, 2007.
51. Barone S Jr, RC Brown, S Euling, E Cohen Hubal, CA Kimmel, S Makris, J Moya, SG Selevan, B Sonawane, T Thomas, C Thompson. (2006) Vision General de al Evaluacion del Riesgo en Salud Infantil Empleando un Enfouque por Etapas de Desarrollo [Overview of a Life Stage Approach to Children's Health Risk Assessment] Acta Toxicologica Argentina. 14(supplement) 7-10.
52. Keshava, N, G. M. Woodall, S. Rice, B. Sonawane and Cote, I. (2006). An evaluation of the mutagenic mode of action for four environmental carcinogens. SOT Abstract #1635.
53. DeVito MJ, Evans MV, Okino MS, Sonawane BR, Thompson CM, Birnbaum LS, Yokley K, Corton C, Lee J, and Geller AM. Evaluation of altered sensitivity of older adults to environmental agents using physiologically based pharmacokinetic (PBPK) modeling. EPA Science Forum 2006, Washington, DC (2006).
54. Kimmel, C.A. Selevan, S.G. Mendola, P. Kimmel, G.L. Children's Environmental Health: Critical windows of development and susceptibility. In: Encyclopedia of Environmental Health. B. Sonawane and R. Brown, eds. Elsevier Ltd., Oxford, UK.
55. Keshava N, Jinot J, Sonawane B; An evaluation of mutagenic mode of action for carcinogenicity: Ethylene Oxide. Environmental Mutagen Society; Thirty-Seventh Annual Meeting; Hyatt Regency. Vancouver, British Columbia, Canada. September 16-20, 2006.
56. Barone, R. Brown, S. Euling, E. Cohen-Hubal, C.A. Kimmel, S. Makris, J. Moya, S. Selevan, B. Sonawane, T. Thomas, C.M. Thompson. Development of A Children's Health Risk

Assessment Framework Using a Life-Stage Approach S. Society of Toxicology. March 6-10, 2005. New Orleans, LA.

57. Barone, S, R. Brown, S. Euling, E. Cohen-Hubal, C. A. Kimmel, S. Makris, J. Moya, S. G. Selevan, B. Sonawane, T. Thomas, and C. M. Thompson. (2005). Development of a children's health risk assessment framework using a life-stage approach. SOT Abstract #378.
58. Hattis, D, G. Ginsberg, B. Sonawane and K. Walker. (2005). Development of physiologically-based toxicokinetic models for early life stages: implications for differential toxicant susceptibility. SOT Abstract #1978.
59. Sonawane, B, G Ginsberg, Population Distribution and Implications of Paraoxonase (PON1) Polymorphism for Risk Assessment; Presented at Twenty-first International Neurotoxicology Conference, Hawaii, February 10 -13, 2004.
60. Ginsburg, G., Hattis, D., Sonawane, B., Russ, A., Banati, P., Kozlak, M., Smolenski, S., Neurotoxicity studies submitted to the U.S. EPA, Abstract No. 630, Presented at 43rd annual meeting of the Society of Toxicology, Baltimore, MD. March 21-28, 2004.
61. Ginsberg, G, D. Hattis, and B. Sonawane Incorporating Pharmacokinetic Differences Between Children and Adults in a Risk Assessment Framework. Presented at the Society for Risk Analysis 2001 Annual Meeting, Seattle, WA, December 2-5, 2001.
62. Sonawane, B, P. Landrigan, and S. Olin; Children's Environmental Health Risk Assessment: Issues and Challenges. Presented at the Society for Risk Analysis 2001 Annual Meeting, Seattle, WA, December 2-5, 2001.
63. Choudhury, H., C. T. De Rosa, B. R. Sonawane and W. Stiteler. 1990. Risk Assessment of systemic and reproductive toxicity studies. Toxicologist: 10(1), Society of Toxicology, Washington, D.C.
64. De Rosa, C. T., H. Choudhury, S. Irene, A. Bathija, D. Sonawane and R. Rubenstein. 1989. Subchronic toxicity study of 2,3-dichloropropanol in rats. Toxicologist: 9(1), Society of Toxicology, Washington, D.C.
65. Rubenstein, R., S. Irene, B. Sonawane, C. T. De Rosa and A. Bathija. 1989. 3D-and 90 day rat oral toxicity study of propargyl alcohol. Toxicologist: 9(1), Society of Toxicology, Washington, D.C.
66. Sonawane, B., R. Rubenstein, C. T. De Rosa, A. Bathija, H. Choudhury and S. Irene. 1989. Subchronic toxicity of beta-chloronaphthalene in mice. Toxicologist: 9(1), Society of Toxicology, Washington, D.C.
67. Choudhury, H., C. T. De Rosa, S. Irene, A. Bathija, B. Sonawane and R. Rubenstein. 1989. Subchronic toxicity of brucine and strychnine in rats. Toxicologist: 9(1), Society of Toxicology, Washington, D.C.
68. Bathija, A., R. Rubenstein, B. R. Sonawane, H. Choudhury, C. T. De Rosa and S. Irene. 1989. Subchronic toxicity study in rats with thallium. Toxicologist: 9(1), Society of Toxicology, Washington, D.C.

69. De Rosa, C. T., H. Choudhury, R. Rubenstein, A. Bathija and B. Sonawane. 1988. Sub chronic toxicity of pyridine in rats. Toxicologist: 8(1), Society of Toxicology, Washington, D.C.
70. Sonawane, B., R. Rubenstein, A. Bathija; C. T. De Rosa, G. Wolfe and E. N. Albert. 1988. Subchronic toxicity studies in rats with m- and p- xylenes. Toxicologist: 8(1), Society of Toxicology, Washington, D. C.
71. Bathija, A. T., B. R. Sonawane, C. T. De Rosa and R. Rubenstein. 1988. Toxicity studies of 2,3,4,6- tetrachlorophenol. Toxicologist: 8(1), Society of Toxicology, Washington, D.C.
72. Levine, B. S., D. D. Dietz, R. B. Sonawane, R. Rubenstein and C. T. De Rosa. 1987. Subchronic toxicity of methyl isobutyl ketone in Sprague-Dawley rats. Toxicologist: 7(1), Society of Toxicology, Washington, D.C.
73. Sonawane, B., R. Rubenstein, C. T. De Rosa, E. C. Thompkins and D. Dietz, 1987. Comparative toxicity of crotonaldehyde in rats. Toxicologist: 7(1), Society of Toxicology, Washington, D.C.
74. Dietz, D. D., B. S. Levine, R. B. Sonawane, R. Rubenstein and C. T. De Rosa. 1987. Comparative toxicity of cresol isomers. Toxicologist: 7(1), Society of Toxicology, Washington, D.C.
75. De Rosa, C. T., B. Sonawane, R. Rubenstein, D. A. Mayhew and D. Dietz. 1986. Subchronic toxicity study of oral ethyl acetate administration to rats. In: Proceedings of 7th Annual Meeting of American College of Toxicology, Philadelphia, PA.
76. Sonawane, B. R, Rubenstein, C. T. De Rosa and M. Otto. 1986. Subchronic toxicity testing of chemicals in support of the Resource Conservation and Recovery Act. Toxicologist: 6(1), Society of Toxicology, Washington, D.C.
77. Sonawane, B; de Rosa, C; Rubenstein, R. (1986) Estimation of reference dose (RfD) for oral exposure of acetone. 7th Annual Meeting, American College of Toxicology, November 16-19, 1986.

RESEARCH GRANTS (As Principal Investigator and/or Co-Principal Investigator)

1. Biotransformation of Drugs and Other Xenobiotics During Postnatal Development of Food Producing Animals: Project Officer FDA/CVM, $225,000 (1985).
2. Regulation of Drug Metabolism During Development: Principal Investigator, NIH-HD 17475, $57,336 (1982-1983).
3. Developmental Organizers of Drug Metabolism and Action: Co-Principal Investigator, NIH Grant, $128,000 (1980-1983).
4. Training Program – Pediatric Clinical Pharmacology and Anesthesiology: Preceptor, NIH-NIGMS T-32GM7514, $545,067 (1977-1982).
5. Travel Grant, 1981 – To present paper to the International Congress Pharmacology (IUPHR), Paris, France (Supported by McNeil Laboratories, Ft. Washington, PA).

6. Regulation of Drug Metabolism During Development: Co-Principal Investigator, NIH-HD 10063, (1978-1980).
7. Drug Disposition in Sick Infants and Children: Co-investigator, NIH-HD 10053 (1975-1977).
8. Regulation of Drug Metabolism During Development: Co-investigator, NIH-HD 10063, (1977)
9. Biochemical Genetics of Drug Induced Cleft Palate Teratogenesis: Investigator, NIH-Dental Institute, (197710 Developmental Aspects of Acetaminophen Hepatotoxicity: Principal Investigator, McNeil Laboratories, Ft. Washington, PA

PROMOTED PROFESSIONAL DEVELOPMENT OF STAFF

Stanley Barone, Jr. Ph.D., Acting Assistant Center Director for Planning, NCEA/ORD
Maureen Gwinn, Ph.D. Acting Assistant Center Director for Risk Analysis, NCEA/ORD
Kathryn Guyton, Ph.D. Acting Deputy Director for HHRA/ORD, NCEA/ORD
Kathleen Raffaele, Ph.D. Acting Assistant Director for Risk Analysis, NCEA/ORD

PROFESSIONAL TRAINING OPPORTUNTIES PROVIDED TO SCIENTISTS (Served as mentor/advisor):

Academia

Vinod Bhutani, M.D. (1981)
Chanda Gupta, Ph.D. (1978-1981)
David Kowalczyk, DVM, Ph.D. (1976-1977)
Robert Mancini, Ph.D. (1977-1978)
Larry Andrews, Ph.D. (1976)

American Association for Advancement of Science (AAAS) Fellows

Lynn Adams, Ph.D. (2011 - 2012)
Sarah- Burgess Herbert, Ph.D. (2010-2011)
Scott Glabberman, Ph.D. (2010-2011)
Chadwick Thompson, Ph.D. (2004 - 2005)
Nancy Beck, Ph.D. (2003 - 2005)
Susan Euling, Ph.D. (2001 - 2003)
Dennis Brown, Ph.D. (1998)

NCEA – ORD Post-doctoral Fellows

Douglas Johns, Ph. D. (2006 - 2007)
Maureen Gwinn, Ph. D. (2004 - 2006)
Sederick Rice, Ph. D. (2004 - 2005)
Andrea Kim, Ph.D. (2004 - 2005)

Marquea King, Ph.D. (2003 - 2005)

Oak Ridge Institute for Science and Education (ORISE) Post-doctoral Fellows

Carol Starkey (2015)
Larissa Pardo (2015)
Mary Kushman, Ph. D. (2012 - 2013)
David Szabo, Ph.D. (2011 -2012)
Connie Kang-Sickel Ph.D. (2011)
Brian Pachkowski, Ph.D. (2008 - 2012)
Andrew Kraft, Ph. D. (2010 - 2011)

Oak Ridge Institute for Science and Education (ORISE) Faculty Fellows

Ivan Rusyn, Ph.D., Professor, Univ. North Carolina, Chapel Hill (2011 - 2013)
Gary Ginsberg, Ph.D., Assoc. Professor, Univ. of Connecticut/Yale Univ. (2009 - 2012)
David Eastmond, Ph.D., Professor, Univ. of California, Riverside (2009 - 2012)

Association of Schools of Public Health (ASPH) Fellows

Rebecca Brown, MPH, MES
Cecilia Alcala, MPH (2014 -2015))
Jennifer Mall, MPH (2011 - 2012)
Christine Robel, MPH (2005)
Margaret Adgent, MPH (2004-205)
Ann Bradley, MPH (2004-2005)

Congressional Fellows

Elaine Francis, Ph.D. with Senator Joseph Lieberman
Rebecca Brown, MPH, MES with Congresswoman Diana DeGette and Congressman Henry Waxman

Detail Assignments- Opportunities Provided and Served as Mentor

Don Bergfeld, Ph.D. (OPPT)
Hugh Tilson, Ph.D. (NHEERL-ORD)
Mike Luster, Ph.D. (NIOSH)
Prof. Zoltan Anneu, Ph.D., (Johns Hopkins Univ., Baltimore, MD as an IPA)

MANAGEMENT TRAINING: Examples

- Completed the Federal Executive Institute (FEI) Training – "Leaders for a Democratic Society" Elected by the FEI Class 271 to moderate two sessions of the Executive Forum (August 2001)
- Invited to attend training course "Managing People for Maximum Performance and Promoting Innovation and Organizational Change", Wellesley, MA (July, 2000)
- Promoting Innovation and Organizational Change and Managing People for Maximum Performance Program – Harvard University – John F. Kennedy School of Government, Boston, MA (July 16-20, 2000)
- EPA Senior Executive Service Mentoring Program – Mentor—Alan Sielen, Deputy Assistant Administrator, Office of International Activities/EPA (1999)
- Course on Managing the Federal Employee Discipline and Performance Process offered by the Brookings Institution – Center for Public Policy Education (1998)
- American Business Management Training Course: A five day MPA program (August, 1996)
- Project Officer Course – (1984, 1988, 1996)
- How to Handle Difficult People (June, 1995)
- OPM – Science, Technology and Public Policy – Lancaster, PA (March 6-17, 1995)
- Federal Managers' Financial Integrity Act (FMFIA) Training (March, 1994)
- EPA – Resolving Team Conflicts Course (February, 1994)
- Brookings Institute: Issues in Science and Technology Policy – Williamsburg, VA (December, 1993)
- OPM Government Affairs Institute – Congress and Money (April 12-13, 1993)
- Preventing Sexual Harassment (1993)
- Office of Personnel Management (OPM), Management Development Seminar – Lancaster, PA (March 2-13, 1992)
- ORD Resource Management and Integrity Training (1992)
- Contracts Administration Training for Supervisors and Managers (1992)
- Managing Multiple Projects, Objectives and Deadlines, Skillpath, Inc. (1992)
- Effective Listening and Memory Development (1992)
- Pre-TQM Course – John Pickering (1991)
- Executive Potential Program – OPM (One year training 1990) – William H. Farland (SES Advisor); Developmental Assessments: Edward Johnson, Associate Administrator, OIA/EPA and Michele Mercier (Manager), WHO – IPCS, Geneva, Switzerland (July – August, 1989) and William Jordan, OPPTS/EPA (May- June, 1989). Shadowing Assignment with Joseph Merenda, OPPTS (January, 1990)
- Negotiation Skills Training (1990)
- Position Description Writing for Supervisors and Managers – OPM (1984)
- Effective Writing Course – FDA (1984)

HONORS AND AWARDS: (Examples)

- USEPA Distinguished Special Service Citation in Recognition of 34 Years Dedicated Service to the Citizens of This Great Nation awarded by Administrator of EPA (Gina McCarthy, January, 2016)
- EPA Gold Medal- Libby Amphibole Asbestos Assessment (2015)
- Invited Member of the Nomination Committee for Heinz Foundation Awards, 2011-2013.
- Member of the Advisory Committee on: UC Berkeley NIEHS Superfund Research Center (2012-2014) and Mount Sinai School of Medicine NIEHS Superfund Research program (2000)
- Bronze Medal (2006) awarded by ORD in recognition for preparing approaches for application of PBPK models supporting data in risk assessment.
- Science and Technology Achievement Award – Level II for publication co-authored in Toxicological sciences 66 (2): 185-200 (2002).
- Office of Pesticides Program Bronze Medal for Harmonization of Reproductive and Developmental Toxicology Testing Protocols (1999)
- Office of International Activities/EPA Silver Medal Award for Technical Support on International Lead (Pb) Program (1996)
- EPA's Bronze Medal Award for Lead (Pb) Capacity Building in Developing Countries (1996)
- Recipient of Office of Pesticides and Toxic Substances "Joseph Seifter Memorial Award in Regulatory Science" for higher commitment to scientific excellence and understanding of the public impact of regulatory science (June, 1991).
- Special Act Award for Human Resources Development as Acting deputy Director, ECAO – Cincinnati (1991)
-
- Achievement Awards, OPPT, In recognition of contribution to developing the first set of 25 ATSDR Toxicological Profiles (1987)

- **CERTIFICATES AND LETTERS OF APPRECIATION (Examples):** The President of Fetus as a Patient Institute, Philippines (FPIP) in sincere recognition of contribution as Speaker during the Annual Convention/Postgraduate Course (1996).
- Invited guest speaker – Institute of Children's Hospital Philippines, Manila, Philippines; Awarded Certificate of Appreciation for teaching post-graduate course to physicians and medical support staff as well as for addressing the Third Annual convention of Pediatricians of Philippines (1993, 1996)
- MTBE Risk Assessment effort by OHEA (1995)
- A letter of Appreciation by Dr. James O. Mason, Assistant Secretary for Health Assistance in preparing the US Report to UNICEF to the World Summit on Children (1992)

- Great Lakes Association of Clinical Medicine – Recognized and Honored for participation in teaching Metal Binding in Medicine at the XVIII International Congress, Grand Rapids, Michigan (1992)
- A letter of Appreciation from Assistant Secretary for Health and Human Services – Public service participation in health and Medical Sciences Working Groups of the Indo-US Joint Sub-commission for Cooperation in Science and Technology (1991)
- A letter of Appreciation by Linda Fisher AA/OPTS to recognize contribution in developing the Agency response to the TSCA Section 21 petition on carpet-related health problems at EPA HQ (1990)
- A Special Appreciation Certificate for organizing and contributing to Asian Pacific American heritage Week at FDA by EEO/PHS Director (1984)
- Appreciation of Assignment as Acting Deputy Director of ECAO-Cincinnati – Commendable Efforts for Support both Administratively and technically (1991)

Management of Extramural Resources in support of several research projects, sponsorship of symposia/meetings, workshops, conferences etc (some examples):

Grants and Cooperative Research agreements

- WHO-IPCS, Geneva, Switzerland
- University of Arkansas, Little Rock, AK
- University of Illinois, Chicago, IL
- Mt. Sinai School of Medicine, New York, NY
- Clark University, Worcester, MA
- University of Rochester, Rochester, NY
- International Federation of Teratology Societies, Tokyo (Japan)
- Indiana School of Medicine, Indianapolis, IN
- Georgetown University, Washington, DC
- Cornell University, Ithaca, NY
- Penn State Medical Center, Hershey, PA
- Johns Hopkins University, Baltimore, MD
- Children's Environmental Health Network, Washington, DC
- Teratology Society
- Neurobehavioral Teratology Society
- Environmental Mutagen and Genomics Society
- Society of Risk Analysis, Reston, VA
- Endocrine Society, Washington, DC
- University of Wisconsin, Madison, Wisconsin
- University of Vermont, Burlington, VT

- California Public Health Foundation
- University of Maryland, Catonsville, MD
- University of Washington, Seattle, WA
- University of Virginia, Richmond, VA
- International Life Science Institute- Health and Environmental Sciences (ILSI-HESI)
- Albany Medical College, Albany, NY

Research Contracts: Examples

- University of California, Riverside, CA
- University of San Francisco, San Francisco, CA
- Versar, Inc., Vienna, VA
- Eastern Research Group, Boston, MA
- Syracuse Research Corporation
- Sciences International Inc.
- University of California, Berkley, CA
- Science Applications International Corporation (SAIC)
- Department of Pharmacology, State of Maine
- Department of Environment, New Jersey
- Mitre Corporation, Washington DC
- University of Pittsburgh, Pittsburgh, PA
- University of Arkansas, Little Rock, AK
- Tulane University, New Orleans, LA
- Summit Toxicology
- ENVIRON

Interagency Agreements (National and International): Examples

- National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC
- National Institute of Occupational Health and Safety (NIOSH), Morgantown, WV
- Agency for Toxic Substance and Disease Registry (ATSDR), Atlanta, GA
- National Center for Toxicological Research (NCTR), Food and Drug Administration, Jefferson, AK and Rockville, MD
- HESI- Risk Sciences Institute, Washington, DC
- Ministry of Health, National Institute of Occupational Health, Ahmadabad, India
- Industrial Toxicology Research Center (ITRC), Lucknow; CSIR, New Delhi, India
- Indian Council of Medical Research, Ministry of Health, New Delhi, India

Auditor Report: SciPinion Scientific Review Package NSA3

Independence of the Auditor

The identity of the Sponsor of this peer review was not conveyed by SciPinion or by NeutralScience to this Auditor.

Panel Expertise Verification

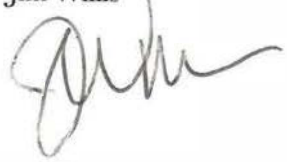
I have reviewed the expertise of the 22 candidates identified by Candidate ID # in the **Audit Summary Table** below. My review has included CV's, publications, indicators of experience available through independent sources, web pages of home institutions, selected manuscripts and the descriptions, data and minimum experience criteria for the peer review panel as described by SciPinion through NeutralScience on April 15, 2016. Based on my review and comparison to minimum expertise criteria and the peer review needed, I have provided a score for each candidate of **0** (does not meet minimum criteria), **1** (meets minimum criteria) or **2** (meets minimum criteria and is also well qualified for this peer review).

Audit Summary Table

Candidate ID #	Name	**Review outcome***
571	[redacted]	1
1003		2
1019		1
1039		0
1032		0
531		2
1291		2
669		0
601		1
1292		1
831		0
547		2
818		1
1280		1
1297		2
1304		0
1309		0
1312		0
1319		1
48		0
518		1
539		0

Notes on each candidate appear in Appendix 1 to this report.

Auditor: Jim Willis

Signature: 

Date: April 20, 2016

Appendix 1

Auditor comments

Candidate ID #	Name	Review outcome*	Auditor comments
571	[redacted]	1	Some rodents, respiratory, pulmonary, oral; no cardiovascular.
1003		2	Rodents, inhalation toxicology, nasal, cardiovascular. Broad experience in all areas.
1019		1	Rodents, oral, inhalation, general toxicology; limited experience in other required areas
1039		0	Very limited experience in required areas; some oral and inhalation.
1032		0	Limited toxicology, oral, inhalation; experience in cardiovascular impacts.
531		2	Extensive rodents, inhalation, aerosols, oral, toxicology; cardiovascular less clear.
1291		2	Rodents, inhalation toxicology, respiratory, cardiovascular, systems toxicology; other areas less clear.
669		0	Predominately particulates and nanomaterials; more occupational/exposure/hygiene than toxicology; some inhalation, respiratory, cardiovascular and -omics, no rodents.
601		1	Some rodents, inhalation toxicology, extensive in vivo. No cardiovascular or nasal/oral toxicology.
1292		1	Extensive cardiovascular, some rodents; limited toxicology, oral inhalation exposure.
831		0	Limited toxicology, cardiovascular, respiratory or exposure routes. Some rodents.
547		2	Considerable mouse, inhalation toxicity, cardiovascular and respiratory/airway; limited oral.
818		1	Some mouse, inhalation, respiratory, and in vivo; cardiovascular, endothelial, oral unclear. Mainly nanomaterials.
1280		1	Rodents, oral, systems toxicology; limited inhalation, cardiovascular, other areas.
1297		2	Rodents, inhalation, respiratory,

	[redacted]		aerosols, cardiovascular and systems toxicology. Limited oral.
1304		0	Limited/no demonstrated expertise in required areas (e.g., mice, cardiovascular, respiratory, inhalation toxicology)
1309		0	Not clear if there is any experience in the required areas.
1312		0	Mainly informatics and genomics; not clear there is direct experience in required areas other than -omics.
1319		1	Rodents, some cardiovascular; oral and respiratory unclear; more radiation than chemical.
48		0	Some rodents; general toxicology. Not clear there is expertise on other required areas.
518		1	Mainly respiratory and –omics, toxicology. Cardiovascular, oral/nasal and rodent work unclear/limited.
539		0	Some in vitro, inhalation, respiratory and rodents. Limited oral and nasal toxicology or cardiovascular. Focus on lung disease/COPD.

* Score of 0 indicates that the candidate did not meet the minimum expertise criteria, 1 indicates that the candidate met the criteria, and 2 indicates that in addition to meeting the criteria the candidate was well qualified for the described task.

Jim Willis
1308 Rockland Terrace
McLean, Virginia, 22101 USA
Telephone: (mobile) 1-571-286-2442
e-mail: zheeeem@gmail.com
US Citizen

WORK EXPERIENCE

1. **President, Jim Willis. LLC**
   McLean, VA USA
   April, 2014 – present

Consultant on nanotechnology regulatory policy and practice and on international chemicals and waste policy, with a particular focus on the policies, procedures and requirements of SAICM and the Basel, Rotterdam and Stockholm Conventions.

2. **Executive Secretary, Basel, Rotterdam and Stockholm Conventions**
   UN Environment Programme (UNEP)
   Geneva, Switzerland
   April, 2011 – April, 2014

Executive Secretary of the three pre-eminent global treaties to ensure the sound management of chemicals and wastes. Responsible for directing a secretariat of 65 staff in holding Conferences of the Parties and subsidiary body meetings and implementing the legal, scientific and policy decisions of the parties and the relevant provisions of the treaties.

3. Director, Chemical Control Division (Senior Executive Service)
October 2004 – April, 2011
Office of Prevention, Pesticides and Toxic Substances
United States Environment Protection Agency
1200 Pennsylvania Avenue NW
Washington DC 20460

Responsible for managing EPA's chemical control program under the Toxic Substances Control Act (TSCA). Elements of this program include review of all new chemicals prior to their entry into commerce, review of existing chemicals for possible risk, administering the chemical testing program, and developing and implementing EPA's program to review and manage nanoscale materials. I also provided input into EPA's international portfolio, including substantive input into the Rotterdam Convention, Stockholm Convention, LRTAP POPs Protocol and OECD Chemicals Committee, of which I chaired the Working Party on Manufactured Nanomaterials from its inception in 2006 until 2009.

4. Director, Chemicals (D-1)
November, 1995 – October 2004
United Nations Environment Programme (UNEP)
15 Chemin des Anemones
CH-1219 Geneva, Switzerland

Responsible for the implementation of UNEP's Chemicals programme, including:

- Executive Secretary of the Rotterdam Convention on the Prior Informed Consent (PIC) Procedure for Certain Hazardous Chemicals and Pesticides in International Trade and the Stockholm Convention on Persistent Organic Pollutants, and manager of the secretariat responsible for the negotiations of these treaties.

- Executive Secretary of the Stockholm Convention on Persistent Organic Pollutants (POPs) and manager of the secretariat responsible for negotiations of this treaty (adopted in 2001

- Managing UNEP's global programmes to reduce the risks of mercury, lead and other heavy metals and initiation of UNEP's global mercury programme, as well as UNEP's programme to assist countries to reduce environmental releases of POPs.

- Design and implementation of the process and negotiations leading to the Strategic Approach to International Chemicals Management (SAICM).

- Administering UNEP's chemical safety capacity building programme, including GEF projects related to chemicals in the international waters and POPs focal areas and mobilizing tens of millions of US$ annually for international chemical safety capacity building.

- Diverse other activities to promote global chemical and pesticides safety, including assessing the global and regional risks of chemicals, issuing publications on chemical safety, developing technical guidelines on POPs and other chemicals, managing projects to build the capacities of developing countries to manage chemicals safely, implementing information clearinghouses and facilitating cooperative programmes with intergovernmental organizations, other convention secretariats, NGOs, and national governments.

- Convening (23) major 6-language intergovernmental meetings to develop global chemicals policy and over 500 regional or national workshops to train and assist. national staff to take actions to identify and reduce chemical risks.

5. Deputy Director, Environmental Assistance Division (GM-15)
(**Acting Director October 1994 – April, 1995**)
October 1992 – November, 1995
Office of Pesticides and Toxic Substances
United States Environment Protection Agency

Responsible for managing EPA's Toxics Release Inventory programme, EPA's "33/50" voluntary emissions reduction programme, and communications, outreach, congressional and regional relations functions for EPA's Office of Pollution Prevention and Toxics.

6. Deputy Director, Existing Chemical Assessment Division (GM-15)
March, 1990 – October, 1992
Office of Pesticides and Toxic Substances
United States Environment Protection Agency

Responsible for managing EPA's risk assessment, risk management, chemical testing, information gathering, and export programme under the Toxic Substances Control Act.

7. Special Assistant to the Director (GM-14)
August, 1988 - March, 1990

Office of Pesticides and Toxic Substances
United States Environment Protection Agency

Assisted the Office Director in managing EPA's biotechnology, risk assessment, risk management, chemical testing, new chemicals and asbestos programmes.

8. Environmental Protection Specialist (GS-13)
April, 1984 – August, 1988
Existing Chemical Assessment Division
Office of Pesticides and Toxic Substances
United States Environment Protection Agency

Staff policy expert and regulatory programme manager for EPA's biotechnology, chemical export programme, information collection regulations, and chemical control regulations under TSCA.

9. Project Manager
September, 1982 – April, 1984
Science Applications International Corporation
McLean, VA 22102

Managed staff and contracts for systems development and scientific support for the US Navy and EPA.

10. Systems Analyst
February, 1981 - September, 1982
CRC Systems
Fairfax, VA 22032

Developed scientific computer systems for EPA pesticides and toxic substances programs.

11. Scientific Analyst
October, 1978 – February, 1981
Raven Systems and Research
Washington DC 20460

Developed and implemented scientific information systems for EPA's pesticide registration program.

12. Chemist
March, 1977 – October, 1978
Tracor, Inc.
Rockville, MD

Developed technical reports and risk assessments for US pesticide program and other government departments.

13. Chemist
June, 1976 – March, 1977
Blue Plains Wastewater Treatment Plant
Washington DC

Analyzed pesticides and industrial chemicals in wastewater and environmental media.

14. Teaching Assistant
June, 1975 – June, 1977
American University
Washington DC

Lab instructor and teaching assistant for analytical chemistry and biochemistry courses.

EDUCATION

Langley High School
McLean VA, Diploma 1971

American University
Washington DC, B.S. Chemistry, 1975
Advanced studies in chemistry, 1975-1977

Verification of Expert Participation

Furthermore, upon review of the information provided to me for the SciPinion user accounts and correspondence for this peer review, I attest that to the best of my knowledge the review comments and judgments of the peer reviewers provided in "Peer Review of Data for a Modified Risk Tobacco Product (THS 2.2, PMI Study Number 15015): Part 2 Mixed Expertise" (signed by Sean M. Hays and Christopher R. Kirman and dated August 30, 2016) belong to the identities indicated in **Table 2**.

Table 2.

Sonawane	Willis	Composite	Candidate ID #	Name
2	1	3	571	[redacted]
2	2	4	1003	
2	0	2	1039	
2	2	4	531	
2	2	4	547	
1	1	2	818	
2	2	4	1297	
2	0	2	1312	
2	1	3	1319	
2	0	2	48	

Signature: **Date:**

8/30/2016

Print:

Richard Canady, PhD

Affiliation:

Managing Director,
NeutralScience, L3C

Appendix D: Editor's Report

SciPinion Editor's Report: Questions for Panels Reviewing *In Vitro* and *In Vivo* Studies Assessing THS2.2

Ted Simon, Ph.D., DABT



Ted Simon LLC
http://www.tedsimon-toxicology.com ted@tedsimon-toxicology.com

April 18, 2016

Introduction

This report provides comments on the document listed in the title. These questions will be used in a SciPinion peer review of research on modified risk tobacco products (MRTPs) conducted by Phillip Morris International Research and Development.

All the questions fall into one of the three categories listed in the *Editor Instructions for SciPinion Peer Reviews*, hereafter referred to as the Editor Instructions.

Accompanying this report is a "track-changes" version of the MS-Word file with editorial revisions and comments on the set of peer review questions. The first section of this report provides substantive changes needed to improve the clarity of the questions and to minimize the use of leading language. The second section provides suggested edits that may improve the questions but are not substantive or necessary. These two sections are consistent with the Editor Instructions. In addition, other very minor changes are provided in the "track-changes" version but not discussed in this report.

This report is provided in MS-Word format to be included in the draft and final SciPi reports per the Editor Instructions.

1. Major Substantive Revisions needed for the SciPi to move forward

The majority of these revisions deal with the questions geared towards eliciting the reviewers' opinions. Revisions were made with the goal of ensuring the questions are neither biased nor leading, fostering clarity and providing the reviewers sufficient information.

First Page

The following text was added to indicate the additional reading for question 29:

> *To answer question 29, reviewers will need to read two papers available for free on PubMed. These are:*
>
> - *Potteaux et al. 2011 Suppressed monocyte recruitment drives macrophage removal from atherosclerotic plaques of Apoe-/- mice during disease regression. J Clin Invest 121(5):2025-36. PMID: 21505265*
> - *Konakahara et al. 2011 A neuronal transmembrane protein LRFN4 induces monocyte/macrophage migration via actin cytoskeleton reorganization. FEBS Lett 585(14):2377-84. PMID: 21704618*

Section 3. Study design, endpoints, and data quality
The description of the two forms of tobacco product—smoke vs. aerosol—was not specific enough in the questions. Hence, text was added at the beginning of the section and individual questions were changed.

At the beginning of the section, the following text was added:

> *In questions 7 - 12 in this section, the word "aerosol" refers to aerosol from the modified tobacco product THS2.2 and the word "smoke" refers to conventional cigarette smoke.*

In questions 7-10, the word "exposure-related" was changed to "aerosol-related" to describe the material applied to the biological test system

In addition, the order of questions #10 and #11 were switched to keep the four "aerosol-related" questions together and the two "smoke-related" questions together.

Section 2. Potential Conflict of Interest or Bias.
Text was added to the expert witness question to include all activities in which an expert providing litigation support might engage. The question now reads:

- *Have you ever served as an expert witness, including deposition and testimony, or served as a consulting (non-testifying) expert in a tobacco related case? (yes/no)*

Question 28
The listed effects referring to the Poussin et al and Van der Toorn studies were reworded to be more consistent with the endpoint descriptions in those studies. The two listed items now read:

- *Monocyte-endothelial cell adhesion / events relevant to atherogenesis in Poussin et al. 2016*
- *Monocyte chemotaxis and migration / events relevant to atherosclerotic changes in Van der Toorn et al. 2016*

Question 29
The following item was added to the list of considered items to be inclusive:

- *Another mechanism not mentioned above (please explain in the comment box)*

Question 35
The word "transversal" seemed confusing. Hence, the question and list of considerations were made more specific for clarity as follows:

> *Do the study designs and models allow a mechanism-based evaluation for mechanisms such as oxidative stress and inflammation across endpoints and between studies?*

- *Yes, the systems approach applied in each study enhances the understanding of pathomechanisms across the various endpoints and between the various studies*
- *Yes, a systems approach can potentially enhance the understanding of pathomechanisms across endpoints and between different studies, but considering the current studies, additional aspects would be required in order to make the* evaluation *of pathomechanisms more conclusive: (please indicate missing aspects in the text box)*
- *No, comparisons across endpoints or different model systems are not reliable; the only reliable approach to understand pathomechanisms is to use separate, mechanism-specific investigations in each of that various dedicated model systems*
- *I cannot answer (please explain in the comment box)*

Question 41
This comment is for consideration. The comment appears in the track-changes version but no actual edit was made. The phrase "human situation" did not seem specific enough. Perhaps this phrase should be made more specific as follows:

> *… human situation, e.g. either substituting tobacco aerosol/vapor inhalation (vaping) for cigarette smoking or quitting tobacco use altogether.*

Question 48
The first two considerations in the list were edited for clarity.

- *The approach was indeed efficient and should be further considered in future studies*
- *Some compromises in design (please identify these and describe in the comment box) are not weighed out by the benefits, and hence separate models would be more efficient*

2. Suggested Edits for Consideration

First Page
Added the phrase "… and manuscripts" to note that the Iskander et al. and Zanetti et al. works are not yet published.

Changed "… to accomplish the following:" to "… to accomplish the following tasks:"

Question 13
The first listed consideration now reads:

- *It is useful for particulate, aerosol, and gaseous exposure*

Question 23
The word "publications" was changed to "manuscripts." The question now reads:

> *Having inspected the study data in the various repositories provided, as well as in the Supplementary materials provided with the Iskandar et al. and Zanetti et al.*

manuscripts reporting assessment of test items on organotypic tissue models, how do you rate the quality of the data, its annotation, and its presentation (1: insufficient, 2: partially sufficient, 3: sufficient). Please comment if rated 1 or 2.

Question 24
The third criterion was reworded as follows:

Sufficient detail in the narrative description of the methods

Question 36
The three sub-questions are clearly related and need to be kept together. The narrative of this question, although clear, is information-dense. No changes were made in the document, but the entire question might be easier for reviewers if the sub-questions were presented in a bulleted list as was done in Question 35.

Question 38
A slight clarification was made. The question now reads:

To demonstrate a reduced level of potentially toxic effects of MRTPs compared with conventional cigarettes, please rate the weight of the following evidence on a 1-5 scale (5 representing strong weight). Please insert any relevant comment in the box below.

Question 41
Prepositional phrase "… on the following studies …" moved for clarity.

Questions 42 – 44
The phrase "investigated further" was substituted for the word "capture." Please see track-changes version.

Question 48
The word "please" was spelled out in the third item

- *Further reduction of animals could be envisioned by the following accomplishments: (please specify)*

Question 52, Grid Title
"Detailed enough" changed to "Sufficient Detail."

Question 54
The name "Garuda" was added in the question text and the URL provided.

Question 55
Added the word "workbook" to the question for clarity.

Question 57
The text of the third listed consideration now reads:

> *Both enough and appropriate information were provided in the question pages to answer to the questions.*

Questions for panels reviewing in vitro and in vivo studies assessing THS2.2

Questions should enable the panelists to address key points in the studies and verify relevance of the study design, methods, and interpretation of the results presented in the following publications and manuscripts:

1. Phillips, B. *et al.* An 8-Month Systems Toxicology Inhalation/Cessation Study in Apoe$^{-/-}$ Mice to Investigate Cardiovascular and Respiratory Exposure Effects of a Candidate Modified Risk Tobacco Product, THS 2.2, Compared with Conventional Cigarettes. *Toxicological Sciences 149, 411-432 (2016).*
2. Poussin et al. Systems toxicology-based assessment of the candidate modified risk tobacco product THS2.2 for the adhesion of monocytic cells to human coronary arterial endothelial cells. *Toxicology* 339:73-86 (2016)
3. van der Toorn et al. Aerosol from a candidate modified risk tobacco product has reduced effects on chemotaxis and transendothelial migration compared to combustion of conventional cigarettes. *Food Chem Toxicol* 86:81-87 (2015).
4. Organotypic nasal manuscript (Iskandar et al)
5. Organotypic oral manuscript (Zanetti et al)

To answer question 29, reviewers will need to read two papers available for free on PubMed. These are:

- Potteaux et al. 2011 Suppressed monocyte recruitment drives macrophage removal from atherosclerotic plaques of Apoe-/- mice during disease regression. J Clin Invest 121(5):2025-36. PMID: 21505265
- Konakahara et al. 2011 A neuronal transmembrane protein LRFN4 induces monocyte/macrophage migration via actin cytoskeleton reorganization. FEBS Lett 585(14):2377-84. PMID: 21704618

Peer reviewers are expected to accomplish the following tasks:

- read in detail the above-mentioned references;
- access the systox webportal to access additional information;
- download some of the data from the systox webportal and review the quality of data annotation;
- provide commentary and appropriate rationale for all answers, including answers to multiple choice questions and any reason why a reviewer cannot answer a particular question;
- review the results in light of existing publications in the same field and cite appropriate references when justifying their answers; and
- read additional publications/documents as necessary to answer some of the questions;

1. Demographics

1. **What is your experience (in years) in the respective fields?**

- General toxicology (0,1-3, 3-10, >10)
- Molecular toxicology (0,1-3, 3-10, >10)
- Systems toxicology (0,1-3, 3-10, >10)
- Risk assessment (0,1-3, 3-10, >10)
- Public health research (0,1-3, 3-10, >10
- The conduct and/or interpretation of tobacco-related studies (0,1-3, 3-10, >10)
- Histopathology (0,1-3, 3-10, >10)
- Other?

2. **Have you been involved in inhalation toxicology studies?**

- Yes, as an author
- Yes, as a reviewer
- No

3. **Are you familiar with 21st Century Toxicology concepts?**

4. **Are you familiar with *in vitro* test methods for respiratory toxicology?**

5. **Have you been involved in regulatory toxicological review, read-across panels, or other activity sponsored by a regulatory agency?**

6. **How would you rate your confidence in leveraging or interpreting results obtained from omics data to compare the toxicological effects of different treatments or substances?**

- Very high
- High
- Medium
- Low
- It depends on the type of omics data being generated.

2. Potential Conflict of Interest or Bias

- *Currently, what percentage of your funding comes from either grant or contract work from (<10%, 10-50%, 50-75%, >75% of my funding)?*
  - *Governments*
  - *Industry*
  - *Non-profit organizations*
  - *Tobacco companies*
  - *Other*

Comment [BS1]: Not for German panel

- *Either in the past or currently, have you received funding from (select all that apply)?*
  - *The tobacco company Philip Morris International*
  - *Another tobacco company*
  - *US FDA*
  - *US EPA*
  - *NIEHS*
  - *NSF*
  - *DKFZ (German cancer research center)*
  - *European Commission*
  - *Anti-tobacco non-profit organizations*
  - *Animal rights organizations*

Comment [BS2]: Possibly add only for German panel

- *Have you ever been an employee of:*
  - *A tobacco company*
  - *The US FDA*
  - *Any regulatory agency that had jurisdiction over tobacco*

- *Have you ever served as an expert witness, including deposition and testimony, or served as a consulting (non-testifying) expert in a tobacco related case? (yes/no)*

Comment [BS3]: Not for German panel

- *Do you know of any reason that you might be unable to provide impartial advice on this or any reason that your impartiality in the matter might be questioned?*

- *Have you had any previous involvement with the document(s) under consideration including authorship, collaboration with the authors, or previous peer review functions? If so, please identify and describe that involvement. (text box)*

- *Have you served on previous advisory panels, committees or subcommittees that have addressed the topic under consideration? If so, please identify those activities. (Text box)*

- *Do you own stock in a tobacco company?*

- *Is anyone in your immediate family an employee of:*
  - *A tobacco company (y/n)*
  - *FDA (y/n)*
  - *An anti-smoking non-profit organization (y/n)*

- *What is your smoking status?*
  - *Nonsmoker*
  - *Former smoker*
  - *Current smoker*

Comment [BS4]: Not for German panel

3. Study design, end points, and data quality

In questions 7 - 12 in this section, the word "aerosol" refers to aerosol from the modified tobacco product THS2.2 and the word "smoke" refers to conventional cigarette smoke.

7. For the evaluation of aerosol-related respiratory disease endpoints reported by Phillips et al. (2016) in an *in vivo* mouse model, how confident are you that the following criteria are met?

Deleted: exposure

- Appropriate choice of model, including species, strain, and gender
- Appropriate choice of controls
- Appropriate number of replicates in respective groups
- Appropriate choice of exposure modality
- Appropriate duration of the exposure
- Appropriate choice of endpoints
- Appropriate choice of analytical methods

8. For the evaluation of aerosol-related effect on the nasal epithelium in an *in vitro* human model reported by Iskandar et al. (2016), how confident are you that the following criteria are met?

Deleted: exposure

- Appropriate choice of model
- Appropriate choice of controls
- Appropriate number of biological and technical replicates in respective groups
- Appropriate choice of exposure modality, including exposure equipment
- Appropriate duration of the exposure
- Appropriate choice of endpoints
- Appropriate choice of analytical methods

9. For the evaluation of aerosol-related effect on the oral mucosa in an *in vitro* human model reported by Zanetti et al. (2016), how confident are you that the following criteria are met?

Deleted: exposure

- Appropriate choice of model
- Appropriate choice of controls
- Appropriate number of biological and technical replicates in respective groups
- Appropriate choice of exposure modality, including exposure equipment
- Appropriate duration of the exposure
- Appropriate choice of endpoints
- Appropriate choice of analytical methods

10. For the evaluation of aerosol-related effect on the adhesion of monocytes on endothelial cells, an atherosclerosis-relevant mechanism, in a human *in vitro* model reported by Poussin et al. (2016), how confident are you that the following criteria are met?

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- Appropriate choice of model, including species, strain, and gender
- Appropriate choice of controls

- Appropriate number of replicates in respective groups
- Appropriate choice of exposure modality
- Appropriate duration of the exposure
- Appropriate choice of endpoints
- Appropriate choice of analytical methods

11. **For the evaluation of smoke-related cardiovascular disease endpoints in an *in vivo* mouse model reported by Phillips et al. (2016), how confident are you that these criteria are met?**

- Appropriate choice of cell types
- Appropriate choice of controls
- Appropriate number of replicates in respective groups
- Appropriate choice of exposure modality
- Appropriate choice of endpoints
- Appropriate choice of analytical methods

12. **For the evaluation of smoke-related effect on monocyte migration, a key atherosclerosis-relevant mechanism, in a human *in vitro* model reported by Van der Toorn et al. (2016), how confident are you that the following criteria are met?**

- Appropriate choice of cell types
- Appropriate choice of controls
- Appropriate number of replicates in respective groups
- Appropriate choice of exposure modality
- Appropriate choice of endpoints
- Appropriate choice of analytical methods

Comment [TS5]: The order of these two questions was switched.

13. **In your opinion what is the utility domain of the Vitrocell Exposure system?**

- It is useful for particulate, aerosol, and gaseous exposure
- It is adaptable to different smoking regimes
- It is suitable of different types of aerosols
- Other (please specify in the comment box)
- I cannot answer (please explain in the comment box)

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14. **Given the scientific question and the available data generated in the studies, do you see any important gap(s) not covered by the authors? Please provide comments below.**

- In the 8-month inhalation study reported in Phillips et al. 2016?
- In the *in vitro* assessment of adhesion reported in Poussin et al. 2016?
- In the in *vitro* assessment of transendothelial migration reported in Van der Toorn et al. 2016?
- In the *in vitro* assessment of effect on respiratory tissue reported in Iskandar et al?
- In the *in vitro* assessment of effect on oral tissue reported in Zanetti et al?

15. **How confident are you that the following criteria related to smoke/aerosol generation and characterization in terms of reproducibility were met in the publications? Please comment in the box below whether more details should be given in every single manuscript.**

Comment [BS6]: 1 column per study – drop down menu of answers: Excellent, sufficient, poor, I cannot answer

- Sufficient level of details and explanation regarding smoke/aerosol generation
- Sufficient level of details and explanation regarding smoke/aerosol characterization

16. **Is the approach in the organotypic *in vitro* studies and *in vivo* study to match aerosol exposure levels on the basis of nicotine, instead of TPM concentration reasonable?**

- Yes (Please specify)
- No (Please specify)
- I cannot answer (please explain in the comment box)

17. **Is the approach in the *in vitro* studies by Poussin et al and Van der Toorn et al. to match the aerosol extract exposure levels on the basis of puffs/mL, instead of nicotine or other constituent concentrations, reasonable?**

- Yes (Please specify)
- No (Please specify)
- I cannot answer (please explain in the comment box)

18. **In your opinion, which tissue characteristics, if any, are missing from the 3D reconstructed buccal mucosa to support an assessment that sufficiently reflects the human response to exposure (e.g. cigarette smoke, aerosols)?**

- Free text

19. **In your opinion, which tissue characteristics, if any, are missing from the 3D reconstructed nasal epithelium to support an assessment that sufficiently reflects the human response to exposure (e.g. cigarette smoke, aerosols)?**

- Free text

20. **On a scale from 1 to 5 (5 being the highest), how relevant, informative, and intuitive are the various types of computational analyses (related to transcriptomics, lipidomics, proteomics) reported in the respective manuscripts?**

	Relevant (1-5)	Informative (1-5)	Intuitive (1-5)
transcriptomics: computational network modeling and NPA calculation			
transcriptomics: gene set enrichment analysis			
miRNA analysis			
proteomics: functional clustering			
Other (please specify)			

21. Having inspected the study data in the various repositories provided, as well as in the Supplementary materials provided with the Phillips et al publication, and supplementary material provided on the systox portal, how do you rate the quality of the data, its annotation, and its presentation (1: insufficient, 2: partially sufficient, 3: sufficient). Please comment if rated 1 or 2.

- In-life observations, mortality, body weight, gross pathology
- Biomarkers of exposure
- Hematology and clinical chemistry (biomarkers of effect & lipoproteins)
- Respiratory physiology
- BAL fluid analysis (free lung cells and inflammatory mediators)
- Histopathology and histomorphometry
- Plaque size measurements (planimetry and microCT)
- Transcriptomics analysis
- Proteomics analysis
- Lipidomics analysis

22. Having inspected the study data in the various repositories provided, as well as in the Supplementary materials provided with the Poussin et al and Van der Toorn et al. publications reporting *in vitro* mechanistic assays, how do you rate the quality of the data, its annotation, and its presentation (1: insufficient, 2: partially sufficient, 3: sufficient). Please comment if rated 1 or 2.

- *Poussin et al.* Functional adhesion assay
- *Poussin et al.* Inflammatory mediators
- *Poussin et al.* Transcriptomics analysis
- *Van der Toorn et al.* Transendothelial migration assay
- *Van der Toorn et al.* Endothelial barrier integrity
- *Van der Toorn et al.* Cytotoxicity and inflammatory behavior of THP-1 cells

23. Having inspected the study data in the various repositories provided, as well as in the Supplementary materials provided with the Iskandar et al. and Zanetti et al. manuscripts reporting assessment of test items on organotypic tissue models, how do you rate the quality of the data, its annotation, and its presentation (1: insufficient, 2: partially sufficient, 3: sufficient). Please comment if rated 1 or 2.

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- *Iskandar et al.* Histopathological evaluation
- *Iskandar et al.* Cytotoxicity (AK assay)
- *Iskandar et al.* CYP1A1/B1 activity assay
- *Iskandar et al.* Characterization of pro-inflammatory mediators
- *Iskandar et al.* Transcriptomics analysis
- *Iskandar et al.* miRNA analysis

- *Zanetti et al.* Histopathological evaluation
- *Zanetti et al.* Cytotoxicity (AK assay)
- *Zanetti et al.* CYP1A1/B1 activity assay
- *Zanetti et al.* Characterization of pro-inflammatory mediators
- *Zanetti et al.* Transcriptomics analysis
- *Zanetti et al.* miRNA analysis
- *Zanetti et al.* Endpoint reproducibility/variability analysis

24. The standards for presenting omics data are still evolving. Regarding the publication of results from omics data in the studies evaluated, please rate the importance of each of the following criteria in determining the suitability of these data for their intended purposes and your confidence (scale of 1-5, 5 being highest confidence) that these criteria were met in the study (please download some files to judge the quality of the annotations and format)? Please also discuss in the text box any other criteria you deem useful or appropriate.

	Importance and fitness for purpose (1-5)	Confidence that criteria were met(1 - 5)
Availability of raw data		
Annotation of the metadata		
Sufficient detail in the narrative description of the methods		
Availability of the source code and/or software		
Possibility to run the code and/or software on own datasets to judge the quality of the tool		
Other (please specify		

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4. Data evaluation and interpretation

25. Are the results in the publication reporting the *in vivo* study (Phillips et al.) presented with sufficient detail, and were they clearly presented?

- In-life observations, mortality, body weight, gross pathology
- Biomarkers of exposure
- Hematology and clinical chemistry (biomarkers of effect & lipoproteins)
- Respiratory physiology
- BAL fluid analysis (free lung cells and inflammatory mediators)
- Histopathology and histomorphometry
- Plaque size measurements (planimetry and microCT)
- Transcriptomics analysis
- Proteomics analysis
- Lipidomics analysis

26. Are the results in the publications reporting the *in vitro* mechanistic assays (Poussin et al., Van der Toorn et al.) presented with sufficient detail, and were they clearly presented?

- *Poussin et al.* Functional adhesion assay
- *Poussin et al.* Inflammatory mediators
- *Poussin et al.* Transcriptomics analysis
- *Van der Toorn et al.* Transendothelial migration assay
- *Van der Toorn et al.* Endothelial barrier integrity
- *Van der Toorn et al.* Cytotoxicity and inflammatory behavior of THP-1 cells

27. Are the results in the publications reporting the *in vitro* assessment with the organotypic systems (Iskandar et al., Zanetti et al.) presented with sufficient detail, and were they clearly presented?

- *Iskandar et al.* Histopathological evaluation
- *Iskandar et al.* Cytotoxicity (AK assay)
- *Iskandar et al.* CYP1A1/B1 activity assay
- *Iskandar et al.* Ciliary beating frequency assay
- *Iskandar et al.* Characterization of pro-inflammatory mediators
- *Iskandar et al.* Transcriptomics analysis
- Iskandar et al. miRNA analysis
- Iskandar et al. Endpoint reproducibility/variability analysis
- *Zanetti et al.* Histopathological evaluation
- *Zanetti et al.* Cytotoxicity (AK assay)
- *Zanetti et al.* CYP1A1/B1 activity assay
- *Zanetti et al.* Characterization of pro-inflammatory mediators
- *Zanetti et al.* Transcriptomics analysis
- Zanetti et al. miRNA analysis
- *Zanetti et al.* Endpoint reproducibility/variability analysis

Comment [BS7]: table format (rows below), column headings: "Sufficient detail?" and "Clarity of presentation", choices: insufficient, partially sufficient, sufficient

Comment [BS8]: table format (rows below), column headings: "Sufficient detail?" and "Clarity of presentation", choices: insufficient, partially sufficient, sufficient

Comment [BS9]: table format (rows below), column headings: "Sufficient detail?" and "Clarity of presentation", choices: insufficient, partially sufficient, sufficient

28. Are the interpretations and conclusions in the publications regarding the effects of exposure to 3R4F cigarette and THS2.2 aerosol in the different models reasonably supported by the results?

- Lung / respiratory effects in Phillips et al. 2016
- Vascular / atherosclerotic effects in Phillips et al. 2016
- Clinical chemistry and systemic effects in Phillips et al. 2016
- Monocyte-endothelial cell adhesion / events relevant to atherogenesis in Poussin et al. 2016
- Monocyte chemotaxis and migration / events relevant to atherosclerotic changes in Van der Toorn et al. 2016
- Respiratory effects in Iskandar et al. 2016
- Oral effects in Zanetti et al. 2016

29. In the transendothelial migration assay, how would you explain the unexpected decrease in monocyte transmigration upon smoke/aerosol extract exposure? Please specify evidence in the comment box below.

- This response is likely a consequence of the leukemic origin of the THP-1 cells and can be expected to be opposite when primary monocytes are employed
- The model design is only suitable to address reverse intravasation of monocytes/macrophages from the subendothelial space back into the vascular lumen as described by Potteaux et al., 2011 (PMID: 21505265)
- Apparently a general cytotoxic or a more specific anti-locomotory effect (e.g., on the organization of the actin cytoskeleton- cf. Konakahara et al., 2011 (PMID: 21704618) of the exposure antagonized the expected increase in transendothelial migration
- The exposure modality (pre-treatment of monocytic and endothelial cells or common exposure during the transmigration phase determines whether CSE exposure is stimulatory or inhibitory
- Transmigration assays mimic *in vivo* mechanisms only under flow conditions and results from static assays are not representative for the initiation of atherosclerosis
- Another mechanism not mentioned above (please explain in the comment box)
- I cannot answer (please explain in the comment box)

30. Considering the results derived from the repetition of the assessment in the nasal organotypic model across multiple phases, how do you rate the reproducibility of this model and consequently its usefulness in product assessment?

- The model is useful to get an overall idea of relative effects
- The model allows quantitative analyses and assessment
- The model is appropriate for mechanistic understanding, but not for product assessment
- The model is not appropriate for assessment of inhaled toxicants
- I cannot answer (please explain in the comment box)

31. Causal biological networks have been developed for understanding and interpreting omics data using a systems toxicology approach (http://causalbionet.com). These networks are developed based on *a priori* biological knowledge about causal signaling pathways. How confident are you that the specific application of the causal network model by Phillips et al.

Comment [BS10]: dropdown menu: Reasonable and well supported by the data, Interpretation goes beyond what the data will support, Unsupported assertions or gaps in the interpretation

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Comment [BS11]: Responses: strongly agree, agree, disagree, strongly disagree

and Iskandar et al. provides an appropriate high-level evaluation of the biological impact (direct or indirect) of an exposure on the transcriptome of tissues investigated?

- Extremely confident
- Very confident
- Confident
- Somewhat confident
- Not confident
- I cannot answer (please explain in the comment box)

32. How critical and informative do you consider the following endpoints for assessing the relative impact of various tobacco products on the *in vivo* model system considered?

Comment [BS12]: Grid answer: critical (low, medium, high, cannot answer); informative (low, medium, high, cannot answer)

- In-life observations, mortality, body weight, gross pathology
- Biomarkers of exposure
- Hematology and clinical chemistry (biomarkers of effect & lipoproteins)
- Respiratory physiology
- BAL fluid analysis (free lung cells and inflammatory mediators)
- Histopathology and histomorphometry
- Plaque size measurements (planimetry and microCT)
- Transcriptomics analysis
- Proteomics analysis
- Lipidomics analysis
- The totality of the data, collectively

33. How critical and informative do you consider the following endpoints for assessing the relative impact of various tobacco products on early steps of atherogenesis?

Comment [BS13]: Grid answer: critical (low, medium, high, cannot answer); informative (low, medium, high, cannot answer)

- *Poussin et al.* Functional adhesion assay
- *Poussin et al.* Inflammatory mediators
- *Poussin et al.* Transcriptomics analysis
- *Van der Toorn et al.* Transendothelial migration assay
- *Van der Toorn et al.* Endothelial barrier integrity
- *Van der Toorn et al.* Cytotoxicity and inflammatory behavior of THP-1 cells
- The totality of the data, collectively

34. How critical and informative do you consider the following endpoints for assessing the relative impact of various tobacco products on the respiratory tract?

Comment [BS14]: Grid answer: critical (low, medium, high, cannot answer); informative (low, medium, high, cannot answer)

- *Iskandar et al.* Histopathological evaluation
- *Iskandar et al.* Cytotoxicity (AK assay)
- *Iskandar et al.* CYP1A1/B1 activity assay
- *Iskandar et al.* Ciliary beating frequency assay
- *Iskandar et al.* Characterization of pro-inflammatory mediators
- *Iskandar et al.* Transcriptomics analysis
- *Iskandar et al.* miRNA analysis
- *Zanetti et al.* Histopathological evaluation

- *Zanetti et al.* Cytotoxicity (AK assay)
- *Zanetti et al.* CYP1A1/B1 activity assay
- *Zanetti et al.* Characterization of pro-inflammatory mediators
- *Zanetti et al.* Transcriptomics analysis
- *Zanetti et al.* miRNA analysis
- *Zanetti et al.* Endpoint reproducibility/variability analysis
- The totality of the data, collectively

35. Do the study designs and models allow a mechanism-based evaluation for mechanisms such as oxidative stress and inflammation across endpoints and between studies?

- Yes, the systems approach applied in each study enhances the understanding of pathomechanisms across the various endpoints and between the various studies
- Yes, a systems approach can potentially enhance the understanding of pathomechanisms across endpoints and between different studies, but considering the current studies, additional aspects would be required in order to make the evaluation of pathomechanisms more conclusive: (please indicate missing aspects in the text box)
- No, comparisons across endpoints or different model systems are not reliable; the only reliable approach to understand pathomechanisms is to use separate, mechanism-specific investigations in each of that various dedicated model systems
- I cannot answer (please explain in the comment box)

36. The analysis of lung transcriptomics data using the causal network model (Philips et al. 2016) predicted a robust CS-induced increase in extracellular matrix (ECM) degradation that disappears upon cessation. Are the quantified CS exposure-related histopathological changes in the lung tissue consistent with ECM degradation throughout CS exposure? Are the inflammatory mediators in BALF reflective of an observed increase in ECM degradation during CS exposure? Are any of the lung functional parameters likely reflective of ECM/tissue degradation?

37. In your opinion, how useful is the assessment of CYP1A1/CYP1B1 activity levels of the *in vitro* nasal and oral epithelial models following cigarette smoke/aerosol exposure in deducing the potential harmful and deleterious effects for cells/tissues of cigarette smoke/aerosol exposure? Do you believe other CYP enzymes should be monitored as well?

38. To demonstrate a reduced level of potentially toxic effects of MRTPs compared with conventional cigarettes, please rate the weight of the following evidence on a 1-5 scale (5 representing strong weight). Please insert any relevant comment in the box below.

- Integration of diverse data modalities within a single study for one biological system (e.g., respiratory)

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Comment [BS15]: If possible, free text box for each question, but keep the 3 "subquestions" together.

Comment [TS16]: Bulleted list for sub-questions? Please see Editor's Report.

- Analysis of the effects of the products on multiple systems (respiratory, cardiovascular) in a single model (Apoe$^{-/-}$ mice)
- Reproducibility of results across full experiment repetitions (see Iskandar et al.)
- Concordance of results for specific mechanisms (e.g. oxidative stress or inflammation) across diverse studies and model systems (Apoe$^{-/-}$ mouse and *in vitro* assays)

39. Can any of the inflammatory networks from the BIF analysis or secreted inflammatory mediators in BALF serve to explain the type of inflammatory cell infiltration observed during the histopathological evaluation in the cessation and switch group in the Apoe$^{-/-}$ THS switching study reported in Phillips et al, 2016?

- Free text

40. Based on your evaluation of the data, is there any additional observation regarding THS2.2 or switch groups that is relevant for the interpretation of the results, but has not been discussed in the manuscripts?

5. Translatability and outlook

41. Do the exposure conditions (dose/regimen/duration) and the biomarkers of exposure allow for extrapolation to the human situation? Please comment on the following studies and cite any appropriate reference.

- *Phillips et al. 2016* - Apoe$^{-/-}$ THS switching study, respiratory effects
- *Phillips et al. 2016* - Apoe$^{-/-}$ THS switching study, vascular effects
- *Phillips et al. 2016* - Apoe$^{-/-}$ THS switching study, systemic effects
- *Poussin et al. 2016 - In vitro* adhesion assay: indirect condition
- *Poussin et al. 2016 - In vitro* adhesion assay: direct condition
- *Poussin et al. 2016 - In vitro* adhesion assay: fresh direct condition
- *Van der Toorn et al. 2016 - In vitro* transendothelial migration assay
- *Iskandar et al. 2016* – Organotypic nasal tissue THS2.2 assessment
- *Zanetti et al. 2016* – Organotypic oral tissue THS2.2 assessment

42. For future studies, which change(s) and/or additional experiment(s) would you suggest to investigate further the effects of both 3R4F and THS2.2 / other MRTP aerosol exposure on respiratory and/or oral tissues?

- Free text

43. For future studies, which change(s) and/or additional experiment(s) would you suggest to investigate further the effects of both 3R4F and THS2.2 / other MRTP aerosol exposure on cardiovascular tissues?

- Free text

44. For future studies, which change(s) and/or additional experiment(s) would you suggest to investigate further systemic effects of both 3R4F and THS2.2 / other MRTP aerosol exposure?

- Free text

45. Considering the *in vivo* study and the underlying evidence you have reviewed, how does the biological impact of switching to THS2.2 compare with the biological impact from smoking cessation?

- Switching leads to a greater biological impact than cessation
- Switching leads to a smaller biological impact than cessation
- Both switching and cessation lead to similar biological impacts.
- Those two groups cannot be compared (please explain the comment box)
- I cannot answer (please explain in the comment box)

Comment [TS17]: Perhaps this phrase should be made more specific as follows: "… human situation, e.g. either substituting tobacco aerosol exposure (vaping) cigarette smoking or quitting tobacco use altogether.

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Comment [BS18]: Columns:
- Dose
- Regimen
- Duration
- Biomarkers of exposure

Possible answers: Not appropriate, moderately appropriate, very appropriate.

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46. Can the approaches reported for the assessment of cigarette smoke or THS2.2 aerosols be applied for a risk assessment of other inhaled toxicants, e.g. environmental air pollution, workplace exposure, etc.?

- Free text

47. What are the most important biological effects of cigarette smoke exposure that are relevant to human disease (COPD and CVD) and considering the 5 publications under review, how strong is the evidence supporting a reduction in effects from THS2.2 exposure compared with 3R4F exposure? (grid answer: importance (1-5) / evidence strength for reduction (1-5))

- Inflammation
- Oxidative stress
- Extracellular matrix (ECM) synthesis and degradation
- Metabolic alterations
- Other (please specify)

48. The authors of the studies reported in the 5 publications under review aimed at reducing and refining the exposure of animals *in vivo* by assessing two human disease models in the same inhalation toxicity study and to maximize the use of various tissues from the same animals within an array of classical toxicity end points plus transcriptomics, proteomics, and lipidomics. Moreover, they have developed *in vitro* models to enable detailed comparative assessment and mechanistic evaluations. Do you think this approach was efficient and worthwhile, and how could it be further improved?

- The approach was indeed efficient and should be further considered in future studies
- Some compromises in design (please identify these and describe in the comment box) are not weighed out by the benefits, and hence separate models would be more efficient
- Further reduction of animals could be envisioned by the following accomplishments: (please specify)
- I cannot answer (please explain in the comment box)

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49. Considering the mechanistic *in vitro* assays and the assessment using *in vitro* organotypic models, how would you characterize each measurement regarding its usefulness for comparison to human clinical exposure-response studies?

- *Poussin et al.* Functional adhesion assay
- *Poussin et al.* Inflammatory mediators
- *Poussin et al.* Transcriptomics analysis
- *Van der Toorn et al.* Transendothelial migration assay
- *Van der Toorn et al.* Endothelial barrier integrity
- *Van der Toorn et al.* Cytotoxicity and inflammatory behavior of THP-1 cells

Comment [BS19]: Dropdown menu: very useful, useful, not useful, cannot answer (please justify your responses in the comment box below)
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50. Considering the assessment using *in vitro* organotypic models, how would you characterize each measurement regarding its usefulness for comparison to human clinical exposure response studies?

Comment [BS20]: Dropdown menu: very useful, useful, not useful, cannot answer (please justify your responses in the comment box below)

- *Iskandar et al.* Histopathological evaluation
- *Iskandar et al.* Cytotoxicity (AK assay)
- *Iskandar et al.* CYP1A1/B1 activity assay
- *Iskandar et al.* Ciliary beating frequency assay
- *Iskandar et al.* Characterization of pro-inflammatory mediators
- *Iskandar et al.* Transcriptomics analysis
- *Iskandar et al.* miRNA analysis
- *Zanetti et al.* Histopathological evaluation
- *Zanetti et al.* Cytotoxicity (AK assay)
- *Zanetti et al.* CYP1A1/B1 activity assay
- *Zanetti et al.* Characterization of pro-inflammatory mediators
- *Zanetti et al.* Transcriptomics analysis
- *Zanetti et al.* miRNA analysis
- *Zanetti et al.* Endpoint reproducibility/variability analysis

6. Tools and Review Process

51. Was the access to the digitalized slides in the Aperio platform useful for your review?

- Yes
- No
- I did not visit the portal
- Optional Comment

52. How would you qualify the annotation (e.g. use of ontologies, richness of annotation), and the structure (isa-tab format) of the data available in the underlying database (via the sbv IMPROVER systems toxicology Web Portal?

	Sufficient Detail (1-5)	Intuitive (1-5)	Informative / Helpful (1-5)
Data annotation			
Description of protocols (M&M)			
Use of ontologies			
Raw data format			
Processed data format			
ISA tab format			
Illustrations in M&M			

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53. In collaboration with Selventa, the SDPplus platform has been made available for this review. The SDPplus platform allows users to evaluate transcriptomics data in

reverse causal analyses, including the network perturbation amplitude analyses. Algorithms built into this platform use measurable biology to infer casual mechanisms or biological impact on causal network models. Based on your experience with the SDPplus platform, please select all that apply:

- SDPplus allows users to assess which key processes or pathways are most impacted by a given treatment
- SDPplus allows users to explore large-scale omics study datasets in a comprehensive manner
- The implementation of the network perturbation amplitude (NPA) and Biological Impact Factor (BIF) in SDPplus allows users to assess and compare transcriptomics profiles in an insightful manner
- SDPplus is intuitive and easy to use and benefit from
- A platform such as SDPplus is very useful and should be made available to the whole scientific community
- I did not understand the intended purpose of the platform and/or the presentation of the results, and therefore did not benefit from it
- I cannot comment on SDPplus (please explain in the comment box)
- None of the above

54. In collaboration with the Systems Biology Institute in Japan, custom-built software called Garuda gadgets (http://ec2-54-95-52-79.ap-northeast-1.compute.amazonaws.com/GarudaWebNew/index.html#home), have been made available for this review. Follow the instructions in the details section below to be able to install and use the gadgets. Based on your experience with the gadgets, please select all that apply:

- Garuda gadgets allows users to assess which key processes or pathways are enriched for biological functions for a given set of genes under a specific condition of study
- Garuda gadgets allows users to explore large-scale omics study datasets and perform enrichment for genes and proteins
- Garuda gadgets allows users to assess and compare the transcriptomics profiles for a specific gene or set of genes between two study conditions
- Garuda gadgets allow the visualization of pathways associated with the genes in transcriptomics profile data
- Garuda gadgets are very useful and should be made available to the whole scientific community
- I cannot comment on Garuda (please explain in the comment box)
- None of the above

55. Tableau is a software for visualization and data analytics (http://www.tableau.com). Usually, authors of scientific publications choose specific data visualization (figure) to convey a particular message. Reviewers and readers may want to view the data in other ways to fully grasp the relevance of it. To allow for interactive browsing of study results, a Tableau workbook was prepared (see question-specific link in the Introduction). In your opinion, this workbook (select all that apply):

- Is superfluous given the images that are already provided in the publication

- Could be useful but is not easy to understand
- Is useful and should be included as supplementary material for publication of large studies whenever possible

56. What additional material/information would you wish to have to better assess the relevance of the studies and their results?

57. Regarding the information provided on the sbv IMPROVER systems toxicology Web Portal (select all that apply)

- The navigation on the website was intuitive and I found the information I needed easily
- The content was not easily accessible
- Background information provided in the question pages was misleading
- Both enough and appropriate information were provided in the question pages to answer to the questions
- The tutorial videos were useful to efficiently review the material
- The tutorial videos and tools were distracting for the review purposes
- I was lacking information and had to go back to the article reprint on the publisher's website
- I was missing key functionalities (please specify below)
- I did not visit the portal

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58. How could this peer review process be improved?

59. Your participation in this peer review is completely anonymous. For future peer reviews, how important was anonymity to your participation in this peer review?

- Very important: I don't want anyone to know that I took part in this peer review
- Somewhat important: I would be comfortable having my name disclosed, but want my individual responses to the questions to remain undisclosed.
- Not important: I would be comfortable having my name and individual responses to the questions disclosed.

60. Your participation in this peer review was completely independent. For future peer reviews, would you prefer to have anonymous interaction with other panel members (e.g., online comment/debate)? Please indicate the reasons for your answer in the text box.

- Yes
- No

61. Your participation included an offer of compensation. For future peer reviews, how important is compensation for your participation?

- Not important: I would be willing to participate for free
- Somewhat important: I prefer some level of compensation for my participation, but this could be less than received for this effort

- Important: I require compensation for my participation, and the level of compensation is appropriate
- Very Important: I require compensation for my participation, but the level of compensation would need to be increased

***** *Please make sure to review the associated information before selecting this answer*