Peer Review of Data for a Modified Risk Tobacco Product (THS 2.2, PMI Study Number 15015): Part 1 In Vivo Study - Histopathology Focus

Prepared for:

Philip Morris International

Prepared by:

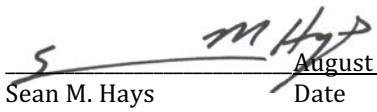


August 30, 2016

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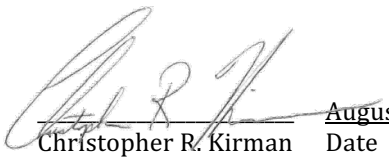
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The undersigned certify that the contents of the report provide an accurate description of the opinions of the peer review panel members.



August 30, 2016

Sean M. Hays Date
President, SciPinion



August 30, 2016

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Vice President, SciPinion

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1. Survey_65_results.csv

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 PMI Study Number 15015, which included five published manuscripts:
   a. 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).
   b. Titz, B. et al. Effects of cigarette smoke, cessation and switching to two heat-not-burn tobacco products on lung lipid metabolism in C57BL/6 and Apoe-/- mice – an integrative systems toxicology analysis. Toxicological Sciences. 149, 441-457, doi:10.1093/toxsci/kfv244 (2016).
   c. Lo Sasso, G. et al. Effects of cigarette smoke, cessation and switching to a candidate modified risk tobacco product on the liver in Apoe−/− mice – a systems toxicology analysis. Inhalation toxicology – in press.
   d. Szostak, J. et al. Long term exposure to cigarette smoke impacts the expression of genes involved in cardiac muscle structure and function in Apoe-/- mouse while exposure to the aerosol of a Tobacco Heating System does not. BMC Medical Genomics – submitted
   e. Lo Sasso, G. et al. The Apoe−/− mouse model: a suitable animal model to study cigarette smoke-induced cardiovascular and respiratory diseases. J. Translat. Sci – submitted.

   In addition, the sponsor provided web portals to access histopathology slides and 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 (see **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.

Table 1. Candidate Experts for Peer Review Panel

Expertise Criteria (souce of information):														
				Gender	Highest Degree	Board of Pathology (e.g., DACVP, ECVP, ESVP)	Year of Degree	Years Experince	Geographic Location (CV)	Employment Sector	# Papers/ Reports (CV or online)	# First/Last Author (CV or online)	Participation in Pathology Peer Review	Pathology Society/Association Memberships
			Minimum Value:	*NA*	***PhD/DVM/DVSc/MD***	*NA*	*NA*	*5*	Diverse	NA	2	NA	NA	NA
Candidate Values:														
Candidate #	**Name (Last, First)**	**Email**	**Title/Position**	**Gender**	***PhD/DVM/DVSc/MD***	**Board of Pathology (e.g., DACVP, ECVP, ESVP)**	**Year of Degree**	**Years Experince**	**Location (CV)**	**Employment Sector**	**# Papers/ Reports (CV or online)**	**# First/Last Author (CV or online)**	**Participation in Pathology Peer Review / Workgroup**	**Pathology Society/Association Memberships**
1048	*Redacted*			M	Ph.D/DVSc.	DACVP	2000	16	Singapore	Research Institute	13	6	Yes	3
576				M	Ph.D		2002	14	Brazil	Academia	79	16		
1012				F	DVM/PhD	DACVP	1996	20	US	Academia	113	8		3
1098				F	DVM	DACVP	2006	10	US	Academia	15	5		1
1046				M	PhD	VR, MRCVS, FRCpath, DECVP	1994	22	West Indies	Academia	91	26		3
1031				F	PhD	ECVP, DESV	2008	8	France	Contract Laboratory	7	1		2
1043				F	PhD/ DVM	ESVP	2007	9	Romania	Contract Laboratory	16	8		3
1003				M	PhD	DACVP	2009	7	US	Contract Laboratory	23	12	Yes	4
1021				F	DVM, DVSc	CVMA, NAVLE	2003	13	Canada	Hospital/ Medical Research	22	1		4
1028				F	PhD/DVM	ECVP, ESVP	2002	14	Australia	Academia	67	33	Yes	10
1053				M	PhD/DVM	DACVP	1977	39	US	Consultant	36	8		6
532				F	DVM	DACVP, NVMB	1996	20	US	Academia	94	28	Yes	6
514				M	PhD		2009	7	India	Research Institute	49	18		
534				M	DVM/PhD	DACVP	1983	33	US	Hospital/ Medical Research	130	38		6
1034				F	BSc	Candidate not evaluated further								

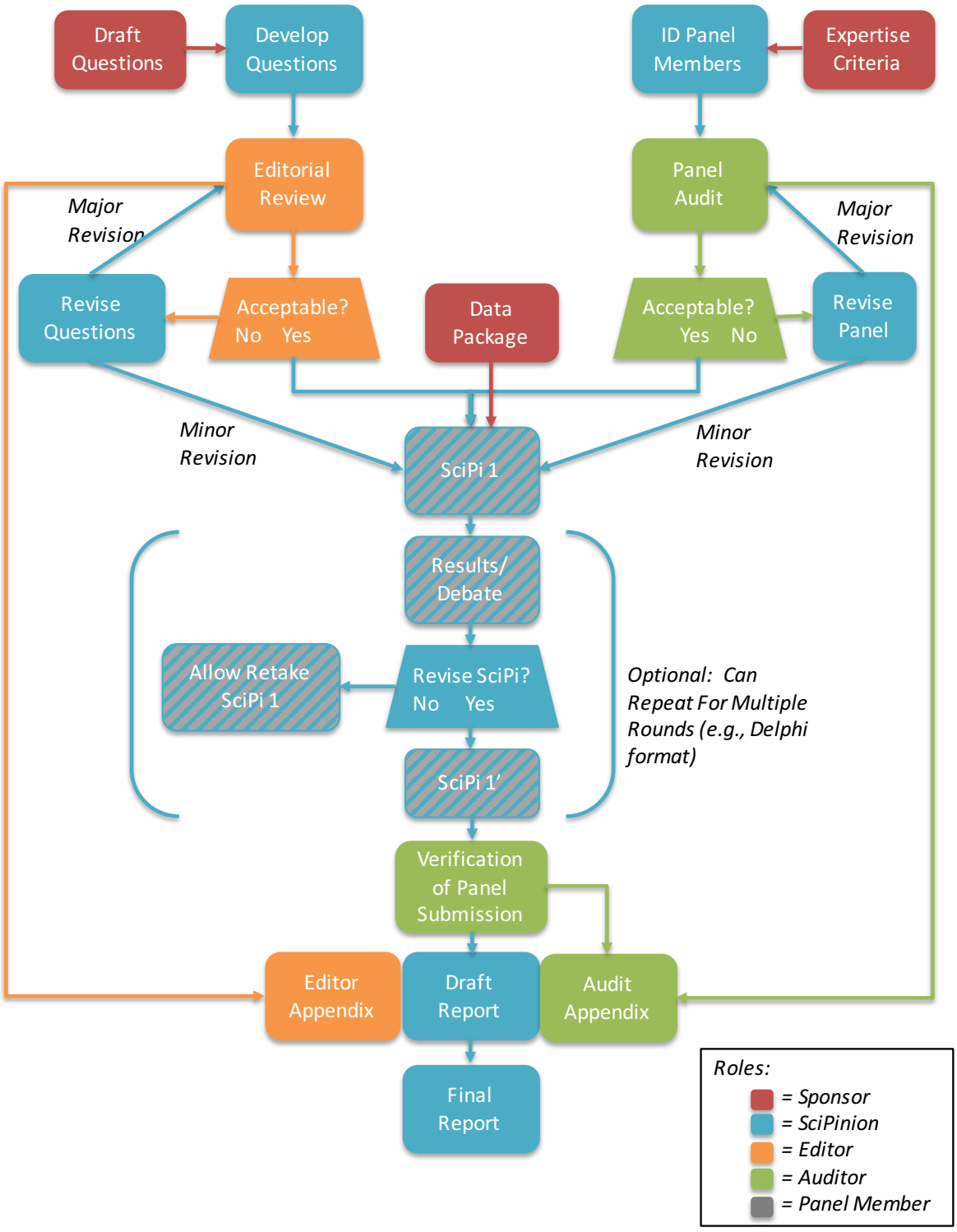
2. Methods

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:

- ***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 independent 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 histopathology 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**). Six peer review panel members (five initial members plus one replacement) 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 545 potential candidates were identified as having relevant experience in histopathology 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 545 potential candidates, requesting interested candidates to volunteer on www.scipinion and upload a copy of their CV/resume.

Panel Expertise Verification

Based on the recruitment effort, 15 candidates volunteered. 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 12 candidates.

Panel Selection

Information relevant to panel selection criteria was extracted from CVs from the 12 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. Five 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 (-13%), this decrease was offset by a large increase in the diversity score (+40%) for the panel. Prior to placing under contract, one selected member dropped out (illness), and was replaced by a candidate that yielded the next highest expertise-diversity score. After an outlier analysis was performed on the panel member responses (see below), one panel member responses were flagged, and a replacement panel member was identified based on providing the next highest expert-diversity score. This additional engaged expert completed the peer review.

Panel Engagement

Six panel members were placed under contract, and the email addresses corresponding to their SciPinion accounts were verified as belonging to the experts. All five panel members participated in the peer review. The peer review period was started on March 22, 2016 and completed on July 15, 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 -ciPi questions are provided in graphical format in **Appendix B** and the raw data are available in the file survey_results_65.csv (see attached) and for download from www.scipinion.com as a delimited text file for the five panel members retained. Peer reviewer responses for the panel member identified as a potential outlier are presented in **Appendix E**.

Evaluation of Potential Outliers

For question responses that can be readily codified to a numeric value (e.g., ratings of 1-5, confidence statements 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 - value is higher than the nominal number of questions, since each cell in the grid-formatted 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 with +/- 0.5 grade units of the mean 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 are less than the mean - 0.5 grade units 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 the mean + 0.5 grade units for other members). The results of this evaluation are provided in **Table 2**. Based on this evaluation one panel member (Expert 843) was flagged based on criteria 2. Only 1% of their responses were less confident than the other panel members, as compared to ~52% of their responses were more confident than the other members (i.e., this expert is ~54 times more likely to be more confident than less confident, when compared to the other panel members). Upon inspection of the demographics responses for this panel member, it was learned that their self-reported years of experience falls below the minimum required for panel selection (1-3 years), which for panel selection purposes was estimated from the year an advanced degree received (this panel member received both PhD and DVM, which can yield different estimates for years of experience). For this reason, the response pattern observed for this panel member may be attributed to a lack of relevant experience. Based on this evaluation, the responses from this panel member have been flagged as potential outliers, and are presented separately from those from the remaining panel (**Appendix E**).

Table 2. Evaluation of Potential Outliers

	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)**
1166	31.4%	49.8%	18.8%
875	29.0%	53.6%	17.4%
842	23.7%	61.8%	14.5%
876	29.0%	46.9%	24.2%
843	***0.97%****	46.9%	52.2%
846	47.3%	47.3%	5.3%

*Flagged as a potential outlier

Flag	<5%	<30%	<5%
Number flags	1	0	0

Demographics

Excluding one potential outlier, the expert panel consisted of five scientists. In addition to the demographics information presented in **Appendix B**, the following information was obtained from panel member CVs:

- Gender: Male (3), Female (2)
- Countries of origin (unique): 3
- Total person-years of experience: 127
- Years of experience: 25±10 years
- Number of publications: 78±47
- Number of first or last author publications: 16±15

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). However, our panel had a nominally lower number of publications compared to typical FDA advisory panels (170±146; ERG, 2007[1]), but this was intentional since 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), so this may not be an apples-to-apples comparison.

[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.

Potential Conflicts of Interest

Based on the information presented in **Appendix B**, no potential conflicts of interests are identified for any of the panel members.

Science

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

- *Overall* - Overall the results were positive, with 64% of responses being either supportive or very supportive, 18% of responses being neutral/weak, and 17% of responses being not supportive (**Figure 2A**).
- *Study Design* - With respect to study design/methods, 86% of responses being either supportive or very supportive, 12% of responses being neutral/weak, and 2% of responses being not supportive (**Figure 2B**).
- *Data Evaluation/Translatability* - For data evaluation/translatability, 62% of responses being either supportive or very supportive, 19% of responses being neutral/weak, and 19% of responses being not supportive (**Figure 2C**).

The summary of codified results in **Figure 2** for the panel contrast sharply with that prepared for the excluded panel member in **Appendix E**. The panel members' opinions on specific science issues are provided in **Appendix B**. A few notable items are provided below:

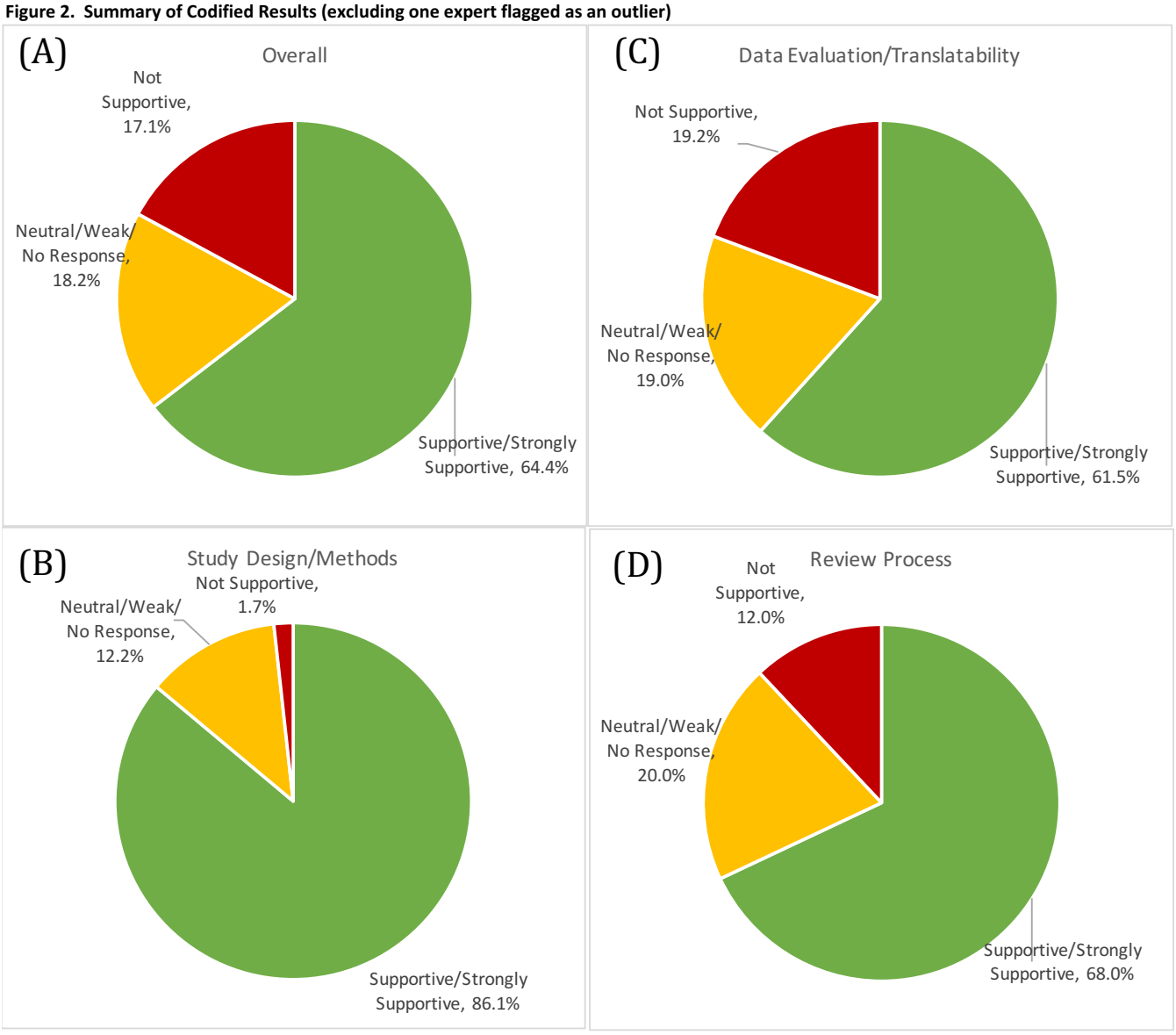
- A majority of panel members (4/5 – 5/5) believe that the approach used for histopathogical evaluation and morphometry were an example of "state of the art" scientific technique.
- A majority of panel members (3/5 – 5/5) believe that the interpretations and conclusions of the study authors were reasonably supported by the data with respect to exposure to cigarette smoke and THS2.2 aerosol.
- A majority of panel members (4/5 – 5/5) believe that the liver, aortic arch, and upper respiratory tract histopathology results were correctly interpreted by the study authors.
- 4/5 panel members concluded there was no difference between cessation and switch groups with respect to histopathology and histomorphometry.

Review Process

For results that can be readily codified for the review process, the results again are positive with 68% of responses being either supportive or very supportive, 20% of responses being neutral/weak, and 12% of responses being not supportive (**Figure 2D**). Overall, panel members felt that the tools used in the review (Aperio, SBVImprover) were helpful in their review. The reviewers were generally in favor of including some degree of anonymity and compensation as part of the review

structure, however, a majority (3/5) would prefer to have had anonymous interaction with other panel members.

Figure 2. Summary of Codified Results (excluding one expert flagged as an outlier)



Appendix A. Survey Questions

SURVEY PREVIEW

Veterinary Histopathologist Wanted

Paid Peer Review Opportunity #65 $7000 for ~56 hrs

Generated: 2016-05-31 12:17:10 +0000
URL: https://scipinion.com/survey_preview/65

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: **PLEASE CLICK TO DOWNLOAD PAPERS**

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. Titz, B. et al. Effects of cigarette smoke, cessation and switching to two heat-not-burn tobacco products on lung lipid metabolism in C57BL/6 and Apoe-/- mice – an integrative systems toxicology analysis. Toxicological Sciences. 149, 441-457, doi:10.1093/toxsci/kfv244 (2016).
3. Lo Sasso, G. et al. Effects of cigarette smoke, cessation and switching to a candidate modified risk tobacco product on the liver in Apoe−/− mice – a systems toxicology analysis. Inhalation toxicology – in press
4. Szostak, J. et al. Long term exposure to cigarette smoke impacts the expression of genes involved in cardiac muscle structure and function in Apoe-/- mouse while exposure to the aerosol of a Tobacco Heating System does not. BMC Medical Genomics - submitted
5. Lo Sasso, G. et al. The Apoe−/− mouse model: a suitable animal model to study cigarette smoke-induced cardiovascular and respiratory diseases. J. Translat. Sci – submitted.

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). Peer reviewers are expected to accomplish the following:

- 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;
- read additional publications/documents as necessary to answer some of the questions; and
- review images of lung and liver sections.

Because many of the questions include hyperlinks to additional information, it is strongly recommended that this peer review be conducted using a desktop or laptop computer (rather than tablet or cell phone). Peer reviewers are able to save their progress so that they may complete.

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

Thank you for your participation!

SECTION 1: DEMOGRAPHICS

ID #1769

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

Field	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 #1770

2) Have you been involved in inhalation toxicology studies?

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

Please explain your answer(s)

ID #1771

3) 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 #1772

4) How would you rate your confidence in leveraging or interpreting results obtained from omics data to compare and contrast 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 #1773

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

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

Please explain your answer(s)

ID #1833

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

- [ ] A tobacco company
- [ ] Philip Morris International
- [ ] US FDA
- [ ] US EPA
- [ ] NIEHS
- [ ] NSF
- [ ] Anti-tobacco non-profits
- [ ] European Commission

Please explain your answer(s)

ID #1834

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 #1776

4) Have you ever served as an expert witness in a tobacco related case?

- ◯ Yes
- ◯ No

Please explain your answer(s)

ID #1777

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 #1778

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.

ID #1779

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

ID #1780

8) Do you own stock in a tobacco company?

- Yes
- No

Please explain your answer(s)

ID #1781

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

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

Please explain your answer(s)

ID #1782

10) What is your smoking status?

- ○ Nonsmoker
- ○ Former smoker
- ○ Current smoker

Please explain your answer(s)

ID #1783

11) Please use the text box to provide a brief statement about your potential biases that may influence your scientific opinion. Biases are not necessarily based on financial relationships or past employment. Also, If you believe you are without any bias that might influence you in this review, please indicate so.

SECTION 3: STUDY DESIGN, END POINTS, AND DATA QUALITY

ID #1784

1) For the evaluation of respiratory disease endpoints, how confident are you that the following criteria are met by Phillips et al. (2016)?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	Very confident	Confident	Somewhat confident	Not confident	I cannot answer based on the information provided
Appropriate choice of animal 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 #1785

2) For the evaluation of cardiovascular disease endpoints, how confident are you that these criteria are met by Phillips et al. (2016)?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	Very confident	Confident	Somewhat confident	Not confident	I cannot answer based on the information provided
Appropriate choice of animal 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 #1786

3) The study authors chose to evaluate the data in each treatment group versus sham at each respective time point rather than across time because different pools of animals were used to measure endpoints at the different time points. How do you judge this approach?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ◯ It is satisfactory and sufficient
- ◯ It is appropriate but not comprehensive enough (please specify what you believe is missing)
- ◯ Some measurements that can be done longitudinally should complement the study design (please specify which)
- ◯ It is not appropriate (please specify)
- ◯ I cannot answer this question

Please explain your answer(s)

ID #1787

4) Are the cessation and switching groups appropriately designed to achieve the proposed aims? In particular, do you think that 2 months of exposure to 3R4F cigarette aerosol prior to cessation or switching is appropriate? Would you expect different results if the cessation or switching time point was set to 3 or 4 months?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1788

5) Is the approach used for histopathological evaluation and morphometry an example of a "state of the art" scientific technique and consistent with published literature/guidelines regarding (please provide any relevant comments and suggestions for improvements in the text box below):

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	Yes	No	I cannot answer
tissue preparation, including type of staining (for lung)	○	○	○
tissue preparation, including type of staining (for lung)	○	○	○
number of sections evaluated	○	○	○
blinding / randomization	○	○	○
scoring system	○	○	○
histomorphometry methods	○	○	○
statistical evaluation	○	○	○
data visualization	○	○	○

Please explain your answer(s)

ID #1789

6) Is the histopathological liver analysis presented in Lo Sasso et al 2016 appropriate to detect possible hepatotoxic effect? Would you recommend additional observations or analyses?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

SECTION 4: DATA EVALUATION AND INTERPRETATION

ID #1790

1) Are the results in the four publications presented with sufficient detail, and were they clearly presented?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

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

	Sufficient Detail?	Clarity of Presentation
In-life observations, mortality, body weight, gross pathology		
Biomarkers of exposure, urine		
Biomarkers of exposure, blood, plasma		
Clinical chemistry, including blood lipoproteins		
Hematology		
Respiratory physiology		
BAL fluid - inflammatory mediators		
BAL cells (free lung cells) - identification and activation markers		
Histopathology, upper respiratory tract		
Histopathology, lung		
Histopathology, liver		
Histopathology, other organs/tissues		
Histomorphometry, lung		

Plaque size measurement, planimetry		
Plaque size measurement, micro-CT		
Dataset, transcriptomics		
Dataset, lipidomics		
Dataset, proteomics		

Please explain your answer(s)

ID #1792

2) Are the interpretations and conclusions in the publications regarding the effects of exposure to 3R4F cigarette and THS2.2 aerosol in the Apoe-/- mouse model 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 Titz et al. 2016	○	○	○
Lung / respiratory effects in Phillips et al. 2016	○	○	○
Vascular / atherosclerotic effects in Phillips et al. 2016	○	○	○
Cardiac effects in Szostak et al. 2016	○	○	○
Clinical chemistry and systemic effects in Phillips et al. 2016	○	○	○
Hepatic effects in Lo Sasso et al. 2016	○	○	○

Please explain your answer(s)

ID #1793

3) How critical and informative do you consider each endpoint shown in Philips et al. for assessing the relative impact of various tobacco products?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

Answer Options: *High* *Medium* *Low* *I cannot answer*

	Critical	Informative
Clinical chemistry (Figure 2, Supplementary Fig. 8, and supplementary results file 2)		
Aortic arch planimetric plaque measurements (Figure 3)		
microCT-based aortic arch plaque measurements (Figure 4, Suppl. Fig. 9, and supplementary files)		
Plasma and aortic arch lipidomics (Figure 5)		
Respiratory physiology (Figure 6)		
Lung volume measurements (Figure 7)		
Bronchoalveolar lavage fluid – FACS analysis of free lung cells (Figure 8)		
Bronchoalveolar lavage fluid – Multianalyte Profiling (Figure 9, supplementary Fig. 12)		
Nasal epithelium histopathological evaluation (Figure 10)		
Lung histology (Figure 11, Supplementary Results File 2)		
Lung histomorphometry (Figure 12)		
Lung transcriptomics results – Differentially expressed genes (Figure 13, Supplementary Fig. 13)		
Lung transcriptomics results – NPA/BIF analysis (Figure 14)		
Lung proteomics analysis (Figure 15, Supplementary Figs. 14 to 16)		
The totality of the data, collectively (All figures)		

Please explain your answer(s)

ID #1795

4) Does this study design allow a transversal evaluation of a number of mechanisms, such as oxidative stress and inflammation?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ◯ Yes, this systems approach demonstrated the potential to enhance the understanding of pathomechanisms also in a transversal manner
- ◯ Yes, a systems approach can potentially enhance the understanding of pathomechanisms also in a transversal manner, but in the current study, the following additional aspects would be required in order to make it more conclusive: (pls. indicate missing aspects in the text box)
- ◯ No, the only reliable approach to understand pathomechanisms is to use separate, mechanism-specific investigations in various dedicated model systems
- ◯ I cannot answer the question

Please explain your answer(s)

ID #1796

5) Do the aortic arch plaque measurements (planimetry and micro-CT imaging) support the authors' statement that cessation or switching to THS2.2 halted the progression of atherosclerotic plaques?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ◯ Yes, both plaque measurements confirm the statement
- ◯ Only the 2D measurements confirm the statement
- ◯ Only the CT imaging confirm the statement
- ◯ No, the statement is not supported by these data
- ◯ I cannot answer this question

Please explain your answer(s)

ID #1797

6) To demonstrate reduced effect of RRPs 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
Side-by-side analysis of effects observed in two independent studies for two different RRPs: 1) an early prototype RRP (pMRTP) and 2) a later RRP using a newer tobacco heating system (THS2.2) (see, Titz et al. 2016)	○	○	○	○	○
Integration of diverse data modalities (e.g. proteomics and lipidomics data in Titz et al. 2016) within a single study	○	○	○	○	○
Analysis of the effects of the products on multiple systems (respiratory, cardiovascular) in a single model	○	○	○	○	○
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 #1798

7) 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?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1799

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

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1800

9) Looking at lung slides on the Aperio platform for the different groups, do you agree that the histopathological evaluation and results support the authors' (Phillips et al. 2016) conclusion that:

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	Yes	No	I cannot answer
"THS2.2 aerosol-exposed lungs were indistinguishable from sham"	○	○	○
"switching or cessation inhibited the further progression of emphysema-related parameters, leading to a convergence with the sham/THS2.2 group levels"	○	○	○

Please explain your answer(s)

ID #1801

10) Are the liver, aortic arch, and upper respiratory tract histopathology results correctly interpreted by the authors regarding the effects of exposure to aerosol from 3R4F cigarettes and THS2.2 in this mouse model?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	Yes	No	I cannot answer
Liver	○	○	○
Aortic arch	○	○	○
Upper respiratory tract	○	○	○

Please explain your answer(s)

ID #1869

11) Do histopathological, morphometric, functional (pulmonary function) and other investigations indicate emphysematous changes at 2 months of 3R4F cigarette smoke exposure?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ○ Yes
- ○ No
- ○ I cannot answer

Please explain your answer(s)

ID #1803

12) Given the lung slides provided in the Aperio tool, please rate the magnitude of change (1-5 scale, 5 being the largest magnitude of change) you observe between 3R4F aerosol and sham (please specify the ID numbers of slides you have looked at and based your evaluation on)?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	1	2	3	4	5
Presence/number of lymphocytic cells and neutrophilic granulocytes in the intra-alveolar space	○	○	○	○	○
Presence/number of non-pigmented macrophages in the alveolar lumen	○	○	○	○	○
Presence/number of macrophages with yellow-brownish pigmentation in the alveolar lumen	○	○	○	○	○
Presence/number of pigmented macrophages that are arranged in small intra-alveolar clusters (described as pigmented macrophage nests) in the alveolar lumen	○	○	○	○	○
Presence/number of macrophages that appear to be enlarged or multi-nucleated cells, and in part contain PAS-positive material in the cytoplasm, in the alveolar lumen	○	○	○	○	○
Presence/number of aggregates of lymphocytic cells in the lung interstitium; i.e., alveolar interstitium, peri-vascular area, interstitium at main bronchus	○	○	○	○	○
Amount of non-cellular eosinophilic material in the alveolar lumen	○	○	○	○	○
Emphysematous lesions characterized morphologically by alveolar septal destruction as well as by mild enlargement of the distal airways	○	○	○	○	○
Multifocal alveolar emphysema	○	○	○	○	○
Emphysema locally associated with mild hyperplasia/hypertrophy of the alveolar epithelium	○	○	○	○	○
Emphysema locally associated with the accumulation of inflammatory cells	○	○	○	○	○
Hyperplasia of the bronchial epithelium	○	○	○	○	○
Hyperplasia of the bronchial goblet cells	○	○	○	○	○

Please explain your answer(s)

ID #1805

13) Given the lung slides provided in the Aperio tool, please rate the magnitude of change (1-5 scale, 5 being the largest magnitude of change) you observe between THS2.2 and 3R4F aerosols (please specify the ID numbers of slides you have looked at and based your evaluation on)?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	1	2	3	4	5
Presence/number of lymphocytic cells and neutrophilic granulocytes in the intra-alveolar space	○	○	○	○	○
Presence/number of non-pigmented macrophages in the alveolar lumen	○	○	○	○	○
Presence/number of macrophages with yellow-brownish pigmentation in the alveolar lumen	○	○	○	○	○
Presence/number of pigmented macrophages that are arranged in small intra-alveolar clusters (described as pigmented macrophage nests) in the alveolar lumen	○	○	○	○	○
Presence/number of macrophages that appear to be enlarged or multi-nucleated cells, and in part contain PAS-positive material in the cytoplasm, in the alveolar lumen	○	○	○	○	○
Presence/number of aggregates of lymphocytic cells in the lung interstitium; i.e., alveolar interstitium, peri-vascular area, interstitium at main bronchus	○	○	○	○	○
Amount of non-cellular eosinophilic material in the alveolar lumen	○	○	○	○	○
Emphysematous lesions characterized morphologically by alveolar septal destruction as well as by mild enlargement of the distal airways	○	○	○	○	○
Multifocal alveolar emphysema	○	○	○	○	○
Emphysema locally associated with mild hyperplasia/hypertrophy of the alveolar epithelium	○	○	○	○	○
Emphysema locally associated with the accumulation of inflammatory cells	○	○	○	○	○

Hyperplasia of the bronchial epithelium	○	○	○	○	○
Hyperplasia of the bronchial goblet cells	○	○	○	○	○

Please explain your answer(s)

ID #1807

14) Given the lung slides provided in the Aperio tool, please rate the magnitude of change (1-5 scale, 5 being the largest magnitude of change) you observe between switch and cessation (please specify the ID numbers of slides you have looked at and based your evaluation on)?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	1	2	3	4	5
Presence/number of lymphocytic cells and neutrophilic granulocytes in the intra-alveolar space	○	○	○	○	○
Presence/number of non-pigmented macrophages in the alveolar lumen	○	○	○	○	○
Presence/number of macrophages with yellow-brownish pigmentation in the alveolar lumen	○	○	○	○	○
Presence/number of pigmented macrophages that are arranged in small intra-alveolar clusters (described as pigmented macrophage nests) in the alveolar lumen	○	○	○	○	○
Presence/number of macrophages that appear to be enlarged or multi-nucleated cells, and in part contain PAS-positive material in the cytoplasm, in the alveolar lumen	○	○	○	○	○
Presence/number of aggregates of lymphocytic cells in the lung interstitium; i.e., alveolar interstitium, peri-vascular area, interstitium at main bronchus	○	○	○	○	○
Amount of non-cellular eosinophilic material in the alveolar lumen	○	○	○	○	○
Emphysematous lesions characterized morphologically by alveolar septal destruction as well as by mild enlargement of the distal airways	○	○	○	○	○

Multifocal alveolar emphysema	○	○	○	○	○
Emphysema locally associated with mild hyperplasia/hypertrophy of the alveolar epithelium	○	○	○	○	○
Emphysema locally associated with the accumulation of inflammatory cells	○	○	○	○	○
Hyperplasia of the bronchial epithelium	○	○	○	○	○
Hyperplasia of the bronchial goblet cells	○	○	○	○	○

Please explain your answer(s)

ID #1809

15) For each exposure group, 10 animals were included for the histopathology analysis. Within single animal lungs, 5 different section levels are available on the Aperio platform with H&E staining, and 1 level with AB-PAS staining. How do you judge the homogeneity of the findings within lungs and across animals from the same groups? Please specify the finding and which slides you considered in your evaluation in the text box below.

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

Answer Options: *High consistency* *Moderate consistency* *Low consistency*

	Intra-animal homogeneity	Inter-animal homogeneity
Presence/number of lymphocytic cells and neutrophilic granulocytes in the intra-alveolar space		
Presence/number of non-pigmented macrophages in the alveolar lumen		
Presence/number of macrophages with yellow-brownish pigmentation in the alveolar lumen		
Presence/number of pigmented macrophages that are arranged in small intra-alveolar clusters (described as pigmented macrophage nests) in the alveolar lumen		

Presence/number of macrophages that appear to be enlarged or multi-nucleated cells, and in part contain PAS-positive material in the cytoplasm, in the alveolar lumen		
Presence/number of aggregates of lymphocytic cells in the lung interstitium; i.e., alveolar interstitium, peri-vascular area, interstitium at main bronchus		
Amount of non-cellular eosinophilic material in the alveolar lumen		
Emphysematous lesions characterized morphologically by alveolar septal destruction as well as by mild enlargement of the distal airways		
Multifocal alveolar emphysema		
Emphysema locally associated with mild hyperplasia/hypertrophy of the alveolar epithelium		
Emphysema locally associated with the accumulation of inflammatory cells		
Hyperplasia of the bronchial epithelium		
Hyperplasia of the bronchial goblet cells		

Please explain your answer(s)

ID #1811

16) Is there any other substantial finding that you would report for the THS2.2 or switch group based on the analysis of the slides you performed on the Aperio tool?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1813

17) Given the histopathology and histomorphometry results, how substantial would you consider the difference between the cessation and switch groups?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ◯ Large difference
- ◯ Moderate difference
- ◯ Minimal difference
- ◯ No difference at all

ID #1814

18) Do you find evidence for any substantial effect in the liver tissue linked to exposure to THS2.2 aerosol? Please specify.

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

SECTION 5: TRANSLATABILITY AND OUTLOOK

ID #1815

1) How confident are you that the lung histopathological evaluation in mice as conducted in the present study allows translation to human pathology? Which finding is most suitable? (please indicate in the comment box)

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	Extremely confident	Very confident	Confident	Somewhat confident	Not confident	I cannot answer
Presence/number of lymphocytic cells and neutrophilic granulocytes in the intra-alveolar space	○	○	○	○	○	○
Presence/number of non-pigmented macrophages in the alveolar lumen	○	○	○	○	○	○
Presence/number of macrophages with yellow-brownish pigmentation in the alveolar lumen	○	○	○	○	○	○
Presence/number of pigmented macrophages that are arranged in small intra-alveolar clusters (described as pigmented macrophage nests) in the alveolar lumen	○	○	○	○	○	○
Presence/number of macrophages that appear to be enlarged or multi-nucleated cells, and in part contain PAS-positive material in the cytoplasm, in the alveolar lumen	○	○	○	○	○	○
Presence/number of aggregates of lymphocytic cells in the lung interstitium; i.e., alveolar interstitium, peri-vascular area, interstitium at main bronchus	○	○	○	○	○	○
Amount of non-cellular eosinophilic material in the alveolar lumen	○	○	○	○	○	○
Emphysematous lesions characterized morphologically by alveolar septal destruction as well as by mild enlargement of the distal airways	○	○	○	○	○	○
Multifocal alveolar emphysema	○	○	○	○	○	○
Emphysema locally associated with mild hyperplasia/hypertrophy of the alveolar epithelium	○	○	○	○	○	○
Emphysema locally associated with the accumulation of inflammatory cells	○	○	○	○	○	○
Hyperplasia of the bronchial epithelium	○	○	○	○	○	○

Hyperplasia of the bronchial goblet cells	○	○	○	○	○	○

Please explain your answer(s)

ID #1817

2) For future studies, would you suggest alternative methodologies to capture the effects of both 3R4F and THS2.2 / other MRTP aerosol exposure on the respiratory tissues and if so, which ones?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1818

3) In your opinion, can the cigarette smoke effect findings be leveraged or translated to risk assessment of other inhaled toxicants, such as environmental air pollution, workplace exposures, etc.? Please cite appropriate references to justify your answer.

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1819

4) For future studies, would you suggest examination of other tissues or biological samples to increase the overall level of scientific confidence and translatability to humans?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1820

5) Which type of study do you deem appropriate to study the impact of switching to THS2.2 in smokers? Select all that apply, and please indicate in the comment box which in vitro or in vivo model might apply.

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	Extremely confident	Very confident	Confident	Somewhat confident	Not confident	I cannot answer (please explain in the comment box)
Clinical, long term	○	○	○	○	○	○
Clinical, short term	○	○	○	○	○	○
In vivo	○	○	○	○	○	○
In vitro	○	○	○	○	○	○

Please explain your answer(s)

ID #1821

6) What are the most important biological effects of cigarette smoke exposure on the lung that are relevant to human disease (i.e., COPD) and how strong is the evidence supporting a reduction in effects from THS2.2 compared with 3R4F (1-5; 5 = most important or strongest)?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

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

	Importance	Strength
Inflammation		
Oxidative stress		
Extracellular matrix (ECM) production, deposition and degradation		
Metabolic alterations		
Other (please specify)		

Please explain your answer(s)

ID #1822

7) The authors of these studies aimed at reducing and refining the exposure of animals in vivo by assessing two human diseases in the same inhalation toxicity study and to maximize the use of various tissues from the same animals to an array of classical toxicity end points plus transcriptomics, proteomics, and lipidomics. Do you think this approach was efficient and worthwhile, and how could it be further improved?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ◯ Efficient approach, should be further considered in future studies
- ◯ Some compromises in design (which ones?) 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)

Please explain your answer(s)

SECTION 6: TOOLS AND REVIEW PROCESS

ID #1823

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

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

Please explain your answer(s)

ID #1824

2) Tableau is a software for visualization and data analytics (http://www.tableau.com). Showing some of the results in a particular manner in a scientific article may not allow readers to grasp the full relevance of the results. To allow for interactive browsing of study results, a Tableau workbook was prepared (see link below). In your opinion this (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 #1825

3) What additional material/information would you need to obtain to better assess the relevance of the study and its results?

ID #1826

4) 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
- [ ] Enough and appropriate information was 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 #1827

5) How could this peer review process be improved?

ID #1828

6) How could the sbv IMPROVER systems toxicology Web Portal be made more valuable in responding to the questions?

ID #1829

7) 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 #1830

8) 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 #1831

9) 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

Veterinary Histopathologist Wanted

Paid Peer Review Opportunity #65 $7000 for ~56 hrs

Generated: 2016-07-21 12:55:19 +0000
URL: https://scipinion.com/question_groups/65/results

SECTION: DEMOGRAPHICS

ID #1769 (https://scipinion.com/surveys/65/results/1769)

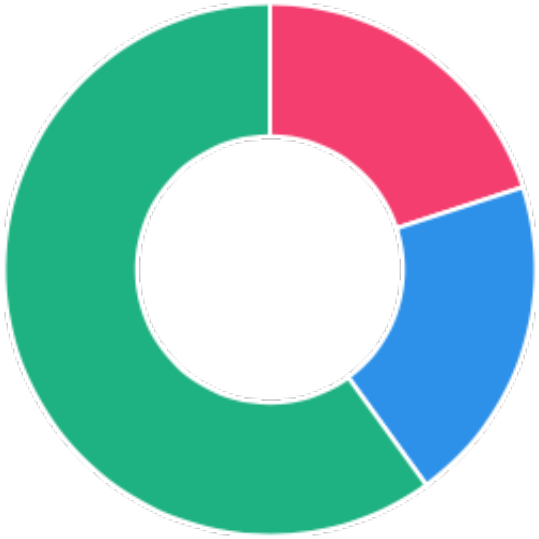
What is your experience (in years) in the respective fields?

Field	0 years	1-3 years	3-10 years	>10 years	Total
General toxicology	25.00% 1	25.00% 1	25.00% 1	25.00% 1	4
Molecular toxicology	50.00% 2	0.00% 0	25.00% 1	25.00% 1	4
Systems toxicology	25.00% 1	50.00% 2	0.00% 0	25.00% 1	4
Risk assessment	0.00% 0	50.00% 2	0.00% 0	50.00% 2	4
Public health research	25.00% 1	25.00% 1	25.00% 1	25.00% 1	4
The conduct and/or interpretation of tobacco-related studies	100.00% 4	0.00% 0	0.00% 0	0.00% 0	4
Histopathology	0.00% 0	0.00% 0	20.00% 1	80.00% 4	5
Other	0.00% 0	0.00% 0	0.00% 0	100.00% 1	1

Appendix B: Survey Results

ID #1770 (https://scipinion.com/surveys/65/results/1770)

Have you been involved in inhalation toxicology studies?



Legend

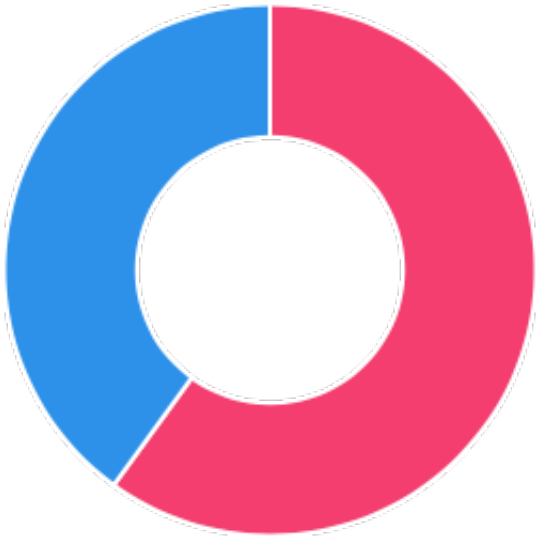
- Yes, as an author: 1 *(20%)*
- Yes, as a reviewer: 1 *(20%)*
- No: 3 *(60%)*

answers: 5
skips: 0

Appendix B: Survey Results

ID #1771 (https://scipinion.com/surveys/65/results/1771)

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



Legend

- Yes: 3 *(60%)*
- No: 2 *(40%)*

answers: 5

skips: 0

Appendix B: Survey Results

ID #1772 (https://scipinion.com/surveys/65/results/1772)

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



Legend

- Very high: 0 *(0%)*
- High: 0 *(0%)*
- Medium: 5 *(100%)*
- Low: 0 *(0%)*
- It depends on the type of omics data being generated: 0 *(0%)*

answers: 5

skips: 0

Appendix B: Survey Results

SECTION: POTENTIAL CONFLICT OF INTEREST OR BIAS

ID #1773 (https://scipinion.com/surveys/65/results/1773)

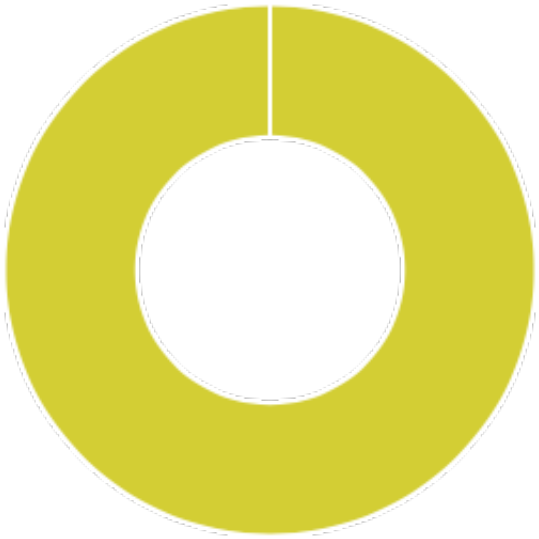
Currently, what percentage of your funding comes from either grant or contract work from the following

	<10%	10-50%	50-75%	>75%	Total
Governments	33.33% 1	33.33% 1	33.33% 1	0.00% 0	3
Industry	50.00% 2	25.00% 1	25.00% 1	0.00% 0	4
Non-profit organizations	0.00% 0	0.00% 0	50.00% 1	50.00% 1	2
Tobacco companies	100.00% 2	0.00% 0	0.00% 0	0.00% 0	2
Other	0.00% 0	100.00% 2	0.00% 0	0.00% 0	2

Appendix B: Survey Results

ID #1833 (https://scipinion.com/surveys/65/results/1833)

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



Legend

- A tobacco company: 0 *(0%)*
- Philip Morris International: 0 *(0%)*
- US FDA: 0 *(0%)*
- US EPA: 0 *(0%)*
- NIEHS: 0 *(0%)*
- NSF: 0 *(0%)*
- Anti-tobacco non-profits: 0 *(0%)*
- European Commission: 1 *(100%)*

answers: 1

skips: 4

Appendix B: Survey Results

ID #1834 (https://scipinion.com/surveys/65/results/1834)

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: 0 *(0%)*

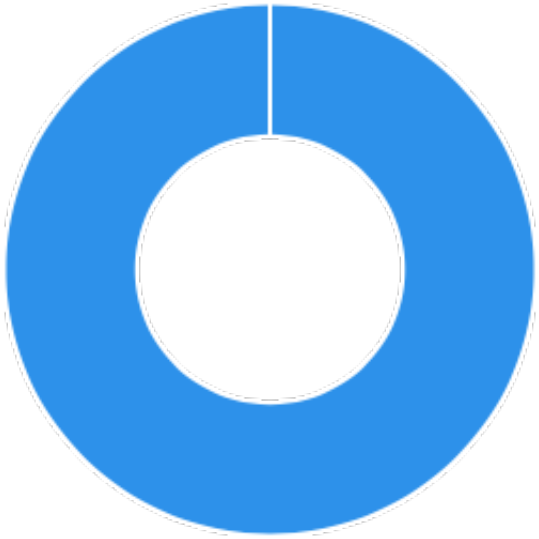
answers: 0

skips: 5

Appendix B: Survey Results

ID #1776 (https://scipinion.com/surveys/65/results/1776)

Have you ever served as an expert witness in a tobacco related case?



Legend

Yes: 0 *(0%)*

No: 5 *(100%)*

answers: 5

skips: 0

ID #1777 (https://scipinion.com/surveys/65/results/1777)

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?

> No. I have no conflict of interest.

> No

> No

> No, this seems a straightforward scientific review and I have no strong feelings.

> No: my knowledge would mainly focus on histopathology, study design and interpretation of omics data relevant to histopathological findings. I am a histopathologist rather than a toxicologist

ID #1778 (https://scipinion.com/surveys/65/results/1778)

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.

> No.

> No

> No

> I've had no involvement with any of the work in these papers or any of the listed authors to my knowledge.

> No

ID #1779 (https://scipinion.com/surveys/65/results/1779)

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

> No.

> No

> No

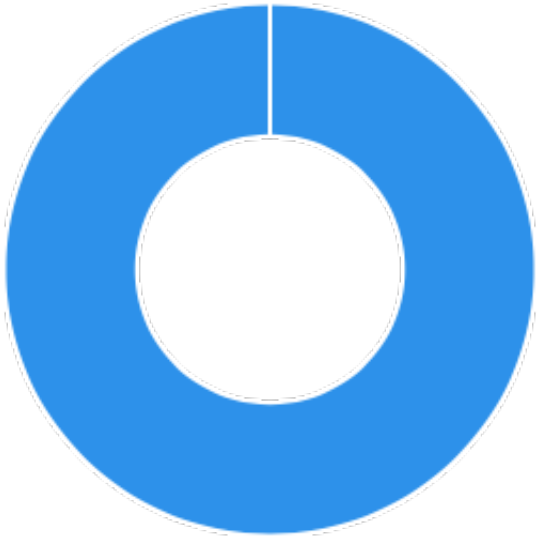
> No.

> No

Appendix B: Survey Results

ID #1780 (https://scipinion.com/surveys/65/results/1780)

Do you own stock in a tobacco company?



Legend

Yes: 0 *(0%)*

No: 5 *(100%)*

answers: 5

skips: 0

ID #1781 (https://scipinion.com/surveys/65/results/1781)

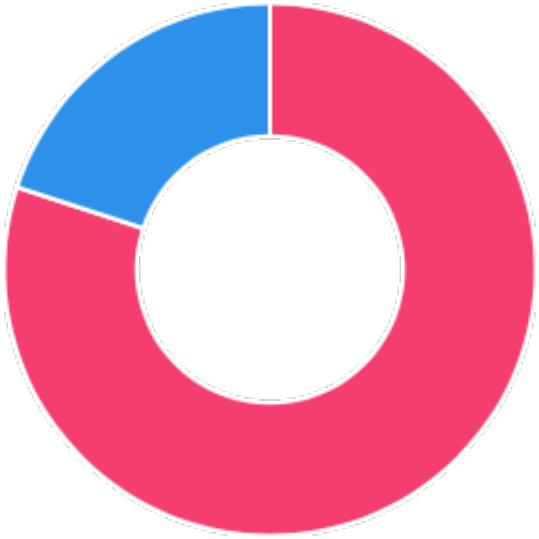
Is anyone in your immediate family an employee of?

	Yes	No	Total
A tobacco company	0.00% 0	100.00% 5	5
FDA	0.00% 0	100.00% 5	5
An anti-smoking non-profit	0.00% 0	100.00% 5	5

Appendix B: Survey Results

ID #1782 (https://scipinion.com/surveys/65/results/1782)

What is your smoking status?



Legend

- Nonsmoker: 4 *(80%)*
- Former smoker: 1 *(20%)*
- Current smoker: 0 *(0%)*

answers: 5
skips: 0

ID #1783 (https://scipinion.com/surveys/65/results/1783)

Please use the text box to provide a brief statement about your potential biases that may influence your scientific opinion. Biases are not necessarily based on financial relationships or past employment. Also, If you believe you are without any bias that might influence you in this review, please indicate so.

> I do not believe that I have any bias that would influence this review.

> I believe that I have no bias in the interpretation and evaluation of the documents provided

> I am fully aware that tobacco smoke is damaging to the lung and that prolonged use of cigarettes will substantially increase the risk of lung cancer and/or emphysema. However, I am interested in learning more about the effects on the lung of products designed to reduce these risks.

> I do not believe I harbor any personal feelings that would influence my scientific judgement on these matters.

> I am a histopathologist and have designed many projects and understand the interpretation of data. I am not a toxicologist, but have experience as a histopathologist working in a toxicological environment and also I worked on a number of studies focused on "cause and effect", so therefore I may have a different bias in knowledge

Appendix B: Survey Results

SECTION: STUDY DESIGN, END POINTS, AND DATA QUALITY

ID #1784 (https://scipinion.com/surveys/65/results/1784)

For the evaluation of respiratory disease endpoints, how confident are you that the following criteria are met by Phillips et al. (2016)?

	Very confident	Confident	Somewhat confident	Not confident	I cannot answer based on the information provided	Total
Appropriate choice of animal model, including species, strain, and gender	40.00% 2	60.00% 3	0.00% 0	0.00% 0	0.00% 0	5
Appropriate choice of controls	60.00% 3	40.00% 2	0.00% 0	0.00% 0	0.00% 0	5
Appropriate number of replicates in respective groups	60.00% 3	20.00% 1	20.00% 1	0.00% 0	0.00% 0	5
Appropriate choice of exposure modality	60.00% 3	40.00% 2	0.00% 0	0.00% 0	0.00% 0	5
Appropriate duration of the exposure	0.00% 0	40.00% 2	60.00% 3	0.00% 0	0.00% 0	5
Appropriate choice of endpoints	40.00% 2	60.00% 3	0.00% 0	0.00% 0	0.00% 0	5
Appropriate choice of analytical methods	20.00% 1	40.00% 2	40.00% 2	0.00% 0	0.00% 0	5

Appendix B: Survey Results

ID #1785 (https://scipinion.com/surveys/65/results/1785)

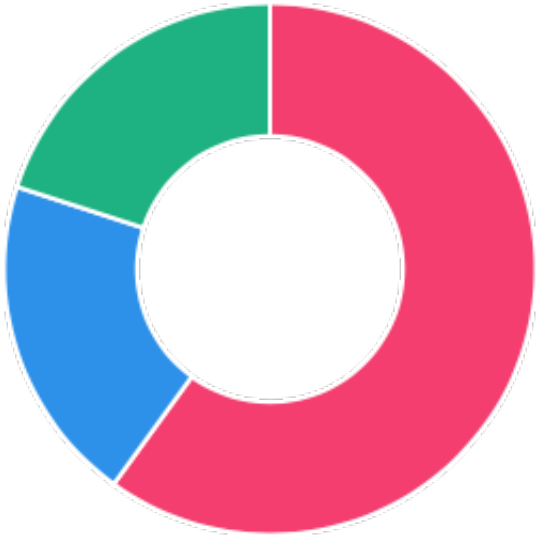
For the evaluation of cardiovascular disease endpoints, how confident are you that these criteria are met by Phillips et al. (2016)?

	Very confident	Confident	Somewhat confident	Not confident	I cannot answer based on the information provided	Total
Appropriate choice of animal model, including species, strain, and gender	20.00% 1	80.00% 4	0.00% 0	0.00% 0	0.00% 0	5
Appropriate choice of controls	60.00% 3	40.00% 2	0.00% 0	0.00% 0	0.00% 0	5
Appropriate number of replicates in respective groups	60.00% 3	20.00% 1	20.00% 1	0.00% 0	0.00% 0	5
Appropriate choice of exposure modality	40.00% 2	60.00% 3	0.00% 0	0.00% 0	0.00% 0	5
Appropriate duration of the exposure	20.00% 1	60.00% 3	20.00% 1	0.00% 0	0.00% 0	5
Appropriate choice of endpoints	40.00% 2	40.00% 2	20.00% 1	0.00% 0	0.00% 0	5
Appropriate choice of analytical methods	40.00% 2	20.00% 1	40.00% 2	0.00% 0	0.00% 0	5

Appendix B: Survey Results

ID #1786 (https://scipinion.com/surveys/65/results/1786)

The study authors chose to evaluate the data in each treatment group versus sham at each respective time point rather than across time because different pools of animals were used to measure endpoints at the different time points. How do you judge this approach?



Legend

- It is satisfactory and sufficient: 3 *(60%)*
- It is appropriate but not comprehensive enough (please specify what you believe is missing): 1 *(20%)*
- Some measurements that can be done longitudinally should complement the study design (please specify which): 1 *(20%)*
- It is not appropriate (please specify): 0 *(0%)*
- I cannot answer this question: 0 *(0%)*

answers: 5

skips: 0

ID #1787 (https://scipinion.com/surveys/65/results/1787)

Are the cessation and switching groups appropriately designed to achieve the proposed aims? In particular, do you think that 2 months of exposure to 3R4F cigarette aerosol prior to cessation or switching is appropriate? Would you expect different results if the cessation or switching time point was set to 3 or 4 months?

> As mentioned above, based on the data that I have seen and from reading a few additional papers in this field, I think that a longer smoking period could potentially change the results that were observed.

> There may be slightly different results due to more lung damage if left until 3 to 4 months.

> The cessation and switching groups do not seem to establish either reduced risk nor safety of THS compared to CS. Conversely, the data from these groups was very helpful many times to identify the presence of systematic variation (time of year, cohort differences, whatever) in the measured responses. I immediately identified the presence of variation of (time point) columns of transcript/proteomic data in heat maps and other measurements. Certainly I would expect cessation or switching after 3-4 months of exposure to CS to take longer for many data (lung pigmented macrophages, etc.) to resolve and revert to levels near control. That said, the Apoe KO mouse is a short-lived model, longer experiments might have been even more complicated by the model's abnormally rapid senescence and mortality. It would still be a valid design and the results would add to the knowledge of this mouse model of human disease.

> From a histopathology standpoint yes the cessation and switching at 2 months is likely to be appropriate. By two months we have observed CS associated changes such as nasal respiratory epithelium reserve cell hyperplasia and metaplasia, in addition to BALF and alveolar lumen inflammatory cell infiltrations. The study clearly highlights that such changes are reversed after this time and thus highlight that even if smoking cannot be stopped the switching to a MRTP would have a relatively similar effect as cessation. However all these findings are in the context of a relatively short term exposure study, albeit in a model which has accelerated diseases processes. Would you expect different results if cessation or switch was at 3-4 months? There is a potential possibility that following a more prolonged exposure that inflammatory, hyperplasia and metaplastic changes may be more resistant to being reversed or may progress to more dramatic lesions or cause greater destruction of underlying tissues. This was also alluded to in Phillips et al 2016 where it was commented on during the in-life results for weight changes that animals in a previous sudy where cessation happened after 3 months only partial weight recovery was achieved (Lietz et al, 2013). This may suggest some additional or more sustained pathogenic mechanisms may exist by 3 months. Therefore for completeness such studies should be extended, but require fine balancing with the natural rapid pathology changes within this model.

> Longer term exposure to 3R4F would likely have resulted in more severe pulmonary lesions (emphysema and inflammation) that would be less reversible in the cessation and switching groups. However, I believe that the 2 month exposure was probably sufficient to demonstrate the similar

> effects of cessation and switching on pulmonary pathology.

Appendix B: Survey Results

ID #1788 (https://scipinion.com/surveys/65/results/1788)

Is the approach used for histopathological evaluation and morphometry an example of a "state of the art" scientific technique and consistent with published literature/guidelines regarding (please provide any relevant comments and suggestions for improvements in the text box below):

	Yes	No	I cannot answer	Total
tissue preparation, including type of staining (for lung)	100.00% 5	0.00% 0	0.00% 0	5
tissue preparation, including type of staining (for lung)	100.00% 5	0.00% 0	0.00% 0	5
number of sections evaluated	100.00% 5	0.00% 0	0.00% 0	5
blinding / randomization	100.00% 5	0.00% 0	0.00% 0	5
scoring system	80.00% 4	20.00% 1	0.00% 0	5
histomorphometry methods	80.00% 4	0.00% 0	20.00% 1	5
statistical evaluation	80.00% 4	0.00% 0	20.00% 1	5
data visualization	80.00% 4	20.00% 1	0.00% 0	5

ID #1789 (https://scipinion.com/surveys/65/results/1789)

Is the histopathological liver analysis presented in Lo Sasso et al 2016 appropriate to detect possible hepatotoxic effect? Would you recommend additional observations or analyses?

The pathology scoring criteria are poorly described in the methods including the estimate of the glycogen content. However, in the results, they described the lesions that they scored and that was helpful. One of the lesions that they are scoring is increased collagen. To adequately score increased collagen, additional staining with a Trichrome, Picrosirius red or immunohistochemistry for specific collagens should have been done. Inclusion of representative images of the lesions would be appropriate.

The methods for analyzing hepatotoxic effects are appropriate. H&E is the gold standard for analyzing micro anatomical changes in liver tissue. Many changes due to toxicity, i.e.. enzyme changes, detoxification processes, ribosome malfunctions, storage disorders, are readily detectable on H&E sections of liver tissue. Fat and glycogen are immediately noticeable on H&E sections, however, they even better visualized, and even quantifiable using special stains: Oil Red O on frozen sections and PAS and PASD on paraffin sections. The authors clearly understand the power and usefulness of these techniques and in my opinion have used them appropriately. Quantification methods were strong.

It is difficult to evaluate from the data presented. I cold not access supplemental figure S1. Histopath only identified reduced vacuolation in CS, probably due to the anorexiogenic effects, and slightly more collagen (fibrosis). I would say the histopath was probably reasonable and the results believable for a subchronic (probably chronic for this animal model), but more extensive evaluation of liver xenobiotic metabolism(IHC, enzymatic, proteomic, transcriptomic and/or other assays for primary and secondary metabolism) would be necessary for convincing toxicologic analysis. It is diffiult to compare the results of an 8-month study of mice to humans with much different metabolism with years of exposure.

The evaluation of hepatocyte size, fat and glycogen content, in addition to overall evaluation of portal and center lobular areas would be typical in the histopathological evaluation for early stage toxic changes. In this study there was no specific comment on individual hepatocyte hypertrophy or atrophy. While not a definitive indicator of toxicity, it would have been nice to see a comment on this within the text.

Similar to the histopathological analysis methods used in evaluating the lung, the amount of lipid and glycogen in the liver were estimated and scored according to stated criteria. Although this methodology is still widely used and considered to be both standard and acceptable, I believe that quantitative rather than subjective measurements of these parameters could have been obtained using image analysis methods from ALL available liver sections from each animal instead of the more subjective estimates. Again, the method that was actually used certainly was acceptable and sufficient but quantitative data obtained from whole slide image analysis would have be "state of the art".

Appendix B: Survey Results

SECTION: DATA EVALUATION AND INTERPRETATION

ID #1790 (https://scipinion.com/surveys/65/results/1790)

Are the results in the four publications presented with sufficient detail, and were they clearly presented?

	Sufficient Detail?	Clarity of Presentation	Total
In-life observations, mortality, body weight, gross pathology	*Sufficient* **100.00%** 5	*Sufficient* **100.00%** 5	5
Biomarkers of exposure, urine	*Partially sufficient* **20.00%** 1 *Sufficient* **80.00%** 4	*Partially sufficient* **20.00%** 1 *Sufficient* **80.00%** 4	5
Biomarkers of exposure, blood, plasma	*Sufficient* **100.00%** 5	*Sufficient* **100.00%** 5	5
Clinical chemistry, including blood lipoproteins	*Sufficient* **100.00%** 5	*Sufficient* **100.00%** 5	5
Hematology	*Sufficient* **100.00%** 5	*Sufficient* **100.00%** 5	5
Respiratory physiology	*Sufficient* **100.00%** 5	*Sufficient* **100.00%** 5	5
BAL fluid - inflammatory mediators	*Sufficient* **100.00%** 5	*Sufficient* **100.00%** 5	5
BAL cells (free lung cells) - identification and activation markers	*Sufficient* **100.00%** 5	*Sufficient* **100.00%** 5	5
	Sufficient **100.00%**	*Partially sufficient* **20.00%** 1	

Appendix B: Survey Results

Histopathology, upper respiratory tract	5	*Sufficient* **80.00%** 4	5
Histopathology, lung	*Partially sufficient* **20.00%** 1 *Sufficient* **80.00%** 4	*Partially sufficient* **20.00%** 1 *Sufficient* **80.00%** 4	5
Histopathology, liver	*Partially sufficient* **20.00%** 1 *Sufficient* **80.00%** 4	*Partially sufficient* **20.00%** 1 *Sufficient* **80.00%** 4	5
Histopathology, other organs/tissues	*Sufficient* **100.00%** 5	*Sufficient* **100.00%** 5	5
Histomorphometry, lung	*Sufficient* **100.00%** 5	*Sufficient* **100.00%** 5	5
Plaque size measurement, planimetry	*Partially sufficient* **20.00%** 1 *Sufficient* **80.00%** 4	*Partially sufficient* **20.00%** 1 *Sufficient* **80.00%** 4	5
Plaque size measurement, micro-CT	*Sufficient* **100.00%** 5	*Sufficient* **100.00%** 5	5
Dataset, transcriptomics	*Sufficient* **100.00%** 5	*Partially sufficient* **20.00%** 1 *Sufficient* **80.00%** 4	5
	Sufficient	*Partially sufficient* **20.00%**	

Dataset, lipidomics	**100.00%** 5	1 *Sufficient* **80.00%** 4	5
Dataset, proteomics	*Sufficient* **100.00%** 5	*Partially sufficient* **20.00%** 1 *Sufficient* **80.00%** 4	5

Appendix B: Survey Results

ID #1792 (https://scipinion.com/surveys/65/results/1792)

Are the interpretations and conclusions in the publications regarding the effects of exposure to 3R4F cigarette and THS2.2 aerosol in the Apoe-/- mouse model 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 Titz et al. 2016	**100.00%** 5	**0.00%** 0	**0.00%** 0	5
Lung / respiratory effects in Phillips et al. 2016	**100.00%** 5	**0.00%** 0	**0.00%** 0	5
Vascular / atherosclerotic effects in Phillips et al. 2016	**80.00%** 4	**0.00%** 0	**20.00%** 1	5
Cardiac effects in Szostak et al. 2016	**60.00%** 3	**20.00%** 1	**20.00%** 1	5
Clinical chemistry and systemic effects in Phillips et al. 2016	**100.00%** 5	**0.00%** 0	**0.00%** 0	5
Hepatic effects in Lo Sasso et al. 2016	**80.00%** 4	**20.00%** 1	**0.00%** 0	5

Appendix B: Survey Results

ID #1793 (https://scipinion.com/surveys/65/results/1793)

How critical and informative do you consider each endpoint shown in Philips et al. for assessing the relative impact of various tobacco products?

	Critical	Informative	Total
Clinical chemistry (Figure 2, Supplementary Fig. 8, and supplementary results file 2)	*High* **40.00%** 2 *Medium* **60.00%** 3	*High* **20.00%** 1 *Medium* **80.00%** 4	5
Aortic arch planimetric plaque measurements (Figure 3)	*High* **60.00%** 3 *Medium* **40.00%** 2	*High* **60.00%** 3 *Medium* **40.00%** 2	5
microCT-based aortic arch plaque measurements (Figure 4, Suppl. Fig. 9, and supplementary files)	*High* **60.00%** 3 *Low* **20.00%** 1 *Medium* **20.00%** 1	*High* **40.00%** 2 *Medium* **60.00%** 3	5
Plasma and aortic arch lipidomics (Figure 5)	*Medium* **100.00%** 5	*Medium* **100.00%** 5	5
Respiratory physiology (Figure 6)	*High* **60.00%** 3 *Medium* **40.00%** 2	*High* **60.00%** 3 *Medium* **40.00%** 2	5
Lung volume measurements (Figure 7)	*High* **80.00%** 4 *Low*	*High* **80.00%** 4 *Low*	5

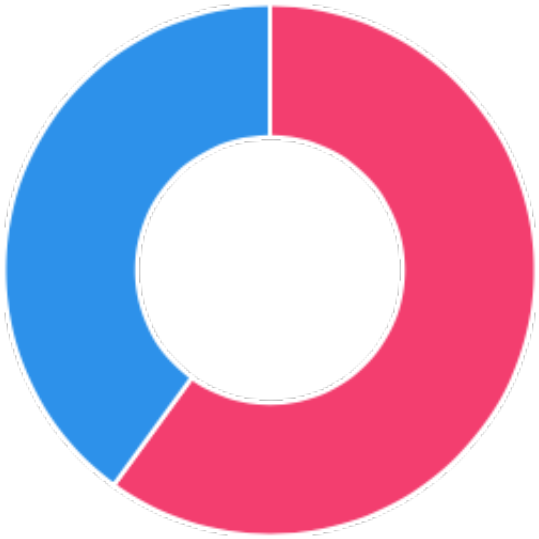
	20.00% 1	**20.00%** 1	
Bronchoalveolar lavage fluid – FACS analysis of free lung cells (Figure 8)	*High* **100.00%** 5	*High* **100.00%** 5	5
Bronchoalveolar lavage fluid – Multianalyte Profiling (Figure 9, supplementary Fig. 12)	*High* **80.00%** 4 *Medium* **20.00%** 1	*High* **80.00%** 4 *Medium* **20.00%** 1	5
Nasal epithelium histopathological evaluation (Figure 10)	*High* **60.00%** 3 *Low* **20.00%** 1 *Medium* **20.00%** 1	*High* **60.00%** 3 *Low* **20.00%** 1 *Medium* **20.00%** 1	5
Lung histology (Figure 11, Supplementary Results File 2)	*High* **100.00%** 5	*High* **80.00%** 4 *Low* **20.00%** 1	5
Lung histomorphometry (Figure 12)	*High* **100.00%** 5	*High* **100.00%** 5	5
Lung transcriptomics results – Differentially expressed genes (Figure 13, Supplementary Fig. 13)	*High* **40.00%** 2 *Medium* **60.00%** 3	*High* **40.00%** 2 *Medium* **60.00%** 3	5
Lung transcriptomics results – NPA/BIF analysis (Figure 14)	*High* **60.00%** 3 *Medium*	*High* **60.00%** 3 *Medium*	5

	40.00% 2	**40.00%** 2	
Lung proteomics analysis (Figure 15, Supplementary Figs. 14 to 16)	*High* **60.00%** 3 *I cannot answer* **20.00%** 1 *Medium* **20.00%** 1	*High* **60.00%** 3 *I cannot answer* **20.00%** 1 *Medium* **20.00%** 1	5
The totality of the data, collectively (All figures)	*High* **100.00%** 5	*High* **100.00%** 5	5

Appendix B: Survey Results

ID #1795 (https://scipinion.com/surveys/65/results/1795)

Does this study design allow a transversal evaluation of a number of mechanisms, such as oxidative stress and inflammation?



Legend

Yes, this systems approach demonstrated the potential to enhance the understanding of pathomechanisms also in a transversal manner: 3 *(60%)*

Yes, a systems approach can potentially enhance the understanding of pathomechanisms also in a transversal manner, but in the current study, the following additional aspects would be required in order to make it more conclusive: (pls. indicate missing aspects in the text box): 2 *(40%)*

No, the only reliable approach to understand pathomechanisms is to use separate, mechanism-specific investigations in various dedicated model systems: 0 *(0%)*

I cannot answer the question: 0 *(0%)*

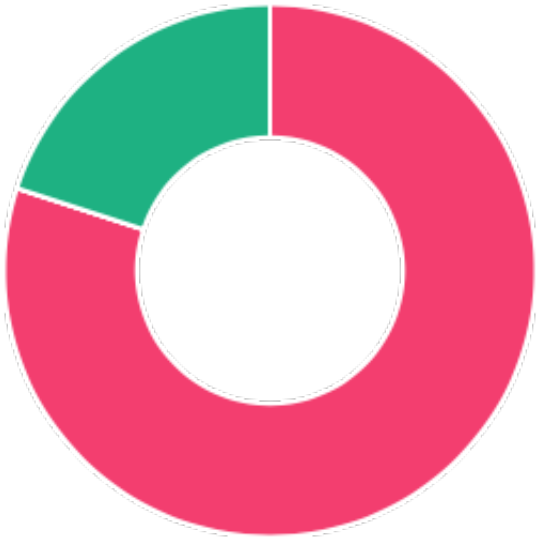
answers: 5

skips: 0

Appendix B: Survey Results

ID #1796 (https://scipinion.com/surveys/65/results/1796)

Do the aortic arch plaque measurements (planimetry and micro-CT imaging) support the authors' statement that cessation or switching to THS2.2 halted the progression of atherosclerotic plaques?



Legend

- Yes, both plaque measurements confirm the statement: 4 *(80%)*
- Only the 2D measurements confirm the statement: 0 *(0%)*
- Only the CT imaging confirm the statement: 1 *(20%)*
- No, the statement is not supported by these data: 0 *(0%)*
- I cannot answer this question: 0 *(0%)*

answers: 5

skips: 0

Appendix B: Survey Results

ID #1797 (https://scipinion.com/surveys/65/results/1797)

To demonstrate reduced effect of RRPs 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
Side-by-side analysis of effects observed in two independent studies for two different RRPs: 1) an early prototype RRP (pMRTP) and 2) a later RRP using a newer tobacco heating system (THS2.2) (see, Titz et al. 2016)	**0.00%** 0	**0.00%** 0	**0.00%** 0	**80.00%** 4	**20.00%** 1	5
Integration of diverse data modalities (e.g. proteomics and lipidomics data in Titz et al. 2016) within a single study	**0.00%** 0	**0.00%** 0	**40.00%** 2	**60.00%** 3	**0.00%** 0	5
Analysis of the effects of the products on multiple systems (respiratory, cardiovascular) in a single model	**0.00%** 0	**0.00%** 0	**0.00%** 0	**60.00%** 3	**40.00%** 2	5
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	**20.00%** 1	**20.00%** 1	**40.00%** 2	**20.00%** 1	5

ID #1798 (https://scipinion.com/surveys/65/results/1798)

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?

> The inflammatory networks that are increased in the multianalyte profile correlate with the leukocyte infiltrate reported in these two groups.

> This is too broad of a data set to make a c definitive conclusion such a complicated biological process to relate to about histopathological details in tissue sections.

> It is difficult to conceive of an explanation of why cessation resulted in persistent BIF perturbations whereas THS and switching resolved to near control. Certainly BALF components are associated with inflammatory migration (e.g. IL1) and persistence (MIPs) in the lung; however, the persistence of BIF perturbations (derived from lung transcriptomic data) in cessation and switching are not supported by BALF cell counts at 6 and 8 months. The persistence of IgA and plasma cells (representing the persistence of protection against historical challenge), as well as dendritic cells and macrophages (resolving/cleaning up foreign matter and damaged cells) after a 2 month exposure to CS is not surprising.

> Yes... the increased macrophages and granulocytes

> Yes, I believe the observations of genes related to the inflammatory processes correlate well with the the observations of inflammatory cells within lung tissue and the then expected population within the BALF. Particularly in the area of lymphocyte, neutrophil, dendritic and macrophage cell signaling. The heat map scores and the relatively biological impacts also highlighted the strength of such signaling in association with CS. Again it would have been nice to have this discussed in more detail in the discussion portion of the paper

ID #1799 (https://scipinion.com/surveys/65/results/1799)

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

> Looking at the BALF multianalyte profiling, it's interesting that there are several analytes that are downregulated in the THS2.2 compared to sham. I don't recall seeing this point addressed.

> Not that i can see.

> No

> Generally that the effects of THS2.2 seem below the threshold of discovery or "significance" despite the extensive metrics deployed.

> As indicated in my answer to question 7 above, there was scope for discussion of the findings of transcriptomic data. Sadly the discussion was more a summary of the transcriptomic results, rather than a relevance of the findings.

Appendix B: Survey Results

ID #1800 (https://scipinion.com/surveys/65/results/1800)

Looking at lung slides on the Aperio platform for the different groups, do you agree that the histopathological evaluation and results support the authors' (Phillips et al. 2016) conclusion that:

	Yes	No	I cannot answer	Total
"THS2.2 aerosol-exposed lungs were indistinguishable from sham"	**100.00%** 5	**0.00%** 0	**0.00%** 0	5
"switching or cessation inhibited the further progression of emphysema-related parameters, leading to a convergence with the sham/THS2.2 group levels"	**80.00%** 4	**20.00%** 1	**0.00%** 0	5

ID #1801 (https://scipinion.com/surveys/65/results/1801)

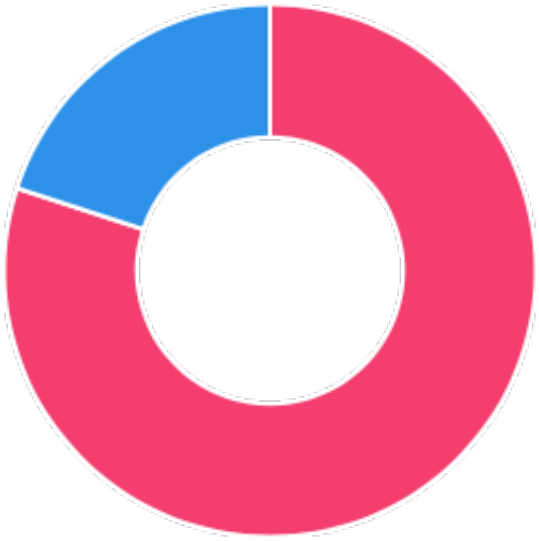
Are the liver, aortic arch, and upper respiratory tract histopathology results correctly interpreted by the authors regarding the effects of exposure to aerosol from 3R4F cigarettes and THS2.2 in this mouse model?

	Yes	No	I cannot answer	Total
Liver	100.00% 5	0.00% 0	0.00% 0	5
Aortic arch	80.00% 4	0.00% 0	20.00% 1	5
Upper respiratory tract	80.00% 4	0.00% 0	20.00% 1	5

Appendix B: Survey Results

ID #1869 (https://scipinion.com/surveys/65/results/1869)

Do histopathological, morphometric, functional (pulmonary function) and other investigations indicate emphysematous changes at 2 months of 3R4F cigarette smoke exposure?



Legend

- Yes: 4 *(80%)*
- No: 1 *(20%)*
- I cannot answer: 0 *(0%)*

answers: 5

skips: 0

Appendix B: Survey Results

ID #1803 (https://scipinion.com/surveys/65/results/1803)

Given the lung slides provided in the Aperio tool, please rate the magnitude of change (1-5 scale, 5 being the largest magnitude of change) you observe between 3R4F aerosol and sham (please specify the ID numbers of slides you have looked at and based your evaluation on)?

	1	2	3	4	5	Total
Presence/number of lymphocytic cells and neutrophilic granulocytes in the intra-alveolar space	0.00% 0	20.00% 1	0.00% 0	40.00% 2	40.00% 2	5
Presence/number of non-pigmented macrophages in the alveolar lumen	0.00% 0	0.00% 0	20.00% 1	40.00% 2	40.00% 2	5
Presence/number of macrophages with yellow-brownish pigmentation in the alveolar lumen	0.00% 0	0.00% 0	0.00% 0	80.00% 4	20.00% 1	5
Presence/number of pigmented macrophages that are arranged in small intra-alveolar clusters (described as pigmented macrophage nests) in the alveolar lumen	0.00% 0	20.00% 1	20.00% 1	20.00% 1	40.00% 2	5
Presence/number of macrophages that appear to be enlarged or multi-nucleated cells, and in part contain PAS-positive material in the cytoplasm, in the alveolar lumen	20.00% 1	20.00% 1	20.00% 1	40.00% 2	0.00% 0	5
Presence/number of aggregates of lymphocytic cells in the lung interstitium; i.e., alveolar interstitium, peri-vascular area, interstitium at main bronchus	0.00% 0	40.00% 2	20.00% 1	20.00% 1	20.00% 1	5
Amount of non-cellular eosinophilic material in the alveolar lumen	60.00% 3	20.00% 1	20.00% 1	0.00% 0	0.00% 0	5
Emphysematous lesions characterized morphologically by alveolar septal destruction as well as by mild enlargement of the distal airways	0.00% 0	20.00% 1	60.00% 3	20.00% 1	0.00% 0	5
Multifocal alveolar emphysema	0.00% 0	20.00% 1	60.00% 3	20.00% 1	0.00% 0	5
Emphysema locally associated with mild hyperplasia/hypertrophy of the alveolar epithelium	0.00% 0	60.00% 3	20.00% 1	0.00% 0	20.00% 1	5
Emphysema locally associated with the accumulation of inflammatory cells	20.00% 1	20.00% 1	40.00% 2	0.00% 0	20.00% 1	5
Hyperplasia of the bronchial epithelium	20.00% 1	20.00% 1	20.00% 1	20.00% 1	20.00% 1	5
	40.00%	20.00%	20.00%	20.00%	0.00%	

Hyperplasia of the bronchial goblet cells	2	1	1	1	0	5

Appendix B: Survey Results

ID #1805 (https://scipinion.com/surveys/65/results/1805)

Given the lung slides provided in the Aperio tool, please rate the magnitude of change (1-5 scale, 5 being the largest magnitude of change) you observe between THS2.2 and 3R4F aerosols (please specify the ID numbers of slides you have looked at and based your evaluation on)?

	1	2	3	4	5	Total
Presence/number of lymphocytic cells and neutrophilic granulocytes in the intra-alveolar space	0.00% 0	20.00% 1	20.00% 1	0.00% 0	60.00% 3	5
Presence/number of non-pigmented macrophages in the alveolar lumen	0.00% 0	0.00% 0	40.00% 2	20.00% 1	40.00% 2	5
Presence/number of macrophages with yellow-brownish pigmentation in the alveolar lumen	0.00% 0	0.00% 0	0.00% 0	40.00% 2	60.00% 3	5
Presence/number of pigmented macrophages that are arranged in small intra-alveolar clusters (described as pigmented macrophage nests) in the alveolar lumen	0.00% 0	40.00% 2	20.00% 1	0.00% 0	40.00% 2	5
Presence/number of macrophages that appear to be enlarged or multi-nucleated cells, and in part contain PAS-positive material in the cytoplasm, in the alveolar lumen	20.00% 1	20.00% 1	0.00% 0	60.00% 3	0.00% 0	5
Presence/number of aggregates of lymphocytic cells in the lung interstitium; i.e., alveolar interstitium, peri-vascular area, interstitium at main bronchus	0.00% 0	40.00% 2	20.00% 1	20.00% 1	20.00% 1	5
Amount of non-cellular eosinophilic material in the alveolar lumen	60.00% 3	20.00% 1	20.00% 1	0.00% 0	0.00% 0	5
Emphysematous lesions characterized morphologically by alveolar septal destruction as well as by mild enlargement of the distal airways	0.00% 0	40.00% 2	20.00% 1	40.00% 2	0.00% 0	5
Multifocal alveolar emphysema	0.00% 0	40.00% 2	20.00% 1	40.00% 2	0.00% 0	5
Emphysema locally associated with mild hyperplasia/hypertrophy of the alveolar epithelium	20.00% 1	40.00% 2	20.00% 1	0.00% 0	20.00% 1	5
Emphysema locally associated with the accumulation of inflammatory cells	40.00% 2	20.00% 1	20.00% 1	20.00% 1	0.00% 0	5
Hyperplasia of the bronchial epithelium	20.00% 1	0.00% 0	20.00% 1	20.00% 1	40.00% 2	5
	40.00%	0.00%	20.00%	40.00%	0.00%	

Hyperplasia of the bronchial goblet cells	2	0	1	2	0	5

Appendix B: Survey Results

ID #1807 (https://scipinion.com/surveys/65/results/1807)

Given the lung slides provided in the Aperio tool, please rate the magnitude of change (1-5 scale, 5 being the largest magnitude of change) you observe between switch and cessation (please specify the ID numbers of slides you have looked at and based your evaluation on)?

	1	2	3	4	5	Total
Presence/number of lymphocytic cells and neutrophilic granulocytes in the intra-alveolar space	80.00% 4	0.00% 0	0.00% 0	20.00% 1	0.00% 0	5
Presence/number of non-pigmented macrophages in the alveolar lumen	80.00% 4	0.00% 0	0.00% 0	20.00% 1	0.00% 0	5
Presence/number of macrophages with yellow-brownish pigmentation in the alveolar lumen	80.00% 4	0.00% 0	0.00% 0	20.00% 1	0.00% 0	5
Presence/number of pigmented macrophages that are arranged in small intra-alveolar clusters (described as pigmented macrophage nests) in the alveolar lumen	80.00% 4	0.00% 0	20.00% 1	0.00% 0	0.00% 0	5
Presence/number of macrophages that appear to be enlarged or multi-nucleated cells, and in part contain PAS-positive material in the cytoplasm, in the alveolar lumen	80.00% 4	20.00% 1	0.00% 0	0.00% 0	0.00% 0	5
Presence/number of aggregates of lymphocytic cells in the lung interstitium; i.e., alveolar interstitium, peri-vascular area, interstitium at main bronchus	80.00% 4	20.00% 1	0.00% 0	0.00% 0	0.00% 0	5
Amount of non-cellular eosinophilic material in the alveolar lumen	80.00% 4	0.00% 0	20.00% 1	0.00% 0	0.00% 0	5
Emphysematous lesions characterized morphologically by alveolar septal destruction as well as by mild enlargement of the distal airways	80.00% 4	0.00% 0	20.00% 1	0.00% 0	0.00% 0	5
Multifocal alveolar emphysema	80.00% 4	0.00% 0	0.00% 0	20.00% 1	0.00% 0	5
Emphysema locally associated with mild hyperplasia/hypertrophy of the alveolar epithelium	80.00% 4	0.00% 0	0.00% 0	20.00% 1	0.00% 0	5
Emphysema locally associated with the accumulation of inflammatory cells	80.00% 4	0.00% 0	0.00% 0	20.00% 1	0.00% 0	5
Hyperplasia of the bronchial epithelium	80.00% 4	0.00% 0	0.00% 0	20.00% 1	0.00% 0	5
	80.00%	0.00%	0.00%	20.00%	0.00%	

Hyperplasia of the bronchial goblet cells	4	0	0	1	0	5

Appendix B: Survey Results

ID #1809 (https://scipinion.com/surveys/65/results/1809)

For each exposure group, 10 animals were included for the histopathology analysis. Within single animal lungs, 5 different section levels are available on the Aperio platform with H&E staining, and 1 level with AB-PAS staining. How do you judge the homogeneity of the findings within lungs and across animals from the same groups? Please specify the finding and which slides you considered in your evaluation in the text box below.

	Intra-animal homogeneity	Inter-animal homogeneity	Total
Presence/number of lymphocytic cells and neutrophilic granulocytes in the intra-alveolar space	*High consistency* **60.00%** 3 *Moderate consistency* **40.00%** 2	*Low consistency* **40.00%** 2 *Moderate consistency* **60.00%** 3	5
Presence/number of non-pigmented macrophages in the alveolar lumen	*High consistency* **60.00%** 3 *Moderate consistency* **40.00%** 2	*Low consistency* **40.00%** 2 *Moderate consistency* **60.00%** 3	5
Presence/number of macrophages with yellow-brownish pigmentation in the alveolar lumen	*High consistency* **80.00%** 4 *Moderate consistency* **20.00%** 1	*High consistency* **20.00%** 1 *Low consistency* **20.00%** 1 *Moderate consistency* **60.00%** 3	5
	High	*High consistency* **20.00%**	

Presence/number of pigmented macrophages that are arranged in small intra-alveolar clusters (described as pigmented macrophage nests) in the alveolar lumen	*consistency* **60.00%** 3 *Low consistency* **40.00%** 2	1 *Low consistency* **40.00%** 2 *Moderate consistency* **40.00%** 2	5
Presence/number of macrophages that appear to be enlarged or multi-nucleated cells, and in part contain PAS-positive material in the cytoplasm, in the alveolar lumen	*High consistency* **80.00%** 4 *Moderate consistency* **20.00%** 1	*High consistency* **40.00%** 2 *Low consistency* **40.00%** 2 *Moderate consistency* **20.00%** 1	5
Presence/number of aggregates of lymphocytic cells in the lung interstitium; i.e., alveolar interstitium, peri-vascular area, interstitium at main bronchus	*High consistency* **60.00%** 3 *Moderate consistency* **40.00%** 2	*Low consistency* **60.00%** 3 *Moderate consistency* **40.00%** 2	5
Amount of non-cellular eosinophilic material in the alveolar lumen	*High consistency* **75.00%** 3 *Moderate consistency* **25.00%** 1	*High consistency* **75.00%** 3 *Moderate consistency* **25.00%** 1	4
	High consistency **40.00%** 2	*High consistency* **40.00%** 2	

Emphysematous lesions characterized morphologically by alveolar septal destruction as well as by mild enlargement of the distal airways	*Low consistency* **20.00%** 1 *Moderate consistency* **40.00%** 2	*Low consistency* **40.00%** 2 *Moderate consistency* **20.00%** 1	5
Multifocal alveolar emphysema	*High consistency* **40.00%** 2 *Low consistency* **40.00%** 2 *Moderate consistency* **20.00%** 1	*High consistency* **40.00%** 2 *Low consistency* **40.00%** 2 *Moderate consistency* **20.00%** 1	5
Emphysema locally associated with mild hyperplasia/hypertrophy of the alveolar epithelium	*High consistency* **40.00%** 2 *Low consistency* **40.00%** 2 *Moderate consistency* **20.00%** 1	*High consistency* **40.00%** 2 *Low consistency* **40.00%** 2 *Moderate consistency* **20.00%** 1	5
Emphysema locally associated with the accumulation of inflammatory cells	*High consistency* **40.00%** 2 *Low consistency* **40.00%** 2 *Moderate*	*High consistency* **40.00%** 2 *Low consistency* **40.00%** 2 *Moderate*	5

	consistency **20.00%** 1	*consistency* **20.00%** 1	
Hyperplasia of the bronchial epithelium	*High consistency* **60.00%** 3 *Moderate consistency* **40.00%** 2	*High consistency* **60.00%** 3 *Moderate consistency* **40.00%** 2	5
Hyperplasia of the bronchial goblet cells	*High consistency* **60.00%** 3 *Moderate consistency* **40.00%** 2	*High consistency* **40.00%** 2 *Low consistency* **20.00%** 1 *Moderate consistency* **40.00%** 2	5

ID #1811 (https://scipinion.com/surveys/65/results/1811)

Is there any other substantial finding that you would report for the THS2.2 or switch group based on the analysis of the slides you performed on the Aperio tool?

> I did not find any additional substantial findings.

> Although the aperio tool is clearly lower in resolution, the ability to evaluate a greater number of slides gives me confidence to report that I found no clear effect of THS2.2 when compared to control.

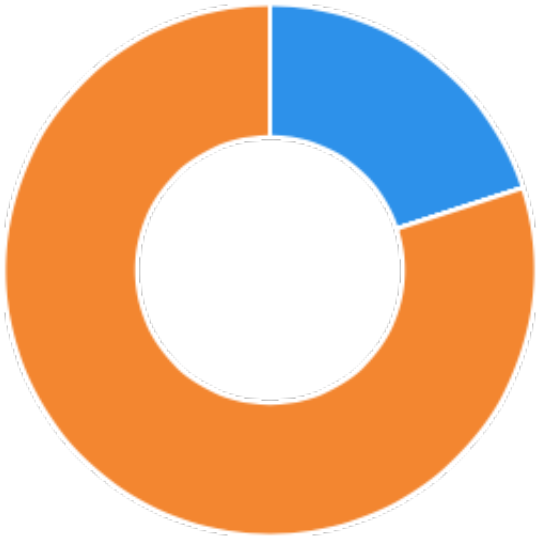
> Changes in the THS2.2 group were generally indistinguishable from the Sham group. The changes in the switch group at 3 and especially at the 8 month time point were milder than those seen at 2 months in the 3R4F group.

> No substantial findings other than switching would seem to provide similar histopathological changes to those experienced in animals that no longer have access to CS.

Appendix B: Survey Results

ID #1813 (https://scipinion.com/surveys/65/results/1813)

Given the histopathology and histomorphometry results, how substantial would you consider the difference between the cessation and switch groups?



Legend

- Large difference: 0 *(0%)*
- Moderate difference: 1 *(20%)*
- Minimal difference: 0 *(0%)*
- No difference at all: 4 *(80%)*

answers: 5

skips: 0

ID #1814 (https://scipinion.com/surveys/65/results/1814)

Do you find evidence for any substantial effect in the liver tissue linked to exposure to THS2.2 aerosol? Please specify.

> There is nothing in any of the studies to suggest an affect on the liver.

> No. Only glycogen deposition and fat infiltration as described in the studies which are both not a treatment related effect. There are no other signs of toxicity histologically i.e.. areas of eosonphilic or basophilic foci, pyknotic nuclei, degeneration, apoptosis or necrosis.

> Based on paper of Sasso et al 2016,, while there are hepatic changes which are mouse-strain related there is no evidence to suggest hepatotoxicity. This was clear in the histopathological evaluation and further supported by proteomic and transcriptomic data. While the latter data was suggestive of impaired biological pathways, there is not enough information to translate this to a toxic effect (i.e it could be an adaptive effect?).

> The trascriptomic and proteomic data indicate believable effects of THS2.2 on the liver; however, I find no clear adverse effect either from these data nor from histopathology (discounting potentially decreased liver glycogen as a likely anorexiogenic or alteration of feeding times and not clearly toxic nor substantial in magnitude).

> No

SECTION: TRANSLATABILITY AND OUTLOOK

ID #1815 (https://scipinion.com/surveys/65/results/1815)

How confident are you that the lung histopathological evaluation in mice as conducted in the present study allows translation to human pathology? Which finding is most suitable? (please indicate in the comment box)

	Extremely confident	Very confident	Confident	Somewhat confident	Not confident	I cannot answer	Total
Presence/number of lymphocytic cells and neutrophilic granulocytes in the intra-alveolar space	0.00% 0	40.00% 2	20.00% 1	40.00% 2	0.00% 0	0.00% 0	5
Presence/number of non-pigmented macrophages in the alveolar lumen	20.00% 1	20.00% 1	40.00% 2	0.00% 0	20.00% 1	0.00% 0	5
Presence/number of macrophages with yellow-brownish pigmentation in the alveolar lumen	20.00% 1	20.00% 1	40.00% 2	20.00% 1	0.00% 0	0.00% 0	5
Presence/number of pigmented macrophages that are arranged in small intra-alveolar clusters (described as pigmented macrophage nests) in the alveolar lumen	0.00% 0	20.00% 1	60.00% 3	20.00% 1	0.00% 0	0.00% 0	5
Presence/number of macrophages that appear to be enlarged or multi-nucleated cells, and in part contain PAS-positive material in the cytoplasm, in the alveolar lumen	20.00% 1	0.00% 0	40.00% 2	20.00% 1	20.00% 1	0.00% 0	5
Presence/number of aggregates of lymphocytic cells in the lung interstitium; i.e., alveolar interstitium, peri-vascular area, interstitium at main bronchus	20.00% 1	0.00% 0	60.00% 3	0.00% 0	20.00% 1	0.00% 0	5
Amount of non-cellular eosinophilic material in the alveolar lumen	0.00% 0	20.00% 1	20.00% 1	0.00% 0	60.00% 3	0.00% 0	5
Emphysematous lesions characterized morphologically by alveolar septal destruction as well as	0.00% 0	40.00% 2	40.00% 2	20.00% 1	0.00% 0	0.00% 0	5

Appendix B: Survey Results

by mild enlargement of the distal airways							
Multifocal alveolar emphysema	0.00% 0	40.00% 2	20.00% 1	0.00% 0	40.00% 2	0.00% 0	5
Emphysema locally associated with mild hyperplasia/hypertrophy of the alveolar epithelium	0.00% 0	40.00% 2	40.00% 2	0.00% 0	20.00% 1	0.00% 0	5
Emphysema locally associated with the accumulation of inflammatory cells	0.00% 0	60.00% 3	20.00% 1	0.00% 0	20.00% 1	0.00% 0	5
Hyperplasia of the bronchial epithelium	0.00% 0	40.00% 2	0.00% 0	40.00% 2	20.00% 1	0.00% 0	5
Hyperplasia of the bronchial goblet cells	0.00% 0	20.00% 1	0.00% 0	40.00% 2	40.00% 2	0.00% 0	5

ID #1817 (https://scipinion.com/surveys/65/results/1817)

For future studies, would you suggest alternative methodologies to capture the effects of both 3R4F and THS2.2 / other MRTP aerosol exposure on the respiratory tissues and if so, which ones?

> I don't have suggestions for other tissues but long term studies to evaluation carcinogenicity must be done.

> In the future, it would be good to see other methods of administration. I am not familiar with cigarette smoke administration in mice, however, am wondering if using whole body plethysmography can be more effectively used and would give in life lung function assessments to complement the current methods.

> I believe that image analysis methods could and should be used to provide quantitative measures of pigmented and non-pigmented macrophages, multinucleated cells and lymphocytes

> A subtractive study of 3R4F CS or any other test substance might be useful. For example, the effect of removal of CO or some specific HPHS might enhance the interpretation of Omics effects. Consider also dosing THS2.2 at multiples of the nicotine exposure in control CS as one might routinely do in escalating dose trials.

> I don't suggest additional methods

ID #1818 (https://scipinion.com/surveys/65/results/1818)

In your opinion, can the cigarette smoke effect findings be leveraged or translated to risk assessment of other inhaled toxicants, such as environmental air pollution, workplace exposures, etc.? Please cite appropriate references to justify your answer.

I don't experience in this area and so I am not sure. I don't know how one would translate cigarette smoke to risk assessments of other toxins since other toxins have different mechanisms of action. If the question is can this systems approach feasible for studying other inhaled toxins, then the answer would be yes.

They can be correlated, however, I feel a thorough analysis of individual pollutants/irritants need to be separately assessed before making a presumptive correlation. I believe the findings do give leverage to the types of inflammation that results, the damage to lung tissue that ensues and how cessation can thwart further damage.

As indicated earlier, this is not my field of research. A number of factors would need to be taken into account e.g. the nature of the pollutant? whether it is chonic or acute exposure, whether it is an addictive product etc... In addition while the Aope -/- model is seems ideal for CS smoke studies, it may not suit/or acurately predict toxic changes in association with other pollutants/toxicants. What I will say is that study design is very applicable, robust and could be translated in the investigation of other inhaled toxicants and air pollutants. No references

Interesting question. Leveraging results would probably be possible by comparing BALF analyses from human smokers and comparing them to those of mice in this study. As well, BALF from humans exposed environmentally or experimentally (Am J Respir Crit Care Med. 2001 Aug 15;164(4):704-8 “Inflammatory lung injury after bronchial instillation of air pollution particles” Ghio AJ, Devlin RB) could also be compared to those of mice given similar exposure. Mice would allow full postmortem examination looking for toxic effects (of pollutants, similar to that done in these studies) and comparison to human smokers gives a well-documented human model with extensive clinical history. As particulate matter has also been implicated in the development of atherosclerosis (Biochim Biophys Acta. 2016 May 5. pii: S0304-4165(16)30150-7. doi: 10.1016/j.bbagen.2016.04.030. [Epub ahead of print] “Fine Particulate Matter Air Pollution and Atherosclerosis: Mechanistic Insights” Bai Y1, Sun Q2.) Studies might be run to identify similarities between CS components and environmental pollutants . As mentioned above, altering CS by removing specific components might be informative. Indeed ash particles from internal combustion engines might be added back to CS from which the particulate matter had been filtered, demonstrating whether PM in CS has similar effects to fly ash from diesel engines. For example, PM/ash has been suggested to be the major inducer of inflammation and oxidative damage indicators (rather than CO) (Inhal Toxicol. 2003 Nov;15(13):1327-46 “Oxidative stress mediates air pollution particle-induced acute lung injury and molecular pathology” Roberts ES1, Richards JH, Jaskot R, Dreher KL.).

> Yes. Pulmonary toxicology studies have been used for these purposes for many years.

ID #1819 (https://scipinion.com/surveys/65/results/1819)

For future studies, would you suggest examination of other tissues or biological samples to increase the overall level of scientific confidence and translatability to humans?

> As I mentioned in earlier comments, additional functional longitudinal studies of lung and heart would be useful for increasing scientific confidence and also translating the work to the human condition.

> Lung function assays can help in prediction of outcome. I would suggest using whole body plethysmography to compliment SciReq studies. This way results are more translatable to the human physiology and everyday breathing dynamics.

> In general in pharmaceutical studies, multiple organs are evaluated to assess toxic effects. So for completeness it may be advisable to undertake histopathological evaluations of kidney and brain in particular, to complement the knowledge we now have on lung, liver and cardiac tissue. (Some federal agencies are likely to ask for the full gamut of organ evaluations. Another factor to consider is fetal toxic effects.

> An escalating dose toxicity study on THS2.2 might generate relevant targets (with extensive tissue evaluation as in FDA submissions). At some point, nicotine might be lethal, but one could escalate the dose of other components by reducing or eliminating nicotine to identify tissues or cells sensitive to the effects of other THS2.2 components. Since skin was exposed it might be worth a look (ears, eyes, tail and haired skin).

> No

Appendix B: Survey Results

ID #1820 (https://scipinion.com/surveys/65/results/1820)

Which type of study do you deem appropriate to study the impact of switching to THS2.2 in smokers? Select all that apply, and please indicate in the comment box which in vitro or in vivo model might apply.

	Extremely confident	Very confident	Confident	Somewhat confident	Not confident	I cannot answer (please explain in the comment box)	Total
Clinical, long term	60.00% 3	40.00% 2	0.00% 0	0.00% 0	0.00% 0	0.00% 0	5
Clinical, short term	40.00% 2	40.00% 2	20.00% 1	0.00% 0	0.00% 0	0.00% 0	5
In vivo	25.00% 1	75.00% 3	0.00% 0	0.00% 0	0.00% 0	0.00% 0	4
In vitro	0.00% 0	0.00% 0	0.00% 0	40.00% 2	40.00% 2	20.00% 1	5

Appendix B: Survey Results

ID #1821 (https://scipinion.com/surveys/65/results/1821)

What are the most important biological effects of cigarette smoke exposure on the lung that are relevant to human disease (i.e., COPD) and how strong is the evidence supporting a reduction in effects from THS2.2 compared with 3R4F (1-5; 5 = most important or strongest)?

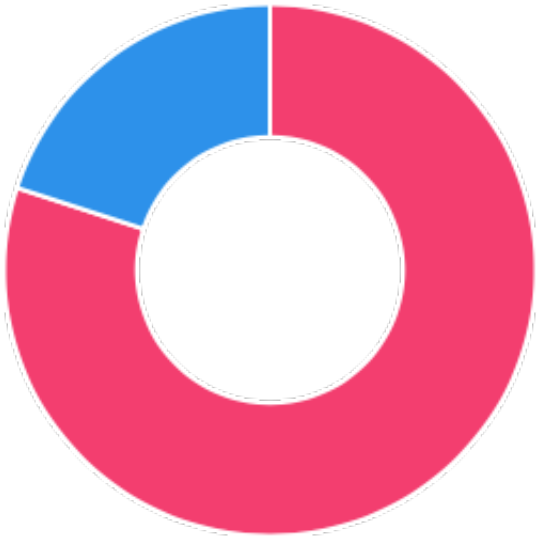
	Importance	Strength	Total
Inflammation	*4* **40.00%** 2 *5* **60.00%** 3	*4* **20.00%** 1 *5* **80.00%** 4	5
Oxidative stress	*3* **80.00%** 4 *4* **20.00%** 1	*2* **20.00%** 1 *3* **40.00%** 2 *4* **20.00%** 1 *5* **20.00%** 1	5
Extracellular matrix (ECM) production, deposition and degradation	*1* **20.00%** 1 *2* **40.00%** 2 *3* **20.00%** 1 *5* **20.00%** 1	*2* **60.00%** 3 *3* **20.00%** 1 *5* **20.00%** 1	5
	1 **20.00%** 1	*2* **40.00%**	

Metabolic alterations	*2* **40.00%** 2 *3* **40.00%** 2	2 *3* **60.00%** 3	5
Other (please specify)	*3* **50.00%** 1 *4* **50.00%** 1	*3* **50.00%** 1 *4* **50.00%** 1	2

Appendix B: Survey Results

ID #1822 (https://scipinion.com/surveys/65/results/1822)

The authors of these studies aimed at reducing and refining the exposure of animals in vivo by assessing two human diseases in the same inhalation toxicity study and to maximize the use of various tissues from the same animals to an array of classical toxicity end points plus transcriptomics, proteomics, and lipidomics. Do you think this approach was efficient and worthwhile, and how could it be further improved?



Legend

- Efficient approach, should be further considered in future studies: 4 *(80%)*
- Some compromises in design (which ones?) are not weighed out by the benefits, and hence separate models would be more efficient: 1 *(20%)*
- Further reduction of animals could be envisioned by the following accomplishments: (please specify): 0 *(0%)*

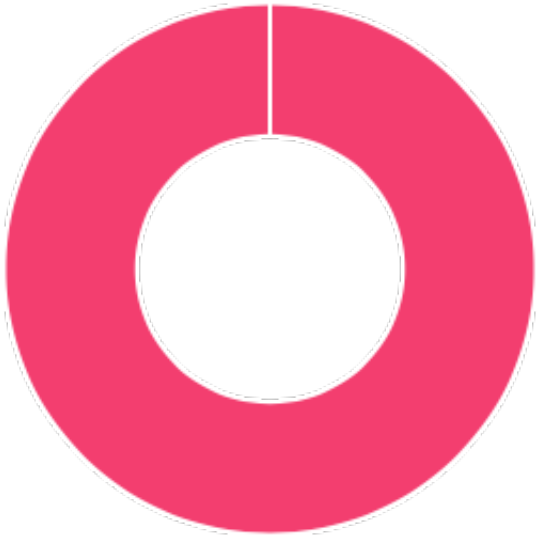
answers: 5

skips: 0

SECTION: TOOLS AND REVIEW PROCESS

ID #1823 (https://scipinion.com/surveys/65/results/1823)

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



Legend

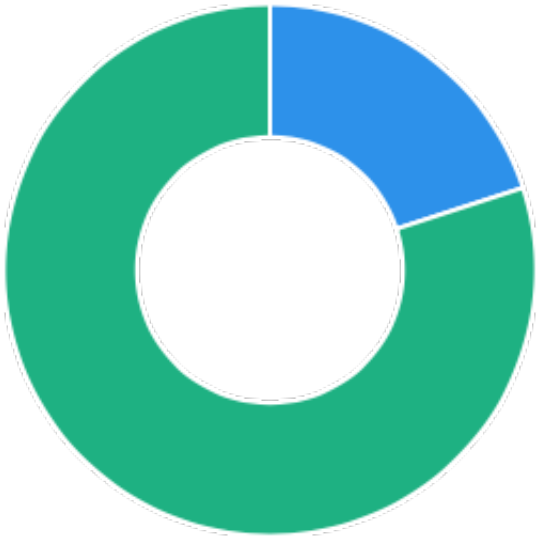
- Yes: 5 *(100%)*
- No: 0 *(0%)*
- I did not visit the portal: 0 *(0%)*

answers: 5
skips: 0

Appendix B: Survey Results

ID #1824 (https://scipinion.com/surveys/65/results/1824)

Tableau is a software for visualization and data analytics (http://www.tableau.com). Showing some of the results in a particular manner in a scientific article may not allow readers to grasp the full relevance of the results. To allow for interactive browsing of study results, a Tableau workbook was prepared (see link below). In your opinion this (select all that apply):



Legend

- Is superfluous given the images that are already provided in the publication: 0 *(0%)*
- Could be useful but is not easy to understand: 1 *(20%)*
- Is useful and should be included as supplementary material for publication of large studies whenever possible: 4 *(80%)*

answers: 5

skips: 0

ID #1825 (https://scipinion.com/surveys/65/results/1825)

What additional material/information would you need to obtain to better assess the relevance of the study and its results?

> I had everything I needed.

> Would be good to have some similar studies i.e. reference and papers, to compare methods and results, in order to assess if state of the art methods were used in the current studies, as I am not an expert at all modalities ie. micro CT

> I think all of the information given was important, if not a little overwhelming. Any additional information should be carefully considered so as not to make it even more overwhelming.

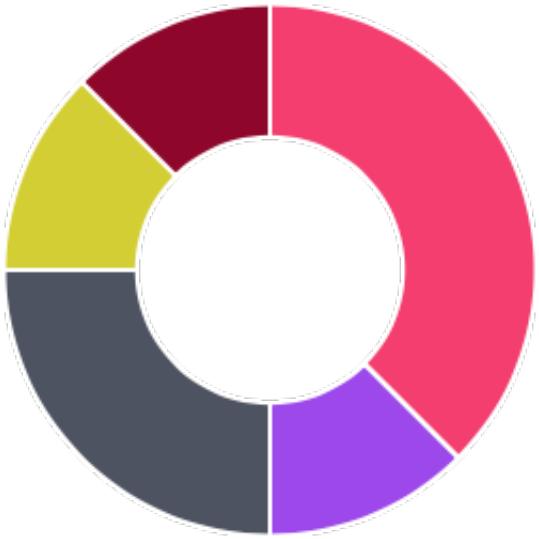
> It would be very nice to see parallel results in another species. For example, BALF results from human smokers showing similar effects and biological impacts on-omic data. Additional evidence of similar species responses to toxic and/or inflammatory perturbations would improve the translatability of these data.

> None that I can think of.

Appendix B: Survey Results

ID #1826 (https://scipinion.com/surveys/65/results/1826)

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: 3 *(38%)*
- The content was not easily accessible: 0 *(0%)*
- Background information provided in the question pages was misleading: 0 *(0%)*
- Enough and appropriate information was provided in the question pages to answer to the questions: 0 *(0%)*
- The tutorial videos were useful to efficiently review the material: 1 *(13%)*
- The tutorial videos and tools were distracting for the review purposes: 0 *(0%)*
- I was lacking information and had to go back to the article reprint on the publisher's website: 2 *(25%)*
- I was missing key functionalities (please specify below): 1 *(13%)*
- I did not visit the portal: 1 *(13%)*

answers: 5
skips: 0

ID #1827 (https://scipinion.com/surveys/65/results/1827)

How could this peer review process be improved?

> This worked great for me. I have not worked in such an easy to use system before. The only suggestion I have for improvement is that when you save your progress that it keeps you on the same page. I would periodically save my work and it would take me all the way out to the home page. That is not a big deal. This system is terrific.

> Peer review might be more valuable as an iterative process whereby reviewers provide evaluations, opinions and feedback that in turn can be reviewed by others in the group. I would suspect that diverse answers to some of the questions might be confusing or inconsistent. Multiple choice answers can force choices that obscure the reviewer's actual conclusions (much the same as in testing students).

> I felt the process worked well. I did have difficulties downloading the papers and Aperio became unreliable. Also, because it is important to annotate on the papers and continue to read them off line, it is important for me to print them. One improvement, given that when we enter this website, we are known, would be a one-click approach to aperio and avoid the need for further usernames and passwords.

> Give a tutorial or phone intro on the portals in order to use less of my dedicated peer review time learning this system. However, once learned, the system is very good.

> Make it easier to find the Aperio slide portal

ID #1828 (https://scipinion.com/surveys/65/results/1828)

How could the sbv IMPROVER systems toxicology Web Portal be made more valuable in responding to the questions?

> No suggestions.

> From the standpoint of a histopathologist I probably didn't find this helpful, but others who delve more deeper into toxicological pathways, proteomics, transcriptomics may do so.

> As mentioned above, an iterative interactive process could be envisioned where key topics could be discussed among reviewers to glean major similarities as well as differences of opinion. Such a task might well be more limited in scope and more intensive in time commitment to get discussions effectively done.

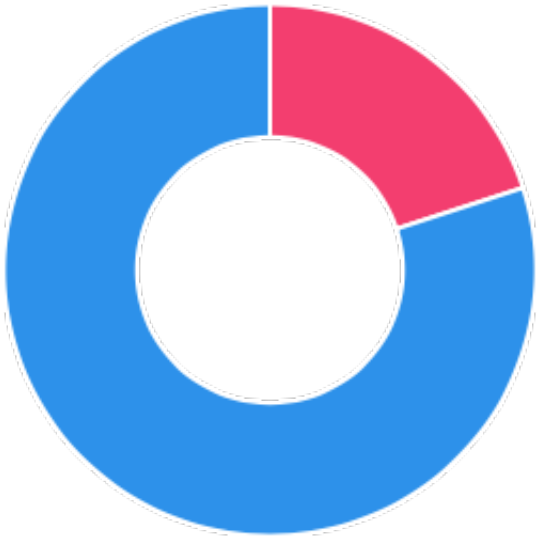
> worked well for me

> I thought it was very well designed.

Appendix B: Survey Results

ID #1829 (https://scipinion.com/surveys/65/results/1829)

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: 1 *(20%)*

Somewhat important: I would be comfortable having my name disclosed, but want my individual responses to the questions to remain undisclosed.: 4 *(80%)*

Not important: I would be comfortable having my name and individual responses to the questions disclosed.: 0 *(0%)*

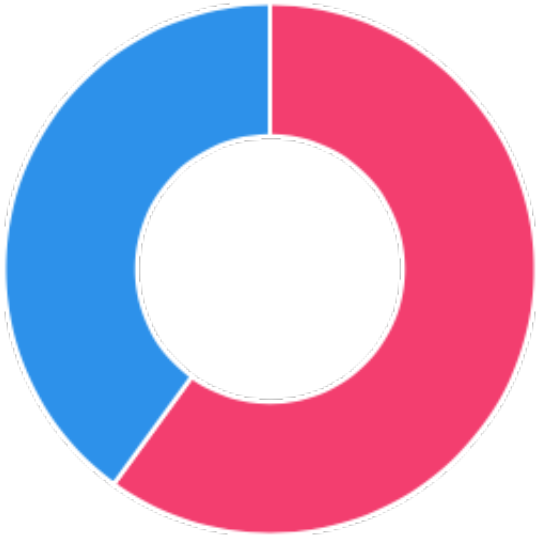
answers: 5

skips: 0

Appendix B: Survey Results

ID #1830 (https://scipinion.com/surveys/65/results/1830)

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: 3 *(60%)*
- No: 2 *(40%)*

answers: 5

skips: 0

Appendix B: Survey Results

ID #1831 (https://scipinion.com/surveys/65/results/1831)

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: 0 *(0%)*

Important: I require compensation for my participation, and the level of compensation is appropriate: 5 *(100%)*

Very Important: I require compensation for my participation, but the level of compensation would need to be increased: 0 *(0%)*

answers: 5

skips: 0

Appendix C: Auditor's Report
NeutralScience Audit 1 (NSA1) for SciPinion Expert Panel

26 February 2016

Documents received for review

Audit package documents were received from SciPinion on 19th February, 2016 with a replacement "Table 1" on 24th February, 2016 providing SciPinion ID numbers for the panel candidates. The complete package as received using the replacement for Table 1 is included as Appendix 1 to this report.

Auditors selected

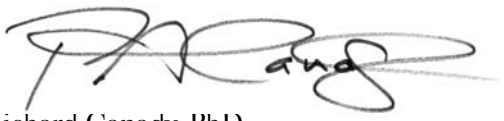
William Farland, PhD, ATS (link provided to LinkedIn profile)

Christopher Portier, PhD (link provided to LinkedIn profile)

Audit findings

The individual audits are provided in Appendix 2 with supporting tables that provide comments on each candidate review. The auditors each described wide variation in the quality of the fit to purpose for the candidates. Therefore, for this audit a 3 bin 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.

SciPinion ID	Name	Decision Bin		
		Portier	Farland	Composite
1012	[redacted]	2	2	4
1021	[redacted]	2	2	4
1053	[redacted]	2	2	4
532	[redacted]	2	2	4
1048	[redacted]	2	1	3
1046	[redacted]	2	1	3
1003	[redacted]	2	1	3
1028	[redacted]	2	1	3
534	[redacted]	1	2	3
1098	[redacted]	1	1	2
1031	[redacted]	1	1	2
1043	[redacted]	1	1	2
1034	[redacted]	0	1	1
576	[redacted]	0	0	0
514	[redacted]	0	0	0



Richard Canady, PhD
NeutralScience L3C rcanady@neutralscience.org

Appendix 1 – Documents received from SciPinion for Audit including associated Excel file for "Table 1"

Histopathology Panel Summary for the Auditor

SciPinion LLC is conducting a scientific peer review of a data package. For this review, Scipinion was provided with three items from the sponsor:

(1) A data package, which consists of five manuscripts and web portals to access histopathology slides and raw data. The data package was reviewed to help understand science issue of interest and the types of expertise needed for the panel. The papers include the following:
   a. Systems Toxicology Inhalation Study in Apoe-/- Mice to Investigate Cardiovascular and Respiratory Exposure Effects of xxx. Toxicological Sciences.
   b. Effects of xxx on lung lipid metabolism in C57BL/6 and Apoe-/- mice – an integrative systems toxicology analysis. Toxicological Sciences.
   c. Effects of xxx on the liver in Apoe−/− mice – a systems toxicology analysis. Inhalation Toxicology.
   d. Long term exposure to xxx ... the expression of genes involved in cardiac muscle structure and function in Apoe-/- mouse BMC Medical Genomics
   e. The Apoe−/− mouse model: a suitable animal model to xxx cardiovascular and respiratory diseases. J. Translat. Sci.

(2) Minimum expertise criteria for panel members, which are included at the top of **Table 1**; and

(3) Suggested questions to ask the experts. These questions have been reviewed and revised by SciPinion, and by an independent editor.

This panel is being tasked specifically to focus on reviewing the histopathology components of the data package with emphasis on the respiratory tract, and secondarily on the hepatic system. Additional panels will be 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**). Peer review panel candidates were identified and screened as 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 545 potential candidates were identified as having relevant experience in histopathology 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 545 potential candidates (see **Supplement 1**), requesting interested candidates to forward a copy of their CV/resume.

- *Panel Screening* – Based on the recruitment effort, 15 candidates volunteered. CVs were collected and reviewed (**Supplement 2**), and information relevant to panel selection criteria was extracted (**Table 1**). The source of panel candidates had no bearing on panel selection (i.e., candidates from all five sources listed above were treated equally). Three candidates were found not to meet the minimum experience requirements for one or more criteria, and were therefore excluded (hi-lited in yellow in **Table 1**). The remaining 12 candidates were identified as viable candidates for the peer review, subject to approval by the auditor(s). Upon auditor approval, SciPinion will select five candidates to serve as peer review panel members based upon a consideration of their expertise and upon panel diversity (e.g., geographic, age).

Letter sent to candidates for the panel by SciPinion

Dear Colleague,

SciPinion is seeking experienced veterinary histopathologists, particularly those with expertise in the rodent respiratory tract, liver, and cardiovascular system, for a paid peer review opportunity. The specific details of this opportunity are as follows:

- **Compensation: $7,000 (USD) per reviewer**
- Desired Expertise: Mouse respiratory tract, liver, cardiovascular system
- Recruitment Period: Present - 02/21/16
- Review Format: Level 3 (in depth), Double-blinded, Single round
- Work Description: Review 3-5 manuscript, Online access to histopathology slides and raw data, Answer ~50 charge questions
- Level of Effort: ~56 hrs (spread out over the review period at reviewers discretion)
- Review Period: 03/01/16 - 05/01/16
- Travel: None (all interaction conducted online)
- Number of reviewers: 5

SciPinion was developed by scientists, for scientists, to provide an anonymous platform to voice their opinions on scientific matters to support decision-making. SciPinion is managing the peer review of a systems toxicology-based inhalation study performed in rodents. The intention of this SciPinion review is to go beyond classical peer review. You are being requested to participate in a ''SciPi'' (e.g., a

collection of **Sci**entific o**Pi**nions), which serves as a new model for an in depth review of a complex study by an independent panel of experts. Your identity and the input that you provide will remain anonymous. For more information on the SciPinion peer review process, please visit: https://scipinion.com/pages/faq.

If you are interested in participating in this peer review process, please apply using the following link: https://scipinion.com/question_groups/65. I truly hope you will be interested in participating in this peer review. Please contact me if you need more information. Feel free to forward this email to other scientists with similar expertise who you think might be interested. I look forward to hearing back from you.

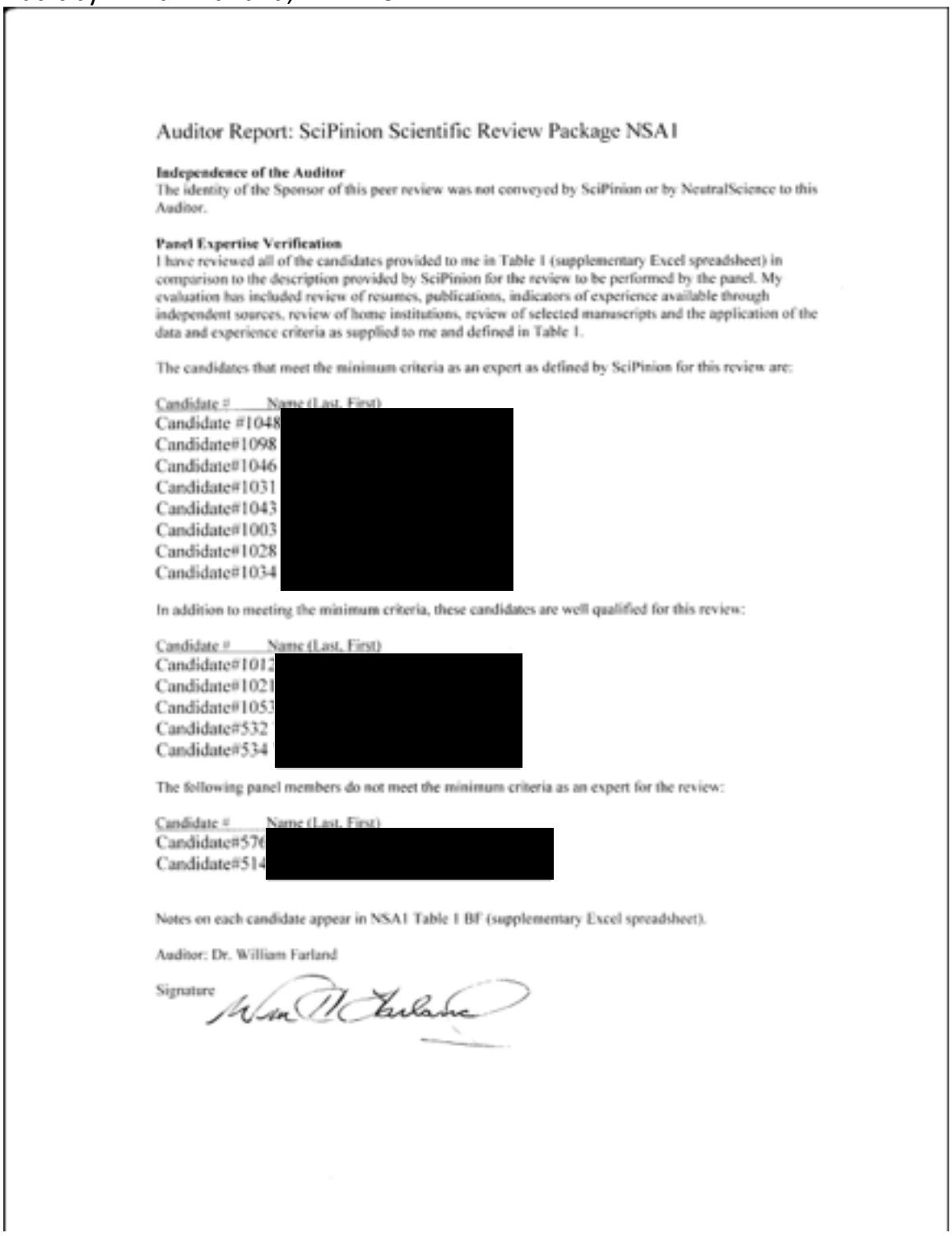
Respectfully,

Sean Hays
President
SciPinion, LLC
PO Box 536
Allenspark, CO 80510
(303) 747-0722
shays@SciPinion.com

(see associated file "Table 1.xlsx")

Appendix 2 – Individual Auditor Reports with reference to associated files in email transmittal to SciPinion February 26, 2016

Audit by William Farland, PhD ATS



Auditor Report: SciPinion Scientific Review Package NSA1

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 all of the candidates provided to me in Table 1 (supplementary Excel spreadsheet) in comparison to the description provided by SciPinion for the review to be performed by the panel. My evaluation has included review of resumes, publications, indicators of experience available through independent sources, review of home institutions, review of selected manuscripts and the application of the data and experience criteria as supplied to me and defined in Table 1.

The candidates that meet the minimum criteria as an expert as defined by SciPinion for this review are:

Candidate # Name (Last, First)
Candidate #1048 [redacted]
Candidate#1098
Candidate#1046
Candidate#1031
Candidate#1043
Candidate#1003
Candidate#1028
Candidate#1034

In addition to meeting the minimum criteria, these candidates are well qualified for this review:

Candidate # Name (Last, First)
Candidate#1012 [redacted]
Candidate#1021
Candidate#1053
Candidate#532
Candidate#534

The following panel members do not meet the minimum criteria as an expert for the review:

Candidate # Name (Last, First)
Candidate#576 [redacted]
Candidate#514

Notes on each candidate appear in NSA1 Table 1 BF (supplementary Excel spreadsheet).

Auditor: Dr. William Farland

Signature

(see associated file "Copy of NSA1 Table 1 BF.xlsx")

Audit by Christopher Portier, PhD

Auditor Report: SciPinion Scientific Review Package NSA1

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 all of the candidates provided to me in Table 1 (supplementary Excel spreadsheet, file NSA1Table1CP.xlsx) in comparison to the description provided by SciPinion for the review to be performed by the panel. My evaluation has included review of resumes, publications, indicators of experience available through independent sources, review of home institutions, review of selected manuscripts and the application of the data and experience criteria as supplied and defined in Table 1.

The candidates that meet the minimum criteria as an expert as defined by SciPinion for this review are:

Candidate #	Name (Last, First)
1098	[redacted]
1031	
1043	
534	
1048	
1012	
1046	
1003	
1021	
1028	
1053	
532	

In addition to meeting the minimum criteria, these candidates are well qualified for this review:

Candidate #	Name (Last, First)
1048	[redacted]
1012	
1046	
1003	
1021	
1028	
1053	
532	

The following panel members do not meet the minimum criteria as an expert for the review:

Candidate #	Name (Last, First)
576	[redacted]
514	
1034	

Notes on each candidate appear in NSA1Table1CP.xlsx (supplementary Excel spreadsheet).

Auditor: Dr. Christopher J. Portier

Signature 

(see associated file NSA1Table1CP.xlsx")

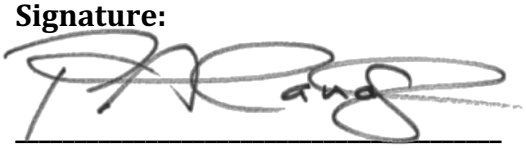
Verification of Expert Participation

Table 2.

SciPinion ID	Name	Decision Bin		
		Portier	Farland	Composite
1012	[redacted]	2	2	4
1021	[redacted]	2	2	4
1053	[redacted]	2	2	4
1046	[redacted]	2	1	3
534	[redacted]	1	2	3

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 1 Histopathology" (signed by Sean M. Hays and Christopher R. Kirman and dated August 8, 2016) belong to the identities indicated in **Table 2**.

Signature: **Date:**



August 17, 2016

Print:

Richard Canady, PhD

Affiliation:

Managing Director,
NeutralScience, L3C

Appendix D

SciPinion Editor's Report: Questions for panels reviewing the Apoe$^{-/-}$ pMRTP/THS2.2 inhalation study

Ted Simon, Ph.D., DABT



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

March 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 for the reviewers, and providing the reviewers sufficient information.

Reviewer Instructions

The following text was added to alert panelists to additional reading:

> For some questions, you will asked about the papers listed above with consideration of:
> 1. Poussin et al. 2016 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
> 2. van der Toom et al. 2015. 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).

3. Stegemann et al. 2011. Comparative Lipidomics Profiling of Human Atheroschlerotic Plaques. *Cirulation: Cardiovascular Genetics* 4:232-242.

The tasks expected of the panelists was also revised as follows:

> The expectation is that panelists/ reviewers will accomplish the following tasks:
> - read in detail the above-mentioned publications
> - 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

Question #4

I reworded the question with additional information to provide greater understanding for the panelists. The question now reads:

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

Section on Potential Conflict of Interest or Bias

- Changed the title to be more encompassing of the reviewers' preconceptions, whatever their source.
- Added a request for a statement on what each reviewer thinks of his/her own biases and the nature of these biases. This might provide additional information for the SciPinion analytics.

Question #12

I reworded the question to make it more open-ended as noted by Stephanie Boue and split it into two distinct questions for increased clarity. The question now reads:

> Which biomarkers of exposure would you recommend using for future studies? Please consider the biomarkers of exposure measured in the Phillips study or additional biomarkers. Biomarkers of exposure were collected in both blood and urine—which medium do you feel provided the most scientifically credible results?

Questions #17, #19

I removed the suggestion of immunohistochemistry and instead suggested "additional observations or analyses" to make the question less leading. The added sentence in #19 has the same rationale. Please see the "track-changes" document for the revised questions.

Question #21

The use of the word "criteria" regarding the evaluation of published omics data suggested that the methods for such evaluation are well established. Such is not the case.[1] Hence, the question was rewritten to establish an appropriate context for identifying these judgment factors as criteria.

> 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 importance and fitness 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 of the quality of the annotation and format)? Please also discuss in the text box any other criteria you deem useful or appropriate.

Question #23

The column headings in the grid box are unclear. Better headings would be:

> "Reasonable and well supported by the data"
> "Interpretation goes beyond what the data will support"
> "Unsupported assertions or gaps in the interpretation"

Changes were not made in the document because the table with these headings was not able to be edited.

Question #24

I added two sentences to make the reference to the causal network model very specific. It took no more than a scan of the references to Phillips et al. to realize that these authors are pioneers in this field. The added text is needed, I think, to give proper context to the question. This causal network model has been evolving for several years and some reviewers may not be sufficiently aware of it. The references in Phillips et al. provide information on the considerable history and scientific acceptance these models enjoy. The revised question is as follows:

> Causal biological networks have been developed for understanding and interpreting omics data using a systems toxicology approach (http://causalbionet.com). These

[1] Tenenbaum JD, Sansone S-A, Haendel M (2014) A sea of standards for omics data: sink or swim? J Am Med Inform Assoc. 21(2):200-3. doi: 10.1136/amiajnl-2013-002066

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. to understand gene expression analysis provides an appropriate high-level evaluation of the biological impact (direct or indirect) of an exposure on the transcriptome of tissues investigated?

Question #31

As written, I did not understand exactly what the word "interference" was supposed to mean. I rewrote the question to ask about the difference in the perturbations of lipid homeostasis between the two strains of mice when exposed to CS. The revised question is as follows:

> Considering the cigarette smoke (CS)-mediated changes in the lung lipidome (Titz et al., 2016), how clear were the differences in the perturbations of lipid homeostasis between ApoE-deficient mice and wild-type C57BL/6 mice?

Question #34

The question seemed a bit leading. The question was rewritten to make it less leading and more open-ended as well as to add clarity.

> 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 is reversed upon cessation. Are the quantified histopathological changes in the lung tissue that occur with CS exposure consistent with ECM degradation throughout CS exposure? Are the inflammatory mediators in BALF reflective of observed increase in ECM degradation during CS exposure? Are any of the lung functional parameters likely reflective of ECM/tissue degradation?

Question #36

The Titz et al. paper considered the differences between the two MRTPs whereas the Phillips et al. paper considered only one. The early prototype MRTP used in Titz et al. was abbreviated pMRTP. The other MRTP called THS2.2 used a different heating system to produce vapor from tobacco. Hence, the use of "pMRTP" in the first line of the question is incorrect—only one of the delivery systems for the MRTPs is referred to as the pMRTP. I also added descriptions to the first bullet for clarity. The revised question and first bullet point are shown below.

> To demonstrate reduced effect 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.

- Side-by-side analysis of effects observed in two independent studies for two different MRTPs: 1) an early prototype MRTP (pMRTP) and 2) a later MRTP using a newer tobacco heating system (THS2.2) (see, Titz et al. 2016)

Question #53

Reviewers were asked to assess their confidence in making two linked comparisons in this question: 1) changes in biological impact of switching from CS to THS2.2 aerosol compared with these changes when CS exposure continues; and 2) changes in biological impact of switching from CS to THS2.2 aerosol compared with the changes that occur with smoking cessation, i.e. switching vs. cessation. The logic of the link between the two comparisons is the wish to understand the comparative biological impact of switching to an MRTP or quitting altogether—an obvious choice for smokers who may enjoy the effects of nicotine but wish to cease cigarette smoking. Ultimately, the sponsor wishes to understand how confident the reviewers feel in distinguishing the biological impact of smoking cessation with that of switching to an MRTP. The question was rewritten to make explicit these two linked comparisons as follows:

> This question is designed to understand how confident you feel in distinguishing the change in biological impact of smoking cessation with the change in biological impact of switching to THS2.2. You will be asked to assess your confidence in two comparisons. First, considering the publications and the underlying evidence from the studies you have reviewed, how confident are you with the conclusion that the systems toxicology approach enables a holistic quantification of the changes in the biological impact due to smoking cessation compared with continuing CS exposure? Second, also considering this systems toxicology approach, how does the change in biological impact of switching to THS2.2 compare with the change in biological impact from smoking cessation?

2. Suggested Edits for Consideration

Title

The title now reads:

> Questions for panels reviewing the Apoe$^{-/-}$ pMRTP/THS2.2 inhalation study

Initial Paragraph

Edits were made in the initial paragraph to clarify the difference between histopathologists and other types of scientists on the panel. The paragraph now reads:

> This document summarizes an initial list of questions that will be addressed by panels of histopathologists and scientists with expertise in other areas (systems biology, toxicology…) about the Apoe$^{-/-}$pMRTP/THS2.2 study. 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):

Question #15
The column headers in the grid box were matched to reflect the order of characteristics (relevant, informative, intuitive) as written in the question.

Question #16
The question was reworded for clarity as follows:

> Is the approach used for histopathological evaluation and morphometry an example of a "state of the art" scientific technique and consistent with published literature/guidelines regarding (please provide any relevant comments and suggestions for improvements in the text box below):

Question #35
Rather than the parenthetical statement, I added a sentence about CYP1A1 as follows;

> CYP1A1 is a major CYP isozyme that occurs in both liver and lung and is induced by CS constituents. Looking at the integrative analysis of the exposure effects on the liver proteome and transcriptome in Lo Sasso et al, what would be a plausible explanation for the lack of an increased CYP1A1 gene expression and protein abundance in the liver?

Question #47
The difficulty I had with this question was due to the placement of the phrase "in the liver tissue. The way the question was originally written, the phrase was placed closer to the noun phrase "exposure to THS2.2 aerosol." This revision, although not considered "major", nonetheless enhances the clarity of the question. The question was rewritten as follows:

> Do you find evidence for any substantial effect in the liver tissue linked to exposure to THS2.2 aerosol? Please specify.

Question #60
I added the two references explicitly.

Question #67
A sentence and a link to Tableau software were added.

Questions for panels reviewing the Apoe$^{-/-}$ pMRTP/THS2.2 inhalation study

This document summarizes an initial list of questions that will be addressed by panels of histopathologists and scientists with expertise in other areas (systems biology, toxicology…) about the Apoe$^{-/-}$ pMRTP/THS2.2 study. 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):

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. Titz, B. *et al.* Effects of cigarette smoke, cessation and switching to two heat-not-burn tobacco products on lung lipid metabolism in C57BL/6 and Apoe$^{-/-}$ mice – an integrative systems toxicology analysis. *Toxicological Sciences. 149, 441-457, doi:10.1093/toxsci/kfv244 (2016).*
3. Lo Sasso, G. *et al.* Effects of cigarette smoke, cessation and switching to a candidate modified risk tobacco product on the liver in Apoe$^{-/-}$ mice – a systems toxicology analysis. *Inhalation toxicology* – in press
4. Szostak, J. et al. Long term exposure to cigarette smoke impacts the expression of genes involved in cardiac muscle structure and function in Apoe-/- mouse while exposure to the aerosol of a Tobacco Heating System does not. *BMC Medical Genomics - submitted*
5. Lo Sasso, G. et al. The Apoe$^{-/-}$ mouse model: a suitable animal model to study cigarette smoke-induced cardiovascular and respiratory diseases. *J. Translat. Sci – submitted*

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

1. Poussin et al. 2016 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
2. van der Toom et al. 2015. 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).
3. Stegemann et al. 2011. Comparative Lipidomics Profiling of Human Atheroschlerotic Plaques. *Cirulation: Cardiovascular Genetics* 4:232-242.

The expectation is that panelists/reviewers will accomplish the following tasks:

- read in detail the above-mentioned publications

- access the systox webportal to access additional information

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- 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

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- review the results in light of existing publications in the same field and cite appropriate references when justifying their answers

- read additional publications/documents as necessary to answer some of the questions

- review >100 images of lung and liver sections, applicable to members of the histopathology panel.

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

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

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

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5. 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*

- *Either in the past or currently, have you received funding from (select all that apply)?*
  - *A tobacco company*

- *Philip Morris International*
- *US FDA*
- *US EPA*
- *NIEHS*
- *NSF*
- *Anti-tobacco non-profits*
- *European Commission*

- *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 in a tobacco related case? (yes/no)*
- *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 (y/n)*
- *What is your smoking status?*
  - *Nonsmoker*
  - *Former smoker*
  - *Current smoker*
- *Please use the text box to provide a brief statement about your potential biases that may influence your scientific opinion. Biases are not necessarily based on financial relationships or past employment. Also, If you believe you are without any bias that might influence you in this review, please indicate so.*

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Comment [TS2]: I don't know if Scipinion could gain additional insight from a short statement about self-recognition of bias, but I included it. If not appropriate, please delete.

6. Study design, end points, and data quality

5. For the evaluation of respiratory disease endpoints, how confident are you that the following criteria are met by Phillips et al. (2016)?

- Appropriate choice of animal 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

How confident are you that these criteria are met in Phillips et al. (2015)	Very confident	Confident	Somewhat confident	Not confident	I cannot answer based on the information provided
Appropriate choice of model, including species, strain, and gender	◯	◯	◯	◯	◯
Appropriate choice of controls	◯	◯	◯	◯	◯
Appropriate choice of exposure modality	◯	◯	◯	◯	◯
Appropriate duration of the experiment	◯	◯	◯	◯	◯
Appropriate choice of endpoints	◯	◯	◯	◯	◯
Appropriate number of replicates	◯	◯	◯	◯	◯
Appropriate choice of analytical methods	◯	◯	◯	◯	◯

6. For the evaluation of cardiovascular disease endpoints, how confident are you that these criteria are met by Phillips et al. (2016)?

- Appropriate choice of animal 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

7. The study authors chose to evaluate the data in each treatment group versus sham at each respective time point rather than across time because different pools of animals were used to measure endpoints at the different time points. How do you judge this approach?

- It is satisfactory and sufficient
- It is appropriate but not comprehensive enough (please specify what you believe is missing)
- Some measurements that can be done longitudinally should complement the study design (please specify which)
- It is not appropriate (please specify)

- I cannot answer this question

8. **Given the amount of the data generated (functional endpoints in addition to microarray-based transcriptomics, untargeted proteomics, and lipidomics data), do you see any important gap(s) not covered by the data? Please provide comments below.**

   - (Free text)

9. **Are the cessation and switching groups appropriately designed to achieve the proposed aims? In particular, do you think that 2 months of exposure to 3R4F cigarette aerosol prior to cessation or switching is appropriate? Would you expect different results if the cessation or switching time point was set to 3 or 4 months?**

   - (Free text)

10. **How confident are you that the following criteria related to aerosol generation and characterization were met in the publications? Please comment in the box below whether more details should be given in every single manuscript. (Potential answer options: Poor, sufficient, excellent, I cannot answer)**

    - Aerosol generation adequately explained with regard to reproducibility
    - Sufficient level of details and explanation regarding aerosol generation
    - Sufficient level of details and explanation regarding aerosol characterization

11. **Is the approach to match the exposures to the aerosols on the basis of a key common constituent, nicotine, instead of TPM reasonable?**

    - Yes (Please specify)
    - No (Please specify)
    - I cannot answer this question*

12. **Which biomarkers of exposure would you recommend using for future studies? Please consider the biomarkers of exposure measured in the Phillips study or additional biomarkers. Biomarkers of exposure were collected in both blood and urine—which medium do you feel provided the most scientifically credible results?**

13. **What would you consider the most appropriate methodology to investigate lipid profiles in these mice?**

14. **Which of the applied parameters in respiratory physiology is most appropriate to demonstrate emphysematous changes in the mouse model?**

    - Snapshot perturbations (measuring resistance, compliance, and elastance)

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- Primewaves (measuring tissue elasticity, tissue damping, tissue hysteresivity, and Newtonian resistance)
- Pressure-volume loops (measuring parameters of the Salazar-Knowles equation, quasi-static compliance, quasi-static elastance, and hysteresis)
- Positive end-expiratory pressure (measuring the end-expiratory lung volume)
- Negative pressure forced expiration (measuring FEV0.1, FEV0.2,FEV0.1/FVC, and FEV0.2/FVC)
- None of the measures is indicative of emphysematous changes
- All of the measures combined
- I cannot answer this question*

15. 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 four manuscripts related to the Apoe$^{-/-}$ THS study?

	Relevant (1-5)	Informative (1-5)	Intuitive (1-5)
transcriptomics: computational network modeling and NPA calculation			
transcriptomics: gene set enrichment analysis			
proteomics: clustering and functional association networks			
proteomics: protein set enrichment analysis			
lipidomics: integration with lipidomics data, PLS plots			
Other (please specify)			

16. Is the approach used for histopathological evaluation and morphometry an example of a "state of the art" scientific technique and consistent with published literature/guidelines regarding (please provide any relevant comments and suggestions for improvements in the text box below):

- tissue preparation, including type of staining (for lung) (yes/no/cannot answer*)
- tissue preparation, including type of staining (for lung) (yes/no/cannot answer*)
- number of sections evaluated (yes/no/cannot answer*)
- blinding / randomization (yes/no/cannot answer*)
- scoring system (yes/no/cannot answer*)
- histomorphometry methods (yes/no/cannot answer*)
- statistical evaluation (yes/no/cannot answer*)
- data visualization (yes/no/cannot answer*)

17. Is the histopathological liver analysis presented in Lo Sasso et al 2016 appropriate to detect possible hepatotoxic effect? Would you recommend additional observations or analyses?

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- Free text

18. How suitable is the Apoe$^{-/-}$ mouse model to detect additional exposure effects in the already challenged liver?

- Free text

19. Do the methods and instruments used to perform lipidomics and proteomics analyses reflect the current state-of-the-art in the field? Are there other approaches you would recommend?

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- Free text

20. Having inspected the study data in the various repositories provided, as well as in the Supplementary materials provided with the publications, 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.

Data category	Data Quality	Annotation quality	Data Quality	Annotation quality
In-life observations, mortality, body weight, gross pathology				
Biomarkers of exposure, urine				
Biomarkers of exposure, blood, plasma				
Clinical chemistry, including blood lipoproteins				
Hematology				
Respiratory physiology				
BAL fluid - inflammatory mediators				
BAL cells (free lung cells) - identification and activation markers				
Histopathology, upper respiratory tract				
Histopathology, lung				
Histopathology, liver				
Histopathology, other organs/tissues				
Histomorphometry, lung				
Plaque size measurement, planimetry				
Plaque size measurement, micro-CT				
Dataset, transcriptomics				
Dataset, lipidomics				
Dataset, proteomics				

21. 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 importance and fitness 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 of the quality of the annotation and format)? Please also discuss in the text box any other criteria you deem useful or appropriate.

Comment [TS4]: Tenenbaum et al. 2014 J Am Med Informatics Assoc. http://dx.doi.org/10.1136/amiajnl-2013-002066
I think you need to acknowledge that these are "critieria" specific to this review and assessing the fitness of these omics for their intended purpose.

	Importance and fitness for purpose (1-5)	Confidence (1 - 5)
Availability of raw data		
Annotation of the metadata		
Detailed enough text 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 of the quality of the tool		
Use of multiple methods on the same data leading to similar conclusions		
Other (please specify		

7. Data evaluation and interpretation

22. Are the results in the four publications presented with sufficient detail, and were they clearly presented (1:insufficient, 2: partially sufficient, 3: sufficient)?

Comment [TS5]: Change the column headings to "Sufficient Detail?" and "Clarity of Presentation"

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Results category	**Completeness** (1: insufficient, 2: partially sufficient, 3: sufficient).	**Pres** (1: i 3: ac
In-life observations, mortality, body weight, gross pathology		
Biomarkers of exposure, urine		
Biomarkers of exposure, blood, plasma		
Clinical chemistry, including blood lipoproteins		
Hematology		
Respiratory physiology		
BAL fluid - inflammatory mediators		
BAL cells (free lung cells) - identification and activation markers		
Histopathology, upper respiratory tract		
Histopathology, lung		
Histopathology, liver		
Histopathology, other organs/tissues		
Histomorphometry, lung		
Plaque size measurement, planimetry		
Plaque size measurement, micro-CT		
Dataset, transcriptomics		
Dataset, lipidomics		
Dataset, proteomics		

23. Are the interpretations and conclusions in the publications regarding the effects of exposure to 3R4F cigarette and THS2.2 aerosol in the Apoe-/- mouse model reasonably supported by the results?

Comment [TS6]: I think the column headings below should be:
"Reasonable and well supported by the data"
"Interpretation goes beyond what the data will support"
"Unsupported assertions or gaps in the interpretation"

	reasonable and founded on the data shown	goes beyond what the data showing
Lung / respiratory effects in Titz et al. 2016		
Lung / respiratory effects in Phillips et al. 2016		
Vascular / atherosclerotic effects in Phillips et al. 2016		
Cardiac effects in Szostak et al. 2016		
Clinical chemistry and systemic effects in Phillips et al. 2016		
Hepatic effects in Lo Sasso et al. 2016		

24. 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. to understand gene expression analysis provides an appropriate high-level evaluation of the biological impact (direct or indirect) of an exposure on the transcriptome of tissues investigated?**

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

25. How critical and informative do you consider each endpoint shown in Philips *et al.* for assessing the relative impact of various tobacco products?

Comment [BS7]: Grid answer: critical (low, medium, high, cannot answer); informative (low, medium, high, cannot answer)

- Clinical chemistry (Figure 2, Supplementary Fig. 8, and supplementary results file 2)
- Aortic arch planimetric plaque measurements (Figure 3)
- microCT-based aortic arch plaque measurements (Figure 4, Suppl. Fig. 9, and supplementary files)
- Plasma and aortic arch lipidomics (Figure 5)
- Respiratory physiology (Figure 6)
- Lung volume measurements (Figure 7)
- Bronchoalveolar lavage fluid – FACS analysis of free lung cells (Figure 8)
- Bronchoalveolar lavage fluid – Multianalyte Profiling (Figure 9, supplementary Fig. 12)
- Nasal epithelium histopathological evaluation (Figure 10)
- Lung histology (Figure 11, Supplementary Results File 2)
- Lung histomorphometry (Figure 12)
- Lung transcriptomics results – Differentially expressed genes (Figure 13, Supplementary Fig. 13)
- Lung transcriptomics results – NPA/BIF analysis (Figure 14)
- Lung proteomics analysis (Figure 15, Supplementary Figs. 14 to 16)
- The totality of the data, collectively (All figures)

26. In your opinion and based on the scientific literature, how could the age of the animals impact the results obtained? For which endpoints would you expect the outcome to look different if the animals were older? Please provide relevant references.

27. Are the lung function results (considering Fig 6 and Suppl. Fig. 10) indicative of emphysematous changes in the 3R4F-exposed animals?

- Yes
- No
- I do not see any indication of airspace enlargement in this animal model
- I cannot answer

28. Considering the lung function results (considering all perturbations, Fig 6 and Suppl. Fig. 10), how do the results from THS2.2 aerosol-exposed animals compare to 3R4F exposed animals?

- Less airspace enlargement
- More airspace enlargement
- No airspace enlargement seen in any of the two exposed groups
- Similar airspace enlargement seen in both exposed groups
- I cannot answer the question*

29. To what extent do you believe the lung transcriptomics data interpretation (NPA/BIF) correspond to physiological changes measured in the animals (e.g., inflammation or emphysema)?

- To a large extent
- To a small extent
- Not at all
- I cannot answer the question*

30. Does this study design allow a transversal evaluation of a number of mechanisms, such as oxidative stress and inflammation?

- Yes, this systems approach demonstrated the potential to enhance the understanding of pathomechanisms also in a transversal manner
- Yes, a systems approach can potentially enhance the understanding of pathomechanisms also in a transversal manner, but in the current study, the following additional aspects would be required in order to make it more conclusive: (pls. indicate missing aspects in the text box)
- No, the only reliable approach to understand pathomechanisms is to use separate, mechanism-specific investigations in various dedicated model systems
- I cannot answer the question*

31. **Considering the cigarette smoke (CS)-mediated changes in the lung lipidome (Titz et al., 2016), how clear were the differences in the perturbations of lipid homeostasis between ApoE-deficient mice and wild-type C57BL/6 mice?**

    - Free text

32. **Do the aortic arch plaque measurements (planimetry and micro-CT imaging) support the authors' statement that cessation or switching to THS2.2 halted the progression of atherosclerotic plaques?**

    - Yes, both plaque measurements confirm the statement
    - Only the 2D measurements confirm the statement
    - Only the CT imaging confirm the statement
    - No, the statement is not supported by these data
    - I cannot answer this question*

33. **Do the gene/protein set analyses results presented in Titz et al., and Lo Sasso et al. effectively contribute to the assessment and do they give adequate information (qualitative and quantitative) on the protein/gene pathways involvement?**

    - Free text

34. **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 is reversed upon cessation. Are the quantified histopathological changes in the lung tissue that occur with CS exposure consistent with ECM degradation throughout CS exposure? Are the inflammatory mediators in BALF reflective of observed increase in ECM degradation during CS exposure? Are any of the lung functional parameters likely reflective of ECM/tissue degradation?**

35. **CYP1A1 is a major CYP isozyme that occurs in both liver and lung and is induced by CS constituents. Looking at the integrative analysis of the exposure effects on the liver proteome and transcriptome in Lo Sasso et al, what would be a plausible explanation for the lack of an increased CYP1A1 gene expression and protein abundance in the liver?**

    - CS constituents are too diluted to induce CYPs when they reach the liver
    - in contrast to the lung where CYP1A1 is induced by typical substrates, there is constitutive CYP1A1 expression in the liver that may be sufficient to metabolize the systemically delivered CS constituents
    - other (pls. explain)
    - I cannot answer this question*

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Comment [BS9]: If possible, free text box for each question, but keep the 3 "subquestions" together.
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36. **To demonstrate reduced effect 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.**

- Side-by-side analysis of effects observed in two independent studies for two different MRTPs: 1) an early prototype MRTP (pMRTP) and 2) a later MRTP using a newer tobacco heating system (THS2.2) (see, Titz et al. 2016)
- Integration of diverse data modalities (e.g. proteomics and lipidomics data in Titz et al. 2016) within a single study
- Analysis of the effects of the products on multiple systems (respiratory, cardiovascular) in a single model
- Concordance of results for specific mechanisms (e.g. oxidative stress or inflammation) across diverse studies and model systems (Apoe$^{-/-}$ mouse and in vitro assays)

37. **Are the lipidomics and proteomics results shown in Titz et al. 2016 providing complementary mechanistic information that contributes to the understanding of the pathomechanisms and the resulting morphological and functional changes? If yes, please indicate 1 to 3 examples where this is obviously the case. If not: what should be changed?**

38. **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?**

- Free text

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

40. **Looking at lung slides on the Aperio platform for the different groups, do you agree that the histopathological evaluation and results support the authors' (Phillips et al. 2015) conclusion that:**

- "THS2.2 aerosol-exposed lungs were indistinguishable from sham" (yes/no/cannot answer)
- "switching or cessation inhibited the further progression of emphysema-related parameters, leading to a convergence with the sham/THS2.2 group levels (yes/no/cannot answer)

41. **Are the liver, aortic arch, and upper respiratory tract histopathology results correctly interpreted by the authors regarding the effects of exposure to aerosol from 3R4F cigarettes and THS2.2 in this mouse model?**

- Yes

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Comment [BS10]: Grid answers – 1 for each tissue type

- No
- I cannot answer

42. Do histopathological, morphometric, functional (pulmonary function) and other investigations indicate emphysematous changes at 2 months of 3R4F cigarette smoke exposure?

43. Given the lung slides provided in the Aperio tool, please rate the magnitude of change (1-5 scale, 5 being the largest magnitude of change) you observe between the different groups for each finding (i) 3R4F aerosol and sham, (ii) THS2.2 and 3R4F aerosols, (iii) switch and cessation (please specify the ID numbers of slides you have looked at and based your evaluation on)?

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Comment [BS11]: It is fine to keep 1 box of slides number overall (we know to which group belongs each slide)

- Presence/number of lymphocytic cells and neutrophilic granulocytes in the intra-alveolar space
- Presence/number of non-pigmented macrophages in the alveolar lumen
- Presence/number of macrophages with yellow-brownish pigmentation in the alveolar lumen
- Presence/number of pigmented macrophages that are arranged in small intra-alveolar clusters (described as pigmented macrophage nests) in the alveolar lumen
- Presence/number of macrophages that appear to be enlarged or multi-nucleated cells, and in part contain PAS-positive material in the cytoplasm, in the alveolar lumen
- Presence/number of aggregates of lymphocytic cells in the lung interstitium; i.e., alveolar interstitium, peri-vascular area, interstitium at main bronchus
- Amount of non-cellular eosinophilic material in the alveolar lumen
- Emphysematous lesions characterized morphologically by alveolar septal destruction as well as by mild enlargement of the distal airways
- Multifocal alveolar emphysema
- Emphysema locally associated with mild hyperplasia/hypertrophy of the alveolar epithelium
- Emphysema locally associated with the accumulation of inflammatory cells
- Hyperplasia of the bronchial epithelium
- Hyperplasia of the bronchial goblet cells

44. For each exposure group, 10 animals were included for the histopathology analysis. Within single animal lungs, 5 different section levels are available on the Aperio platform with H&E staining, and 1 level with AB-PAS staining. How do you judge the homogeneity of the findings within lungs and across animals from the same groups? Please specify the finding and which slides you considered in your evaluation in the text box below.

Comment [BS12]: Grid answer: within animals: high consistency, moderate consistency, low consistency – across animals from the same group: high consistency, moderate consistency, low consistency

- Presence/number of lymphocytic cells and neutrophilic granulocytes in the intra-alveolar space
- Presence/number of non-pigmented macrophages in the alveolar lumen
- Presence/number of macrophages with yellow-brownish pigmentation in the alveolar lumen

- Presence/number of pigmented macrophages that are arranged in small intra-alveolar clusters (described as pigmented macrophage nests) in the alveolar lumen
- Presence/number of macrophages that appear to be enlarged or multi-nucleated cells, and in part contain PAS-positive material in the cytoplasm, in the alveolar lumen
- Presence/number of aggregates of lymphocytic cells in the lung interstitium; i.e., alveolar interstitium, peri-vascular area, interstitium at main bronchus
- Amount of non-cellular eosinophilic material in the alveolar lumen
- Emphysematous lesions characterized morphologically by alveolar septal destruction as well as by mild enlargement of the distal airways
- Multifocal alveolar emphysema
- Emphysema locally associated with mild hyperplasia/hypertrophy of the alveolar epithelium
- Emphysema locally associated with the accumulation of inflammatory cells
- Hyperplasia of the bronchial epithelium
- Hyperplasia of the bronchial goblet cells

45. Is there any other substantial finding that you would report for the THS2.2 or switch group based on the analysis of the slides you performed on the Aperio tool?

- Free text

46. Given the histopathology and histomorphometry results, how substantial would you consider the difference between the cessation and switch groups?

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- Large difference
- Moderate difference
- Minimal difference
- No difference at all

47. Do you find evidence for any substantial effect in the liver tissue linked to exposure to THS2.2 aerosol? Please specify.

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8. Translatability and outlook

48. After reviewing the evidences in the diverse publications using Apoe$^{-/-}$ mice to study responses to smoke inhalation (see also review by Lo Sasso et al., 2016b), for which of the following therapeutic areas do you consider the Apoe$^{-/-}$ mouse strain to be a good model, and where do you see the limitations of the model (please comment below)?

- Inflammatory diseases
- Respiratory diseases
- Cardiovascular diseases
- Neurological diseases
- Hepatotoxicity
- Metabolic syndrome / diabetes

Comment [BS13]: Grid format. Good model, poor model, text box for explanation

49. How confident are you that the lung histopathological evaluation in mice as conducted in the present study allows translation to human pathology? Which finding is most suitable? (please indicate in the comment box – grid answers: Extremely confident, very confident, confident, somewhat confident, not confident, I cannot answer)

- Presence/number of lymphocytic cells and neutrophilic granulocytes in the intra-alveolar space
- Presence/number of non-pigmented macrophages in the alveolar lumen
- Presence/number of macrophages with yellow-brownish pigmentation in the alveolar lumen
- Presence/number of pigmented macrophages that are arranged in small intra-alveolar clusters (described as pigmented macrophage nests) in the alveolar lumen
- Presence/number of macrophages that appear to be enlarged or multi-nucleated cells, and in part contain PAS-positive material in the cytoplasm, in the alveolar lumen
- Presence/number of aggregates of lymphocytic cells in the lung interstitium; i.e., alveolar interstitium, peri-vascular area, interstitium at main bronchus
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- Multifocal alveolar emphysema
- Emphysema locally associated with mild hyperplasia/hypertrophy of the alveolar epithelium
- Emphysema locally associated with the accumulation of inflammatory cells
- Hyperplasia of the bronchial epithelium
- Hyperplasia of the bronchial goblet cells

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

- Dose

Comment [BS14]: Grid answers: Not appropriate, moderately appropriate, very appropriate.

- Regimen
- Duration
- Biomarkers of exposure

51. In your opinion, which of the mechanisms involved in the development and/or progression of human disease are recapitulated in this nonclinical model of cigarette smoke (CS) exposure-related changes, i.e. which are the major disease mechanisms in mice that are translatable to humans?

- Free text

52. For future studies, would you suggest alternative methodologies to capture the effects of both 3R4F and THS2.2 / other MRTP aerosol exposure on the respiratory tissues and if so, which ones?

- Free text

53. This question is designed to understand how confident you feel in distinguishing the change in biological impact of smoking cessation with the change in biological impact of switching to THS2.2. You will be asked to assess your confidence in two comparisons. First, considering the publications and the underlying evidence from the studies you have reviewed, how confident are you with the conclusion that the systems toxicology approach enables a holistic quantification of the changes in the biological impact due to smoking cessation compared with continuing CS exposure? Second, also considering this systems toxicology approach, how does the change in biological impact of switching to THS2.2 compare with the change in biological impact from smoking cessation?

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

54. How confident are you that COPD or CVD drug targets can be identified using a broader systems approach that includes the mechanisms identified in response to cigarette smoke exposure as observed in the study?

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

55. **In your opinion, can the cigarette smoke effect findings be leveraged or translated to risk assessment of other inhaled toxicants, such as environmental air pollution, workplace exposures, etc.? Please cite appropriate references to justify your answer**

- Free text

56. **Stegemann and coworkers analyzed the lipid composition of human atherosclerotic plaques from carotid endarterectomies and reported a list of plaque-enriched lipid species, with a strong contribution from sterol lipids, sphingolipids, and glycerophospholipids [Stegemann et al., 2011]. When comparing these 15 lipids side-by-side with the lipids found in CS-exposed murine aortic arches, 12 of these common lipids were also increased in the mice, while 3 glycerolipids were unchanged. What kind of interpretation would you rate as most appropriate:**

- The correlation between human and murine plaque-enriched lipids is suggestive of related pathomechanisms in plaque formation in this mouse model of atherosclerosis.
- The three unchanged lipids represent the ApoE deficiency-related imbalance in lipid metabolism, particularly the disrupted reverse cholesterol transport.
- Due to the biological differences in human carotid plaques and murine aortic plaques (e.g., the lack of highly unstable plaque morphology in mice) this comparison is meaningless
- Other (pls. explain)

57. **For future studies, would you suggest examination of other tissues or biological samples to increase the overall level of scientific confidence and translatability to humans?**

- Free text

58. **Which type of study do you deem appropriate to study the impact of switching to THS2.2 in smokers? Select all that apply, and please indicate in the comment box which *in vitro* or *in vivo* model might apply.**

- Clinical, long term
- Clinical, short term
- *In vivo*
- *In vitro*

59. **What are the most important biological effects of cigarette smoke exposure on the lung that are relevant to human disease (i.e., COPD) and how strong is the evidence supporting a reduction in effects from THS2.2 compared with 3R4F? (grid answer: importance (1-5) / evidence strength (1-5))**

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

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Comment [BS15]: Grid with confidence rating
- •Extremely confident
- •Very confident
- •Confident
- •Somewhat confident
- •Not confident
- •I cannot answer (please explain in the comment box)

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60. **Specific disease mechanisms can be appropriately studied in *in vitro* models. PMI tested MRTPs with assays that recapitulate two mechanisms relevant to atherosclerosis, namely adhesion of monocyte to endothelial cells and transendothelial migration (Poussin et al. 2016 Toxicology 339(2):73-86; van der Toom et al. 2015. Food Chem Toxicol 86:81-87). After reviewing the results of these publications and in light of the plaque measurements reported in Philips et al, could you please comment on the conclusions you would reach regarding (i) the relative atherogenic effect of THS2.2 and 3R4F aerosol exposure, (ii) the usefulness of the *in vitro* assays to recapitulate atherogenic effects seen *in vivo*.**

61. **The authors of these studies aimed at reducing and refining the exposure of animals *in vivo* by assessing two human diseases in the same inhalation toxicity study and to maximize the use of various tissues from the same animals to an array of classical toxicity end points plus transcriptomics, proteomics, and lipidomics. Do you think this approach was efficient and worthwhile, and how could it be further improved?**

- Efficient approach, should be further considered in future studies
- Some compromises in design (which ones?) 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: (pls. specify)

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9. Tools and Review Process

63. Could you please provide one additional question that you would like to see the peer reviewers address during round 2 of the SciPi?

64. 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

65. 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)?

	Rich (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			

66. In collaboration with the Systems Biology Institute in Japan, gadgets for the Garuda platform (see question-specific link in the Introduction) have been created for the review of data hosted in the Systox Web Portal (see Tools section for SBX Garuda). Please download the application to answer the following question. Alternatively, if you cannot download the application, browse the slides/tutorials to understand the utility of the gadgets. The Garuda gadgets (select all that apply):

- Allow readers to assess which key processes or pathways are enriched for biological functions for a given set of genes under a specific condition of study?
- Automatically interpret large omics datasets
- Allow readers to assess how transcriptomics profiles compare between two studies in terms of a specific set of genes?
- Allow readers to compare the enrichment of biological functions for a specific set of genes between the transcriptomic profiles of two study conditions.
- Are very useful and should be made available to the whole scientific community
- I cannot comment on Garuda (please explain in the following comment box)
- None of the above

67. Tableau is new software for visualization and data analytics (http://www.tableau.com). Showing some of the results in a particular manner in a scientific article may not allow readers to grasp the full relevance of the results. To allow for interactive browsing of study results, a Tableau workbook was prepared (see question-specific link in the Introduction). In your opinion this (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

68. What additional material/information would you need to obtain to better assess the relevance of the study and its results?

69. 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
- Enough and appropriate information was 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

70. How could this peer review process be improved?

71. How could the sbv IMPROVER systems toxicology Web Portal be made more valuable in responding to the questions?

72. 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.

73. 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

74. 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*

Appendix E. Summary of Codified Results (for one expert flagged as an outlier)

