Peer Review of Data for a Modified Risk Tobacco Product (THS 2.2, PMI Study Number 15015): Part 2 In Vivo Study - Mixed Expertise

Prepared for:

Philip Morris International

Prepared by:

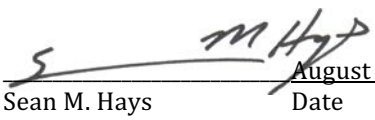


August 30, 2016

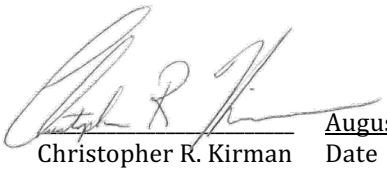
SciPinion, L.L.C.
PO Box 3209
Bozeman, MT 59772

shays@scipinion.com
ckirman@scipinion.com
(406)404-6145

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



August 30, 2016
Sean M. Hays Date
President, SciPinion



August 30, 2016
Christopher R. Kirman Date
Vice President, SciPinion

Table of Contents

1. Introduction 4
2. Methods 4
   2.1 Process Overview 4
   2.2 Peer Review Panel 8
   2.3 SciPi Questions 10
3. Results 10

List of Tables

1. Candidate Experts for Peer Review Panel 5
2. Outlier Evaluation 10

List of Figures

1. SciPinion Review Process 6
2. Summary of Codified Results for Round 3 12

List of Appendices

A. Survey Questions A-1
B. Survey Results for Round 1 ..... B-1
C. Survey Results for Round 3 ..... C-1
D. Auditor Report D-1
E. Editor's Report E-1

List of Files

1. Survey_67_results_Round 1.csv
2. Survey_67_results_Round 3.csv
3. P1 Mixed Results_Comparison between Rounds 1 and 3.xlsx.

1. Introduction

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

(1) *Data Package* - The data package consisted of five published manuscripts:
   a) 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 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.

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.

- ***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

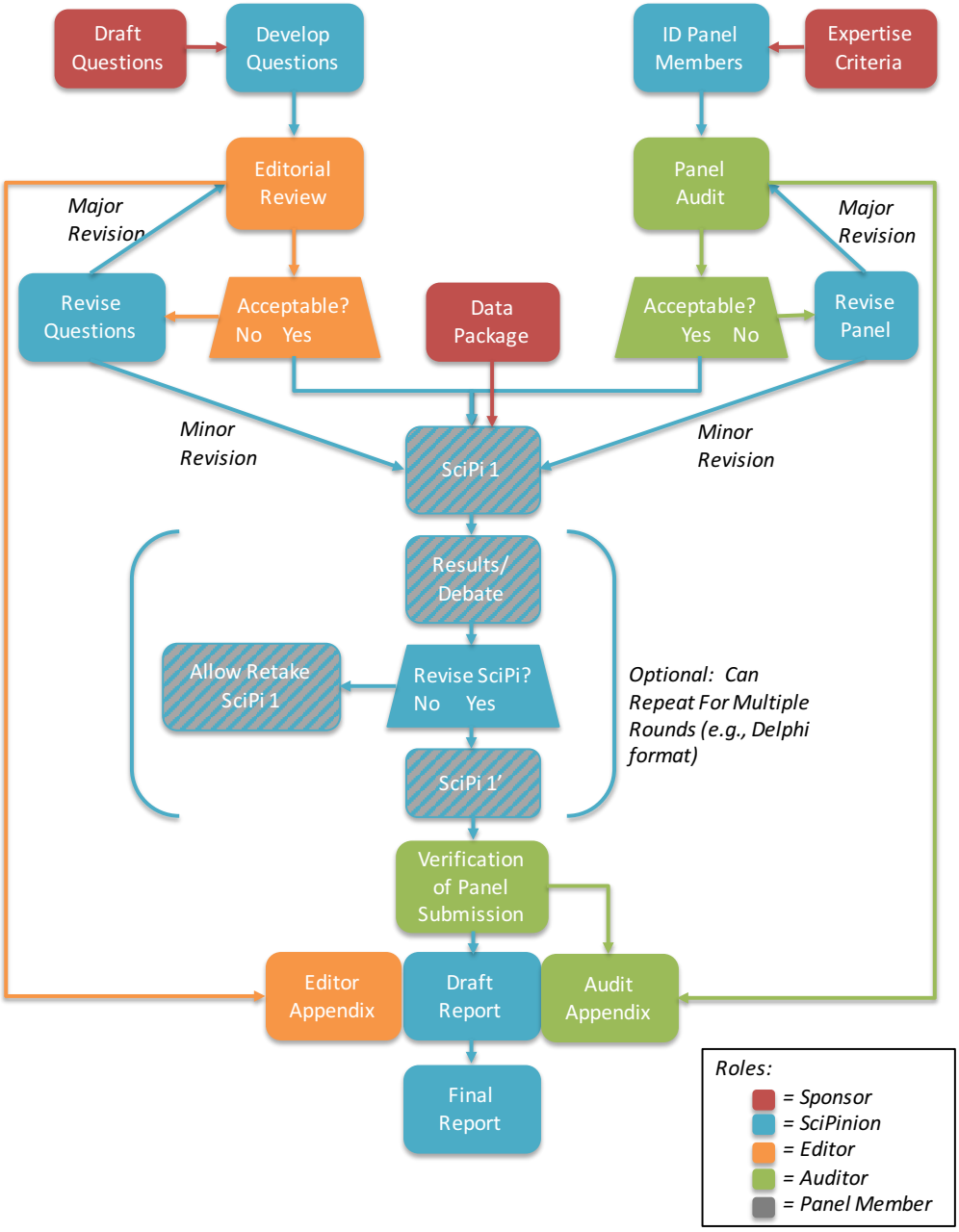
Table 1. Candidate Experts for Peer Review Panel

Expertise Criteria (souce of information):												
				Gender	Highest Degree	Year of Highest Degree (or earlier)	Geographic Location (CV)	# Papers/ Reports (CV or online)	# First/Last Author (CV or online)	h-index	RG score	Professional Society Membership/ Advisory Board Participation (CV)
			Minimum Value:	*NA*	*PhD/DVM /MD*	*2011*	Diverse	10	5	NA	NA	0
Candidate Values:												
Candidate ID #	**Name**	**Email**	Title/Position	**Gender**	***PhD/DVM /MD?***	**Year of Highest Degree**	**Geographic Location (CV)**	**# Papers/ Reports (CV or online)**	**# First/Last Author (CV or online)**	**h-index (self reported)**	**RG Score (self reported)**	**Number of Professional Society Membership/ Advisory Board Participation (CV)**
1002				Male	PhD	1994	USA	208	173			18
518				Male	PhD	2001	USA	36	19			4
815				Male	PhD	1989	Korea	215	176		39.1	20
1006				Male	PhD	2010	USA	13	5	8		2
1009				Male	PhD	2004	USA	30	17			4
1010				Male	PhD	2012	USA	20	6	10	120.28	5
1013				Male	PhD	2011	USA	42	15	19		6
571				Female	PhD	1982	USA	46	23			20
1022				Male	PhD	1986	USA	105	57			2
1023				Male	PhD	2002	USA	30	10	19	30.94	2
1029				Female	PhD	1970	Russia	126	91	11		1
1039				Male	PhD	1987	USA	53	44			12
1041				Male	PhD	1988	USA	26	13	13	28.54	7
48				Male	PhD	1986	USA	53	12			5
1044					PhD	1983	UK	168	81			5
576				Male	PhD	2002	Brazil	79	15	25	35.55	15
1047				Male	PhD	1988	USA	48	35			1
1051				Male	PhD	2003	USA	26	25			12
665				Male	PhD	1983	USA	255	190			15
1053				Male	PhD	1991	USA	36	8			6
1054				Male	PhD	1991	New Zealand	21	5			2
604				Male	PhD	1972	United States	113	88			34
1099				Male	PhD	1986	United States	107	48		34.62	3
326				Male	PhD	1996	Netherlands	71	18	33		1
514				Male	PhD	2009	India	49	17	19		0
1016				Male	PhD	2009	USA	18	4	10		2
1027					PhD	2012	USA	7	5		14.7	3
1014				Male	PhD	2013	USA	9	3	4	24.4	3
1015				Male	PhD	2014	USA	5	4			4
1017				Female	PhD	2015	USA	10	2	6	19.78	1
1018				Male	MSc	2001	USA	3	0			0
1032				Female	PhD	2009	Sweden	15	4		24.95	0
1033				Male	PhD	2008	USA	9	4	5	10.66	2
1034				Female	Bs	2012	Netherlands	16	7	3	21.71	0
1035				Female	PhD	2007	USA	10	4	9		3
1045				Male	MS	2014	France	6	1			0
1049				Male	PhD	2013	UK	2	0			3

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

Figure 1. SciPinion Review Process



Peer Reviewers—to ensure they are free to offer their unbiased opinion, Peer Reviewers remain anonymous to the Sponsor and to any recipients of the report. Only SciPinion and the Auditor know the identities of the Peer Reviewers.

- ***Auditor*** – The role of the Auditor in the peer review is depicted in green in **Figure 1**. An Auditor is retained by SciPinion to independently verify that: (1) Peer Reviewers meet the minimum expertise requirements as defined for the SciPi™; and (2) the submitted responses can be attributed to the SciPinion user accounts belonging to the Peer Reviewers. The Auditor conducts the verification review through an independent audit process not under control of SciPinion or the Sponsor. The Auditor prepares a brief report containing the conclusions of their review. The identity of the Auditor(s) is included in this report.
- ***Editor*** – An independent Editor is retained by SciPinion to ensure that the questions in the SciPi™: (1) focus on the science issues associated with the data package; (2) are clearly written; and (3) are not leading or biased. The Editor prepares a brief report containing the conclusions of his/her review. The identity of the Editor is included in this report.

Importance of Anonymity

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

Importance of Independence

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

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

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

Compensation

It is recognized that the majority of scientific journals rely upon a peer review process in which peer reviewers are not compensated. Instead, peer reviewers volunteer their time (typically a few hours) for this important service. This is the review model with which most scientists are familiar. However, as scientific manuscripts and their underlying data become increasingly large and complex, a few hours may not be sufficient to provide a robust review, and very few reviewers can be found who are qualified, available, and willing to donate a larger amount of their time to the review process. In these situations, the robustness of the peer review can suffer under a volunteer peer review model. For some peer reviews, SciPinion uses a compensated peer review model, in recognition that: (1) large and complex data sets can require a considerable amount of time to review; and (2) the peer reviewer's time is valuable, and is worthy of compensation. SciPinion adopts a compensated peer review model to add value to the peer review process, increasing its robustness both in terms of the number of scientists participating and the time spent per peer reviewer, so that the Sponsor can have confidence that they will receive a high quality peer review to support decision-making. For this peer review, compensation of $7,000 USD/reviewer was offered for an estimate of 56 hours of the reviewers' time.

Confidentiality

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

2.2 Peer Review Panel

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

Panel Recruitment

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

Panel Expertise Verification

Based on the recruitment effort, 44 candidates volunteered by the cutoff date, and after an initial screening of expertise, 23 were retained. Because the panel members for this peer review were anonymous to the sponsor and to the readers of this report, candidate expertise and their CVs were reviewed by a team of two independent auditors (**Appendix D**). Each auditor scored the candidates as either 0 (does not meet minimum requirements), 1 (meets minimum requirements), or 2 (exceed minimum requirements) based on their ability to meet the defined expertise criteria. Three candidates receiving a combined score less than 2 (out of maximum combined score of 4) were excluded from consideration. Expert panel members were selected from the remaining 22 candidates.

Panel Selection

Information relevant to panel selection criteria was extracted from CVs from the 23 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. Twelve 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 (-11%), this decrease was offset by a large increase in the diversity score (+32%) for the panel.

Panel Engagement

Twelve panel members were placed under contract, and the email addresses corresponding to their SciPinion accounts were verified as belonging to the experts. This SciPi was structured to include three rounds (Delphi format):

- Round 1: Review data package and answer questions
- Round 2: Review panel results and participate in an anonymous debate
- Round 3: Revise answers provided in Round 1 (if applicable), and answer new questions.

Round 1 of the peer review was started on May 3, 2016 and completed on May 22, 2016 during which 11 panel members reviewed the data package and answered a series of questions, including providing suggested questions that should be asked of the entire panel of experts during subsequent rounds. One expert did not take part in the peer review because of personal matters. This expert failed to fulfill their responsibility late in the peer review process. The decision was made by the sponsor to proceed with the remaining 11 experts for Rounds 2 and 3.

Round 2 (debate period) started May 25, 2016 and ended June 6, 2016. Experts were given access to the results/opinions of all members. All experts still remained anonymous and the results of all experts were kept anonymous. Experts were encouraged to review all experts' opinions and comment on the collective opinions of the expert panel. Experts were given two rules:

1) "Your participation is anonymous, please do not enter any information that would reveal your identity"
2) "Please keep your comments focused on the science being peer reviewed".

A few clarifying questions from the sponsor were inserted by SciPinion into specific question debates fields. Experts were asked to comment on the clarifying questions from the sponsor.

Upon the completion of Round 2, five new questions from the expert panel were loaded into the SciPi under a new tab. Round 3 started June 6, 2016 and ended June 28, 2016. Experts were instructed to review the debate entries and determine if they wanted to change any of their answers from Round 1.

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 E**), 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 Round 1 of the SciPi questions are provided in **Appendix B**. Peer reviewer responses to Round 3 of the SciPi questions along with the comments inserted during the debate period (Round 2) are provided in **Appendix C**.

Evaluation of Potential Outliers

For question responses that can be readily codified to a numeric value (e.g., ratings of 1-5, confidence of high/medium/low), the results from all panel members were compared to assess whether any member(s) stands apart from the group. Based on a total of 351 responses (note - 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 were within 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 less than the mean minus 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 plus 0.5 grade units for other members). The results of this evaluation are provided in **Table 2**. Based on this evaluation, none of the panel members were flagged as outliers.

Table 2. Outlier Evaluation

	Response Frequency		
Panel Member	Expert is less confident than the other Experts (response<mean-0.5)	Expert confidence is similar to other experts (response within mean+/-0.5)	Expert is more confident than the other experts (response>mean+0.5)
326	15.4%	68.4%	16.2%
514	27.6%	56.6%	15.8%
576	11.4%	63.2%	25.4%
604	28.5%	56.1%	15.4%
665	14.5%	54.8%	30.7%
815	13.2%	72.8%	14.0%
1002	20.6%	62.3%	17.1%
1006	10.5%	75.9%	13.6%
1010	46.1%	38.2%	15.8%
1022	34.6%	53.9%	11.4%
1054	17.1%	71.5%	11.4%

Demographics

The expert panel consisted of 12 scientists. In addition to the demographics information presented in **Appendix B**, the following information was obtained from panel member CVs:

- Gender: Male (11), Female (1). The one female was the one reviewer who did not take part.

- Countries of origin (unique): 7
- Total person-years of experience: 278
- Years of experience: 23±14 years
- Number of publications: 106±82
- Number of first or last author publications: 70±72

On average, the expertise of the peer reviewers engaged in this review compare reasonably well with those typically involved in FDA advisory committees with respect to years of experience (27±13 years; ERG, 2007[1]) and number of publications (170±146; ERG, 2007). The values for this panel are slightly (but not significantly) lower, which likely reflects the fact that this panel was selected specifically to be diverse with respect to years of experience (to help characterize potential correlations between this variable and the results collected).

Potential Conflicts of Interest

One reviewer (665) has received funding in the past from PMI, no other experts received funding from PMI or any other tobacco company. Two reviewers have previously received funding from the US FDA (665, 604). One reviewer has served as an expert witness or non-testifying consultant in a tobacco-related case (expert 665). None of these experts were flagged in the evaluation of potential outliers (see evaluation below). All eleven reviewers stated that they believe they can remain impartial with respect to this peer review.

Opinions on Science Issues

Science questions that can be readily codified as either "supportive/very supportive" (e.g., "yes", "confident" to "very confident") or critical (e.g., "no", "not confident") of the study authors methods and results, as well as for the peer review process, are summarized in **Figure 2** for results from Round 3 (post debate).

- *Overall* - the results were very positive, with 74.1% of responses being either supportive or very supportive, 18.4% of responses being neutral/weak, and 7.9% of responses being not supportive (**Figure 2A**).
- *Study design/methods* - 77.4% of responses being either supportive or very supportive, 16.0% of responses being neutral/weak, and 6.7% of responses being not supportive (**Figure 2B**).
- *Data evaluation/translatability* - 72.2% of responses being either supportive or very supportive, 20.1% of responses being neutral/weak, and 8.2% of responses being not supportive (**Figure 2C**).

The experts' opinions on specific science issues for Round 1 are provided in **Appendix B and survey_67_results_Round 1.csv** and for Round 3 (including debate entries) in **Appendix C** and in the file **survey_67_results_Round 3**. Some highlighted results are below:

1. There was considerable debate amongst the panel on the need for a wild type (WT) mouse control exposure group to compare results to the ApoE-/- exposed mice. The panel was evenly split on this issue. 5 experts thought a WT control was either necessary or would improve the confidence in the interpretation of the ApoE -/- results and 5 experts did not think a WT control exposed group was necessary.
2. There was general agreement amongst the panel that exposers to

[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.

cigarette smoke (CS - 3R4F) longer than 2 months would improve the ability to make predictions in humans given humans may smoke for extended periods before cessation or switching.

3. There was consensus (10/11) that the study authors accurately and adequately characterized aerosol exposures to CS.
4. There was unanimous consensus that the lung transcriptomics data interpretation (NPA/BIF) corresponded to physiological changes measured in the animals (e.g., inflammation and emphysema).

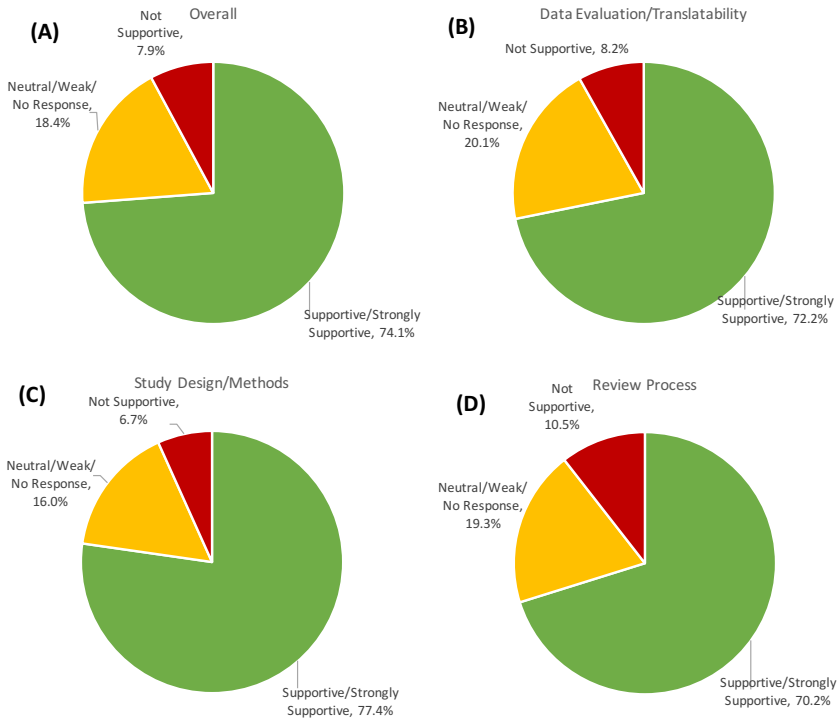
Opinions on Review Process

For results that can be readily codified for the review process, the results again are positive with 70.2% of responses being either supportive or very supportive, 19.3% of responses being neutral/weak, and 10.5% of responses being not supportive **(Figure 2D)**.
Reviewers opinions of the various tools (Aperio, sbv Improver, SDPplus, Garuda gadget, Tableau) were mixed. Reviewers were generally supportive of the inclusion of anonymity and compensation as part of the review process.

Impact of Expert Debate

All 11 experts took part in the Round 2 debate, providing comments on most questions. During Round 3, several experts changed their answers to some questions. Reviewer 665 answered an entire set of questions that they had previously skipped during Round 1. Reviewer 1006 made changes to 2 questions, reviewer 1010 made changes to 23 questions, reviewer 1022 made changes to 1 question and reviewer 1054 made changes to 11 questions. For reviewer 1010, their changes could be best characterized as changes from high confidence (Round 1) to somewhat confident (Round 3) on those questions where changes were made. Reviewer 1054 changed one response from 'very confident' to 'confident', and for another entire set of questions they changed from 'I cannot answer' to 'yes'. All changes can be viewed in the accompanying file: **P1 Mixed Results_Comparison between Rounds 1 and 3.xlsx**. Overall, the debate during Round 2 did not change many opinions of the experts.

Figure 2: Summary of Codified Results for Round 3



SciPinion

Appendix A: Survey Questions

SURVEY PREVIEW

In Vivo Toxicologists, Systems Biologists, and Bioinformatics Scientists Wanted: Panel I

Paid Peer Review Opportunity #67 $7,000 for ~56 hrs CLOSED

Generated: 2016-07-13 19:48:46 +0000
URL: https://scipinion.com/survey_preview/67

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 HERE 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).
   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
3. 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
4. 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):

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

1. Poussin et al. Systems toxicology-based assessment of the candidate modified risk tobacco product THS2.2 for the adhesion of monocytic cells to human coronary arterial endothelial cells. Toxicology 339:73-86 (2016). **PLEASE CLICK HERE TO DOWNLOAD**
2. van der Toorn et al. Aerosol from a candidate modified risk tobacco product has reduced effects on chemotaxis and transendothelial migration compared to combustion of conventional cigarettes. Food Chem Toxicol 86:81-87 (2015). **PLEASE CLICK HERE TO DOWNLOAD**

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
- use the different resources & tools available for the review (in tools section of the SysTox Portal)

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

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 #1864

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 #1865

2) Have you been involved in inhalation toxicology studies?

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

Please explain your answer(s)

ID #1866

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 #1867

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 #1868

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 #1871

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 #1872

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 #1873

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

- Yes
- No

Please explain your answer(s)

ID #1874

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 #1875

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 #1876

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 #1877

8) Do you own stock in a tobacco company?

- Yes
- No

Please explain your answer(s)

ID #1878

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 #1879

10) What is your smoking status?

- ○ Nonsmoker
- ○ Former smoker
- ○ Current smoker

Please explain your answer(s)

ID #1880

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 #1881

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 #1882

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 #1883

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 #1884

4) 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.

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1885

5) 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 #1886

6) 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.

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	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	○	○	○	○

Please explain your answer(s)

ID #1887

7) Is the approach to match the exposures to the aerosols on the basis of a key common constituent, nicotine, instead of TPM reasonable? (please explain below)

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ◯ Yes
- ◯ No
- ◯ I cannot answer this question

Please explain your answer(s)

ID #1888

8) 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?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1889

9) What would you consider the most appropriate methodology to investigate lipid profiles in these mice?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1890

10) Which of the applied parameters in respiratory physiology is most appropriate to demonstrate emphysematous changes in the mouse model?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

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

Please explain your answer(s)

ID #1891

11) 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?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

Answer Options: 1 2 3 4 5

	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 below)			

Please explain your answer(s)

ID #1892

12) 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 #1893

13) 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**

ID #1894

14) How suitable is the Apoe-/- mouse model to detect additional exposure effects in the already challenged liver?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1895

15) 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?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1896

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

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

Answer Options: *1* *2* *3*

Data category	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 ling 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 #1897

17) 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.

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

Answer Options: 1 2 3 4 5

	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)		

Please explain your answer(s)

SECTION 4: DATA EVALUATION AND INTERPRETATION

ID #1898

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

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

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

	Sufficient Detail?	Clarity of Presentation
In-life observations, mortality, body weight, gross pathology		
Biomarkers of exposure, 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 #1899

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 #1900

3) 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?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

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

Please explain your answer(s)

ID #1901

4) 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: *Low* *Medium* *High* *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 #1902

5) 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.

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1903

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

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

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

Please explain your answer(s)

ID #1904

7) 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?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ◯ 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

Please explain your answer(s)

ID #1905

8) 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)?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

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

Please explain your answer(s)

ID #1906

9) 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 #1907

10) 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?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1908

11) 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 #1909

12) 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?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1910

13) The analysis of lung transcriptomics data using the causal network model (Philips et al. 2016) predicted a robust CS-induced increase in extracellular matrix (ECM) degradation that disappears upon cessation.

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	Yes	No	I cannot answer
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?	○	○	○

Please explain your answer(s)

ID #1911

14) 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?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- ○ 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 (please explain)
- ○ I cannot answer this question

Please explain your answer(s)

ID #1912

15) 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 test items: 1) an early prototype MRTP (pMRTP) and 2) the Tobacco Heating System version 2.2 (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 #1913

16) 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?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1914

17) 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 #1915

18) 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 #1943

19) 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 #1917

20) 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 #1918

1) 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)?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	Good model	Poor model	Other (please explain below)
Inflammatory diseases	○	○	○
Respiratory diseases	○	○	○
Cardiovascular diseases	○	○	○
Neurological diseases	○	○	○
Hepatotoxicity	○	○	○
Metabolic syndrome / diabetes	○	○	○

Please explain your answer(s)

ID #1919

2) 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.

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

	Not appropriate	Moderately appropriate	Very appropriate
Dose	○	○	○
Regimen	○	○	○
Duration	○	○	○
Biomarkers of exposure	○	○	○

Please explain your answer(s)

ID #1920

3) 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?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

ID #1921

4) 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 #1922

5) 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. 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?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

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

Please explain your answer(s)

ID #1923

6) 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. 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?

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

Please explain your answer(s)

ID #1924

7) 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?

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

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

Please explain your answer(s)

ID #1925

8) 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 #1926

9) 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:

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

Stegemann et al., 2011

- ○ 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 (please explain)

Please explain your answer(s)

ID #1927

10) 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 #1928

11) 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 #1929

12) 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 (Scale of 1-5, 5 being the highest)?

- **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 #1930

13) Specific disease mechanisms can be appropriately studied in in vitro models. PMI tested RRPs with assays that recapitulate two mechanisms relevant to atherosclerosis, namely adhesion of monocyte to endothelial cells (Poussin et al. 2016 Toxicology 339(2):73-86) and transendothelial migration (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.

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

adhesion of monocyte to endothelial cells
transendothelial migration

ID #1931

14) 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 #1932

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

ID #1933

2) 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 #1934

3) 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) (scale 1-5, 5 being extremely good)?

Answer Options: 1 2 3 4 5

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

Please explain your answer(s)

ID #1935

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

- **PLEASE CLICK FOR ADDITIONAL DETAILS**

- [ ] 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
- [ ] Explore large-scale omics study datasets and perform enrichment for genes and proteins
- [ ] Allow readers to assess and compare the transcriptomics profiles for a specific gene or set of genes between two study conditions
- [ ] Allow the visualisation of pathways associated with the genes in transcriptomics profile data
- [ ] Are very useful and should be made available to the whole scientific community
- [ ] I cannot comment on Garuda (please explain in the comment box)
- [ ] None of the above

Please explain your answer(s)

ID #1936

5) 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 question-specific link in the Introduction). 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 #1937

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

ID #1938

7) 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 #1939

8) How could this peer review process be improved?

ID #1940

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

ID #1941

10) 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 #1942

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

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

Please explain your answer(s)

SECTION 7: POST DEBATE QUESTIONS

ID #2062

1) In your opinion, does the absence of an inflammatory reaction in THS2.2 exposed animals, inferred from the proteomic or transcriptomic analyses, indicate that lung function is normal in these mice?

- ◯ Yes
- ◯ No

Please explain your answer(s)

ID #2063

2) To what extent are the mouse studies on the effect of switching, which are necessarily short term as compared to the human situation, predictive for the effect of switching in smokers? If not, what would be the better alternative?

ID #2064

3) Both CS and THS2.2 contain some cadmium which can both cause production of reactive oxygen species (ROS) and act as a potent inducer of metallothionein (MT) and a number of other stress proteins. Both oxidative stress and MT induction were observed in the reported studies. How could one separate these 2 types of effects and use the information to support Mode of Action(MOA) based risk assessment to further quantify differences in risk between CS and THS2.2?

To view details of Cadmium measurements, please refer to the Aerosol Chemistry page.

ID #2065

4) Two reviewers expressed concern over the lack of a dose-response for CS and THS2.2. They stated: “The difference in concentration-response profiles between 3R4F and TSH2.2 need to be explored further”. “The lack of dose-response in all of these studies is a significant concern”. In your opinion, does the lack of testing for dose-response for both CS and THS2.2 limit the conclusions about the effects of THS2.2 and/or switching in a disease model? Would a 28/90-day inhalation study in rats following OECD guideline 412/413 be sufficient to address this concern?

ID #2066

5) Several experts expressed concerns over the duration of exposure. They stated: “Do you expect similar datasets for cessation or switching after 6 months 3R4F exposure?”. “Would the animal study reflect with more fidelity the comparison with the human CS exposure with longer exposure time?”. QUESTION: Would you expect to see similar responses to CS, THS2.2 and/or switching after longer durations of exposures?

SciPinion

Appendix B: Survey Results for Round 1

SURVEY RESULTS

In Vivo Toxicologists, Systems Biologists, and Bioinformatics Scientists Wanted: Panel I

Paid Peer Review Opportunity #67 $7,000 for ~56 hrs CLOSED

Generated: 2016-07-13 19:54:55 +0000
URL: https://scipinion.com/surveys/67/report.pdf?debug=true

SECTION: DEMOGRAPHICS

ID #1864 (https://scipinion.com/surveys/67/results/1864)

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

Field	0 years	1-3 years	3-10 years	>10 years	Total
General toxicology	9.09% 1	9.09% 1	18.18% 2	63.64% 7	11
Molecular toxicology	0.00% 0	18.18% 2	45.45% 5	36.36% 4	11
Systems toxicology	18.18% 2	18.18% 2	36.36% 4	27.27% 3	11
Risk assessment	36.36% 4	0.00% 0	27.27% 3	36.36% 4	11
Public health research	27.27% 3	0.00% 0	9.09% 1	63.64% 7	11
The conduct and/or interpretation of tobacco-related studies	27.27% 3	45.45% 5	18.18% 2	9.09% 1	11
Histopathology	9.09% 1	9.09% 1	36.36% 4	45.45% 5	11
Other	0.00% 0	0.00% 0	0.00% 0	100.00% 5	5

ID #1865 (https://scipinion.com/surveys/67/results/1865)

Have you been involved in inhalation toxicology studies?



Legend

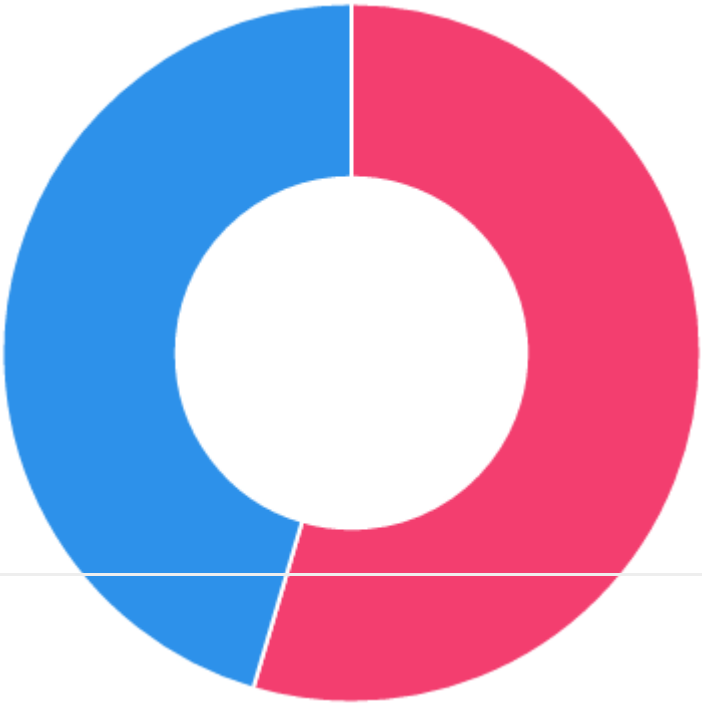
- Yes, as an author: 6 *(55%)*
- Yes, as a reviewer: 2 *(18%)*
- No: 3 *(27%)*

answers: 11

skips: 0

ID #1866 (https://scipinion.com/surveys/67/results/1866)

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



Legend

Yes: 6 *(55%)*

No: 5 *(45%)*

answers: 11

skips: 0

ID #1867 (https://scipinion.com/surveys/67/results/1867)

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: 2 *(18%)*
- High: 3 *(27%)*
- Medium: 3 *(27%)*
- Low: 0 *(0%)*
- It depends on the type of omics data being generated: 3 *(27%)*

answers: 11

skips: 0

SECTION: POTENTIAL CONFLICT OF INTEREST OR BIAS

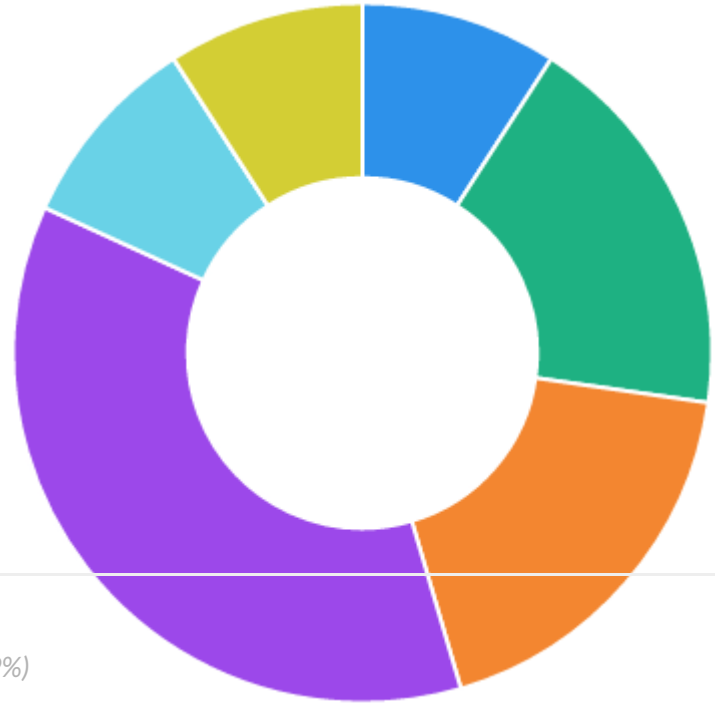
ID #1868 (https://scipinion.com/surveys/67/results/1868)

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

	<10%	10-50%	50-75%	>75%	Total
Governments	36.36% 4	36.36% 4	0.00% 0	27.27% 3	11
Industry	44.44% 4	11.11% 1	22.22% 2	22.22% 2	9
Non-profit organizations	77.78% 7	22.22% 2	0.00% 0	0.00% 0	9
Tobacco companies	100.00% 8	0.00% 0	0.00% 0	0.00% 0	8
Other	100.00% 3	0.00% 0	0.00% 0	0.00% 0	3

ID #1871 (https://scipinion.com/surveys/67/results/1871)

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



Legend

- A tobacco company: 0 *(0%)*
- Philip Morris International: 1 *(9%)*
- US FDA: 2 *(18%)*
- US EPA: 2 *(18%)*
- NIEHS: 4 *(36%)*
- NSF: 1 *(9%)*
- Anti-tobacco non-profits: 0 *(0%)*
- European Commission: 1 *(9%)*

answers: 6

skips: 5

ID #1872 (https://scipinion.com/surveys/67/results/1872)

Have you ever been an employee of:

Legend

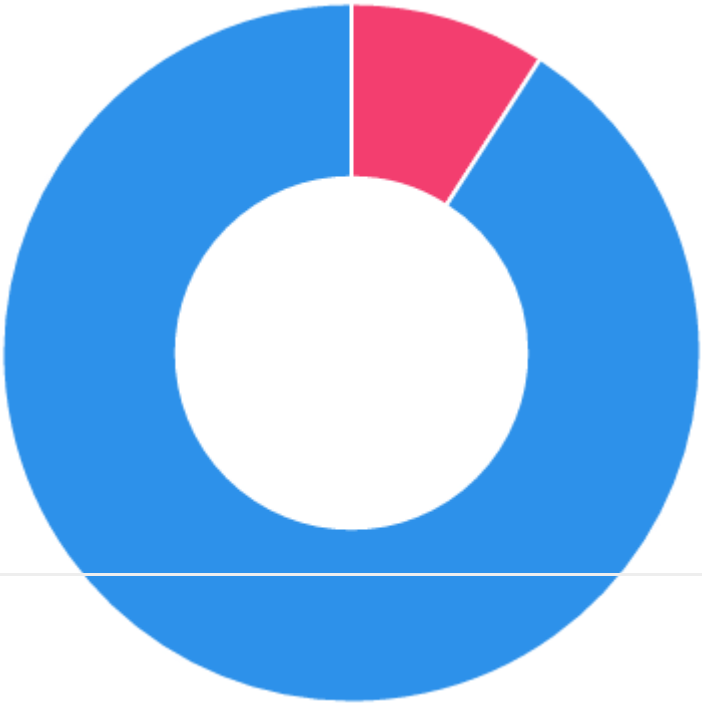
- A tobacco company: 0 *(0%)*
- The US FDA: 0 *(0%)*
- Any regulatory agency that had jurisdiction over tobacco: 0 *(0%)*

answers: 0

skips: 11

ID #1873 (https://scipinion.com/surveys/67/results/1873)

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



Legend

- Yes: 1 *(9%)*
- No: 10 *(91%)*

answers: 11

skips: 0

ID #1874 (https://scipinion.com/surveys/67/results/1874)

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, because being myself a scientist and experienced reviewer of projects, dissertations, theses, articles, I learned how to be impartial despite the subject in question, looking at the scientific facts and not empty arguments.

> no

> Not applicable

> No.

> none

> No

> None

> None

> I do not find any reason for the same.

> None

> no

ID #1875 (https://scipinion.com/surveys/67/results/1875)

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 involvement

> No

> No.

> No

> No

> None

> None

> Currently, I do not have any pending document(s) under consideration. But I have completed the peer review for Philips et al, 2015.

> No

> No.

> no

ID #1876 (https://scipinion.com/surveys/67/results/1876)

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

> None

> None

> No.

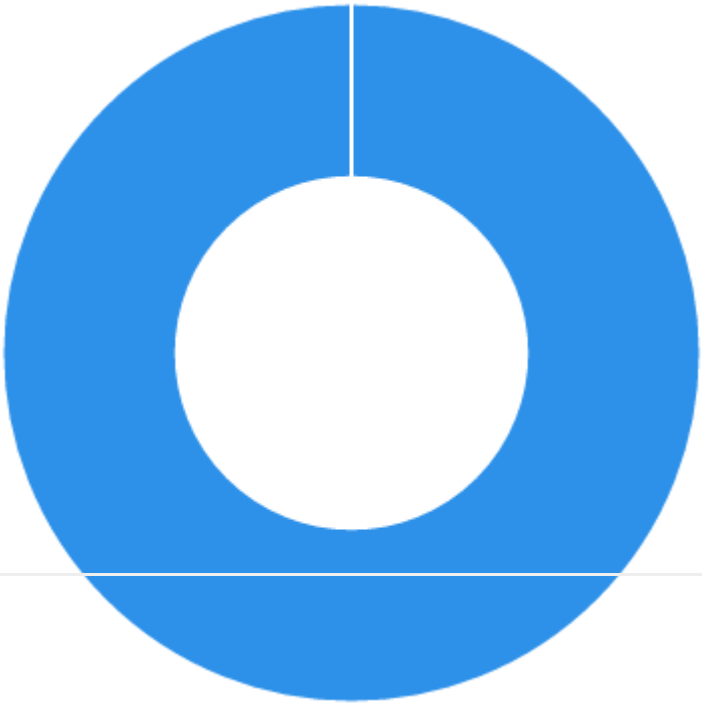
> I did not involve in any of such activities.

> No

> no

ID #1877 (https://scipinion.com/surveys/67/results/1877)

Do you own stock in a tobacco company?



Legend

Yes: 0 *(0%)*

No: 11 *(100%)*

answers: 11

skips: 0

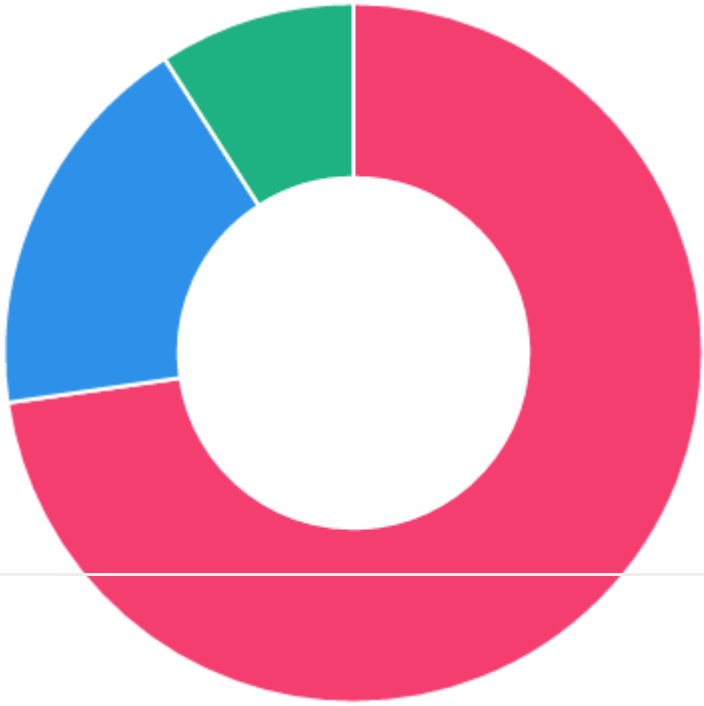
ID #1878 (https://scipinion.com/surveys/67/results/1878)

Is anyone in your immediate family an employee of?

	Yes	No	Total
A tobacco company	**0.00%** 0	**100.00%** 11	11
FDA	**0.00%** 0	**100.00%** 11	11
An anti-smoking non-profit	**0.00%** 0	**100.00%** 11	11

ID #1879 (https://scipinion.com/surveys/67/results/1879)

What is your smoking status?



Legend

- Nonsmoker: 8 *(73%)*
- Former smoker: 2 *(18%)*
- Current smoker: 1 *(9%)*

answers: 11

skips: 0

ID #1880 (https://scipinion.com/surveys/67/results/1880)

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 am sure of having no bias to answer this review. I have been always a university professor, never worked in any private company. If someone ask me my opinion about tobacco products (cigarette, cigars, pipes, etc), as a non-smoker and knowledgeable of the proven side effects of tobacco products, I would recommend everyone to not smoke or try quitting smoking. However, I am a supporter of the people free-will, even if something is harmful for their health. People should be conscious of their act.

> Not applicable

> I have no bias.

> None.

> I have no bias' that may influence my scientific opinion. I call them as I see them.

> No Biases

> Although I perform omics study occasionally, I have still some biases on use of omics study in regulatory field.

> I am without bias in this review

> I do not have any bias that influence my scientific opinion.

> I have no bias and evaluate research on its merits.

> I don't believe I have any bias that would affect my review.

SECTION: STUDY DESIGN, END POINTS, AND DATA QUALITY

ID #1881 (https://scipinion.com/surveys/67/results/1881)

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	**9.09%** 1	**36.36%** 4	**27.27%** 3	**27.27%** 3	**0.00%** 0	11
Appropriate choice of controls	**45.45%** 5	**18.18%** 2	**27.27%** 3	**9.09%** 1	**0.00%** 0	11
Appropriate number of replicates in respective groups	**54.55%** 6	**27.27%** 3	**18.18%** 2	**0.00%** 0	**0.00%** 0	11
Appropriate choice of exposure modality	**27.27%** 3	**45.45%** 5	**27.27%** 3	**0.00%** 0	**0.00%** 0	11
Appropriate duration of the exposure	**9.09%** 1	**45.45%** 5	**36.36%** 4	**9.09%** 1	**0.00%** 0	11
Appropriate choice of endpoints	**36.36%** 4	**54.55%** 6	**9.09%** 1	**0.00%** 0	**0.00%** 0	11
Appropriate choice of analytical methods	**36.36%** 4	**54.55%** 6	**9.09%** 1	**0.00%** 0	**0.00%** 0	11

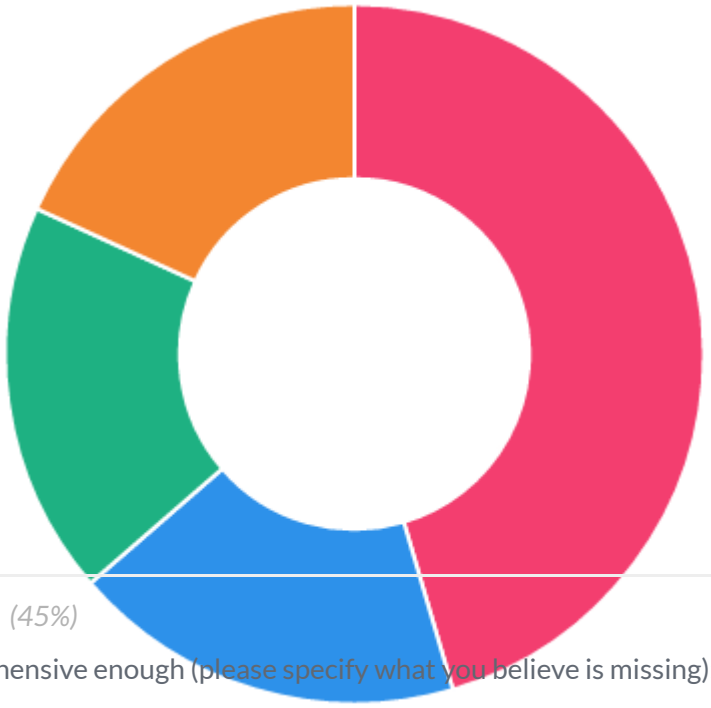
ID #1882 (https://scipinion.com/surveys/67/results/1882)

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	**36.36%** 4	**18.18%** 2	**36.36%** 4	**9.09%** 1	**0.00%** 0	11
Appropriate choice of controls	**36.36%** 4	**27.27%** 3	**27.27%** 3	**9.09%** 1	**0.00%** 0	11
Appropriate number of replicates in respective groups	**54.55%** 6	**27.27%** 3	**18.18%** 2	**0.00%** 0	**0.00%** 0	11
Appropriate choice of exposure modality	**27.27%** 3	**63.64%** 7	**9.09%** 1	**0.00%** 0	**0.00%** 0	11
Appropriate duration of the exposure	**18.18%** 2	**36.36%** 4	**45.45%** 5	**0.00%** 0	**0.00%** 0	11
Appropriate choice of endpoints	**18.18%** 2	**72.73%** 8	**9.09%** 1	**0.00%** 0	**0.00%** 0	11
Appropriate choice of analytical methods	**45.45%** 5	**45.45%** 5	**9.09%** 1	**0.00%** 0	**0.00%** 0	11

ID #1883 (https://scipinion.com/surveys/67/results/1883)

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: 5 *(45%)*
- It is appropriate but not comprehensive enough (please specify what you believe is missing): 2 *(18%)*
- Some measurements that can be done longitudinally should complement the study design (please specify which): 2 *(18%)*
- It is not appropriate (please specify): 2 *(18%)*
- I cannot answer this question: 0 *(0%)*

answers: 11

skips: 0

ID #1884 (https://scipinion.com/surveys/67/results/1884)

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.

As indicated in #2 the quantification of gene expression changes from xenobiotic exposures is dose and time dependent. A single exposure level and single time point post exposure without data to support these choices is problematic.

It would have been informative to see the cellular sites underlying the changes in the lipidome found in the Titz et al. study. Possibly in situ hybridization or immunostaining for some of the critical biomarkers in lung tissue at various time points across conditions.

There is lot of data generated related to transcriptomics and proteomics, however a logical translation of the data in relation to particular respiratory or cardiovascular disease(s) was incomprehensible.

Yes, the absence of a true control and the inappropriate use of statistics are the two most glaring errors.

No.

I don;t knwo well

I do not see important gaps

No important gaps noted.

The authors of respective papers had almost covered whatever they can do. But it would be very nice to use the respective organs like lung, heart, liver for brief validation of few top most or bottom most genes or proteins with RT-PCR, northern, Western or suitable techniques. This validation part is necessary for this kind of study where the study has been done once and 'OMICS" has the tendency to get less reproducibility. In this context, the research community started emphasizing the methods to improve the reproducibility (Kolker et al, OMICS. 2014 Jan 1; 18(1): 10–14., Fisch et al, Bioinformatics. 2015 Jun 1;31(11):1724-8 and Ioannidis et al, Science. 2011; 334: 1230–1232).

Apparently researchers have covered their bases.

Yes, there are still gaps to be filled. Despite the fact that a scientific article is not a book in terms of available space, I would recommend in the next publications, expanding the target tissues or organs to verify the overall effects of THS2.2. I would include eyes, brain, skin, join, muscles, stomach, bladder, intestines.

ID #1885 (https://scipinion.com/surveys/67/results/1885)

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?

I think that this is a difficult time point for the authors to determine, as some of the parameters measured are altered to their maximal effect at month 2; however, certainly using a 3, or 4-month timepoint would have been better for certain parameters, especially the lung function parameters. For example, a maximal effect on lung function/inflammation is not observed until 3-6 months, therefore the cessation/switch effects may be more interesting here. However, in a study of this magnitude, I believe that the timepoint chosen was sufficient for the majority of the analyses.

I am confident in the study design and thought the data were convincing for the ability to see reversals of many endpoints and levels of biomarkers returning to baseline by 6 or 8 months. This was evident in respiratory, cardiovascular and hepatic evaluations. A cited reference indicated that 2 months was sufficient to induce systemic changes, especially for cardiovascular measures. It is possible, or at least difficult to predict, that out to 3 or 4 months exposure, there would be some changes to tissue that might not be reversible in the subsequent switching or cessation period. I would not expect refractoriness in gene expression or protein concentration changes if 3 or 4 months were chosen versus 2. My guess from similar type studies is that these are driven by external signals that need to be present to relay the signal to cellular receptors.

This is an open question. If we see the lipid profiles in the treatment groups, even in the CS group (3R4F group), they return to normalcy overtime. So, therefore, it is only the initial rise(1-2 months). After going through Jobse B.N. et al's work “Impact of inflammation, emphysema, and smoking cessation on V/Q in mouse models of lung obstruction” Respir Res. 2014; 15(1): 42, I am convinced that even a longer exposure would not have made a significant differences in the end points observed, unless the researcher consider looking at different end points such as expression of tumor specific genes etc.

No. The design is not appropriate the achieve the proposed aims. The selection of 2 months is not having proper logic. The authors have cited two earlier papers for this selection. But if one see carefully these two papers, it is disappointing. While Philips et al (2015) had used C57BL/6 mice, Boué et al had measured Lm in 6 months smoke exposed mice (though they have done some indirect measurements on 3 months smoke exposed mice and these indirect measurements may not impact the emphysema). In addition, Philips et al (2015) had shown that there was a maximum Lm after 5 months smoke exposure. Even in this study, maximum Lm has been observed only after 6 months exposure though 2 months exposure is statistically significant. Most of the inflammatory parameters are up-regulated even one month smoke exposure. In addition, most of COPD patients who want to quit smoke will not have mild stage disease and indeed they have severe stage disease. So to translate effectively this research into human, the authors needs to switch after 6 months smoke exposure. I expect the similar results if the cessation or switching time point was set to 3 or 4 months as the disease severity starts only after 5 -6 months. But at the same time I believe that there may not be similar results if switching time point was set to 6 months post 3R4F exposure. So rigorous design is required to select the point of cessation or switching. If needed, one can plan for open questionnaire from the ex-smokers and get the information like after how many years they wanted to quit or they want ed a change ? what are the reasons behind this mind change ? Then converting this knowledge into this design would be ideal. If needed one can plan for multiple time points for

cessation or switching like post 3 months, post 5 months, post 6 months and so on and concentrate only on functional end points like emphysema, aortic arch plaque measurement.

Yes, I think it was appropriate given the life span of laboratory animals. It is possible that results may be different.

Yes the cessation group and switching groups are appropriate to mimic the human situation and does demonstrate and amelioration of the effects compared to CS, however THS2.2 still alters a number of genes/pathways similar to CS when compared to air controls.

Based on the data presented in the papers, the cessation and switching studies seem appropriately designed to achieve the proposed aims. I would expect similar results with switching time point set to 3-4 months. Only differences in severity of effects. Suggest also evaluating male mice for similarities and differences with the females at these later time points.

Two month cessation is appropriate and 3-4 month cessaion or switching may not make big difference.

I would have liked to see longer exposure before switching. Ideally, groups exposed to 2/3 and 6 months before switching to sham or THS2.2 to see the recovery. Smokers are exposed to extended period of smoking before switching and will generate more convincing data.

yes, they are appropriately designed but as mentioned above (Q1) it is possible that more irreversible effects are picked up if cessation/switching is set at a later time point. Conclusion that switching causes very rapid restoration of CS-affected parameters may be overly optimistic in this design. However, even setting the switch time point some months later may not resolve this due to the relative short time span allowed in this model in general.

If the authors started the experiments with 2 months-old mice (not clear in the text), it is equivalent to 20 years for humans. Mice inhaled smoke for 2 months, it means for approximately 6 human years. The recover of the inflammatory indicators after cessation or switching time point is somehow expected at young ages. I would like to see longer exposure times because in humans usually the symptoms of cardiovascular or respiratory problems usually appears after 20-25 years of smoking.

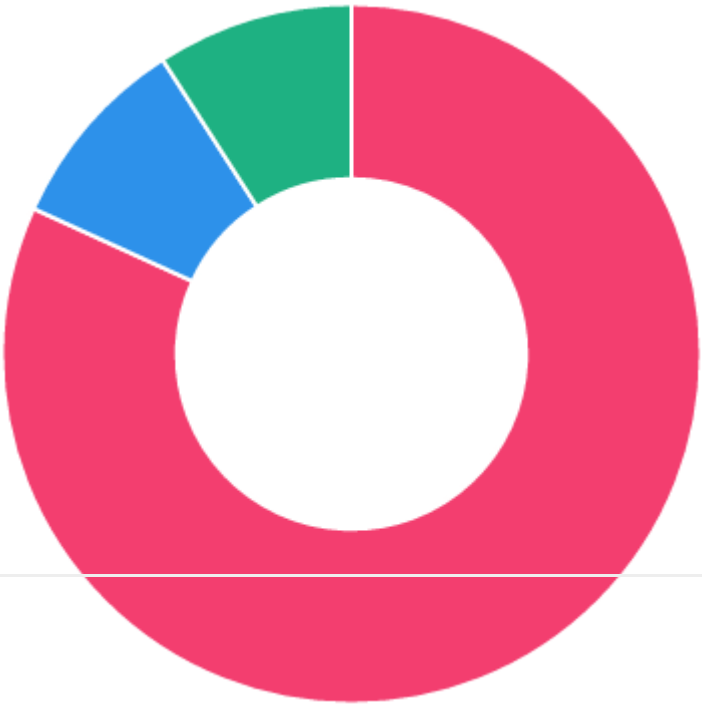
ID #1886 (https://scipinion.com/surveys/67/results/1886)

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.

	Poor	Sufficient	Excellent	I cannot answer	Total
Aerosol generation adequately explained with regard to reproducibility	9.09% 1	63.64% 7	18.18% 2	9.09% 1	11
Sufficient level of details and explanation regarding aerosol generation	0.00% 0	72.73% 8	18.18% 2	9.09% 1	11
Sufficient level of details and explanation regarding aerosol characterization	0.00% 0	54.55% 6	36.36% 4	9.09% 1	11

ID #1887 (https://scipinion.com/surveys/67/results/1887)

Is the approach to match the exposures to the aerosols on the basis of a key common constituent, nicotine, instead of TPM reasonable? (please explain below)



Legend

- Yes: 9 *(82%)*
- No: 1 *(9%)*
- I cannot answer this question: 1 *(9%)*

answers: 11

skips: 0

ID #1888 (https://scipinion.com/surveys/67/results/1888)

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?

> Biomarkers were appropriately selected

> Clearly the most informative are for nicotine and cotinine. Urine collection seems most variable and the plasma levels at 8 months look consistent. These seem to be the most sensitive markers of exposure. Non-nicotine markers will be valuable, and the SPMA and CEMA biomarkers should be evaluated, even though they are far more abundant in CS exposure versus THS2.2 exposure.

> I think that the use of blood and urine based biomarkers was appropriate. The choice really depends on the endpoints being examined.

> This is not clear for CS or for THS2.2 since only a single exposure level was used. Biomarker assessment requires careful dose-response time course studies that were not done for any endpoint measure here. Nicotine blood levels are likely the best biomarker for CS or for THS2.2

> Suggest measurement of urinary cadmium as a marker of exposure on a longitudinal basis since this a major contaminant of tobacco, present in both the CS and to a lesser extent in heated tobacco vapor if I am reading the exposure figures correctly. Such measurements would also permit exposure comparisons with CDC NHANES data which uses urinary cadmium as marker of exposure. Please note that smokers are well known to have much higher kidney concentrations of cadmium than non - smokers (see Nordberg GF et al., Handbook on the Toxicology of Metals, Elsevier, 2015) for a more detailed discussion. The utility of measuring biomarkers of exposure in blood or urine will depend on the chemical of interest. Some chemicals accumulate in blood so that matrix is a good index of exposure for them depending on the duration of exposure while others pass out into urine following metabolism or duration of exposure so that would be the matrix of choice.

> I feel that both the urine and blood yielded useful information, however I think that the primary problem with the biomarker assessment is simply the time that the samples were collected. It is unclear why only chose the 2, 6, and 8 month timepoints for the COHb measurements, while ignoring the 1 and 3 months. The high level of variability in the time with which the biomarkers were measured is the major concern.

> HPMA and total NNAL in urine are good biomarkers that clearly separate 3R4F from CS at 3, 6, and 8 months and are strongly sensitive to switching/cessation. Urine exposure biomarkers appear most robust.

> none suggested

> Urine is susceptible to renal function. Cigarette smoke itself can cause systemic endothelial dysfunction and thus affect the nicotine excretion and thus urine nicotine levels are dependent on kidney function. Blood COHb, Nicotine and Cotinine Measurements in Plasma are recommended biomarkers of exposure.

> If one want to use urine, the parameters should be normalized by creatinine.

> I did not see cotinine levels, which in my opinion will be advantageous, see Marsot and Simon, 2016 for detail.

> I would keep nicotine (and its main metabolites) as the main marker(s). The ideal scenario for a smoker is the nicotine as the only chemical being provided by the cigarette or devices. Most of other chemicals produced are consequences of tobacco heating/combustion. A cigarette varies its nicotine concentration 1-3 mg. One could assume (and limit) the nicotine availability should reach proportional concentrations in the mice (in mg/kg and mg/m3).

ID #1889 (https://scipinion.com/surveys/67/results/1889)

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

I would consider not using ApoE-/- mice to investigate alterations in lipidomic profiles. This is quite evident in the planimetry where even the sham mice display a significant increase in plaque formation at the 8 month timepoint. Comparison to a WT mouse is a necessary control that wasn't utilized. It is also unclear why the authors chose only the 8 month timepoint for the full lipidomic screen. I think that the unbiased screening of plasma and tissue lipids using an LC-MS/MS platform is the most appropriate methodology . The authors did, however, do a nice job presenting gross morphology of the aortic arch plaque formation.

I am unfamiliar with the lipid profiling methodologies used in the study.

The methodology chosen for lipid profiling was appropriate

Metabolomics platforms.

The lipidomics approach as outlined in Boue et al., however as outlined above the lack of dose-response and time course is problematic to make causal links between exposure and response

Mass spectrometry of lipid profiles should yield the most rigorous results assuming no technical problems with the sample preparation methods.

VAP - sufficiently accurate measurement of HDL, LDL, VLDL

The methods used in this study are perfect. For plaque assessment, calcification measurement is also recommended as there may be underestimation of plaque area because of shrinkage due to sclerotic effect.

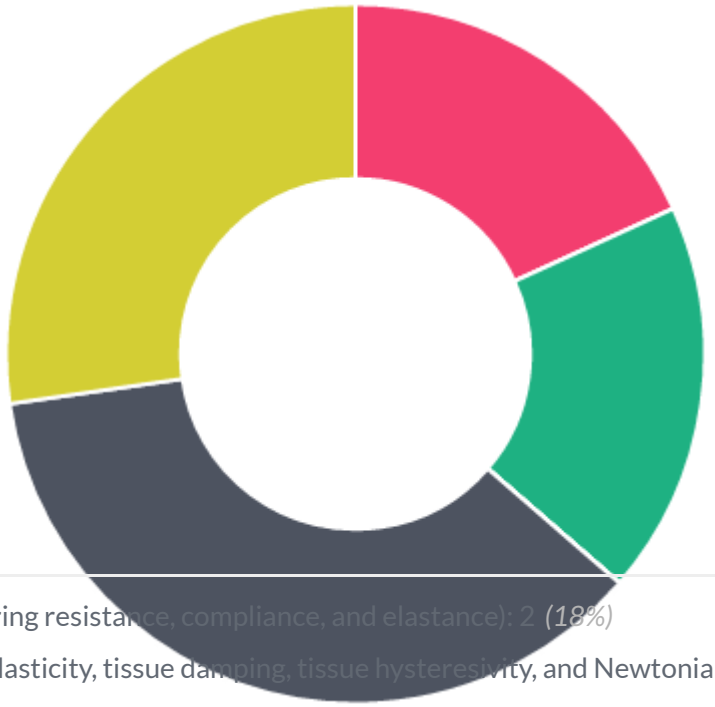
lipidomic analysis

liquid chromatography coupled to tandem mass spectrometry

The same as applied in the paper.

ID #1890 (https://scipinion.com/surveys/67/results/1890)

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



Legend

- Snapshot perturbations (measuring resistance, compliance, and elastance): 2 *(18%)*
- Primewaves (measuring tissue elasticity, tissue damping, tissue hysteresivity, and Newtonian resistance): 0 *(0%)*
- Pressure-volume loops (measuring parameters of the Salazar-Knowles equation, quasi-static compliance, quasi-static elastance, and hysteresis): 2 *(18%)*
- Positive end-expiratory pressure (measuring the end-expiratory lung volume): 0 *(0%)*
- Negative pressure forced expiration (measuring FEV0.1, FEV0.2,FEV0.1/FVC, and FEV0.2/FVC): 0 *(0%)*
- None of the measures is indicative of emphysematous changes: 0 *(0%)*
- All of the measures combined: 4 *(36%)*
- I cannot answer this question: 3 *(27%)*

answers: 11

skips: 0

ID #1891 (https://scipinion.com/surveys/67/results/1891)

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)	Total
transcriptomics: computational network modeling and NPA calculation	*1* **9.09%** 1 *2* **9.09%** 1 *3* **54.55%** 6 *4* **9.09%** 1 *5* **18.18%** 2	*2* **27.27%** 3 *3* **36.36%** 4 *4* **9.09%** 1 *5* **27.27%** 3	*1* **18.18%** 2 *2* **27.27%** 3 *3* **9.09%** 1 *4* **45.45%** 5	11
transcriptomics: gene set enrichment analysis	*1* **9.09%** 1 *2* **18.18%** 2 *3* **18.18%** 2 *4* **45.45%** 5 *5* **9.09%** 1	*2* **18.18%** 2 *3* **9.09%** 1 *4* **54.55%** 6 *5* **18.18%** 2	*1* **9.09%** 1 *2* **27.27%** 3 *3* **18.18%** 2 *4* **45.45%** 5	11
proteomics: clustering and functional association networks	*1* **10.00%** 1 *3* **20.00%** 2 *4* **50.00%**	*3* **30.00%** 3 *4* **30.00%** 3 *5*	*1* **20.00%** 2 *2* **20.00%** 2 *3* **20.00%**	10

	5 *5* **20.00%** 2	**40.00%** 4	2 *4* **40.00%** 4	
proteomics: protein set enrichment analysis	*1* **9.09%** 1 *3* **27.27%** 3 *4* **45.45%** 5 *5* **18.18%** 2	*3* **36.36%** 4 *4* **27.27%** 3 *5* **36.36%** 4	*1* **9.09%** 1 *2* **27.27%** 3 *3* **36.36%** 4 *4* **27.27%** 3	11
lipidomics: integration with lipidomics data, PLS plots	*1* **9.09%** 1 *2* **18.18%** 2 *3* **27.27%** 3 *4* **9.09%** 1 *5* **36.36%** 4	*1* **9.09%** 1 *2* **18.18%** 2 *3* **18.18%** 2 *4* **18.18%** 2 *5* **36.36%** 4	*1* **18.18%** 2 *2* **18.18%** 2 *3* **18.18%** 2 *4* **36.36%** 4 *5* **9.09%** 1	11
Other (please specify below)				0

ID #1892 (https://scipinion.com/surveys/67/results/1892)

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)	**90.91%** 10	**9.09%** 1	**0.00%** 0	11
tissue preparation, including type of staining (for lung)	**90.91%** 10	**9.09%** 1	**0.00%** 0	11
number of sections evaluated	**90.91%** 10	**9.09%** 1	**0.00%** 0	11
blinding / randomization	**100.00%** 11	**0.00%** 0	**0.00%** 0	11
scoring system	**100.00%** 11	**0.00%** 0	**0.00%** 0	11
histomorphometry methods	**100.00%** 11	**0.00%** 0	**0.00%** 0	11
statistical evaluation	**81.82%** 9	**9.09%** 1	**9.09%** 1	11
data visualization	**90.91%** 10	**9.09%** 1	**0.00%** 0	11

ID #1893 (https://scipinion.com/surveys/67/results/1893)

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?

Analysis was appropriate. Inclusion of additional IHC would have been helpful.

No. Standard mice under non-stressed conditions do not spontaneously generate any of the parameters assessed. The need for a wild type control is very evident in these experiments. The authors should also show representative histological sections for each treatment group/time point. This raises further speculation into the relevance of this study to investigate hepatic effects of cigarettes.

I don't have sufficient expertise in liver histopathology to answer definitively, but I would say yes. First, significant changes were seen in an age-dependent manner across several hallmarks of hepatotoxicity. Second, the range of analyses suggested that many types of histopathology were examined and determined to not be significantly altered in the CS or THS2.2 groups compared to same time points in sham group. The changes seen across time in all groups point to sensitive detection methods and consistent, toxicity-related changes.

As there was no overt hepatotoxicity observed even with CS exposure, there is no necessity for additional analyses.

No, this model has rapid accumulation of collagen fibers, degeneration and necrosis, inflammatory cells and bile duct hyperplasia, that has limited value when compared to either wild type mice/rats or human hepatocytes.

Yes- I would consider measurements of heme oxygenase (HSP27) in mice to provide an explanation for the accumulation of Fe which could be occurring from increased turnover of hemoproteins such as the cytochrome P-450s.

It would be helpful to compare conventional hepatotoxcity blood biochemical parameters such as GOT, GPT and APT etc with thes histopaghology.

yes - appropriate. Multiple parameters including collagen fiber density, parenchymal deterioration, inflammatory cells, bile duct hyperplasia are scored using blinded analysis by pathologist.

All these 4 anllytical methods are appropriate. TUNEL assay can be done in paraffin slides to determine the DNA fragmentation. Ultrastructural study can be performed to reveal mild hepatotoxic effects in the form of mitochondrial swelling, ER dilation, etc.

Not entirely. H&E-stained sections, Oil Red and PAS/PAS-D are basic and useful staining for histopathology, However, an ampler analyses of toxic effect on cells should consider labeling of apoptosis, autophagy and ROS markers in situ of different liver areas. The electron microscopy approach is also important to observe the ultrastructure of hepatocytes, such as RER, SER, mitochondria.

ID #1894 (https://scipinion.com/surveys/67/results/1894)

How suitable is the Apoe-/- mouse model to detect additional exposure effects in the already challenged liver?

> Not an excellent model to replicate the human condition but certainly among the best we currently have available.

> suitable: In[de Leon et al J Liver 2:132, 2013] clear effect of cigarette smoke over control can be observed. This indicates that effects are measurable above those determined in the already challenged liver and therefore it provides a model to examine relative effects of 3R4F and THS2.2.

> As previously mentioned, I believe that this is a very poor model, especially to assess hepatic insult. Observing what are likely small hepatic effects resulting from smoking are likely being missed due to the high basal hepatic injury. This study is seriously lacking due to the absence of appropriate controls.

> The model may indeed be more difficult to assess hepatotoxicity than others, although past studies point to a lack of hepatotoxicity by CS. Even so, the xenobiotic response to CS in the Apoe-/- mouse was readily apparent as judged by the transcriptomics data.

> It is my opinion that leptin deficient mouse (ob/ob) or leptin-resistant (db/db) mouse model might have been a better choice for this study.

> First of all, based on the literature, Apoe deficiency did not alter the cholesterol balance across the liver though it affected the hepatic cholesterol metabolism (Kuipers et al, Hepatology. 1996 Jul;24(1):241-7). Interestingly, Apoe deficient mice do not become obese or develop insulin resistance. However, when these mice fed with typical high calory diet such as western diet, these mice develop (Dis Model Mech. 2010 MarApr; 3(34): 156–166) some liver function alteration. So with normal fed condition Apoe deficient mice may not lead to challenged liver condition. Evidently this study also did not find any hepatotoxic effects. In addition, smoke exposure in these mice did not lead to hepatotoxicity though there was significant gene and protein expression these indicate that the observed alteration in gene and protein expression may be necessary to maintain liver homeostasis or amount of smoke or its metabolites did not reach Liver sufficiently. So first cotinine concentrations should be checked in Liver. Depends on the results, one can plan accordingly. In the cotinine concentrations are very low in Liver, it is wise to think twice before using Apoe KO mice model for smoke induced liver toxicity. Evidently smokers are not having significant liver failure. In any event, the knowledge obtained from this study would be helpful in a subset of smokers who also have altered cholesterol metabolism like that of Apoe deficiency. In summary, additional smoke exposure to Apoe-/- mouse model may not be suitable. This may be suitable when these mice fed high calorie diet/

> Not valuable unless the study is concerned with the <5% of the population that are equivalent to the ApoE-/- mouse.

> Apoe -/- animal model is suitable to detect addtiional expsoure effectes based on several published papers

> Again, The Apoe-/- mouse model is useful but suggest including male mice in the studies for the reasons noted above.

> Apoe-/- mouse model is very specific to develop CVD even spontaneously when feed basal diet and therefore best suited for CVD. It is logical to use the same animals to evaluate liver effects along with CVD; however, it will provide almost the same information which can be gleaned from normal mice. I am not sure about the extent of impact/sensitivity of the lipids in liver as this knockout mouse model as it affect lipids. Bottomline, Apod-/-knockout mice is to better understand the inflammatory background of atherosclerosis.

> According to the literature, Apoe KO is a good model to observe the toxic effects of CS on the lungs, cardiovascular system and liver; however, WT animals cannot be neglected.

ID #1895 (https://scipinion.com/surveys/67/results/1895)

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?

Yes. Not at this time.

Both methodologies are appropriate according to current standards. For proteomics a SILAC approach (SILAC mouse: [Cell. 2008 Jul 25;134(2):353-64] and subsequent papers from the M Mann group) could be used to facilitate quantification of global proteomic changes but this is highly expensive and the approach taken here is sufficiently informative.

The proteomics platform is well done. The use of a QExactive is considered "state-of-the-art". The lipidomics platform, while complete, is a bit unclear. The IS should have been spiked into the homogenization buffer (i.e. 70% MeOH +BHT) prior to performing the extraction. The only other possible issue is the limit of detection on the QTRAP 5500. The sensitivity on the 6500 is orders of magnitude greater and thus, potentially important lipids may be missed using their "Shotgun" platform.

The LC-MS methods appeared in line with most studies referenced. There are alternative methods and instruments that might be used, such as UHPLC coupled with ICR-FT/MS.

Yes, the approaches are appropriate

Yes the lipidomics and proteomics used states of the art technology. I have n o other recommendations. The flaw in this study is not the methodology rather is the lack of a dose-response to make causal links between exposures and disease outcomes.

I believe that the mass spectrometry instruments and approaches for evaluating lipidomics and proteomics are presently state -of -the- art. One might consider RIA approaches for specific proteins once target molecules of interest have been identified by mass-spectral screening.

The methods reflects the current state of the art in the field

Yes. These methods and instruments are reflecting the current state-of-the-art in the field. The authors have done lipid related proteome in Lungs (Titz et al, 2016). This is the best biological validation. In future ,this combined approach can be used for every lipidomic study.

Yes, it does. I don't have any additional comment.

As far as I know about the instruments, the ones used are definitively up-to-date. I think it is the best available and precise method for the omics approach.

ID #1896 (https://scipinion.com/surveys/67/results/1896)

Having inspected the study data in the various repositories provided, as well as in the Supplementary materials provided with the publications, and 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	Total
In-life observations, mortality, body weight, gross pathology	*2* **9.09%** 1 *3* **90.91%** 10	*2* **9.09%** 1 *3* **90.91%** 10	11
Biomarkers of exposure, urine	*2* **18.18%** 2 *3* **81.82%** 9	*2* **18.18%** 2 *3* **81.82%** 9	11
Biomarkers of exposure, blood, plasma	*2* **9.09%** 1 *3* **90.91%** 10	*2* **9.09%** 1 *3* **90.91%** 10	11
Clinical chemistry, including blood lipoproteins	*2* **27.27%** 3 *3* **72.73%** 8	*2* **18.18%** 2 *3* **81.82%** 9	11
Hematology	*3* **100.00%** 11	*3* **100.00%** 11	11
Respiratory physiology	*1* **9.09%** 1 *2* **18.18%** 2 *3* **72.73%** 8	*1* **9.09%** 1 *2* **18.18%** 2 *3* **72.73%** 8	11

BAL fluid - inflammatory mediators	*2* **18.18%** 2 *3* **81.82%** 9	*2* **18.18%** 2 *3* **81.82%** 9	11
BAL cells (free ling cells) - identification and activation markers	*2* **18.18%** 2 *3* **81.82%** 9	*2* **18.18%** 2 *3* **81.82%** 9	11
Histopathology, upper respiratory tract	*2* **27.27%** 3 *3* **72.73%** 8	*2* **9.09%** 1 *3* **90.91%** 10	11
Histopathology, lung	*2* **30.00%** 3 *3* **70.00%** 7	*2* **18.18%** 2 *3* **81.82%** 9	11
Histopathology, liver	*1* **9.09%** 1 *2* **18.18%** 2 *3* **72.73%** 8	*1* **9.09%** 1 *2* **18.18%** 2 *3* **72.73%** 8	11
Histopathology, other organs/tissues	*2* **27.27%** 3 *3* **72.73%** 8	*2* **18.18%** 2 *3* **81.82%** 9	11
Histomorphometry, lung	*3* **100.00%** 11	*2* **9.09%** 1 *3* **90.91%** 10	11

Plaque size measurement, planimetry	*1* **9.09%** 1 *2* **9.09%** 1 *3* **81.82%** 9	*2* **18.18%** 2 *3* **81.82%** 9	11
Plaque size measurement, micro-CT	*2* **9.09%** 1 *3* **90.91%** 10	*3* **100.00%** 11	11
Dataset, transcriptomics	*2* **18.18%** 2 *3* **81.82%** 9	*1* **9.09%** 1 *2* **18.18%** 2 *3* **72.73%** 8	11
Dataset, lipidomics	*2* **18.18%** 2 *3* **81.82%** 9	*2* **18.18%** 2 *3* **81.82%** 9	11
Dataset, proteomics	*3* **100.00%** 11	*2* **9.09%** 1 *3* **90.91%** 10	11

ID #1897 (https://scipinion.com/surveys/67/results/1897)

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.

	Importance and fitness for purpose (1-5)	Confidence (1-5)	Total
Availability of raw data	*4* **27.27%** 3 *5* **72.73%** 8	*3* **9.09%** 1 *4* **36.36%** 4 *5* **54.55%** 6	11
Annotation of the metadata	*4* **36.36%** 4 *5* **63.64%** 7	*3* **18.18%** 2 *4* **54.55%** 6 *5* **27.27%** 3	11
Detailed enough text description of the methods	*2* **9.09%** 1 *4* **18.18%** 2 *5* **72.73%** 8	*2* **9.09%** 1 *3* **9.09%** 1 *4* **27.27%** 3 *5* **54.55%** 6	11
	2 **9.09%**	*2* **9.09%**	

Availability of the source code and/or software	1 *3* **18.18%** 2 *4* **18.18%** 2 *5* **54.55%** 6	1 *3* **18.18%** 2 *4* **27.27%** 3 *5* **45.45%** 5	11
Possibility to run the code and/or software on own datasets to judge of the quality of the tool	*3* **18.18%** 2 *4* **36.36%** 4 *5* **45.45%** 5	*2* **9.09%** 1 *3* **27.27%** 3 *4* **18.18%** 2 *5* **45.45%** 5	11
Use of multiple methods on the same data leading to similar conclusions	*3* **9.09%** 1 *4* **45.45%** 5 *5* **45.45%** 5	*1* **9.09%** 1 *2* **9.09%** 1 *3* **18.18%** 2 *4* **27.27%** 3 *5* **36.36%** 4	11
Other (please specify)	*4* **100.00%** 1	*4* **100.00%** 1	1

SECTION: DATA EVALUATION AND INTERPRETATION

ID #1898 (https://scipinion.com/surveys/67/results/1898)

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	*Insufficient* **9.09%** 1 *Partially sufficient* **18.18%** 2 *sufficient* **72.73%** 8	*Insufficient* **9.09%** 1 *Partially sufficient* **9.09%** 1 *sufficient* **81.82%** 9	11
Biomarkers of exposure, urine	*Partially sufficient* **18.18%** 2 *sufficient* **81.82%** 9	*Partially sufficient* **9.09%** 1 *sufficient* **90.91%** 10	11
Biomarkers of exposure, blood, plasma	*Partially sufficient* **9.09%** 1 *sufficient* **90.91%** 10	*sufficient* **100.00%** 11	11
Clinical chemistry, including blood lipoproteins	*Insufficient* **9.09%** 1 *Partially sufficient* **9.09%** 1 *sufficient* **81.82%** 9	*Insufficient* **9.09%** 1 *Partially sufficient* **9.09%** 1 *sufficient* **81.82%** 9	11
Hematology	*Insufficient* **9.09%** 1 *sufficient* **90.91%** 10	*Insufficient* **9.09%** 1 *Partially sufficient* **9.09%** 1 *sufficient* **81.82%** 9	11

Respiratory physiology	*Partially sufficient* **18.18%** 2 *sufficient* **81.82%** 9	*Partially sufficient* **18.18%** 2 *sufficient* **81.82%** 9	11
BAL fluid - inflammatory mediators	*Partially sufficient* **18.18%** 2 *sufficient* **81.82%** 9	*sufficient* **100.00%** 11	11
BAL cells (free lung cells) - identification and activation markers	*Partially sufficient* **9.09%** 1 *sufficient* **90.91%** 10	*sufficient* **100.00%** 11	11
Histopathology, upper respiratory tract	*Insufficient* **18.18%** 2 *Partially sufficient* **9.09%** 1 *sufficient* **72.73%** 8	*Insufficient* **9.09%** 1 *Partially sufficient* **18.18%** 2 *sufficient* **72.73%** 8	11
Histopathology, lung	*Insufficient* **9.09%** 1 *Partially sufficient* **18.18%** 2 *sufficient* **72.73%** 8	*Insufficient* **9.09%** 1 *Partially sufficient* **18.18%** 2 *sufficient* **72.73%** 8	11
Histopathology, liver	*Insufficient* **18.18%** 2 *Partially sufficient* **9.09%** 1 *sufficient* **72.73%** 8	*Insufficient* **18.18%** 2 *Partially sufficient* **9.09%** 1 *sufficient* **72.73%** 8	11
	Insufficient	*Insufficient*	

Histopathology, other organs/tissues	**27.27%** 3 *Partially sufficient* **27.27%** 3 *sufficient* **45.45%** 5	**18.18%** 2 *Partially sufficient* **27.27%** 3 *sufficient* **54.55%** 6	11
Histomorphometry, lung	*Partially sufficient* **9.09%** 1 *sufficient* **90.91%** 10	*Partially sufficient* **9.09%** 1 *sufficient* **90.91%** 10	11
Plaque size measurement, planimetry	*Partially sufficient* **9.09%** 1 *sufficient* **90.91%** 10	*Partially sufficient* **9.09%** 1 *sufficient* **90.91%** 10	11
Plaque size measurement, micro-CT	*sufficient* **100.00%** 11	*sufficient* **100.00%** 11	11
Dataset, transcriptomics	*Partially sufficient* **18.18%** 2 *sufficient* **81.82%** 9	*Insufficient* **9.09%** 1 *Partially sufficient* **18.18%** 2 *sufficient* **72.73%** 8	11
Dataset, lipidomics	*Partially sufficient* **18.18%** 2 *sufficient* **81.82%** 9	*Insufficient* **9.09%** 1 *Partially sufficient* **18.18%** 2 *sufficient* **72.73%** 8	11
Dataset, proteomics	*Partially sufficient* **9.09%** 1 *sufficient* **90.91%**	*Partially sufficient* **9.09%** 1 *sufficient* **90.91%**	11

	10	10	

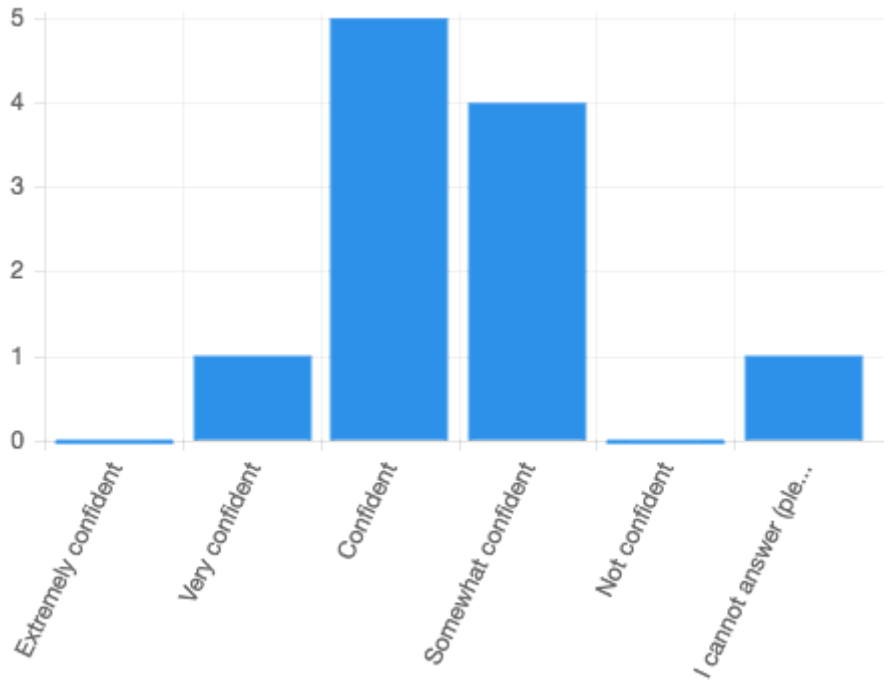
ID #1899 (https://scipinion.com/surveys/67/results/1899)

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	**90.91%** 10	**9.09%** 1	**0.00%** 0	11
Lung / respiratory effects in Phillips et al. 2016	**81.82%** 9	**9.09%** 1	**9.09%** 1	11
Vascular / atherosclerotic effects in Phillips et al. 2016	**81.82%** 9	**9.09%** 1	**9.09%** 1	11
Cardiac effects in Szostak et al. 2016	**63.64%** 7	**36.36%** 4	**0.00%** 0	11
Clinical chemistry and systemic effects in Phillips et al. 2016	**81.82%** 9	**18.18%** 2	**0.00%** 0	11
Hepatic effects in Lo Sasso et al. 2016	**72.73%** 8	**18.18%** 2	**9.09%** 1	11

ID #1900 (https://scipinion.com/surveys/67/results/1900)

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?



Legend

answers: 11

skips: 0

ID #1901 (https://scipinion.com/surveys/67/results/1901)

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* **45.45%** 5 *Low* **27.27%** 3 *Medium* **27.27%** 3	*High* **45.45%** 5 *Low* **27.27%** 3 *Medium* **27.27%** 3	11
Aortic arch planimetric plaque measurements (Figure 3)	*High* **63.64%** 7 *Low* **9.09%** 1 *Medium* **27.27%** 3	*High* **63.64%** 7 *Low* **18.18%** 2 *Medium* **18.18%** 2	11
microCT-based aortic arch plaque measurements (Figure 4, Suppl. Fig. 9, and supplementary files)	*High* **63.64%** 7 *Low* **9.09%** 1 *Medium* **27.27%** 3	*High* **63.64%** 7 *Low* **18.18%** 2 *Medium* **18.18%** 2	11
Plasma and aortic arch lipidomics (Figure 5)	*High* **45.45%** 5 *Low* **9.09%** 1 *Medium* **45.45%** 5	*Cannot Answer* **9.09%** 1 *High* **27.27%** 3 *Low* **18.18%** 2 *Medium* **45.45%** 5	11

Respiratory physiology (Figure 6)	*Cannot Answer* **9.09%** 1 *High* **72.73%** 8 *Medium* **18.18%** 2	*Cannot Answer* **9.09%** 1 *High* **72.73%** 8 *Low* **9.09%** 1 *Medium* **9.09%** 1	11
Lung volume measurements (Figure 7)	*High* **36.36%** 4 *Low* **27.27%** 3 *Medium* **36.36%** 4	*High* **36.36%** 4 *Low* **27.27%** 3 *Medium* **36.36%** 4	11
Bronchoalveolar lavage fluid – FACS analysis of free lung cells (Figure 8)	*High* **100.00%** 11	*High* **90.91%** 10 *Low* **9.09%** 1	11
Bronchoalveolar lavage fluid – Multianalyte Profiling (Figure 9, supplementary Fig. 12)	*High* **63.64%** 7 *Medium* **36.36%** 4	*High* **54.55%** 6 *Low* **18.18%** 2 *Medium* **27.27%** 3	11
Nasal epithelium histopathological evaluation (Figure 10)	*High* **72.73%** 8 *Low* **18.18%** 2 *Medium* **9.09%** 1	*High* **72.73%** 8 *Low* **18.18%** 2 *Medium* **9.09%** 1	11

Lung histology (Figure 11, Supplementary Results File 2)	*High* **90.91%** 10 *Medium* **9.09%** 1	*High* **81.82%** 9 *Low* **9.09%** 1 *Medium* **9.09%** 1	11
Lung histomorphometry (Figure 12)	*High* **72.73%** 8 *Medium* **27.27%** 3	*High* **72.73%** 8 *Low* **9.09%** 1 *Medium* **18.18%** 2	11
Lung transcriptomics results – Differentially expressed genes (Figure 13, Supplementary Fig. 13)	*High* **54.55%** 6 *Low* **9.09%** 1 *Medium* **36.36%** 4	*High* **54.55%** 6 *Low* **9.09%** 1 *Medium* **36.36%** 4	11
Lung transcriptomics results – NPA/BIF analysis (Figure 14)	*High* **54.55%** 6 *Medium* **45.45%** 5	*High* **36.36%** 4 *Low* **9.09%** 1 *Medium* **54.55%** 6	11
Lung proteomics analysis (Figure 15, Supplementary Figs. 14 to 16)	*High* **72.73%** 8 *Medium* **27.27%** 3	*High* **54.55%** 6 *Low* **9.09%** 1 *Medium* **36.36%** 4	11
	High	*High*	

The totality of the data, collectively (All figures)	**90.91%** 10 *Medium* **9.09%** 1	**90.91%** 10 *Low* **9.09%** 1	11

ID #1902 (https://scipinion.com/surveys/67/results/1902)

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.

Age is an important variable that needs to be carefully integrated into the design of studies of this nature. It is reasonable that age was not integrated given the complexity of the experimental design.

Because the ApoE-/- background results in such stark pathologies simply with age, I would expect nearly all of the timepoints to look different should the correct animal model be utilized. This is especially evident in the aortic arch plaque formation, where the sham group displays "25% plaque formation" by month 8. I do not believe that the age used for these mice is the issue, but rather the model as a whole.

For the endpoints investigated, the age group is appropriate.

Certainly in many commonly used mouse strains there will be an increased incidence of cancer, as in humans. There are likely to be age-related changes in metabolic rate and animal behavior. The increased morbidity may not affect specific endpoints but rather the study as a whole.

The age of the animals could impact the results since a number of biological patterns change in senescent animals due to attenuated sexual hormonal influences. This particularly true for females when they go out of estrus since this life event will undoubtedly influence all the omics data endpoints.

For the ApoE-/- mouse the exposure length was sufficient however, studies done with WT C57/Bl6 a chronic inhalation study will likely be needed for safety assessment of THS2.2 compared to air.

This is a very sensitive part of the study. Mouse metabolism is different from humans and the extrapolation should be conducted very carefully. I would try to make a parallel of human age and mouse age in WT animals that don't suffer the problems of Apoe -/- mouse.

Age in this model will affect cardiovascular aspects. E.g. plaques will already be bigger at later age in Apoe- mice and this could render the animals no longer a good model to analyse cardiovascular effects of CS versus THS2.2.

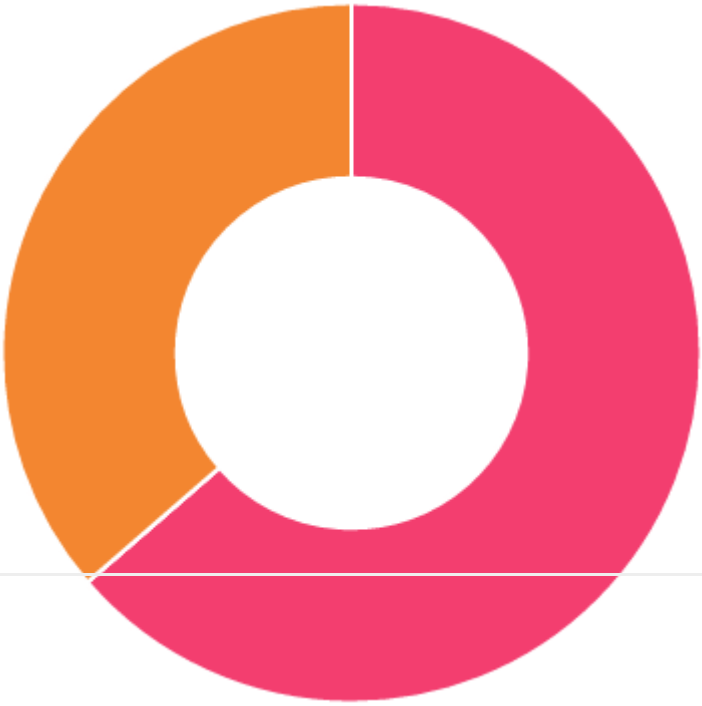
1. Respiratory physiology: Though aging as such cannot directly cause COPD, but it increases the susceptibility of the lung to develop COPD (Proceedings of the American Thoracic Society, Vol. 6, No. 7 (2009), pp. 570-572) 2. Atheosclerosis: This is very obvious to any strain of mice including Apoe KO mice.

Since age affects athreosclerosis development, if animals were older, it may present some difficulties in distingusing test article effect from age effect. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. Y Nakashima, A S Plump, E W Raines, J L Breslow, R Ross Arteriosclerosis, Thrombosis, and Vascular Biology. 1994; 14: 133-140

> I will be most interested in knowing longer-term exposure effects and not starting the exposure in older animals. The reason for this is to see the long-term exposure followed by cessation in older humans and also the combined effects of exposure and age when many biochemical and physiological changes occur.

ID #1903 (https://scipinion.com/surveys/67/results/1903)

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



Legend

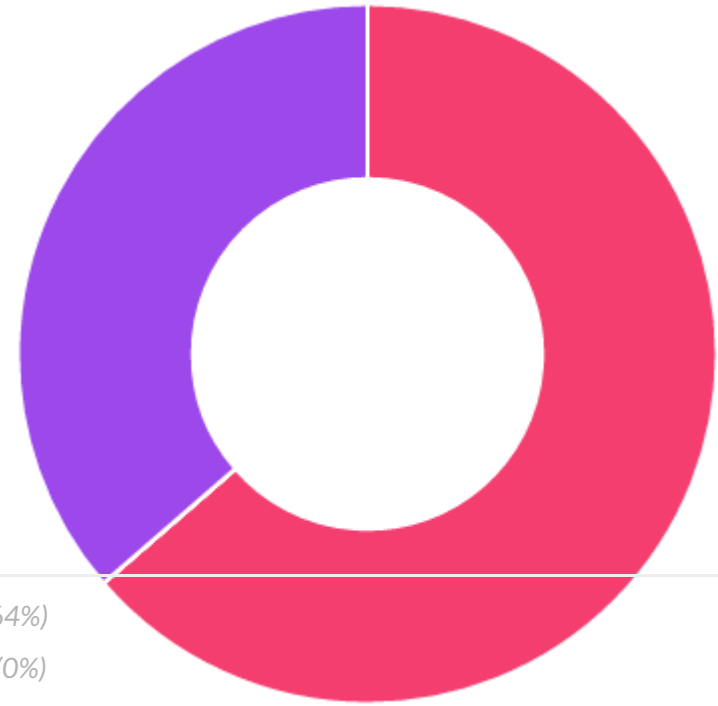
- Yes: 7 *(64%)*
- No: 0 *(0%)*
- I do not see any indication of airspace enlargement in this animal model: 0 *(0%)*
- I cannot answer : 4 *(36%)*

answers: 11

skips: 0

ID #1904 (https://scipinion.com/surveys/67/results/1904)

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?



Legend

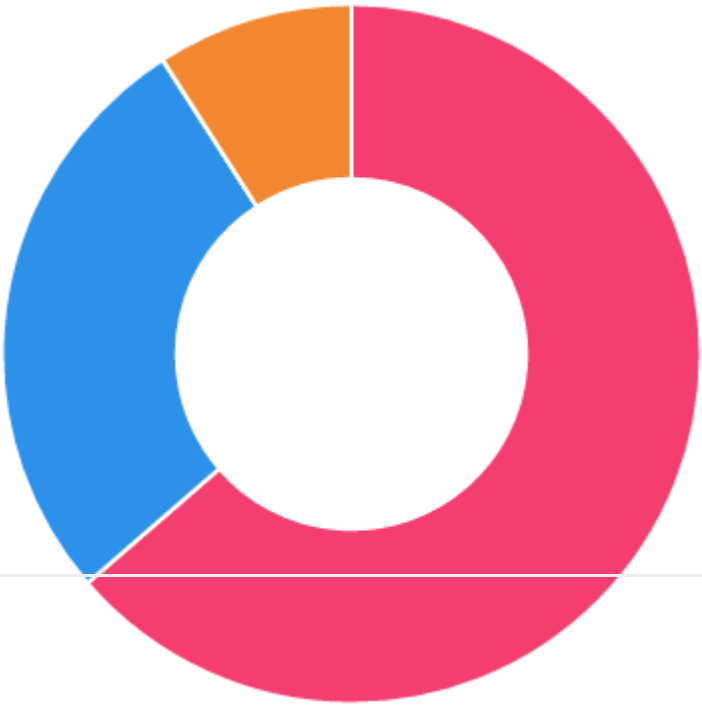
- Less airspace enlargement: 7 *(64%)*
- More airspace enlargement: 0 *(0%)*
- No airspace enlargement seen in any of the two exposed groups: 0 *(0%)*
- Similar airspace enlargement seen in both exposed groups: 0 *(0%)*
- I cannot answer the question: 4 *(36%)*

answers: 11

skips: 0

ID #1905 (https://scipinion.com/surveys/67/results/1905)

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)?



Legend

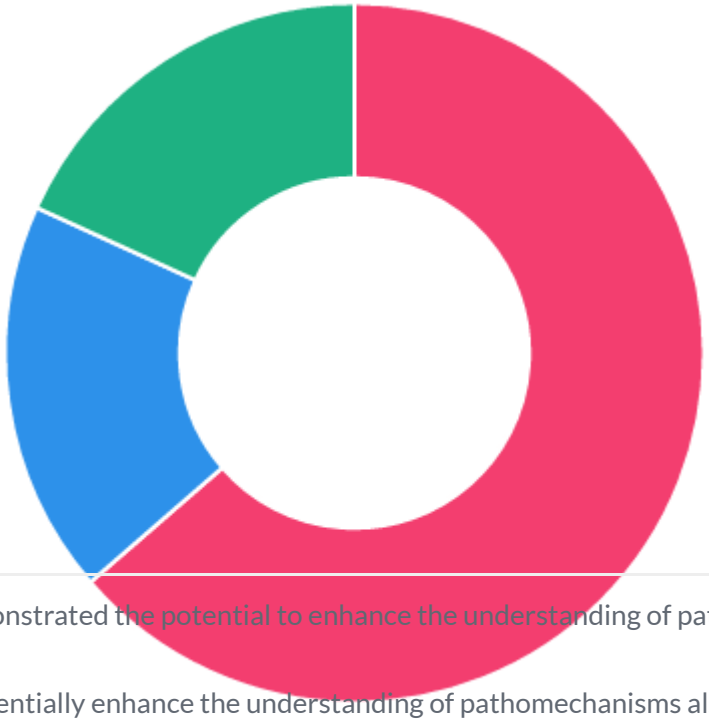
- To a large extent: 7 *(64%)*
- To a small extent: 3 *(27%)*
- Not at all: 0 *(0%)*
- I cannot answer the question: 1 *(9%)*

answers: 11

skips: 0

ID #1906 (https://scipinion.com/surveys/67/results/1906)

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: 7 *(64%)*

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 *(18%)*

No, the only reliable approach to understand pathomechanisms is to use separate, mechanism-specific investigations in various dedicated model systems: 2 *(18%)*

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

answers: 11

skips: 0

ID #1907 (https://scipinion.com/surveys/67/results/1907)

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?

> Clear.

> The question states "How clear were the changes". Addressing this question, the authors did not display the changes in a way that is easy to discern. Using the heat maps, it seems that the authors again are flooding the reader with numbers/figures, rather than highlighting the actual changes. It is evident if you inspect the data closely that there are clear differences between the ApoE-/- and C57 mice. This can be seen "clearly" in the panel E of the Lung Lipidome, where significant (according to their inappropriately done statistics) differences can be seen in the 3R4F groups in the C57 compared to the ApoE-/-. This is especially evident with the Cer and Glc/GalCer classes. This furthers my concern that this is an inappropriate model to use for this study, although there may be some differences due to the inconsistent treatment times between the C57 and ApoE-/- mice (5 vs 6 and 7 vs 8).

> The data in Titz et al. suggested very consistent changes in the lung lipidome in response to CS in these strains. A few genes/proteins were differentially affected, but overall the vast majority of expression differences were preserved across wild-type and apoE-deficient mice.

> It was evident that the changes in lipid profiles was more pronounced in C57BL/6 mice than in Apoe-/- mice between 3R4F and pMRTP/THS2.2

> The differences in the perturbations of lipid homeostasis between ApoE-deficient mice and wild-type C57BL/6 mice (among tested lipids both in C57 and ApoE KO), is minimum. The difference was related to cholesteryl ester. This was also reflected the Steroid biosynthesis and Steroid hormone biosynthesis strikingly as these two events were significantly higher in Apoe KO mice compared to C57 mice (Figure 2B). Interestingly, this striking difference was also observe in liver also (Figure 4D from La Sasso er al, 2016) indicates the importance both Liver and Lung. This difference can make major difference in inflammation as mild difference in steroid can have major effect in body metabolism.

> There are some clear differences between Apoe and WT mice based on KEGG pathways particularly lipid metabolism

> The data presented in Figure 3C would indicate general similarities between the 2 strains and that the observed lipidomic responses appear to be related to a more general physiological process held in common between the 2 strains.

> C57BL/6 mice seem to be more responsive to lung lipid metabolism biomarkers even the Apoe-/- was also responsive to the CS.

> Modest difference with more pronounced effects in APoE- than in C57b6 mice. This is obvious for instance in fig 2a,b where 3R4F causes somewhat more effects. In fig 3c it is clear that effects largely

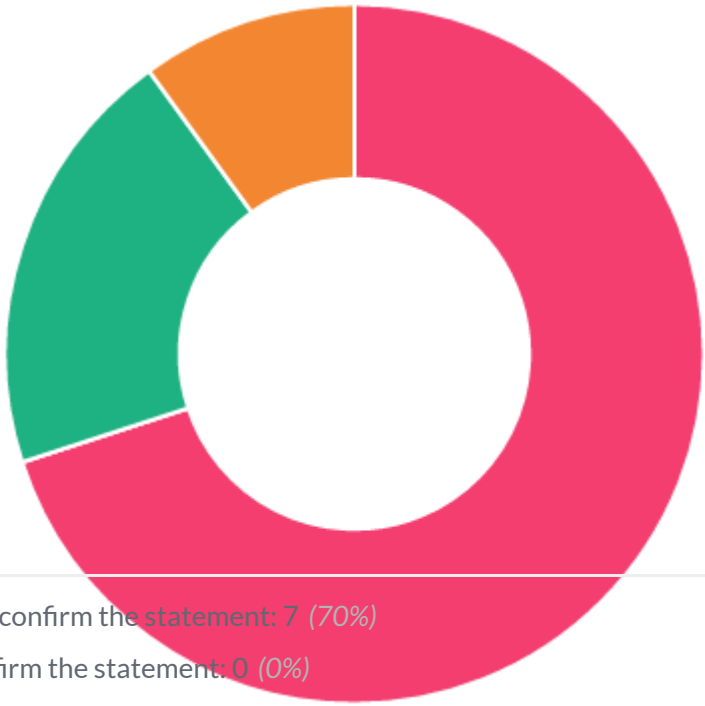
> overlap.

> Little bit clear

> THS2.2 - ApoE -/- 1,2,3,6,7 months pMRTP - C57BL/6 1,2,3,6,8 months Comparing with Sham group, the lipid metabolism was very much clear disturbed in both mouse strains. Comparing both strains, Fig. 1: Lung lipidome showed colesterol and PCO/PCP were higher on Apoe-/-.

ID #1908 (https://scipinion.com/surveys/67/results/1908)

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: 7 *(70%)*
- Only the 2D measurements confirm the statement: 0 *(0%)*
- Only the CT imaging confirm the statement: 2 *(20%)*
- No, the statement is not supported by these data: 1 *(10%)*
- I cannot answer this question: 0 *(0%)*

answers: 10

skips: 1

ID #1909 (https://scipinion.com/surveys/67/results/1909)

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?

> Yes, adequate qualitative information on protein/gene pathways.

> I think the gene/protein level profiling was critical in the assessment. The analysis of changes in the lung in Titz et al. required molecular characterization and beautifully tied together the morphological changes related to emphysema to underlying mechanistic responses such as the ECM network.

> Yes.

> Yes, GSEA and PSEA results of Titz et al., and Lo Sasso et al. effectively contribute to the assessment and they have given adequate information on the protein/gene pathways involvement. However, it seems that the authors have not given the importance to striking pathways like steroid hormone synthesis in Apoe mice. Maybe the aim of the study is not to compare the C57 with ApoE KO mice. But the deep exploration of Steroid biosynthesis and Steroid hormone biosynthesis pathway might give crucial information as most of COPD patients are relatively resistant to steroid.

> Yes

> The gene /protein set analyses presented by Titz etal and Lo Sasso etal. do contribute to the assessment but provide only limited information on the protein/gene pathways involved. In other words, not a comprehensive snapshot of all the protein / gene pathways potentially involved.

> I am not sure about this.

> Somewhat. Of course, many of these effects have been observed previously in inhalation studies (i.e. the inflammatory markers). The comparison between gene and protein is not particularly helpful, however, and the correlation between mRNA and protein expression is typically about 40%. The protein expression does provide some useful information as to what pathways are activated using this model, but I do not find the transriptomics that useful.

> Omits data in Titz et al do not add much to the obvious conclusion that cessation or switching to THS2.2 attenuates effects caused by 3R4F but they provide qualitative info on potential involvement of SREBP-regulated changes in lipid metabolism. The same goes for data in LoSasso et al 2016 where also qualitative info on lipid and xenobiotic metabolism and iron homeostasis is added.

> They give some informaiton on the protein/gene pathways involvement

> Both papers presented extensive data on BALF, lung and liver transcriptomics and proteomics that are adequate to the goals proposed by the authors.

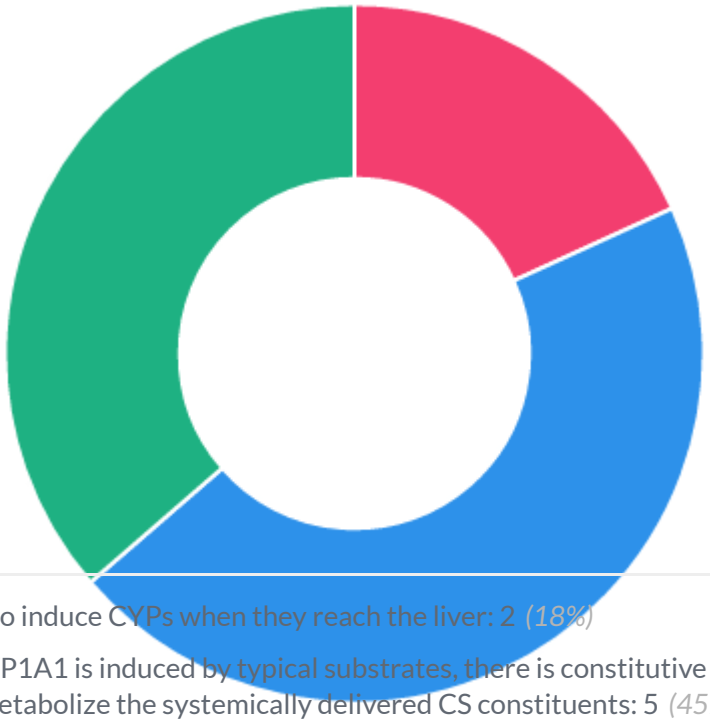
ID #1910 (https://scipinion.com/surveys/67/results/1910)

The analysis of lung transcriptomics data using the causal network model (Philips et al. 2016) predicted a robust CS-induced increase in extracellular matrix (ECM) degradation that disappears upon cessation.

	Yes	No	I cannot answer	Total
Are the quantified histopathological changes in the lung tissue that occur with CS exposure consistent with ECM degradation throughout CS exposure?	**72.73%** 8	**9.09%** 1	**18.18%** 2	11
Are the inflammatory mediators in BALF reflective of observed increase in ECM degradation during CS exposure?	**90.91%** 10	**0.00%** 0	**9.09%** 1	11
Are any of the lung functional parameters likely reflective of ECM/tissue degradation?	**54.55%** 6	**0.00%** 0	**45.45%** 5	11

ID #1911 (https://scipinion.com/surveys/67/results/1911)

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?



Legend

- CS constituents are too diluted to induce CYPs when they reach the liver: 2 *(18%)*
- 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: 5 *(45%)*
- Other (please explain): 4 *(36%)*
- I cannot answer this question: 0 *(0%)*

answers: 11

skips: 0

ID #1912 (https://scipinion.com/surveys/67/results/1912)

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 test items: 1) an early prototype MRTP (pMRTP) and 2) the Tobacco Heating System version 2.2 (see Titz et al. 2016)	**0.00%** 0	**9.09%** 1	**36.36%** 4	**27.27%** 3	**27.27%** 3	11
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	**18.18%** 2	**54.55%** 6	**27.27%** 3	11
Analysis of the effects of the products on multiple systems (respiratory, cardiovascular) in a single model	**0.00%** 0	**9.09%** 1	**9.09%** 1	**36.36%** 4	**45.45%** 5	11
Concordance of results for specific mechanisms (e.g. oxidative stress or inflammation) across diverse studies and model systems (Apoe-/- mouse and in vitro assays)	**0.00%** 0	**0.00%** 0	**18.18%** 2	**54.55%** 6	**27.27%** 3	11

ID #1913 (https://scipinion.com/surveys/67/results/1913)

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?

Yes. 1. The return to control levels upon cessation or switching to THS2.2 or pMRTP. 2. Consistency between proteome and lipidome profiles.

Yes. Arachidonic acid metabolism/ sphingolipid metabolism and proteins implicated in reactive oxygen species have changed.

This study is like a first step for long way to go for a concept of giving complementary mechanistic information as this is really superficial but good study to scan the alteartion of lipid and its related proteome. I could not see any alteration that can explain the pathogenesis of COPD. What should be changed ? 1. Validation of proteomic data is required (western, ELISA, IHC) 2. One best particular pathway can be focussed. 3. Interference studies are required to confirm the mechanistic role of that pathway in the pathogenesis. 4. Cessation and switch can be planned after 5/6 months 3R4F exposure as 2/3 months 3R4F exposure hardly affected the proteome. 5. Steroid biosynthesis and steroid hormone biosynthesis is a novel direction compared to other known directions. This direction can be emphasized after the protein validation experiments.

Yes they examined and had biological overlap in 3 key areas surfactant lipd/protein correspondence alterations in eicosanoids/metabolizing enzyme correspondence sphingolipid alterations and enzyme changes.

Yes. 1. Figure 1 E and F are clearly showing differential profiles in different group. 2. Figure 2 is clearly showing the difference between the two mouse species and among different groups for lipid-related lung proteome. 3. Other figures and plots are showing the same results and very obvious on the effects of CS on all the biomarkers whereas, similar effects are much lower in the other groups including non-traditional (heat-not-burn) smoking devices.

I do not believe so. Without an actual readout as to the functional activity of the changes observed, it is these data do not provide any mechanistic information. The increase in FASN is suggestive of an increase in total 16:0 species; however this is not universal across the board. The biggest question is, what is the consequence of the increases/decreases in any altered lipid species? What is the functional role of an increase in total 16:0? No mechanistic information is really displayed, but rather correlations are drawn between measured alterations in the proteome and lipidome.

Yes, they provide complementary information in the sense of perhaps early and late toxicological mechanisms. The proteomic effects may participate in the initiating events while the lipidomic data may more be more a reflection of cell death and replacement events. Both are important to the overall pathological outcomes. This idea could be evaluated by looking for correlations with morphological events and changes in cell populations over time.

yes: -- Induction of surfactant proteins Sftpa1 and Sftpd combined with increased PC 16:0/16:0, PC 16:0/16:1, and PC 16:0/14:0. -- Increase in Ptgs1(COX1) combined with increased prostaglandins D2/E2

Both approaches indicated that many lung molecules were affected by the exposure of CS. Most of the molecules increased its concentrations. Increase in several lipids were correlated with changes in the surfactant metabolism, phospholipases activity. Phosphatidylcholine was the most affected by far, followed by SM, phosphatidylserine, ether-linked phospholipids (PE O), PG and GD1.

inflammation, antioxidant enzymes, pulmonary funciton

ID #1914 (https://scipinion.com/surveys/67/results/1914)

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?

Several of the networks can explain the profiles observed.

Inflammatory infiltration under CS exposure involved neutrophils > lymphocytes > dendritic cells > macrophages. The numbers of all cell types returned to the sham/THS2.2 group level upon cessation or switching. Likewise, BIF analysis points to 14 inflamation-related subnetwork NPAs. In agreement with inflammatory cells detected, these include Treg Signaling, Th... signaling, Neutrophil Signaling, Macrophage Signaling, Dendritic Cell Signaling, Cytotoxic T−cell Signaling. All these NPAs return to THS2.2 levels after switching or cessation. So indeed, BIF analysis points to inflammatory networks that can explain the type of inflammation.

GM-CSF levels and differences in alveolar dendritic cells between the two groups (this would be one network).

Secreted inflammatory mediators: 1. Lymphotactin (found increased in cessation at 8 months) is known T-cell chemoattractant chemokine (Am J Pathol. 2001 Nov;159(5):1751-61.) and this can explain the increased lymphocytes in cessation. 2. MIP 1 alpha (CCL3) attracts macrophages, monocytes and neutrophils (J Leukoc Biol. 2005 Jul;78(1):167-77). This could explain the presence of neutrophils (also in BAL) and macrophages in cessation ans switch groups. 3. Monocyte chemoattractant protein-1 (MCP-1/CCL2) that regulate migration and infiltration of monocytes and macrophages (J Interferon Cytokine Res. 2009 Jun; 29(6): 313–326./macrophages) could explain the presence of macrophages in both cessation and switch. 4. MPO indicate the presence of active neutrophil (in BAL of cessation and switch groups) 5. M-CSF, chemokine, attract macrophages to the site of inflammation. (Mol. Cell. Biol. May 2005 vol. 25no. 10 4211-4220). This could explain the presence of macrophages in both cessation and switch. 7. IL-1beta induces neutrophil migration (Inflammation. 2008 Feb;31(1):36-46.). This could explain the presence of neutrophil (in BAL of cessation and switch groups). 8. GM-CSF known for its role in recruitment of neutrophil to site of insult (J Leukoc Biol. 2011 Jun;89(6):945-53.), This could explain the presence of neutrophil (in BAL of cessation and switch groups) inflammatory networks from the BIF analysis 1. Th1-Th2 signaling and Th17 signaling could explain the presence of neutrophils and macrophages (Th17 is well known for its neutrophilia and Th2 can induce arginase in macrophages). 2. Neutrophil signaling can explain the presence of neutrophils in BAL of cessation and switching. 3. Macrophages signaling for the presence of macrophges in cessation and switching. 4. Epithelial innate immune activation can explain the presence of variety of inflammatory cells as the epithelial secreted mediators recruits variety of immune cells depends on severity of insult (Nat Immunol. 2010 Aug;11(8):656-65). 5. Cytotoxic T cell and B cell signaling could explain the presence of Lymphocytes in cessation and switching. 6. Epigenetics could indicate the amplification of inflammation or relative persistence of inflammation as smoke induced cytokines may cause the epigenetic changes such a way that there will be inflammation even after quitting (Multidisciplinary Respiratory Medicine 2011, 6:210). The relative persistence of inflammation even after quitting smoking is the major concern (Thorax. 2006 Feb; 61(2): 96–97 and Multidisciplinary Respiratory Medicine 2011, 6:210). This leads to thinking of initiation of autoimmune phenomena that can amplify the inflammation even without fresh insult.

yes, There remain increases in IgA, ILs, monocyte attractants, and MMP-9 and the BALF shows increased neutrophils/macrophages.

Perhaps depending upon when in the inflammatory process samples are collected. Consider looking at changes in BIF and BALF and correlating with histopathology data from early, middle and late phases of the experimental protocol. A time course study of these parameters in relation to histopathology data.

For BIF or RBIF shown in Figure 14 and BALF in Figure 8 show that both in the cessation and switch groups the biological impact never reached above 10% of the biological impact observed in 3R4F CS groups. Similary, the FLC counts almost reached to the sham level within one month and completely returned to the sham levels by 8 months. A similar reversal of the metaplasia and hyperplasia were observed in the nose and of inflamatory changes in the lungs in the switch and cessation groups reaching to sham levels after 6 months. Such effects were never observed in the THS groups. From Figure 11, I can discern mostly unpigmented and pigmented macrophages were responsible for the inflamation.

I do not believe the BIF analysis to be overly useful in this situation. The BALF does assist in determining the type of inflammatory cell infiltration, however standard immunohistochemistry would be a much more useful direct measurement of the type of inflammatory cells that are playing a role .

Even after a careful read of the suggested literature, I didn't accumulate enough knowledge during this review to correlate the results of BIF analysis and type of cell infiltration. However, BALF cell counts showed that alveolar dendritic cells were the majoritarian cell type in the cessation and switch groups comparing to the Sham. Authors could determine the amounts of dendritic subtypes, plasmacytoid and myeloid. Dendritic cells are vital for both the initiation and the maintenance of allergic airway inflammation in asthma.

noe

ID #1915 (https://scipinion.com/surveys/67/results/1915)

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?

Despite the 20X differences reported for THS2.2, it is intriguing that profiles between 3RF4 and THS2.2 can be comparable. This homology should be explored further.

None that I can determine.

The comparison between the two groups has been satisfactory

These studies using single exposure levels of CS compared to THS2.2 qualitatively shows a reduction in risk.

No- the story seems rather complete. My only concern with the use of only female mice in these studies. The rationale for this seems tenuous and not really supported by the cited literature.

The data presented are adequate for the studies conducted. However, I would have liked to see several scenarios of exposure, e.g., longer exposure to all, switching and cessation groups are exposed for longer period of time before switching, use of humanized animal models.

None of particular importance, aside from the differing timepoints in Titz et al.

1. Philips et al, 2016: Aortic arch plaque area measurements: Switching groups (6 months)also had shown the significant (it seems) increase of percentage of Plaque compared to Sham 6 months. Statistical significance was not calculated and it was discussed. 2. Liver histopathology data from La Sasso et al, 2016: The 6 months THS had shown similar harmful effects compared to 3R4F towards bile duct hyperplasia, and inflammatory cell recruitment. These are not discussed properly. 3. Proteomics data from La Sasso et al, 2016: THS has shown the clear upregulation of some Steatosis and Phospholipidosis proteins especially at 8 months. 4. sPLS analysis from La Sasso et al, 2016: 8 months THS exposure and Switch group altered almost of 90 % genes. These data were not given emphasis and statistical tests were not tested. The cumulative effects of these mild to moderate grade gene alteration might have cumulative toxicity. 5. Parenchymal amunt of fat from La Sasso et al, 2016: In contrast to the authors claim (Despite the time-dependent increase of parenchyma fat accumulation in the liver tissue observed in all groups, no statistically significant histopathological differences among exposure groups were observed.), THS both 8 months and Switch 8 months clearly shown the accumulation of parenchymal fat. This is well correlated with Figure 3 where THS had shown the increase of steatosis. Indeed, the authors tested the significant against its own 1 month group. However, they failed to test such a drastic change with Sham groups. 6. Titz et al, 2016: Protein set enrichment analysis: "Synthesis and degradation of ketone bodies" got significantly (seems) altered with Switch and THS groups and this was not discussed.

no

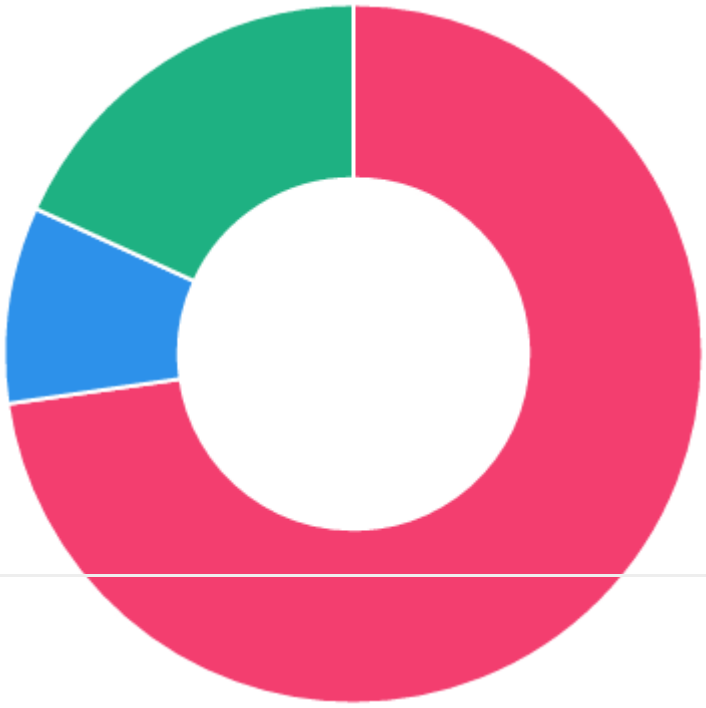
The discussion sections are quite ample in Phillips, Titz, Lo Sasso and Szostak papers. However, I would

> include few sentences discussing the possible allergens response, asthma development and dendritic cells subtypes in Titz. Phillips, Titz, Lo Sasso and Szostak papers are of excellent quality.

> none

ID #1943 (https://scipinion.com/surveys/67/results/1943)

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



Legend

- Yes: 8 *(73%)*
- No: 1 *(9%)*
- I cannot answer: 2 *(18%)*

answers: 11

skips: 0

ID #1917 (https://scipinion.com/surveys/67/results/1917)

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

> No.

> Yes there appears to be some slight induction GSTs (e.g., mu class) that is involved in detoxication of electrophiliic compounds. The lack of Cyp1a2 in the THS2.2 group, known to be induced by nicotine is a concern. Some follow up the microarray data using a second methods for specific suspect genes qRT-PCR would eliminate this concern

> In phillips et al 2016 liver histology does not reveal any exposure-related changes above the age-related effects in apoe- mice. In LoSasso et al 2016, Apoe- mice also show exposure unrelated transcriptomic changes in the liver over time. On top of this, there appears to be a specific downregulation of pyrimidine/purine metabolism in the liver of THS2.2. exposed APoe- mice (LoSasso et al 2016 Figure 4D, proteomics) but besides that effects are negligible.

> I do not; however, as indicated multiple times, I believe the model chosen is masking any effects that may be present.

> I find essentially no evidence based on the data provided and linked to THS2.2.

> The study is based on using Apoe-/- strain, so cannot make conclusive inferences.

> In my opinion, both 3R4F and THS exposures behaved similarly based on the histopathological analysis. In fact, both THS and 3R4F increased the accumulation of collagen fibers, bile duct hyperplasia and infiltration of mononuclear inflammatory cells in periportal and interstitial areas at 6 months time point. Evidently, both THS and 3R4F leads to reduce the gene expression related necrosis (less necrosis may be related more bile duct hyperplasia). However, the authors could not find the clear time-consistent differences in histopathology endpoints. This could be due to the different studies for different time point (different pooling) rather than exposing all mice together and then sacrifice at different time points. Figure 2 from La Sasso et al, 2016: The 6 months harmful effects of both THS and 3R4F is clearly visible in transcriptomics as THS, 3R4F, and switch data all clustered together at 6 and 8 months time points. Figure 3: Proteomics: THS has shown the clear upregulation of some Steatosis and Phospholipidosis proteins especially t 8 months and these upregulations were not visible in Switch groups. This could be due to accumulative toxicity of THS which needs at least 8 months exposure. Figure 4D: It is really strange that though 8 months THS exposure and Switch group altered almost of 90 % genes proteomics could not find them. This indicates the possibility of alteration of set of microRNAs by THS. Figure 6: Similar strange results in Metallothionein 1 and 2 (mt 1 and mt 2). 8 months THS had shown upregulation of these genes (both Switch and THS 8 months) without change in the respective protein. This indicates the possibility of alteration of set of microRNAs by THS to cause such discrepancy. Otherwise THS may have effect on protein degradation machinery such a way that it degrades these proteins. Figure 5B: In contrast to the authors claim, THS both 8 months and Switch 8 months clearly shown the accumulation of parenchymal fat. This is well correlated with Figure 3 where THS had shown the increase of steatosis. Overall, it seems that THS affected the liver lipid metabolism at moderate level compared to 3R4F.

> The finding of periportal interstitial fibrosis in the CS, THS2.2 and cessation groups relative to the sham controls at later (6 momth) time point is suggestive of some mild cell injury process from the THS2.2 aerosol (Lo Sesso etal., 2106). Suggest considering the effects of cadmium which is toxic to vascular endothelial cells.

> Yes. I can see the difference of traditional CS on the induction of molecular response in Figure 4A, induction of protein expression profile related to lipid-metabolism (Figure 5C), induction of oxidative stress related proteins (Figure 6), increased hematocrit to compensate for low oxygen carrying capability from smoking of traditional cigarette (3R4F).

> No, I didn't notice any substantial effect. I do see changes in 3 months porphyrin and fatty acid elongation, 8 months pyrimidine and purine metabolism, and retinal metabolism. But according to the authors, there were no statistical difference comparing to Sham.

> no

SECTION: TRANSLATABILITY AND OUTLOOK

ID #1918 (https://scipinion.com/surveys/67/results/1918)

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)?

	Good model	Poor model	Other (please explain below)	Total
Inflammatory diseases	**72.73%** 8	**18.18%** 2	**9.09%** 1	11
Respiratory diseases	**72.73%** 8	**18.18%** 2	**9.09%** 1	11
Cardiovascular diseases	**90.91%** 10	**0.00%** 0	**9.09%** 1	11
Neurological diseases	**18.18%** 2	**36.36%** 4	**45.45%** 5	11
Hepatotoxicity	**45.45%** 5	**27.27%** 3	**27.27%** 3	11
Metabolic syndrome / diabetes	**36.36%** 4	**27.27%** 3	**36.36%** 4	11

ID #1919 (https://scipinion.com/surveys/67/results/1919)

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.

	Not appropriate	Moderately appropriate	Very appropriate	Total
Dose	**18.18%** 2	**54.55%** 6	**27.27%** 3	11
Regimen	**9.09%** 1	**63.64%** 7	**27.27%** 3	11
Duration	**27.27%** 3	**63.64%** 7	**9.09%** 1	11
Biomarkers of exposure	**9.09%** 1	**27.27%** 3	**63.64%** 7	11

ID #1920 (https://scipinion.com/surveys/67/results/1920)

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?

> None using this model. The measurement of nicotine is likely accurate, however I do not trust the model utilized, so it is hard to extrapolate this to "which mechanisms are relevant for human disease".

> From the data presented in this collection of murine studies, I feel that the CS exposure paradigm revealed both atherogenic mechanisms as well as COPD mechanisms that would be initiated in humans following a period of smoking. Oxidative stress responses, inflammation resulting in plaques in CVD and specific induction of pathways leading to ECM damage in COPD.

> oxidative stress, emphesematous changes, atherosclerosis are all major disease mechanisms

> 1. ECM degradation 2. Oxidative stress 3. Inflammation 4. Aortic arch lipid deposition

> Pathobiological mechanisms are translatable. The inherent resistance of mice to disease complications needs to be investigated as it can serve to better define human response to injury.

> Initiation of inflammation in the lung followed by pulmonary scarring and development of COPD.

> Mechanism of emphysema and chronic bronchitis and to some extent cardiac response.

> Potential CVD effects of CS in individuals with ApoE deficiency. Not sure how this will apply to the general population since this "short cuts" the long term repeated daily inhalation of CS.

> Oxidative stress and UPR together with inflammatory responses contributing to lung emphysema and vascular pathologies (e.g. plaque formation)

> In my opinion, the animal model exposed to CS provides a fairly comparable panorama of the CS effects on humans. Despite the obvious differences in physiology of mouse and humans, mouse biological responses of the lung and liver can be somehow extrapolated to humans. The data presented in all cited papers provided a strong background to move to the research next level: human studies.

> chronic inflammatory reponse

ID #1921 (https://scipinion.com/surveys/67/results/1921)

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?

No. I think these studies have captured most of the aspect of CS of concern and showing the lack of hallmark responses including biomarkers of toxicity when used the modified heat-not-burn tobacco delivery methods. I would like to see if such products can also be made available for "Sheha" and "Hukka" or water-pipe users.

No suggested alternatives

1. Micro CT thorax for the best assessment of emphysema 2. Transthoracic echocardiography, 24 hr BP for cardiovascular function 3. Micro CT for anti-gravity skeletal muscle for skeltal muscle loss 4. fMRI for brain for cognitive dysfunction

Studies that are compliant with OECD test guidance and studies that include dose response to conduct quantitative risk assessment for THS2.2 compared to air. Addition of the informative biomarkers shown here to a guidance study. Unfortunately the rats in the preferred species for inhalation toxicology all of these studies were done in mice that have limited historical controls.

It would be helpful to see a more comprehensive concentration-response analysis.

No - The methodologies seem very comprehensive and should capture the effects of 3R4F and THS2.2 on the respiratory tract. Suggest focusing on which cell types in the lung are responding to the CS and THS2,2 vapors to more accuarately develop a mode of action risk assessment for CS and THS2.2.

I think that the majority of the measurements conducted are reliable. The only major issues are the statistical comparisons and the model utilized.

No additional methodologies suggested - move to clinical studies

An interesting experiment would be testing the effects of nicotine alone (and byproducts). I think in the future the nicotine will be the only chemical available for the lung. I think the other chemical products derived from tobacco heating will be meaningless.

In my opinion the methodologies used in these studies are appropriate

none suggested

ID #1922 (https://scipinion.com/surveys/67/results/1922)

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. 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?



Legend

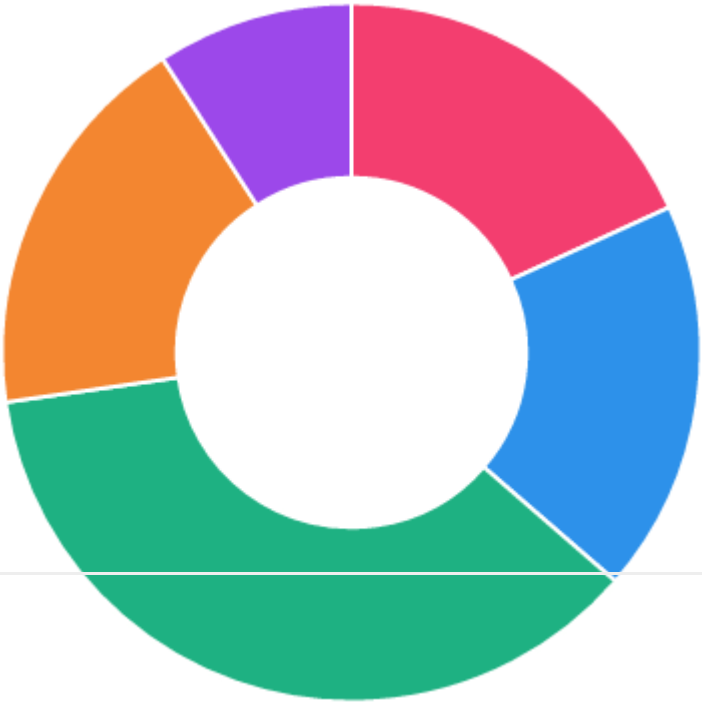
- Extremely confident: 2 *(18%)*
- Very confident: 3 *(27%)*
- Confident: 4 *(36%)*
- Somewhat confident: 1 *(9%)*
- Not confident: 1 *(9%)*
- I cannot answer (please explain in the comment box): 0 *(0%)*

answers: 11

skips: 0

ID #1923 (https://scipinion.com/surveys/67/results/1923)

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. 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?



Legend

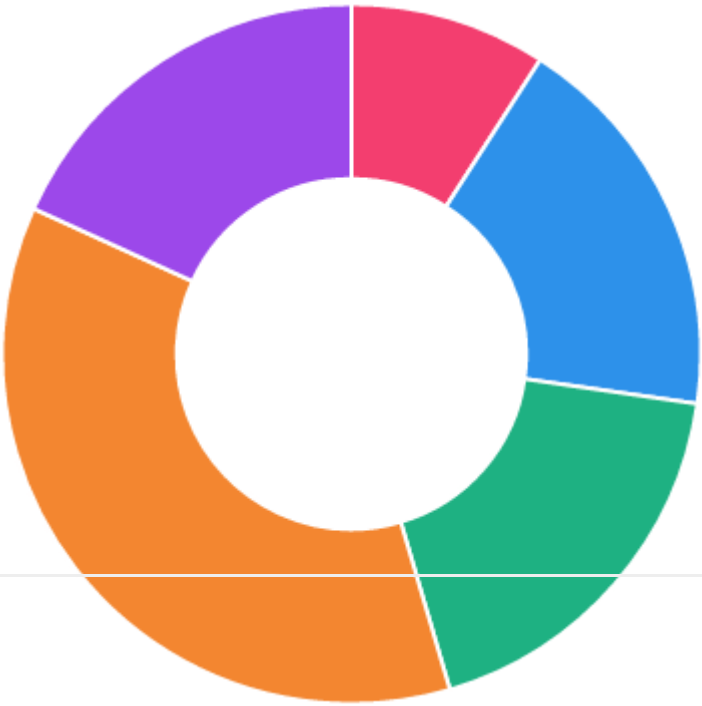
- Extremely confident: 2 *(18%)*
- Very confident: 2 *(18%)*
- Confident: 4 *(36%)*
- Somewhat confident: 2 *(18%)*
- Not confident: 1 *(9%)*
- I cannot answer (please explain in the comment box): 0 *(0%)*

answers: 11

skips: 0

ID #1924 (https://scipinion.com/surveys/67/results/1924)

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?



Legend

- Extremely confident: 1 *(9%)*
- Very confident: 2 *(18%)*
- Confident: 2 *(18%)*
- Somewhat confident: 4 *(36%)*
- Not confident: 2 *(18%)*
- I cannot answer (please explain in the comment box): 0 *(0%)*

answers: 11

skips: 0

ID #1925 (https://scipinion.com/surveys/67/results/1925)

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.

Yes, the findings are translatable to other environmental exposures. For instance, oxidative stress is a common pathway for environmental injury (Miller and Ramos, 2004).

They could be translated to risk assessment of other toxicants. For instance in case of chronic air pollution changes in lipid metabolism and activation of inflammatory responses likewise appears to contribute to aspects of atherosclerosis identified using the same Apoe- model (Circ Res. 2014 Oct 10;115(9):770-80). Likewise, in studies examining "Beijng ambient particle exposure using the Apoe- model, again inflammation, oxidative stress and altered lipid metabolism were identified in parallel with enhanced signs of atherosclerosis (Cell Stress Chaperones. 2014 Sep;19(5):715-24). This points to common mechanisms that can be leveraged for risk assessment purposes.

In my opinion, only for comparison as different environmental air pollutants may be effecting through different mechanism. For example, what to expect if these biomarkers are observed.

Yes, because the cigarette smoke effect findings can be leveraged for risk assessments of the same or other inhaled toxicants found in workplace and environmental exposures. Examples would included fine particulates, organic and inorganic chemical mixtures such as emissions from coal-fired power plant which contain similar types of chemicals. Persons occupationally exposed to PAHs as cooks in the restaurant industry might be another group of concern.

None that I am aware of.

I do not believe so. Each study should be treated uniquely, as there is too much variability in sample preparation, data analysis etc.

Not, not without a dose response

Certainly yes. I would include the particulate matter (PM). 1. Teng TH, Williams TA, Bremner A, Tohira H, Franklin P, Tonkin A, Jacobs I, Finn J. A systematic review of air pollution and incidence of out-of-hospital cardiac arrest. J Epidemiol Community Health. 2014 68(1):37-43 2. Bhatt SP, Dransfield MT. Chronic obstructive pulmonary disease and cardiovascular disease. Transl Res. 2013;162(4):237-51 3. Weichenthal SA, Godri-Pollitt K, Villeneuve PJ. PM2.5, oxidant defence and cardiorespiratory health: a review. Environ Health. 2013, 4;12:40

cigarett smoking may not e comparable with other ihaled toxicants in terms of dose and frequency.

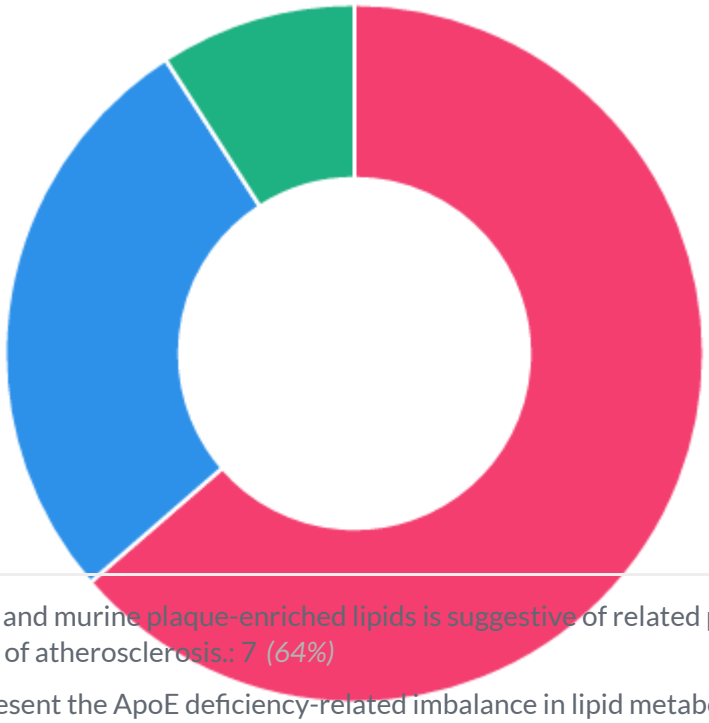
1. Cigarette contains more than 5000 chemicals and its toxicity is further amplified by its burning. If there is any considerable overlap between active chemicals and the burning process, one can confidently leverage the findings of cigarette smoke to risk assessment of other inhaled toxicants, such as

> environmental air pollution. 2. So kitchen smoke and industrial smoke and some level vehicular smoke may get such constituent and process overlap, indeed, Indian women who exposed to biomass fuel used kitchen smoke develop cigarette smoke exposure like COPD (Koul, Lung India. 2013 Jul-Sep; 30(3): 175–177). 3. The workplace exposure mostly has single dominant chemicalwithout burning process like silica, cotton, asbestas etc. Mostly these exposures leads to decline in lung function but in a different way like developing lung injury followed by fibrosis. The site of fibrosis is different in emphysema and these diseases. There is small airway remodeling in COPD whereas parenchymal fibrosis is observed in most of the work exposure or occupational lung diseases. So the translation is difficult in some conditions and partially relevant in some conditions. There is a necessity of comparison and side by side chemical analysis of the components smoke and other toxic inhalants before attempting to such leveraging.

> Yes, particularly for air-pollution related effects.

ID #1926 (https://scipinion.com/surveys/67/results/1926)

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:



Legend

- The correlation between human and murine plaque-enriched lipids is suggestive of related pathomechanisms in plaque formation in this mouse model of atherosclerosis.: 7 *(64%)*
- The three unchanged lipids represent the ApoE deficiency-related imbalance in lipid metabolism, particularly the disrupted reverse cholesterol transport.: 3 *(27%)*
- 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: 1 *(9%)*
- Other (please explain): 0 *(0%)*

answers: 11

skips: 0

ID #1927 (https://scipinion.com/surveys/67/results/1927)

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?

Yes, the more comprehensive the better, especially when considering the introduction of a "safer" alternative.

No, I believe examination of nose/lung (histopathol, omics, and functional) combined with analysis of blood samples and cardiovascular system is in fact sufficient to capture the critical impacts.

I do not think that there are any other tissues/samples that need to be analyzed. Had WT mice been used, I think this study would be much more "translatable". In addition, correct statistical comparisons should have been conducted (previously discussed).

I consider these studies to be quite comprehensive and cannot think of any additional aspects for future studies. However, may like to add or subtract depending upon the nature of the study and underlying questions/issues needing answers.

no other suggestions

Reproductive organ systems are worthy of study since a number of hormones are similar among mammals. These organ systems could respond on either a primary or secondary basis.

Again OECD inhalation study (90 day or chronic) in wild type rats using THS2.2 will likely be needed at some point with examination of 40 tissues.

CS exposure has being implicated in many toxic effects in other organs, tissues and reproduction: eyes, brain, skin, join, muscles, stomach, bladder, intestines, reproductive organs, bone marrow cell production, embryo development problems. Some conditions such as Rheumatoid Arthritis, diabetes, Inflammatory Bowel Disease, Immune Function and Autoimmune Disease, Ectopic Pregnancy, Breast Cancer and others have also been implicated with CS exposure.

1. Skeletal muscle as skeletal muscle loss has been observed in most of the severe COPD patients. In addition, GOLD stage severity has been shown to correlated with loss of particular skeletal muscle like anti-gravity muscle like erector spinae (Tanimura K et al, Ann Am Thorac Soc. 2016 Mar;13(3):334-41). In mice soleus, anti-gravity muscle can be tested and the methods like fMRI or micro CT for assessing cross sectional area, muscle electrophysiology, walking tests along with other omics. 2. Brain: The cognitive dysfunction has been observed in most of COPD patients. The possible reasons could be hypoxia and hypercapnia. Micro CT and fMRI brain and EEG along with other omics approach can be tried.

Tissues examined for the end points are appropriate

ID #1928 (https://scipinion.com/surveys/67/results/1928)

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%** 6	**30.00%** 3	**10.00%** 1	**0.00%** 0	**0.00%** 0	**0.00%** 0	10
Clinical, short term	**27.27%** 3	**45.45%** 5	**27.27%** 3	**0.00%** 0	**0.00%** 0	**0.00%** 0	11
In vivo	**54.55%** 6	**36.36%** 4	**9.09%** 1	**0.00%** 0	**0.00%** 0	**0.00%** 0	11
In vitro	**0.00%** 0	**30.00%** 3	**40.00%** 4	**30.00%** 3	**0.00%** 0	**0.00%** 0	10

ID #1929 (https://scipinion.com/surveys/67/results/1929)

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 (Scale of 1-5, 5 being the highest)?

	Importance	Strength	Total
Inflammation	*4* **9.09%** 1 *5* **90.91%** 10	*2* **9.09%** 1 *4* **36.36%** 4 *5* **54.55%** 6	11
Oxidative stress	*2* **9.09%** 1 *3* **9.09%** 1 *4* **36.36%** 4 *5* **45.45%** 5	*1* **9.09%** 1 *2* **9.09%** 1 *4* **45.45%** 5 *5* **36.36%** 4	11
Extracellular matrix (ECM) production, deposition and degradation	*3* **18.18%** 2 *4* **27.27%** 3 *5* **54.55%** 6	*3* **27.27%** 3 *4* **54.55%** 6 *5* **18.18%** 2	11
Metabolic alterations	*2* **9.09%** 1 *3* **45.45%** 5 *4* **36.36%** 4	*3* **36.36%** 4 *4* **54.55%** 6 *5*	11

	5 **9.09%** 1	**9.09%** 1	
Other (please specify)	*1* **25.00%** 1 *3* **25.00%** 1 *4* **25.00%** 1 *5* **25.00%** 1	*1* **33.33%** 1 *3* **33.33%** 1 *5* **33.33%** 1	4

ID #1930 (https://scipinion.com/surveys/67/results/1930)

Specific disease mechanisms can be appropriately studied in in vitro models. PMI tested RRPs with assays that recapitulate two mechanisms relevant to atherosclerosis, namely adhesion of monocyte to endothelial cells (Poussin et al. 2016 Toxicology 339(2):73-86) and transendothelial migration (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.

> THS2.2 appears to be less atherogenic than 3R4F. In vitro models recapitulate in vivo biology if used properly and if data is interpreted with caution.

> (i). I agree with the authors' conclusions that aqueous aerosol extract from THS2.2 reduced the effects of the monocytic cells adhesion to human coronary endothelial cells comparing with 3R4F. The authors measured 9 different analytes in both tested aqueous extractions, and with exception for nicotine, all chemicals were lower in THS2.2 fluid. (ii). I think it is an elegant experiment especially using human cells to test the atherogenic effects of THS2.2 and 3R4F aerosol exposure.

> From Poussin et al 2016 is is clear that 3R4F promotes adhesion of monocytic cells to coronary arterial endothelial cells and that this is strongly reduced for THS2.2 exposure (some effect at higher concentrations). From van der Toom et al 2015, studying chemotactic migration of monocytic cells through a monolayer of coronary arterial endothelial cells is is clear that treatment with 3R4F induces monocyte production of cytotoxic and inflammatory cytokines and decreased transendothelial migration. Evidence is shown that THS2.2 has significantly reduced effects over 3R4F on these parameters. It can be concluded that THS2.2 exposure shows reduced atherogenic-like effects as compared to 3R4F in vitro. These in vitro studies provide some additional insight to the in vivo atherogenesis studies.

> As indicated, the ApoE background is providing a significant level of background plaque formation in these mice. Overall, this type of assay would be extremely helpful and it is my belief, based on the other data (i.e. the other manuscripts provided) that the effects on plaque formation would be much more drastic in the THS2.2-treated mice if they had been used in a different model. I think that something that is getting lost here is that this is simply a better alternative to the 3R4F cigarettes and should not be considered as "healthy" and completely devoid of the effects typically observed in "3R4F-smoking patients.

> (i) THS2.2 aerosol contains a much lower concentration of atherogenic components than found in CS aerosol, based on the adhesion and migration assays. (ii) The in vitro assays allow for carefully controlled conditions involving specific cell types and experimental designs to investigate exogenous administration of complex mixtures (aerosols). These studies can unambiguously point to chemicals that alter functions or capabilities of specific cell types (e.g. monocyte adhesion to endothelial cells). One of the caveats is that the in vitro 'environment' is not a good reflection of the in vivo complexity and temporal dynamics cannot be modeled easily.

Gene expression changes in vitro are dose and time course dependent and to mimic an inhalation exposure is problematic. In vitro exposure using the aqueous extracts in this assay is a useful tool to allow dose-response assessment. How this can be quantitatively related to in vivo effects is uncertain.

This is a complex question since physically cells are cultured in aqueous or semisolid media to maintain cellular viability and chemicals in CS or THS2.2 are in a vapor phase which creates a problem with regard to how to create an appropriate exposure system that is relevant to in vivo exposures. One would ideally like to know the degree to which chemicals in a vapor phase partition into an in vitro culture media aqueous phase .

As expected traditional cigarette (3R4F) caused an array of effects for both lung and heart and use of the THS significantly reduced, but not eliminated, these effects. Use of in vivo methods is the gold standard; however, a trend could be observed in vitro allowing to further improve the in vivo design before starting the study in order to conserve the use of animals and save resources.

The method of analyzng adhesion of monocyte to endothelical for transendothelial migraion could be very powerful tool to analyze response to THS2.2 and 3R4F

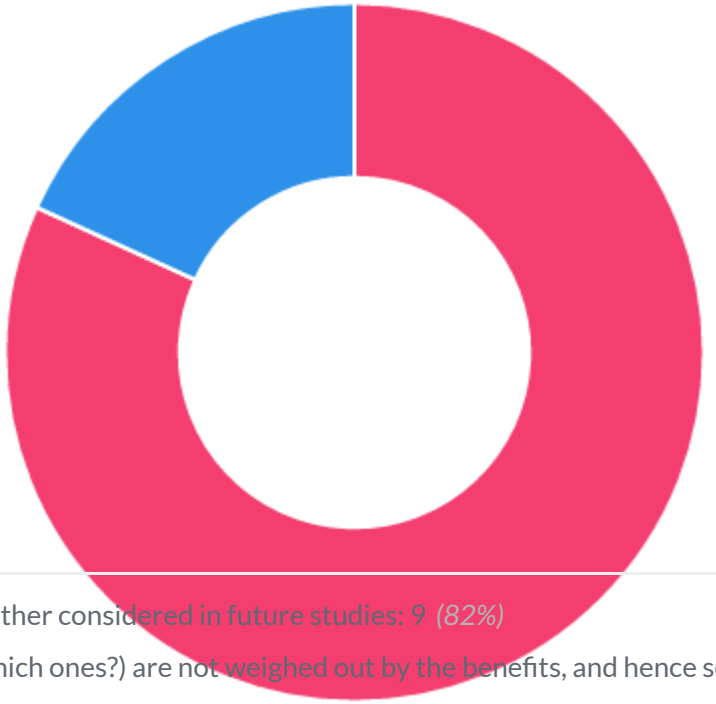
First of all, I would like to mention some drawbacks of the two main in vitro studies mentioned in this review, Poussin C et al, 2016,Toxicology article: 1. Table 2: The concentrations of all 8 carbonyl molecules mentioned in Aerosol Chemistry Page in SysToX Portal (I am not sure how these concentrations were estimated) and Table 2 were different. In any event there will be batch to batch and lot to lot variations. But interestingly concentrations for THS2.2 abPBS of these 8 molecules are more or less similar in Table 2 (except Buyraldehyde) whereas 3R4F concentrations are always higher (Please see comparison Table). I do not know the reason. One needs to consider this discrepancy to avoid unhealthy comparisons. 2. In vitro Adhesion Assay: As per the Materials and Methods, the authors should have used fresh uninduced monocytes for the adhesion assay rather than smoke treated monocytes. In the section 3.2. subtitled “No significant increase of MM6 cell-HCAEC adhesion was observed following fresh direct and indirect exposures to THS2.2 aqueous extract at concentrations exhibiting maximal adhesion with 3R4F aqueous extract“, the authors have interpreted “In the I treatment, the increase of 3R4F aqueous extract concentrations showed a biphasic response curve indicative of cytotoxic effects on MM6 cells beyond a certain concentration range (Supplementary Fig. 2A).” The Supplementary Fig. 2A, had shown increased cell death with increasing the concentrations of 34RF aqueous extract. As per the Materials and Methods the authors have used supernatant of cultured monocytes that were induced with 34RF aqueous extract. But it seems from their statement that they may have used smoke extract induced used monocytes for the adhesion assay because supernatant may not have huge concentrations of TNF-alpha to kill the cells (high concentrations of recombinant TNF−alpha did not cause a reduction in adhesion rate. [Please forgive me if I misunderstood]. 3. The authors have claimed “To achieve similar increases of MM6 cell-HCAEC adhesion with THS2.2 aqueous extract as observed for 3R4F, 10-fold higher concentrations (2.25 puffs/ml) were necessary for FD treatment, and 20–25-fold higher concentrations (1.125 puffs/ml) were required for I treatment (Fig. 1 and Supplementary Fig. 3).” Through this sentence, it seems that the authors had compared 3R4F with THS2.2 inconsistently. Former claim is OK for two reasons: a) no biphasic effect and b) they found similar increase with 2.25 puffs/ml (THS2.2) and 0.225 puffs/ml (3R4F). But latter claim is not perfect for two reasons: a) there was no similar increase between 1.125 with 0.045 and b) there is a biphasic effect/ no dose dependent effect. In this context, Why they did not compare 1.125 with 0.225 ? It seems to be the poor comparison. 4. It is not clear and confusive why the authors had selected 0.06 and 0.225 puffs/ml in I treatment even after the observation of low adhesion rates in these

conditions. Because 0.225 dose had shown very less adhesion rate and the same dose caused more cell death. It seems that the expression analysis (figure 2) was performed with dying and less adhesive cells. This could be strongest reason why these two treatments (0.06 and 0.225 puffs/ml in I treatment) has shown less differential expression when compared to 0.225 dose at FD treatment which had shown very high adhesion rate. Only 0.225 puffs/ml in FD treatment seems to be perfect as this treatment had shown more adhesion rate along with more differential gene expression. All these indicate that there is a poor correlation between the adhesion rate and the gene expression. In this context, there is a less meaning of further analysis. 5. It seems they have not measured any apoptosis parameters and they just relied on the counting of the HCAECs Van der Toorn et al, Food and Chemical Toxicology article: It is not clearly understood why the authors have selected the doses which showed more apoptosis for the chemotaxis studies. It would be very obvious that dying cells move slowly. There is a good correlation between 7-AAD positivity and chemotaxis. For example, 3R4F showed more 7-AAD positive from 0.05 puffs and THS2.2 it is from 1 puffs/ml (Figure 2) and on the other hand the reduction in chemotaxis and TEM starts from 0.05 in case of 3R4F and from 1 in case of THS2.2 (Figure 3). So it seems that the authors had used very high concentration that leads to apoptosis and it did not affect or reduce chemotaxis. The authors had missed an observation of borderline increased TEM with 0.01 dose of THS2.2 (Figure 3B). This indicates that the experiment can be done with lesser concentrations also. In this context, the authors have pointed other hypothesis for substantiate their observations. Question 1: The relative atherogenic effect of THS2.2 and 3R4F aerosol exposure: THS had shown the reduction of both adhesion of monocyte to endothelial cells and TEM. These indirectly suggests that THS may not promote atherogenesis. Question 2: The usefulness of in vitro assays to recapitulate in vivo atherogenic effects: It depends on which in vitro assay has been used for recapitulating in vivo atherogenic effects. For example, the assays like adhesion and chemotaxis of monocytes are very simpler and highly reductionist approach. This approach may be really mimic the atherogenesis. A better model is required to good recapitulation of human atherogenesis. 1. 3D vascular wall model (Robert J et al, PLoS One. 2013 Nov 14;8(11):e79821) 2. Co-culture of arterial wall cells and macrophagemodel Wada Y et al, Yonsei Med J. 2000 Dec;41(6):740-55 3. Coculture model of transmigrant monocytes and foam cell formation (Takaku M et al, Arterioscler Thromb Vasc Biol. 1999 Oct;19(10):2330-9). 4. Co-culture of endothelial cells and smooth muscle cells in modified fibrin gel scaffold to mimic human plaque (Dorweiler B et al, Thromb Haemost. 2006 Jan;95(1):182-9).

The results conclude that the atherogenic potential of 3R4F is much higher than THS2.2. The results also demonstrate the chemotaxis and transendothelial migration to be much lower in THS2.2 treatment group than in 3R4F treatment group. Definitely, the in vitro model system is a surrogate to study atherogenesis.

ID #1931 (https://scipinion.com/surveys/67/results/1931)

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: 9 *(82%)*
- Some compromises in design (which ones?) are not weighed out by the benefits, and hence separate models would be more efficient: 2 *(18%)*
- Further reduction of animals could be envisioned by the following accomplishments: (please specify): 0 *(0%)*

answers: 11

skips: 0

SECTION: TOOLS AND REVIEW PROCESS

ID #1932 (https://scipinion.com/surveys/67/results/1932)

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

> The difference in concentration-response profiles between 3R4F and TSH2.2 need to be explored further. Also, what drives the toxicity of the TSH2.2 exposure?

> Question: Would the animal study reflect with more fidelity the comparison with the human CS exposure with longer exposure time? If the authors started the experiments with 2 months-old mice (not obvious in the Meths.) is more or less equivalent to 20 years in human age. Mice inhaled smoke for 2 months, it means for approximately 6 human years. The recover of the inflammatory indicators after cessation or switching time point is somehow expected at these young ages. I would like to see an exposure time proportionally because in humans usually the symptoms of cardiovascular or respiratory problems come after 20-25 years of smoking.

> To what extent are the mouse studies on the effect of switching, which are necessarily short term as compared to the human situation, predictive for the effect of switching in smokers?

> The lack of dose-response in all of these studies is a significant concern, the lack of a control for THS2.2 (inhaled nicotine?), the use of an ApoE mouse model that is not supported by genomic findings with CS. Will the THS2.2 studies be done in a OECD rat study with a control set of animals inhaling nicotine?

> In your opinion, does the absence of an inflammatory reaction in THS2.2 exposed animals, inferred from the proteomic or transcriptomic analyses, indicate that lung function is normal in these mice?

> Both CS and THS2.2 contain some cadmium which can both cause production reactive oxygen species (ROS) and act as a potent inducer of metallothionein (MT) and a number of other stress proteins. Both oxidative stress and MT induction were observed in the reported studies. How could one separate these 2 types of effects and use the information to support Mode of Action(MOA) based risk assessment to further quantify differences in risk between CS and THS2.2?

> Can the use of modified risk tobacco products be extended beyond traditional cigarette smoking, for example to Shesha and Hukka?

> Do you believe that the THS2.2 is a better alternative to smoking traditional cigarettes? Why? I believe that this is really the question at hand and this was never explicitly stated.

> Do you expect similar datasets for cessation or switching after 6 months 3R4F exposure ?

> Although testing the strong effect of CS on lung cancer using an animal model is a challenge, but it would be informative if a study either on B6C3F1 mouse model or any other appropriate animal model on the carcinogenic potential CS. A more appropriate animal model representing metabolic disease/diabetes or hepatotoxicity such as leptin-deficient or leptin resistant strain to study effects of CS may be more useful.

> I do have question but I forgot. I will try to remember.

ID #1933 (https://scipinion.com/surveys/67/results/1933)

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



Legend

- Yes: 5 *(45%)*
- No: 0 *(0%)*
- I did not visit the portal: 6 *(55%)*

answers: 11

skips: 0

ID #1934 (https://scipinion.com/surveys/67/results/1934)

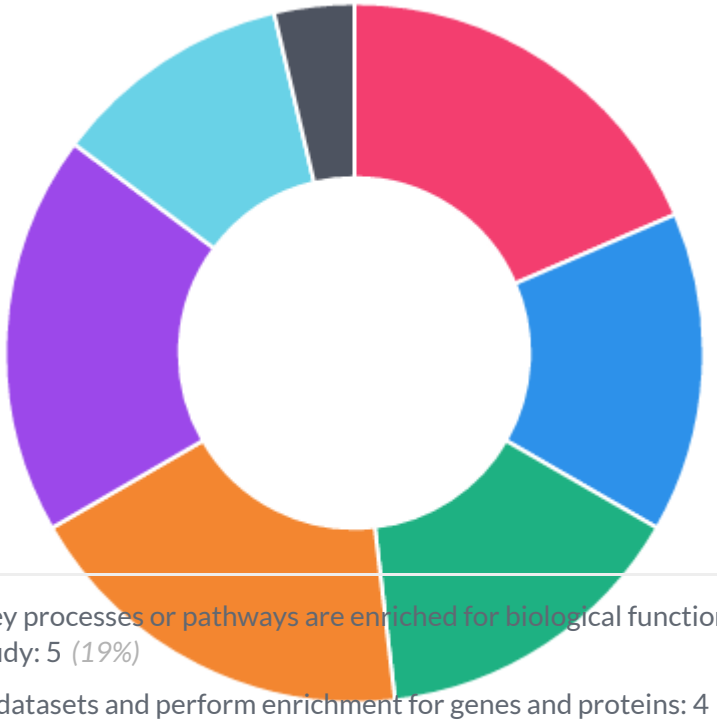
How would you qualify the annotation (e.g. use of ontologies, richness of annotation), and the structure (isa-tab format) of the data available in the underlying database (via the sbv IMPROVER systems toxicology Web Portal) (scale 1-5, 5 being extremely good)?

	Rich	Intuitive	Informative/Helpful	Total
Data annotation	*3* **27.27%** 3 *4* **18.18%** 2 *5* **54.55%** 6	*3* **27.27%** 3 *4* **36.36%** 4 *5* **36.36%** 4	*3* **9.09%** 1 *4* **45.45%** 5 *5* **45.45%** 5	11
Description of protocols (M&M)	*3* **9.09%** 1 *4* **54.55%** 6 *5* **36.36%** 4	*3* **9.09%** 1 *4* **54.55%** 6 *5* **36.36%** 4	*3* **9.09%** 1 *4* **45.45%** 5 *5* **45.45%** 5	11
Use of ontologies	*2* **9.09%** 1 *3* **18.18%** 2 *4* **45.45%** 5 *5* **27.27%** 3	*2* **9.09%** 1 *3* **27.27%** 3 *4* **45.45%** 5 *5* **18.18%** 2	*1* **9.09%** 1 *3* **27.27%** 3 *4* **54.55%** 6 *5* **9.09%** 1	11
Raw data format	*1* **9.09%** 1 *2* **18.18%** 2 *3* **18.18%** 2	*1* **9.09%** 1 *2* **27.27%** 3 *3*	*1* **27.27%** 3 *2* **18.18%** 2 *4*	11

	4 18.18% 2 5 36.36% 4	27.27% 3 4 36.36% 4	45.45% 5 5 9.09% 1	
Processed data format	2 9.09% 1 3 18.18% 2 4 45.45% 5 5 27.27% 3	2 18.18% 2 3 9.09% 1 4 54.55% 6 5 18.18% 2	2 18.18% 2 3 9.09% 1 4 54.55% 6 5 18.18% 2	11
ISA tab format	1 9.09% 1 3 27.27% 3 4 36.36% 4 5 27.27% 3	1 9.09% 1 3 45.45% 5 4 27.27% 3 5 18.18% 2	1 9.09% 1 3 27.27% 3 4 36.36% 4 5 27.27% 3	11
Illustrations in M&M	4 36.36% 4 5 63.64% 7	4 27.27% 3 5 72.73% 8	3 10.00% 1 4 30.00% 3 5 60.00% 6	11

ID #1935 (https://scipinion.com/surveys/67/results/1935)

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



Legend

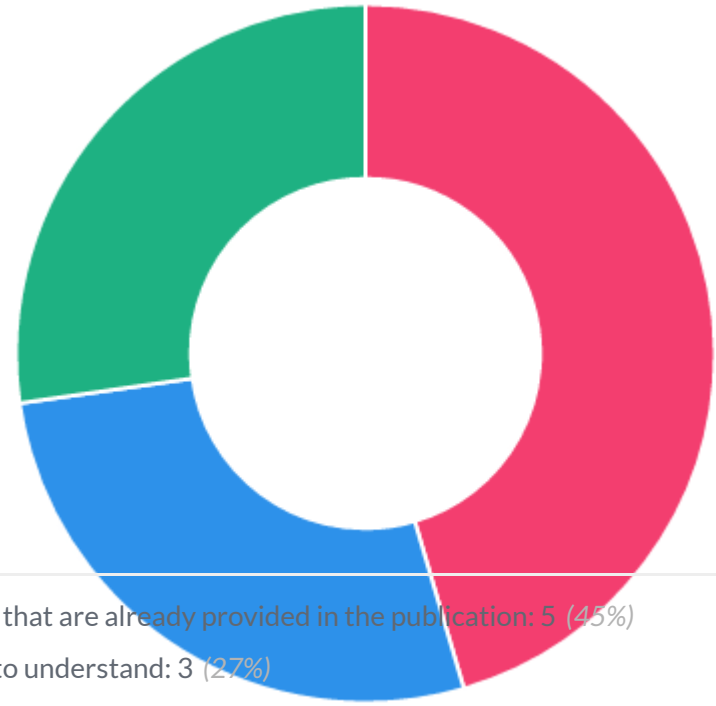
- Allow readers to assess which key processes or pathways are enriched for biological functions for a given set of genes under a specific condition of study: 5 *(19%)*
- Explore large-scale omics study datasets and perform enrichment for genes and proteins: 4 *(15%)*
- Allow readers to assess and compare the transcriptomics profiles for a specific gene or set of genes between two study conditions: 4 *(15%)*
- Allow the visualisation of pathways associated with the genes in transcriptomics profile data: 5 *(19%)*
- Are very useful and should be made available to the whole scientific community: 5 *(19%)*
- I cannot comment on Garuda (please explain in the comment box): 3 *(11%)*
- None of the above: 1 *(4%)*

answers: 11

skips: 0

ID #1936 (https://scipinion.com/surveys/67/results/1936)

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 question-specific link in the Introduction). In your opinion this (select all that apply):



Legend

- Is superfluous given the images that are already provided in the publication: 5 *(45%)*
- Could be useful but is not easy to understand: 3 *(27%)*
- Is useful and should be included as supplementary material for publication of large studies whenever possible: 3 *(27%)*

answers: 11

skips: 0

ID #1937 (https://scipinion.com/surveys/67/results/1937)

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

> no additional info needed

> 1. Studies that help correlate the levels of CS chemicals that mice are chronically exposed to here to levels found in humans over a similar time period. 2. A dose escalation study in mice with both CS and THS2.2. The use of a single dose was a drawback and raises questions about whether THS2.2 becomes more toxic at a higher dose.

> This is sufficient information to demonstrate network connectivity's

> The material presented is fairly complete so cannot think of any additional needed information.

> The data provided in the publications and here were sufficient to understand and make assessment. I don't think any additional information would have changed my understanding of the effects obtained by reading the papers.

> I think videos from the experiment itself could insert a great deal of veracity. Even including the generation of data from a equipment or the execution of an assay. This is based on JoVe journal.

> a) Brief history of study with earlier papers

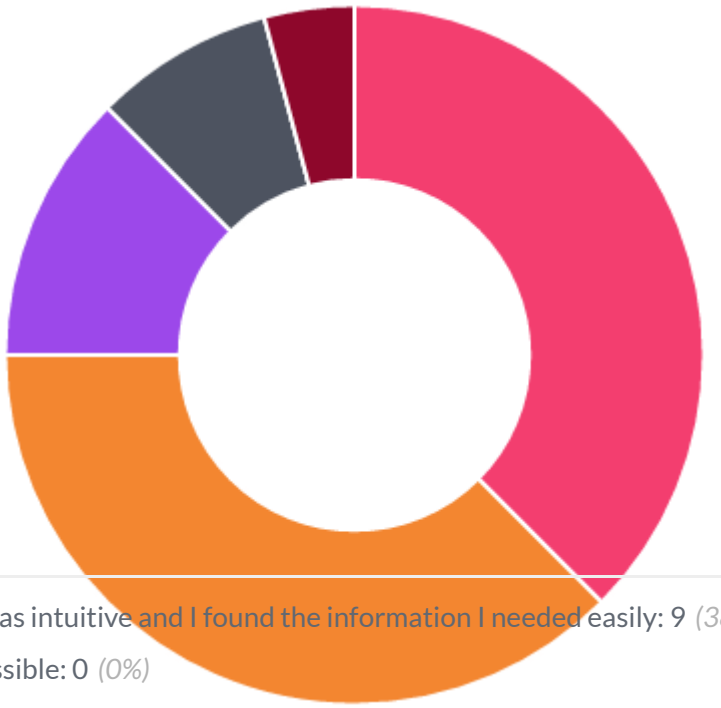
> No additional material needed.

> I think that the portal was rather comprehensive.

> None

ID #1938 (https://scipinion.com/surveys/67/results/1938)

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: 9 *(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: 9 *(38%)*
- The tutorial videos were useful to efficiently review the material: 3 *(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 *(8%)*
- I was missing key functionalities (please specify below): 0 *(0%)*
- I did not visit the portal: 1 *(4%)*

answers: 11

skips: 0

ID #1939 (https://scipinion.com/surveys/67/results/1939)

How could this peer review process be improved?

I think that the way that some of the questions are worded almost places a bias on what the reviewers should be looking for. In a couple of instances, the question pointed out a potential problem that may have otherwise been missed. Also, I felt that a lot of the questions were quite redundant and I found myself using a similar explanation for why the particular study was flawed (i.e. the use of the ApoE-/- mice)

The overall review contains a large amount of data in many figures where the underlying data (i.e. gene/protein array data) are themselves complex. Nearly impossible to examine all in detail.

I think if papers are provided in a format where reviewers can actually write their comments, in addition to or instead of the questions, will be very helpful. Personally, the first-pass in reviewing a paper is to write my thoughts in the margins and then go back to expand them.

This has been a very good process thus far

No Suggestions

no additional suggestions

1. Time can be extended to one more month for effective review.

Less material to read and process at one time. It may have been more valuable to evaluate each one of the papers individually, and to build on the knowledge gained, rather than to read all articles at once and then answer questions for each of them.

N/A

I think one small limitation is the need of a computer connected to the internet. It means one cannot work during a flight, or any situation without internet. It could be interesting working off line.

no suggestion curretly

ID #1940 (https://scipinion.com/surveys/67/results/1940)

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

> No suggestions.

> Provide someway to dovetail into ongoing regulatory processes e.g., AOPs

> No suggestions

> The present format is really nice. I humbly following modifications (my personal opinion) a) Brief history of all studies with earlier papers may be given in Background section b) Mechanisms menu bar (heading) may not be required for an experienced reviewers c) SciPiTM Questions menu bar (heading) may not be required

> see #8 above.

> no additional suggestions

> No comment

> N/A

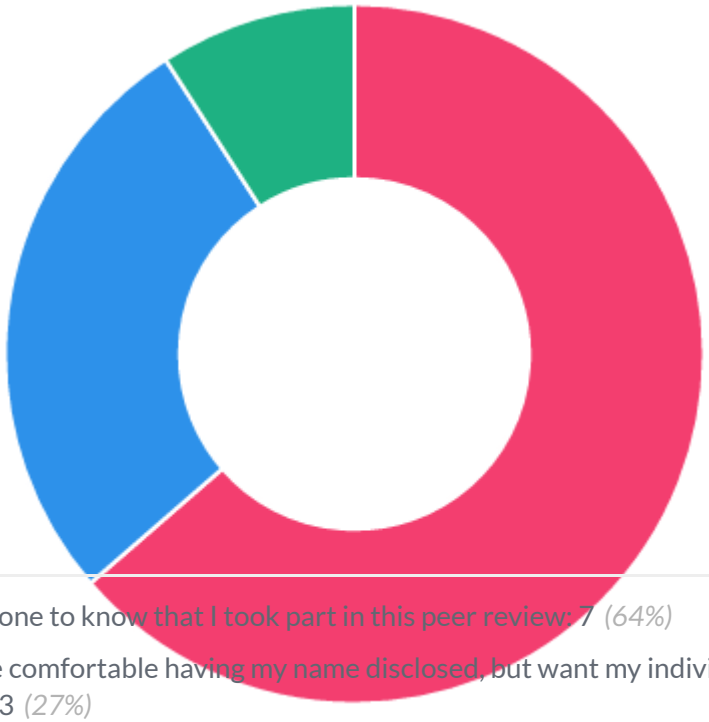
> I think it is very robust and filled all my needs.

> Overall, I do not have any major issues with the IMPROVER web portal.

> no suggestion

ID #1941 (https://scipinion.com/surveys/67/results/1941)

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

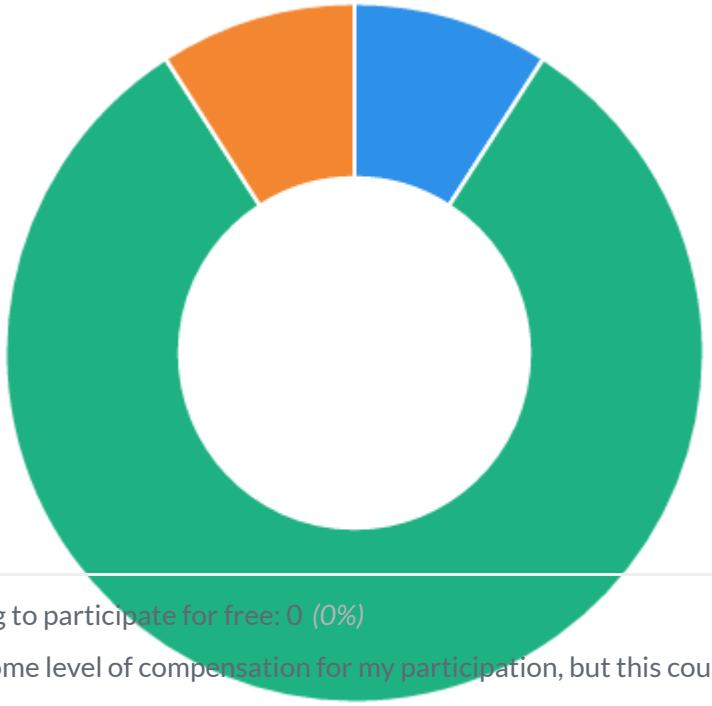
- Very important: I don't want anyone to know that I took part in this peer review.: 7 *(64%)*
- Somewhat important: I would be comfortable having my name disclosed, but want my individual responses to the questions to remain undisclosed.: 3 *(27%)*
- Not important: I would be comfortable having my name and individual responses to the questions disclosed.: 1 *(9%)*

answers: 11

skips: 0

ID #1942 (https://scipinion.com/surveys/67/results/1942)

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



Legend

- Not important: I would be willing to participate for free: 0 *(0%)*
- Somewhat important: I prefer some level of compensation for my participation, but this could be less than received for this effort: 1 *(9%)*
- Important: I require compensation for my participation, and the level of compensation is appropriate: 9 *(82%)*
- Very Important: I require compensation for my participation, but the level of compensation would need to be increased: 1 *(9%)*

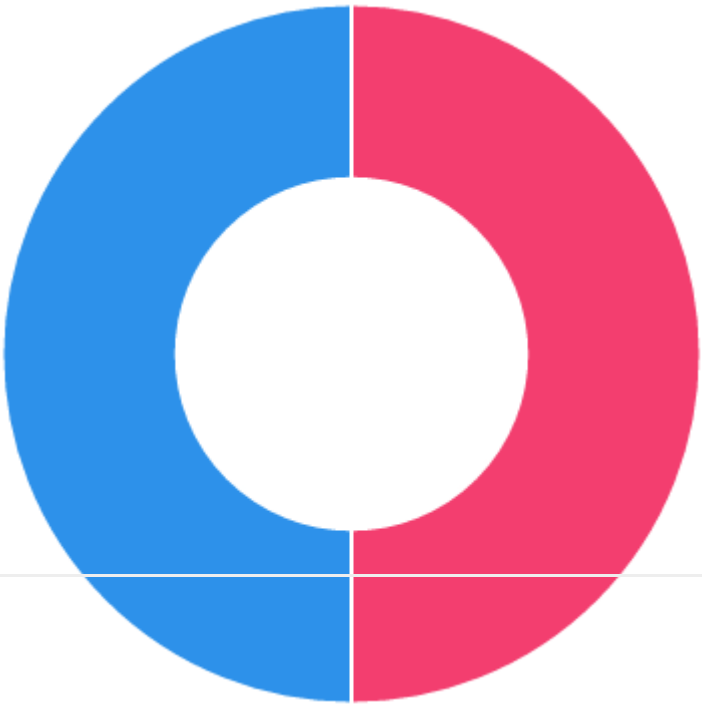
answers: 11

skips: 0

SECTION: POST DEBATE QUESTIONS

ID #2062 (https://scipinion.com/surveys/67/results/2062)

In your opinion, does the absence of an inflammatory reaction in THS2.2 exposed animals, inferred from the proteomic or transcriptomic analyses, indicate that lung function is normal in these mice?



Legend

Yes: 5 *(50%)*

No: 5 *(50%)*

answers: 10

skips: 0

ID #2063 (https://scipinion.com/surveys/67/results/2063)

To what extent are the mouse studies on the effect of switching, which are necessarily short term as compared to the human situation, predictive for the effect of switching in smokers? If not, what would be the better alternative?

Animal models are always limited in reproducing the human condition.

The chemistry of the THS2.2 product and the effect of switching are clear under the conditions used here in this human model of disease. Some of my response to question 1 applies here. Although the authors and the design of the study makes this clear, convincing regulators is entirely different. No DR, and a shortened time frame using a diseased animal model with a high spontaneous disease necessitating shortened timeframes of study is problematic assuming FDA will regulate these product like a small molecule for non-life threatening disease indication. A clinical trial in human would be useful here with some dose-responsive biomarkers.

For recovery of lung function and absence of signs of COPD-related markers the short term study is sufficient. For mutagenesis-related effects it is not.

This was one sensitive point I raised during the survey. Since I study aging, the age equivalence between mouse and human is part of my experimental discussion. They are of course different organisms but is it well accepted in the field an age equivalency. So I think the experiment should had considered longer exposure times for CS to become more comparable to human situation.

a study where animals are exposed for half of their natural expected life and observation up to their death rather than sacrificing at specific study-day

The answer here is complicated by intrinsic differences in longevity and biology of the 2 species. These factors will complicate predictions since one would need to relate mouse years to human years in a defensible way and provide assurances on the similarities between mouse and human lung cells for the selected parameters to be measured. That said, the pulmonary systems between the 2 species are clearly analogous and some useful predictive information should be generated in a broad biological sense. One just must acknowledge the limitations of the model. Any other non-human model will also have similar limitations.

I would be confident that the switching effects on inflammatory responses would carry over to humans. I am less confident about how the vascular changes would be modified.

In my opinion, this is a very illogical comparison. Firstly, we don't have available data or at least data presented to the reviewers on the parameters examined as a comparison between rodent and humans. This is more of subjective question. What is important and significant from the animal studies is the unbiased, controlled exposure treatments between the animal groups and the differences in the results observed among the treatment groups.

Short term switching in mouse models would not be able to predict the real switching effects in humans as

> these short time switching cannot reflect exact human pathology. So long term switching would be the better alternative.

> This is a truly challenging question. Short-term in a mouse is not necessarily short-term in a human. I think the real challenge is interpreting these data in the KO mouse model with out a control. It is hard to say what the true "end-points" should be.

ID #2064 (https://scipinion.com/surveys/67/results/2064)

Both CS and THS2.2 contain some cadmium which can both cause production of reactive oxygen species (ROS) and act as a potent inducer of metallothionein (MT) and a number of other stress proteins. Both oxidative stress and MT induction were observed in the reported studies. How could one separate these 2 types of effects and use the information to support Mode of Action(MOA) based risk assessment to further quantify differences in risk between CS and THS2.2?

> By inclusion of additional controls in the experimental design. Also, use of proxy indicators of non-specific responses.

> Both CS and THS2.2 share in common an number of items that are listed in the FDA HPHC list, e.g., the neurotoxin acrylamide and the heat not burn emissions has not been comprehensively examined rather the focus was on the HPHC list. This needs to be addressed using proper DR study to identify shape of dose-response curve for these effects at the genomic and protein level to identify the TTC based on either oxidative stress of MT induction.

> The correct experiment here would be to expose the mice to Cd-enriched aerosol without tobacco products and revisit all the parameters. Also, estimate the Cd concentrations in blood, kidneys, lung, bone, brain and liver.

> Based the data presented these two effects can not be separated. In vitro studies using lung epithelial cell cultures or a lung-on-a-chip model could be combined with ROS scavengers to dampen ROS production and assess to what extent effects are caused by ROS production.

> Cadmium is a major contaminant of tobacco products (~1-5ug/cigarette) which gets into this crop via the use of cadmium containing phosphate fertilizers and active uptake of this metal by tobacco plants. Other metals such such as mercury are sometimes present at much lower or non-dectectable concentrations. The result of this phenomenon is that smokers are known to have renal cadmium concentrations about 2 times those of non-smokers with females about 2 times higher than males(See CDC NHANES). Cadmium also induces MT and produces oxidative stress. Separating these 2 concomitant types of effects should help support MOA risk assessments in delineating differences in risk between CS and THS2.2 by providing basic information on the roles of MT in mediating the sources of oxidative stress(eg. cadmium versus ROS generated by other CCS/THS2.2 chemicals) in this model system. The use of MT knockout mice, as suggested by another reviewer, should yield some useful information in this regard.

> I think overall it would be hard to discriminate the chemical constituents that drive these events, whether cadmium or others. Testing of each, one by one in ROS assays and then looking at protein expression or MT activity in vitro is one way to obtain MOA.

> The basic principle of toxicology is -Dose makes the poison. It is the relative dose of these metallic constituents in the aerosol that would determine the extent of ROS production or induction of metallothionein. Cells have antioxidant reserves such as glutathione to check and balance ROS. However, how rapidly they are exhausted and replenished would depend on the overall dose of the toxicant (which

could be cadmium or others). When considering the toxicity from aerosol exposure from CS or THS2.2, one needs to consider the relative doses and bioavailabilities of these toxicants in the organ systems examined.

First of all I am not sure why biasedness has been given to cadmium in the question as both CS and THS2.2 contain almost equal quantities of mercury (compared to cadmium) which can cause production of reactive oxygen species (Olivieri G et al, J Neurochem. 2000;7:231-6) and induce metallothionein even more potently than cadmium (Kramer KK et al, Toxicol Appl Pharmacol. 1996;136:94-100). However, the CS or THS exposures to metallothionein knockout mice would separate these two types effects at modest levels though not at complete levels as oxidative stress and MT induction are having feedback controls.

Well, I am not sure that this is that imperative in this sense. While Cd is a potent inducer of both oxidative stress and MT, the amount detected in the THS2.2 is > 2 orders of magnitude lower than that of CS (CS = 63.7 ± 3.3ng and THS2.2 = 0.4 ± 0.01ng). With this massive difference in Cd concentrations between the two groups, there should be a massive difference in both ROS and MT induction. Again, I think that the biggest thing to assess here is that the THS2.2 system is designed to be a modified risk tobacco product, and it seems to me that decreasing the Cd concentrations so drastically should undoubtedly reduce the risk.

ID #2065 (https://scipinion.com/surveys/67/results/2065)

Two reviewers expressed concern over the lack of a dose-response for CS and THS2.2. They stated: “The difference in concentration-response profiles between 3R4F and TSH2.2 need to be explored further”. “The lack of dose-response in all of these studies is a significant concern”. In your opinion, does the lack of testing for dose-response for both CS and THS2.2 limit the conclusions about the effects of THS2.2 and/or switching in a disease model? Would a 28/90-day inhalation study in rats following OECD guideline 412/413 be sufficient to address this concern?

> Dose-response is a tenant of toxicology. Such data always adds credibility and validation. The suggested study does not replace dose response study.

> Qualitative single dose, single time point , shortened study length using diseased animals data as shown in all of these, unfortunately does very little to support the types of quantitative questions that appear to be a significant part of the post-debate. Significant resources used here could have been used to do the proper regulatory studies first then development of appropriate disease models. I still have not seen support based on the genomics data for use of this animal model beyond this narrow application here. There was no down regulation of ApoE in WT mice in the omics data and ApoE mice represent a small fraction of the human population that are smokers. Again, the THS2.2 as regulated by FDA may likely require a full tox package as used for IND up to and beyond phase III clinical trials including a chronic cancer bioassay and a multi-generation repro tox bioassay.

> Dose-response it definitively an important physiological parameter to strengthen the experiment. However, I don't think it limited the conclusions. This new suggested experiment would be great to address this point.

> The suggested inhalation study in rats would indeed address this concern. However, I do not feel that this is in fact a real concern given the strong significant differences between CS and THS2.2 at dosages matched for nicotine exposure.

> qualitatively, no. quantitatively, yes. A dose-response study is needed in order to make stronger argument.

> The lack of dose-response data for CS and THS2.2 is a problem since, in its absence, one not know the shape of the dose-response curve or where data from the present studies might be placed on the curve. As noted by the 2 reviewers, this limits the conclusions which may be drawn for both the effects of THS2,2 and/or switching. I support the addition of dose-response studies for this reason. The OECD 412/413 inhalation studies in rats would not address the concern since such studies would be in another species and the issue here is about dose-response in this special strain of mouse. It would be hard to make comparisons. On the other hand, the guidelines specify rodents which could also include mice so why not use mice for the dose response studies?

> Yes, I believe the lack of additional exposure across a range of particulate concentrations leaves one less confident that THS2.2 has dramatically less biological impact than CS and weakens the argument that

> switching is as useful as cessation.

> In my opinion, testing for dose-response for both CS and THS2.2 does not limit the conclusions of the study about the effects of THS2.2 and/or switching in a disease model. However, the inclusion of does-response would potentially address some of the concerns regarding the toxicant levels and also provide mechanistic insights with regard to the observed physiological/pathological differences between the two groups.

> The problem would persist even one does 28/90-day inhalation study in rats following OECD guideline 412/413 if the authors are not changing the study design. I have expressed my concern in the question "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?". Summary: From the data, it seems that the authors have planned 5 small studies (5 different time points) and each one has multiple groups and multiple endpoints. This may be potent reason for the lack of dose-response relationship. Rather than this approach, with a reduction of some endpoints, common pool experiments like All (all time exposure) mice should be given similar exposure and every time point particular groups will be sacrificed.

> I agree very much with this sentiment. A dose-response would be very informative.

ID #2066 (https://scipinion.com/surveys/67/results/2066)

Several experts expressed concerns over the duration of exposure. They stated: “Do you expect similar datasets for cessation or switching after 6 months 3R4F exposure?”. “Would the animal study reflect with more fidelity the comparison with the human CS exposure with longer exposure time?”. QUESTION: Would you expect to see similar responses to CS, THS2.2 and/or switching after longer durations of exposures?

> As stated earlier, the data may vary as a function of duration of exposure. Needs to be tested.

> The length of exposure can have a profound impact on a number of endpoints. In cancer bioassay with DNA reactive carcinogens like ethylene oxide tumors do not begin to arise until after 1 yr of exposure, this is not unique, a number of compound exposure related effects occur later in rodent. Again a proper dose-response and time course study will likely be needed to convince regulators. In the case of humans it is uncertain when smokers have reached a "point of no return", i.e., no matter what they do progression of disease is irreversible; so at some point switching may have no measurable beneficial effect. Shortened studies like the one reported here leave this unanswered.

> I expect to see a worsening of the physiological parameters and more comparable to the human exposure scenario. Lung cancer is one of the most dramatic consequences of tobacco smoke for humans and this was by far not seen in any animal here with CS exposure only.

> Indeed, as was my concern longer exposures may be needed to more realistically model the effect of switching in humans. However, I do not think moving from 3 to 6 months exposure before switching is useful. The model as it it stands has been used optimally in that respect. The short time nature of these studies in mice will always limit the representativeness for a human situation where switching would occur after many years of smoking.

> It is difficult to predict effects of longer exposure and yes, longer exposure will be more relevant for comparison with humans.

> Yes, I would expect some similar responses to CS and THS2.2 but varying by degree of severity but with cell death and replacement phenomena and scarring superimposed on top. These predictable effects may complicate interpretation of the molecular datasets but provide more information on ultimate biological outcomes. Again, correlative morphological studies should help interpretation by identifying which cell types are involved over time.

> I think you would begin to see permanent tissue- and system function level changes that are difficult to reverse. My guess is that the gene/protein expression changes would reverse even after 6 month exposure.

> To the specific question, would animal study reflect with more fidelity the comparison with human CS exposure, in my opinion, it is an appropriate comparison. Human's smoke intermittently. The breathing rate, tidal volume of a mouse and other physiological parameters of respiration are different and cannot

be equated with Human's. What is important and significant from the animal studies is the unbiased exposure treatments between the animal groups and the differences in the results observed among the treatment groups. After reading Jobse B.N. et al's work “Impact of inflammation, emphysema, and smoking cessation on V/Q in mouse models of lung obstruction” Respir Res. 2014; 15(1): 42, I am convinced that even a longer exposure would not have made a significant differences in the end points observed, unless the researcher consider looking at different end points such as expression of tumor specific genes etc. I am citing this as a reference study from a different group to justify my stand.

I do not expect the similar responses if switching is being done after longer (6 months) CS exposure as there would be severe and irreparable cellular damaging events would have occurred with long term exposure.

This is very similar to my response for question 2. I think this is a hard thing to predict. In the APOE knockout mouse, I don't believe any differences would be observed as the underlying pathogenesis from the genetic manipulation would become overwhelming. The WT mice may provide more information here.

SciPinion

Appendix C: Survey Results for Round 3

SURVEY RESULTS

In Vivo Toxicologists, Systems Biologists, and Bioinformatics Scientists Wanted: Panel I

Paid Peer Review Opportunity #67 $7,000 for ~56 hrs CLOSED

Generated: 2016-08-27 15:40:52 +0000
URL: https://scipinion.com/surveys/67/report.pdf?debug=true

ID #1783 (https://scipinion.com/surveys/67/custom_results/1783)

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	9.09% 1	36.36% 4	27.27% 3	27.27% 3	0.00% 0	11
Appropriate choice of controls	45.45% 5	18.18% 2	27.27% 3	9.09% 1	0.00% 0	11
Appropriate number of replicates in respective groups	54.55% 6	27.27% 3	18.18% 2	0.00% 0	0.00% 0	11
Appropriate choice of exposure modality	27.27% 3	45.45% 5	27.27% 3	0.00% 0	0.00% 0	11
Appropriate duration of the exposure	9.09% 1	45.45% 5	36.36% 4	9.09% 1	0.00% 0	11
Appropriate choice of endpoints	36.36% 4	54.55% 6	9.09% 1	0.00% 0	0.00% 0	11
Appropriate choice of analytical methods	36.36% 4	54.55% 6	9.09% 1	0.00% 0	0.00% 0	11

Comments

SCORE 0 — **user-382348** — *06/02/2016 19:25*
The absence of a true WT control raises more questions then answers. Why was this control not included to begin with? The quantity of data presented in this study is very extensive and it seems a bit suspicious that a true control was not utilized when such care went into both the timepoints and the analyses being conducted at the back end. Suspicions aside, a true WT mouse should have been utilized; as user-922124 pointed out, this is a novel application without a precedence for all of the expected toxicities relating to these exposures. Perhaps the largest need for inclusion of a WT mouse, however, is the observed alterations in the lipidomics profiles. It is VERY likely that any true alterations observed would be much more drastic when the "baseline" phenotype of the ApoE-/- is removed. The phenotype related to this mouse, pertaining to the lipidomics analyses, is likely masking any true fluctuations. These experiments do not need to be repeated with both male and female mice, however, this could yield very interesting results related to toxicity. Assuming that the goal of these studies is to provided a basis for marketing MRTPs, it is unclear why collecting these data would not be beneficial.

SCORE 2 — **ehjd** — *05/27/2016 23:29*
I do not believe repeating all studies in WT mice is required. This C57-derived model for COPD suffices.

SCORE 0 — **user-922124** — *05/29/2016 16:21*
The primary concern here is the use of this model in a novel application. For acceptance there is a lack of historical controls so it is difficult to put the small changes observed in THS2.2 mice. Studies using diesel exposure with and without particulate matter with the same mouse strain used males.

SCORE 0 — **user-509661** — *05/29/2016 20:24*
Opinion to Sponsor's question: The question of using the wild type mice to draw valid conclusions depends on the end points examined. As far as the pulmonary responses are concerned, inclusion of WT control may not be needed. However, some of the results examined, particularly the changes observed with respect to lipid profiles in the ApoE deficient mice, raise inquisitiveness for inclusion of WT as the rise in lipid parameters such as total cholesterol, triglycerides or the HDL levels even in the CS exposure group reach to normalcy after 3-months. What does this initial rise contribute to and how different would the WT controls behave are questions which in my opinion could potentially be addressed by inclusion of WT control mice in the study.

SCORE -1 — **scipi-admin** — *05/25/2016 10:15*
Question from Sponsor: - Is there a necessity to include a wild type control in this study design to draw valid conclusions? If, so would it be necessary to repeat all study arms in wild type strain? - Do the study design and aim require the use of both genders of mice to draw valid conclusions?

SCORE 2 — **user-859026** — *05/27/2016 10:18*
I don't see a sound justification for lack of confidence in the apoE knockout model for examining respiratory endpoints during smoke exposure. Most COPD mouse models use the C57Bl/6 strain, which is the background strain this transgenic is built on. A comprehensive review of COPD models can be found in Leberl, et al. Front. Physiol., 15 May 2013. "C57Bl/6 mice are a common strain for knockout studies and generally a popular model for cigarette smoke induced emphysema (Takubo et al., 2002; Guerassimov et al., 2004; Bartalesi et al., 2005; Yao et al., 2008; Botelho et al., 2010) due to their deficiency in anti-elastase (Gardi et al., 1994; Cavarra et al., 2001)." In light of the clear induction of inflammatory reactions, ephysema-like changes and the cascade of molecular events caused by smoke inhalation, I am curious as to the lack of confidence here.

SCORE -1 — **user-550063** — *05/29/2016 00:01*
Answers for Sponsor's question: I feel that wild type controls are needed to compare the events because it has been shown that Apoe is essential for the alveologenesis (Am J Physiol Lung Cell Mol Physiol. 2008 May;294(5):L991-7 and Pediatr Res. 2011 Nov;70(5):458-61). In this context, one cannot distinguish smoke mediated alveolar disruption and Apoe deficiency mediated alveolar pathology. Some crucial parameters like lung function, histopathology can be checked in WT mice. Since female mice are prone to develop severe emphysema compared to male strain, studies in male mice may not serve the purpose. Since the complete abolition of ApoE is not so common in human, ApoE deficiency may give artifacts. Indeed, ApoE deficient mice with smoke exposure may be good for explaining the pathogenesis of OLDOSA syndrome (overlap syndrome of obesity mediated obstructive sleep apnea and COPD) and obese asthmatics. I am not confident regarding the selection of 2 months (after exposure to 3R4F) for switching or cessation. The use of micro-CT for in vivo demonstration of low attenuation areas due to emphysema would have been the best approach. The crucial gap of any big data is validation due to the inherent problem of repetition. At least the authors can make efforts to validate the few differentially expressed genes or proteins or lipids (by

northern, western, ELISA, IHC etc).

SCORE 2

user-371682 *05/28/2016 09:41*

No, there is not. The same group has published some papers using WT mice, of course, not as complete as in Philips. This paper is very robust, providing an enormous amount of data. The use of both genders is a interesting debate. Most of the animal studies don't use female due to the hormonal cycle. This could neglect important results for the women point of view. Considering that both males and females smoke, the authors should consider including female in the next study to make sure there is or not important differences.

SCORE 1

user-609692 *06/05/2016 09:34*

I agree with most of the above reviewers that there is probably no good reason to conduct another study in another strain of mouse or the WT of the model used in these studies. Such an effort just raise more questions and perhaps generate an endless "do loop" that would not advance the knowledge base.

SCORE 0

user-922124 *05/31/2016 07:52*

user-922124 (me) 05/29/2016 16:21 The primary concern here is the use of this model in a novel application. For acceptance there is a lack of historical controls so it is difficult to put the small changes observed in THS2.2 mice. Studies using diesel exposure with and without particulate matter with the ApoE-/- used males; these studies showed that the gaseous phase not the particulate contributed to the progression of Diesel induced dose-related alterations in gene markers of vascular remodeling and aortic lipid peroxidation; filtration of PM did not significantly alter these vascular responses, indicating that the gaseous portion of the exhaust was a principal driver.

SCORE 1

user-200863 *06/03/2016 08:04*

I don't see any reason to conduct study in another strain or mice including WT of the model used in these studies.

ID #1784 (https://scipinion.com/surveys/67/custom_results/1784)

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	36.36% 4	18.18% 2	36.36% 4	9.09% 1	0.00% 0	11
Appropriate choice of controls	36.36% 4	27.27% 3	27.27% 3	9.09% 1	0.00% 0	11
Appropriate number of replicates in respective groups	54.55% 6	27.27% 3	18.18% 2	0.00% 0	0.00% 0	11
Appropriate choice of exposure modality	27.27% 3	63.64% 7	9.09% 1	0.00% 0	0.00% 0	11
Appropriate duration of the exposure	18.18% 2	36.36% 4	45.45% 5	0.00% 0	0.00% 0	11
Appropriate choice of endpoints	18.18% 2	72.73% 8	9.09% 1	0.00% 0	0.00% 0	11
Appropriate choice of analytical methods	45.45% 5	45.45% 5	9.09% 1	0.00% 0	0.00% 0	11

Comments

SCORE 1 — **scipi-admin** — *05/25/2016 10:17*
Question from Sponsor: - Is there a necessity to include a wild type control in this study design to draw valid conclusions? If, so would it be necessary to repeat all study arms in wild type strain? - Do the study design and aim require the use of both genders of mice to draw valid conclusions?

SCORE 1 — **ehjd** — *05/27/2016 23:30*
For the COPD study no gender comparison needed

SCORE 0 — **user-922124** — *05/28/2016 08:43*
This study in female mice although justified, will likely need to be repeated in a proper male and female dose-response study at the request of the FDA. The "sparse" historical control data at PMI is difficult to be relied upon to compare the "low level-absent" response of the ApoE-/- mouse to CS or air. Scientifically justified but will be difficult if the target is to convince a regulatory agency that THS2.2 has no atherosclerosis risk.

SCORE 0 — **user-371682** — *05/28/2016 09:52*
My answer is pretty much the same as in the previous question. I insist that in general, science has been neglecting female gender in most studies. I am a male and not a feminist, but I think that female physiological responses might be different for CS. I also debate the selected end points. I would like to see an extensive duration of CS exposition mirroring a realistic human condition.

SCORE 2 — **user-859026** — *05/27/2016 10:38*
I do not feel that a strain comparison is needed. The result with a single gender is satisfactory in this case.

SCORE 0 — **user-200863** — *06/06/2016 10:06*
In my opinion, the model is optimized for CV effects and therefore, well suited for the study. I don't see any reason to conduct a separate elaborate study in WT animals. I have reservations on the use of the model for respiratory effects as it is not optimized and may need separate targeted study to confirm/validate the findings.

SCORE 0 — **user-550063** — *05/29/2016 00:12*
Answers for Sponsor's question: Wild type controls are needed to compare the events because one cannot distinguish between smoke mediated atherogenesis and ApoE deficiency mediated. Few critical like atherosclerotic plaque measurement can be done in WT Mice. The role of gender in atherosclerosis in ApoE deficient mice in equivocal as there are mixed reports. In any event, recent study emphasized that female mice develop severe endothelial dysfunction related to atherosclerosis compared male mice (Zhou X et al Microcirculation. 2015 Oct;22(7):518-27). So gender effects may be dissected. I am not confident regarding the selection of 2 months (after exposure to 3R4F) for either for cessation or switching as there is insignificant atherosclerosis after 2 months exposure. If this happens in atherosclerosis prone strain, WT mice may need more time to develop smoke induced significant atherosclerosis. Though the authors have demonstrated the aortic arch plaque lesions, it would be very nice if they can show the plaque pathology using histomorphometric techniques to show the macrophages (IHC with macrophage markers), foam cell lesion (light microscopy), smooch muscle cells (IHC with SMC markers), fibrotic areas (Verhoeff's van gieson staining) and calcific areas (Von Kossa silver staining) along with luminal diameters as these parameters would be able to demonstrate the activeness of plaques. Adding the coagulation Parameters like the measurement of thrombin, tissue facor, factor V would be adding the benefit of study as cigarette smoke leads to hypercoagulation status and further cardiovascular events.

SCORE 0 — **user-609692** — *06/05/2016 09:51*
I agree with most of the above comments that it might be a wise course of action to include males in these studies and it is likely that FDA will ultimately ask for such information.

SCORE 0 — **user-922124** — *05/31/2016 07:55*
I agree with 550063. There seems to be 2 parallel paths here. One is a science based systems biology approach used in a genetically susceptible mouse model. This design suffers from a lack of dose response. If the sponsors intend on using this data as part of a data package for submission to FDA a proper regulatory study in males and females and in WT animals (likely rats) needs to be done.

SCORE 1 — **user-509661** — *05/29/2016 20:34*
My response is same as that of the previous one. As far as inclusion of male mice is concerned, I refer to Leinwand, LA's article "Sex is a potent modifier of the cardiovascular system" J Clin Invest 2003.

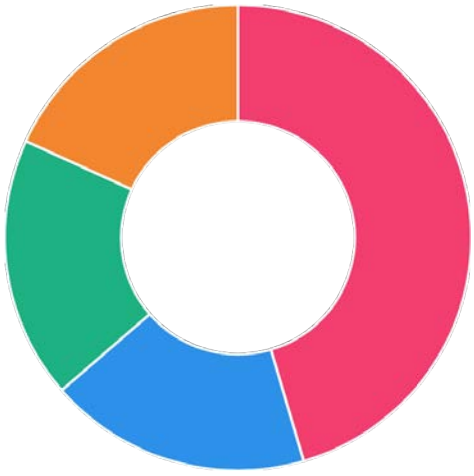
SCORE
-1

user-382348 *06/02/2016 19:31*

I feel strongly about the inclusion of a true WT mouse, as addressed in the previous response. As indicated previously, and now by others, if the goal of these studies is marketing for FDA approval, why risk not presenting the most complete data set. While differences in sex are likely not drastic, these analyses may reveal some potentially important information.

ID #1785 (https://scipinion.com/surveys/67/custom_results/1785)

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: 5 *(45%)*

It is appropriate but not comprehensive enough (please specify what you believe is missing): 2 *(18%)*

Some measurements that can be done longitudinally should complement the study design (please specify which): 2 *(18%)*

It is not appropriate (please specify): 2 *(18%)*

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

answers: 11

skips: 0

Comments

SCORE		
2	**scipi-admin**	*05/25/2016 10:0*
	One reviewer commented that: "The statistical comparisons are not correct. Why were t-tests chosen? A one-way ANOVA should have been performed as this is the appropriate test to compare >2 treatment groups; ALL groups should be compared, not simply one one treatment at one time point (i.e. 1 month sham vs. 1 month 3R4F). This is inappropriate and is skewing the statistical significance of the analyses conducted. It is surprising that this was not caught by the reviewers. " Question from Sponsor: Would the use of an ANOVA statistical model be more appropriate, despite the fact that the variance may not be homogenous across groups? All, please comment.	
1	**ehjd**	*05/27/2016 23:3*
	statistics were god for me	
0	**user-550063**	*05/29/2016 00:1*
	The author's statistics is fine.	
0	**user-200863**	*06/06/2016 10:0*
	ANOVA may be better; however, I wonder if any conclusion will change by conducting a more elaborate statistical analysis.	
1	**user-859026**	*06/01/2016 12:0*
	The study authors outlined their statistical analysis and it appears sound. Unfortunately P values were the only statistical measure given for data interpretation, and these can be highly variable. It would have been preferable to include mean differences with 95% CIs to examine the between-group differences, along with a P value. As to the Sponsor questions: I think the use of an ANOVA model would have been robust to non-homogeneous variance and would have been more appropriate.	
0	**user-922124**	*05/31/2016 08:0*
	Response to sponsor. Prior to choosing stat a test for normality needs to be used for parametric stat if the data are not normal or if there is non-homogenous variance a nonparametric stat should be used. Otherwise stats are OK with me since their is no dose response the data are qualitative non quantitative	
0	**user-609692**	*06/05/2016 12:2*
	The statistics are generally fine but agree with 922124 about the use of non-parametric statistics if there are any problems.	

ID #1786 (https://scipinion.com/surveys/67/custom_results/1786)

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.

As indicated in #2 the quantification of gene expression changes from xenobiotic exposures is dose and time dependent. A single exposure level and single time point post exposure without data to support these choices is problematic.

It would have been informative to see the cellular sites underlying the changes in the lipidome found in the Titz et al. study. Possibly in situ hybridization or immunostaining for some of the critical biomarkers in lung tissue at various time points across conditions.

There is lot of data generated related to transcriptomics and proteomics, however a logical translation of the data in relation to particular respiratory or cardiovascular disease(s) was incomprehensible.

Yes, the absence of a true control and the inappropriate use of statistics are the two most glaring errors.

No.

I don;t knwo well

I do not see important gaps

No important gaps noted.

The authors of respective papers had almost covered whatever they can do. But it would be very nice to use the respective organs like lung, heart, liver for brief validation of few top most or bottom most genes or proteins with RT-PCR, northern, Western or suitable techniques. This validation part is necessary for this kind of study where the study has been done once and 'OMICS" has the tendency to get less reproducibility. In this context, the research community started emphasizing the methods to improve the reproducibility (Kolker et al, OMICS. 2014 Jan 1; 18(1): 10–14., Fisch et al, Bioinformatics. 2015 Jun 1;31(11):1724-8 and Ioannidis et al, Science. 2011; 334: 1230–1232).

Apparently researchers have covered their bases.

Yes, there are still gaps to be filled. Despite the fact that a scientific article is not a book in terms of available space, I would recommend in the next publications, expanding the target tissues or organs to verify the overall effects of THS2.2. I would include eyes, brain, skin, join, muscles, stomach, bladder, intestines.

Comments

SCORE		
2	**ehjd** I missed this but I agree with the comment that verification of OMICS data using qPCR, WB, etc is indeed critical.	*05/27/2016 23:35*
1	**user-371682** I assume the financial support for research is not a problem for the company and they want to make the most accurate science as possible. I recommended other organs that are affected by CS. And I also agree that in situ hybridization and proteins verification by immunoassays for certain molecules would strengthen the OMICS results.	*05/28/2016 10:04*
2	**user-550063** Yes, I further emphasize the importance of verification of few top and bottom most genes, proteins.	*05/29/2016 00:21*
0	**user-200863** I don't see any gap.	*06/06/2016 10:09*
0	**user-609692**	*06/05/2016 12:10*
2	**user-509661** I agree with many of the substantive suggestions listed above. As far as the lipidomics data is concerned, the field itself is in its infancy stages and therefore there is not much scientific information available currently to relate the lipidomic changes observed by the researchers to pathophysiological events. Therefore, it is not surprising that there are gaps in understanding the results/concepts especially underlying the lipidomics data generated by the authors.	*05/29/2016 20:56*
0	**user-922124** Second assay follow up would have been useful particularly for he THS2.2 group that appear to have smaller gene expression studies. Microarrays do not have the dynamic range that qRT-PCR assays have. In addition NGS that has more flexibility and is more quantitative should have been used for these studies.	*05/31/2016 08:07*
0	**user-382348** A secondary approach to validate the gene/protein expression changes (e.g. RT-PCR/Western blotting) would have been much more convincing of the large-scale OMICS study. It would only strengthen the validity of their approaches.	*06/02/2016 19:39*
0	**user-609692** I agree on the need to include the secondary approaches noted by 382348 to help validate OMICS study data.	*06/05/2016 12:26*

ID #1787 (https://scipinion.com/surveys/67/custom_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?

I think that this is a difficult time point for the authors to determine, as some of the parameters measured are altered to their maximal effect at month 2; however, certainly using a 3, or 4-month timepoint would have been better for certain parameters, especially the lung function parameters. For example, a maximal effect on lung function/inflammation is not observed until 3-6 months, therefore the cessation/switch effects may be more interesting here. However, in a study of this magnitude, I believe that the timepoint chosen was sufficient for the majority of the analyses.

I am confident in the study design and thought the data were convincing for the ability to see reversals of many endpoints and levels of biomarkers returning to baseline by 6 or 8 months. This was evident in respiratory, cardiovascular and hepatic evaluations. A cited reference indicated that 2 months was sufficient to induce systemic changes, especially for cardiovascular measures. It is possible, or at least difficult to predict, that out to 3 or 4 months exposure, there would be some changes to tissue that might not be reversible in the subsequent switching or cessation period. I would not expect refractoriness in gene expression or protein concentration changes if 3 or 4 months were chosen versus 2. My guess from similar type studies is that these are driven by external signals that need to be present to relay the signal to cellular receptors.

This is an open question. If we see the lipid profiles in the treatment groups, even in the CS group (3R4F group), they return to normalcy overtime. So, therefore, it is only the initial rise(1-2 months). After going through Jobse B.N. et al's work “Impact of inflammation, emphysema, and smoking cessation on V/Q in mouse models of lung obstruction” Respir Res. 2014; 15(1): 42, I am convinced that even a longer exposure would not have made a significant differences in the end points observed, unless the researcher consider looking at different end points such as expression of tumor specific genes etc.

No. The design is not appropriate the achieve the proposed aims. The selection of 2 months is not having proper logic. The authors have cited two earlier papers for this selection. But if one see carefully these two papers, it is disappointing. While Philips et al (2015) had used C57BL/6 mice, Boué et al had measured Lm in 6 months smoke exposed mice (though they have done some indirect measurements on 3 months smoke exposed mice and these indirect measurements may not impact the emphysema). In addition, Philips et al (2015) had shown that there was a maximum Lm after 5 months smoke exposure. Even in this study, maximum Lm has been observed only after 6 months exposure though 2 months exposure is statistically significant. Most of the inflammatory parameters are up-regulated even one month smoke exposure. In addition, most of COPD patients who want to quit smoke will not have mild stage disease and indeed they have severe stage disease. So to translate effectively this research into human, the authors needs to switch after 6 months smoke exposure. I expect the similar results if the cessation or switching time point was set to 3 or 4 months as the disease severity starts only after 5 -6 months. But at the same time I believe that there may not be similar results if switching time point was set to 6 months post 3R4F exposure. So rigorous design is required to select the point of cessation or switching. If needed, one can plan for open questionnaire from the ex-smokers and get the information like after how many years they wanted to quit or they want ed a change ? what are the reasons behind this mind change ? Then converting this knowledge into this design would be ideal. If needed one can plan for multiple time points for cessation or switching like post 3 months, post 5 months, post 6 months and so on and concentrate only on functional end points like emphysema, aortic arch plaque measurement.

Yes, I think it was appropriate given the life span of laboratory animals. It is possible that results may be different.

Yes the cessation group and switching groups are appropriate to mimic the human situation and does demonstrate and amelioration of the effects compared to CS, however THS2.2 still alters a number of genes/pathways similar to CS when compared to air controls.

Based on the data presented in the papers, the cessation and switching studies seem appropriately designed to achieve the proposed aims. I would expect similar results with switching time point set to 3-4 months. Only differences in severity of effects. Suggest also evaluating male mice for similarities and differences with the females at these later time points.

Two month cessation is appropriate and 3-4 month cessaion or switching may not make big difference.

I would have liked to see longer exposure before switching. Ideally, groups exposed to 2/3 and 6 months before switching to sham or THS2.2 to see the recovery. Smokers are exposed to extended period of smoking before switching and will generate more convincing data.

yes, they are appropriately designed but as mentioned above (Q1) it is possible that more irreversible effects are picked up if cessation/switching is set at a later time point. Conclusion that switching causes very rapid restoration of CS-affected parameters may be overly optimistic in this design. However, even setting the switch time point some months later may not resolve this due to the relative short time span allowed in this model in general.

If the authors started the experiments with 2 months-old mice (not clear in the text), it is equivalent to 20 years for humans. Mice inhaled smoke for 2 months, it means for approximately 6 human years. The recover of the inflammatory indicators after cessation or switching time point is somehow expected at young ages. I would like to see longer exposure times because in humans usually the symptoms of cardiovascular or respiratory problems usually appears after 20-25 years of smoking.

Comments

SCORE 3 — **user-371682** — *05/28/2016 10:13*

Most of the reviewers (6 x 4) agreed longer exposures are needed. However it was not unanimous. I was one of the reviewers that would like to see longer exposures before the switching. I expect more difficulties in recovering some physiological indicators and probably would be more realistic with the human scenario. I understand the mouse lifespan is shorter but there is an age equivalence between them that could be used.

SCORE — **user-550063** — *05/29/2016 00:28*

0

Statistical significance may not be that much relevant to this kind of studies in which future efforts will be done to see the effect of these modified cigarettes on their health and satisfaction. So in the point of biological significance , 2 months exposure have given little effects. So I am further emphasizing the need of at least 5-6 months exposure before cessation or switching.

SCORE
0

user-200863 *06/06/2016 10:15*

I agree with the comments of other reviewers and would like to see a longer exposure before switching - may consider extending the study to the natural death after 1 year of exposure.

SCORE
2

ehjd *05/27/2016 23:39*

The exposure length before cessation/switching is an issue in terms of prediction to humans. It can of course not be really prolonged to better mimic the real case scenario where people have smoked for many years before the cessation/switch due to the short animal life span. Yet, it would be relevant to see if similarly dramatic positive effects of switching are seen in animal exposed much longer (1 year).

SCORE
2

user-922124 *05/31/2016 08:11*

Since smoking can be a lifetime of exposure with many individuals smoking for 20+ yrs prior to switching to a nonsmoker alternative I do not think the smoking cessation and switching occurs in a short time frame as used here. My interpretation was that the shortened time frame was used to compensate for the rapid development of plaques in the ApoE mice.

SCORE
1

user-859026 *06/01/2016 13:29*

One of the study aims was to assess whether switching reduced exposure effects of CS vs. THS2.2. While it is clear in some of these studies that switching after 2 months led to reduction of many physiological responses, especially in lung, to near sham levels, the greatest damage is not seen until 6 to 8 months exposure. In particular, pathogenic responses in the liver and aortic plaque are minimal at 2 months. The lung response is very rapid (1-2 months for most endpoints) but the biggest biological impact is at 6 months, as inferred from the network analysis. Even though I doubt that this longer exposure period would yield different results, I do believe that a longer period is needed to confirm the results.

SCORE
0

user-609692 *06/05/2016 12:33*

I support the need for longer exposure periods prior to switching or cessation in order to allow more time for concrete development of lung disease. That said, this will be balancing act to separate CS induced lung changes from lung changes in older mice. A time-course range finding study might help fine tune the final experiment.

SCORE
0

user-382348 *06/02/2016 20:52*

This was a difficult aspect to the studies performed. I agree with the sentiment that a longer exposure prior to the switching/cessation would have been ideal. However, this is a challenging thing to predict with out prior precedence. In addition, predicting how exposure in a mouse correlates with that of a human on a chronic scale is not feasible. That being said, most humans quit smoking once pathogenesis begins to develop and a doctor suggests this. The 2 month timepoint does not produce any overt differences to the controls, so it is hard to say that cessation is really doing anything.

ID #1788 (https://scipinion.com/surveys/67/custom_results/1788)

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.

	Poor	Sufficient	Excellent	I cannot answer	Total
Aerosol generation adequately explained with regard to reproducibility	9.09% 1	63.64% 7	18.18% 2	9.09% 1	11
Sufficient level of details and explanation regarding aerosol generation	0.00% 0	72.73% 8	18.18% 2	9.09% 1	11
Sufficient level of details and explanation regarding aerosol characterization	0.00% 0	54.55% 6	36.36% 4	9.09% 1	11

Comments

SCORE **-2** — **user-905059** — *05/26/2016 17:39*
Aerosol generation need more characterization such as MMAD (mass median-aerodynamic diameter), particle size distribution, stability of particle generation in real-time during exposure period, and mass concentration as well as particle number concentration.

SCORE **1** — **user-371682** — *05/29/2016 09:01*
More information or details could be included about the aerosol generation; however my opinion is that all animals aspirate the same amount of chemicals produced by the device. I am confident that the animals were exposed to the same experimental conditions and more details are not relevant at this point since this is a article and not a method's book.

SCORE **4** — **user-550063** — *05/29/2016 00:31*
Most of the reviewers including me agree that the Aerosol generation and characterization have been explained in a satisfactory manner.

SCORE **0** — **user-200863** — *06/06/2016 10:22*
Adequately explained. I am fine with whole-body exposure.

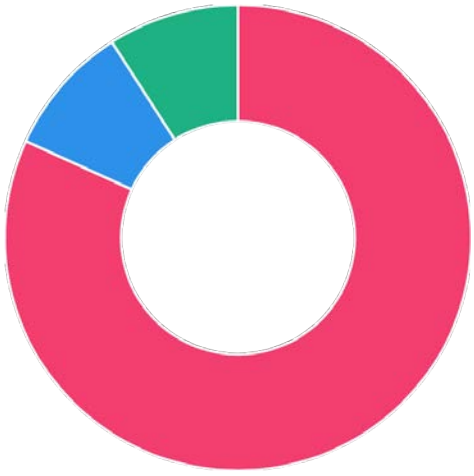
SCORE **0** — **user-609692** — *06/05/2016 12:35*
The Aerosol generation and characterization information is sufficient but agree with 922124 about an oral dose from coat grooming in the mice. Nose only would be more tidy.

SCORE **0** — **user-922124** — *05/31/2016 08:13*
Aerosol generation was generally OK. My concern was the use of whole body exposures that can result in a significant oral dose due to grooming.

SCORE **0** — **user-382348** — *06/02/2016 20:56*
I felt that the Aerosol generation and characterization were explained in sufficient detail.

ID #1789 (https://scipinion.com/surveys/67/custom_results/1789)

Is the approach to match the exposures to the aerosols on the basis of a key common constituent, nicotine, instead of TPM reasonable? (please explain below)



Legend

Yes: 9 *(82%)*

No: 1 *(9%)*

I cannot answer this question: 1 *(9%)*

answers: 11

skips: 0

Comments

SCORE		
3	**ehjd** nicotine is appropriate but I can see that particle characteristics may be different and effect outcome	*05/27/2016 23:41*
1	**user-550063** TPM also contain a number of other known and unknown addictive additives including Nicotine. Though it is convenient to take nicotine as a comparison, this might not lead to give a complete picture. PMI animal research indicated that the combination of nicotine and acetaldehyde increased the addictivness by having more puffs (Am J Public Health. 2007 November; 97(11): 1981–1991). Another study had revealed that TPM is more potent than nicotine in upregulating nicotinic receptors on SH-SY5Y cells (Nicotine Tob Res. 2007 Aug;9(8):793-9.). These indicate that matching the aerosol based on nicotine may not give a complete picture.	*05/29/2016 00:34*
1	**user-371682** Nicotine is in fact the most important marker for CS exposure. However, PM size distribution would be very informative.	*05/29/2016 09:23*
0	**user-200863** Agree with the comments.	*06/06/2016 10:25*
1	**user-509661** Particle size and their dispersion significantly influence the deposition pattern of the inhaled particles within the lung compartments and thus subsequently affect the overall toxicity. Therefore, morphological characterization of the particles is very important.	*05/29/2016 21:33*
1	**user-609692** Agree with the above that nicotine is an appropriate marker but that also important to assess particles.	*06/05/2016 12:37*
3	**user-905059** For the inhalation exposure, it is important to know the size distributio of aerosol particles. The particle size determins depostion of paritcles to lungs. Thus it is important to determine the size distribution of particles from cigarette smoke and particles generated from THS.	*05/26/2016 17:34*
0	**user-922124** Although nicotine matching is nice to consider for this series of experiments it would not hold up to regulatory scrutiny. OECD inhalation studies require defining the MTD then using these data to set doses for the study. Nicotine level matching is not appropriate if the intent is to use these data in support of a FDA MRTP.	*05/31/2016 08:17*
2	**user-382348** Much like ehjd, I think that nicotine is appropriate, but it is still important to monitor the particle characteristics.	*06/03/2016 09:40*

ID #1790 (https://scipinion.com/surveys/67/custom_results/1790)

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?

Biomarkers were appropriately selected

Clearly the most informative are for nicotine and cotinine. Urine collection seems most variable and the plasma levels at 8 months look consistent. These seem to be the most sensitive markers of exposure. Non-nicotine markers will be valuable, and the SPMA and CEMA biomarkers should be evaluated, even though they are far more abundant in CS exposure versus THS2.2 exposure.

I think that the use of blood and urine based biomarkers was appropriate. The choice really depends on the endpoints being examined.

This is not clear for CS or for THS2.2 since only a single exposure level was used. Biomarker assessment requires careful dose-response time course studies that were not done for any endpoint measure here. Nicotine blood levels are likely the best biomarker for CS or for THS2.2

Suggest measurement of urinary cadmium as a marker of exposure on a longitudinal basis since this a major contaminant of tobacco, present in both the CS and to a lesser extent in heated tobacco vapor if I am reading the exposure figures correctly. Such measurements would also permit exposure comparisons with CDC NHANES data which uses urinary cadmium as marker of exposure. Please note that smokers are well known to have much higher kidney concentrations of cadmium than non - smokers (see Nordberg GF et al., Handbook on the Toxicology of Metals, Elsevier, 2015) for a more detailed discussion. The utility of measuring biomarkers of exposure in blood or urine will depend on the chemical of interest. Some chemicals accumulate in blood so that matrix is a good index of exposure for them depending on the duration of exposure while others pass out into urine following metabolism or duration of exposure so that would be the matrix of choice.

I feel that both the urine and blood yielded useful information, however I think that the primary problem with the biomarker assessment is simply the time that the samples were collected. It is unclear why only chose the 2, 6, and 8 month timepoints for the COHb measurements, while ignoring the 1 and 3 months. The high level of variability in the time with which the biomarkers were measured is the major concern.

HPMA and total NNAL in urine are good biomarkers that clearly separate 3R4F from CS at 3, 6, and 8 months and are strongly sensitive to switching/cessation. Urine exposure biomarkers appear most robust.

none suggested

Urine is susceptible to renal function. Cigarette smoke itself can cause systemic endothelial dysfunction and thus affect the nicotine excretion and thus urine nicotine levels are dependent on kidney function. Blood COHb, Nicotine and Cotinine Measurements in Plasma are recommended biomarkers of exposure. If one want to use urine, the parameters should be normalized by creatinine.

I did not see cotinine levels, which in my opinion will be advantageous, see Marsot and Simon, 2016 for detail.

I would keep nicotine (and its main metabolites) as the main marker(s). The ideal scenario for a smoker is the nicotine as the only chemical being provided by the cigarette or devices. Most of other chemicals produced are consequences of tobacco heating/combustion. A cigarette varies its nicotine concentration 1-3 mg. One could assume (and limit) the nicotine availability should reach proportional concentrations in the mice (in mg/kg and mg/m3).

Comments

SCORE		
3	**user-550063** Like many agree, I also agree that Nicotine and Cotinine Measurements would be best biomarkers. In any event, urine Nicotine and Cotinine measurements should be normalized by creatinine.	*05/29/2016 00:37*
0	**user-371682** Analytes in the blood and urine are great indicatives of physiological body conditions.	*05/29/2016 09:33*
1	**user-200863** I agree with the use of Nicotine and Cotinine as biomarkers. I would like to see a dose-response in future studies for better understanding of varying degrees of human SC. For dose-response levels of biomarkers in blood will be good to determine systemic exposure differences; otherwise levels in urine to confirm exposure will be fine. A kinetic study will be wonderful.	*06/06/2016 10:30*
1	**user-922124** No dose response to base the "biomarkers" on. This was a comprehensive net. There was no follow up either using a second method.	*05/31/2016 08:18*
0	**user-609692** I agree with all the above comments.	*06/05/2016 12:38*

ID #1791 (https://scipinion.com/surveys/67/custom_results/1791)

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

I would consider not using ApoE-/- mice to investigate alterations in lipidomic profiles. This is quite evident in the planimetry where even the sham mice display a significant increase in plaque formation at the 8 month timepoint. Comparison to a WT mouse is a necessary control that wasn't utilized. It is also unclear why the authors chose only the 8 month timepoint for the full lipidomic screen. I think that the unbiased screening of plasma and tissue lipids using an LC-MS/MS platform is the most appropriate methodology . The authors did, however, do a nice job presenting gross morphology of the aortic arch plaque formation.

I am unfamiliar with the lipid profiling methodologies used in the study.

The methodology chosen for lipid profiling was appropriate

Metabolomics platforms.

The lipidomics approach as outlined in Boue et al., however as outlined above the lack of dose-response and time course is problematic to make causal links between exposure and response

Mass spectrometry of lipid profiles should yield the most rigorous results assuming no technical problems with the sample preparation methods.

VAP - sufficiently accurate measurement of HDL, LDL, VLDL

The methods used in this study are perfect. For plaque assessment, calcification measurement is also recommended as there may be underestimation of plaque area because of shrinkage due to sclerotic effect.

lipidomic analysis

liquid chromatography coupled to tandem mass spectrometry

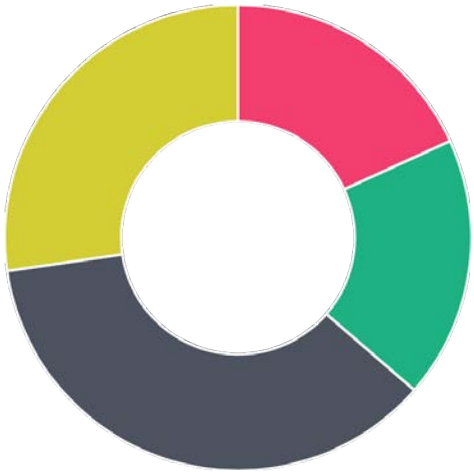
The same as applied in the paper.

Comments

SCORE		
0	**user-509661** I am convinced with the methods used for lipid profiling	*06/03/2016 17:02*
2	**user-550063** The methods mentioned in the studies are effective for assessing lipid profiles.	*05/29/2016 00:39*
2	**user-371682** Very robust methods were applied.	*05/29/2016 09:46*
0	**user-200863** The methods used were appropriate.	*06/06/2016 10:40*
0	**user-922124** no comments	*05/31/2016 08:18*
0	**user-609692** I agree with all the above and nothing further to add.	*06/05/2016 12:39*
0	**user-382348** I agree that the lipidomics screens were very well done and are sufficient for assessing the lipid profiles.	*06/03/2016 09:42*

ID #1792 (https://scipinion.com/surveys/67/custom_results/1792)

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



Legend

Snapshot perturbations (measuring resistance, compliance, and elastance): 2 *(18%)*

Primewaves (measuring tissue elasticity, tissue damping, tissue hysteresivity, and Newtonian resistance): 0 *(0%)*

Pressure-volume loops (measuring parameters of the Salazar-Knowles equation, quasi-static compliance, quasi-static elastance, and hysteresis): 2 *(18%)*

Positive end-expiratory pressure (measuring the end-expiratory lung volume): 0 *(0%)*

Negative pressure forced expiration (measuring FEV0.1, FEV0.2,FEV0.1/FVC, and FEV0.2/FVC): 0 *(0%)*

None of the measures is indicative of emphysematous changes: 0 *(0%)*

All of the measures combined: 4 *(36%)*

I cannot answer this question: 3 *(27%)*

answers: 11

skips: 0

Comments

SCORE		
1	**user-550063** There are three major components that cause expiratory flow limitation in emphysema, They are lung elastic recoil, the tendency of airways to close, and airway resistance. Thus, to effectively assess the emphysema one need to have all the mentioned respiratory parameters.	*05/29/2016 00:43*
0	**user-371682** All three methods bring important information. The problem is to subject the animals to many analyses that can increase pain and suffering. I would use one of them, such as PVL, and cross the results with histopathology of the lungs.	*05/29/2016 11:29*
0	**user-550063** There are invasive airway mechanic apparatuses that can measure all these parameters at a time in an anesthetized mice.	*05/30/2016 02:50*
0	**user-609692** No comment here.	*06/05/2016 12:40*
0	**user-200863** Agree with comments above.	*06/06/2016 10:42*
0	**user-922124** All three components can be useful However the lack of a historical data base makes the detection of minor changes difficult.	*05/31/2016 08:20*

ID #1793 (https://scipinion.com/surveys/67/custom_results/1793)

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)	Total
transcriptomics: computational network modeling and NPA calculation	*1* **9.09%** 1 *2* **9.09%** 1 *3* **54.55%** 6 *4* **9.09%** 1 *5* **18.18%** 2	*2* **27.27%** 3 *3* **36.36%** 4 *4* **9.09%** 1 *5* **27.27%** 3	*1* **18.18%** 2 *2* **27.27%** 3 *3* **9.09%** 1 *4* **45.45%** 5	11
transcriptomics: gene set enrichment analysis	*1* **9.09%** 1 *2* **18.18%** 2 *3* **18.18%** 2 *4* **45.45%** 5 *5* **9.09%** 1	*2* **18.18%** 2 *3* **9.09%** 1 *4* **54.55%** 6 *5* **18.18%** 2	*1* **9.09%** 1 *2* **27.27%** 3 *3* **18.18%** 2 *4* **45.45%** 5	11
proteomics: clustering and functional association networks	*1* **10.00%** 1 *3* **20.00%** 2 *4* **50.00%** 5 *5* **20.00%** 2	*3* **30.00%** 3 *4* **30.00%** 3 *5* **40.00%** 4	*1* **20.00%** 2 *2* **20.00%** 2 *3* **20.00%** 2 *4* **40.00%** 4	10
proteomics: protein set enrichment analysis	*1* **9.09%** 1 *3* **27.27%** 3 *4* **45.45%** 5 *5* **18.18%** 2	*3* **36.36%** 4 *4* **27.27%** 3 *5* **36.36%** 4	*1* **9.09%** 1 *2* **27.27%** 3 *3* **36.36%** 4 *4* **27.27%** 3	11
lipidomics: integration with lipidomics data, PLS plots	*1* **9.09%** 1 *2* **18.18%** 2 *3* **27.27%** 3 *4*	*1* **9.09%** 1 *2* **18.18%** 2 *3* **18.18%** 2 *4*	*1* **18.18%** 2 *2* **18.18%** 2 *3* **18.18%** 2 *4*	11

	9.09% 1 5 36.36% 4	18.18% 2 5 36.36% 4	36.36% 4 5 9.09% 1	
Other (please specify below)				0

Comments

SCORE 0 — **scipi-admin** — *05/25/2016 09:0*
Question from Sponsor: What do you believe would be needed to make the computational network modeling and NPA calculation more informative and more relevant? Would you need further explanations? Do you believe that more biological processes should be covered? Would it be helpful if all tools and the knowledgebase were publically available to allow community testing of the approach?

SCORE 2 — **ehjd** — *05/27/2016 23:4*
it was informative and relevant for me. Certainly public availability would be very helpful

SCORE 1 — **user-371682** — *05/29/2016 11:3*
Bioinformatics is a growing discipline and the knowledge is getting better. It is sometimes controversial just using computational parameters to understand biological functions. It should stand with an extensive discussion to offer trustable conclusions.

SCORE 0 — **user-509661** — *05/29/2016 21:5*
Systems toxicology approach would be more appreciative when directed towards identifying and highlighting changes (could be changes or perturbations in genes, proteins or lipids) at the dose-response network level.

SCORE 1 — **user-922124** — *05/31/2016 08:2*
Response to sponsor: Proper dose-response studies are needed, there can be dramatic changes in gene expression with dose. Needs to be done in male and females A second assay follow up to validate responses. Data need to be publically available.

SCORE 0 — **user-200863** — *06/06/2016 11:0*
A dose-response study is needed to better understand the effects and correlate with SC patterns in human.

SCORE 0 — **user-550063** — *05/31/2016 04:4*
Overall various types of computational analyses are very relevant to 3R4F exposure. However, these computational analyses may not be suitable for THS studies. Because the fruitfulness of NPA calculation and the resultant NPA scoring are based on the magnitude of expression changes. While THS did not alter the gene, protein expression or lipids, there is no much relevance in performing NPA calculation with the aim of identifying biological impact of THS. Since the knowledge of computational analyses in the public is at various levels, public availability may lead to confusions rather than clarifications. So experts may be required for the same.

SCORE 0 — **user-609692** — *06/05/2016 12:4*
Agree with 922124 about the need for proper dose response studies and the inclusion of males in the study design.

SCORE 0 — **user-382348** — *06/03/2016 10:0*
I think that the biggest downside to any bioinformatics analyses is simply the public knowledge of the calculations being run. Public availability is a no-brainer.

ID #1794 (https://scipinion.com/surveys/67/custom_results/1794)

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)	**90.91%** 10	**9.09%** 1	**0.00%** 0	11
tissue preparation, including type of staining (for lung)	**90.91%** 10	**9.09%** 1	**0.00%** 0	11
number of sections evaluated	**90.91%** 10	**9.09%** 1	**0.00%** 0	11
blinding / randomization	**100.00%** 11	**0.00%** 0	**0.00%** 0	11
scoring system	**100.00%** 11	**0.00%** 0	**0.00%** 0	11
histomorphometry methods	**100.00%** 11	**0.00%** 0	**0.00%** 0	11
statistical evaluation	**81.82%** 9	**9.09%** 1	**9.09%** 1	11
data visualization	**90.91%** 10	**9.09%** 1	**0.00%** 0	11

Comments

SCORE **2** — **user-550063** — *05/29/2016 00:5*
There is no doubt that the authors have used “state of the art” scientific techniques in these studies in histopathology. I would be more happy if they would have repeated the experiments more than once for biological reproducibility.

SCORE **0** — **user-371682** — *05/29/2016 11:3*
Everyone agreed about this topic.

SCORE **0** — **user-200863** — *06/06/2016 11:0*
I agree that a dose-response study is needed; however, don't agree with repeating these extremely expensive non-academic study.

SCORE **1** — **user-922124** — *05/31/2016 08:2*
The lack of a dose-response data remains a concern

SCORE **0** — **user-609692** — *06/05/2016 12:4*
I agree with all the above comments

SCORE **0** — **user-382348** — *06/03/2016 10:0*
Reproducibility should have been demonstrated, as user-550063 has indicated.

ID #1795 (https://scipinion.com/surveys/67/custom_results/1795)

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?

Analysis was appropriate. Inclusion of additional IHC would have been helpful.

No. Standard mice under non-stressed conditions do not spontaneously generate any of the parameters assessed. The need for a wild type control is very evident in these experiments. The authors should also show representative histological sections for each treatment group/time point. This raises further speculation into the relevance of this study to investigate hepatic effects of cigarettes.

I don't have sufficient expertise in liver histopathology to answer definitively, but I would say yes. First, significant changes were seen in an age-dependent manner across several hallmarks of hepatotoxicity. Second, the range of analyses suggested that many types of histopathology were examined and determined to not be significantly altered in the CS or THS2.2 groups compared to same time points in sham group. The changes seen across time in all groups point to sensitive detection methods and consistent, toxicity-related changes.

As there was no overt hepatotoxicity observed even with CS exposure, there is no necessity for additional analyses.

No, this model has rapid accumulation of collagen fibers, degeneration and necrosis, inflammatory cells and bile duct hyperplasia, that has limited value when compared to either wild type mice/rats or human hepatocytes.

Yes- I would consider measurements of heme oxygenase (HSP27) in mice to provide an explanation for the accumulation of Fe which could be occurring from increased turnover of hemoproteins such as the cytochrome P-450s.

It would be helpful to compare conventional hepatotoxcity blood biochemical parameters such as GOT, GPT and APT etc with thes histopaghology.

yes - appropriate. Multiple parameters including collagen fiber density, parenchymal deterioration, inflammatory cells, bile duct hyperplasia are scored using blinded analysis by pathologist.

All these 4 anllytical methods are appropriate. TUNEL assay can be done in paraffin slides to determine the DNA fragmentation. Ultrastructural study can be performed to reveal mild hepatotoxic effects in the form of mitochondrial swelling, ER dilation, etc.

Not entirely. H&E-stained sections, Oil Red and PAS/PAS-D are basic and useful staining for histopathology, However, an ampler analyses of toxic effect on cells should consider labeling of apoptosis, autophagy and ROS markers in situ of different liver areas. The electron microscopy approach is also important to observe the ultrastructure of hepatocytes, such as RER, SER, mitochondria.

Comments

SCORE		
0	**user-382348** I further my statement that standard mice under non-stressed conditions should have been used for all of these analyses. WT mice do not spontaneously generate any of the parameters assessed. In addition, representative histological sections for each treatment group/time point should be done.	*06/03/2016 15:32*
1	**user-550063** The assessed analytical methods are sufficient for fully developed hepatotoxicity but these cannot detect minor changes. So ultra-structural studies are required to see minor but significant ongoing hepatotoxicity.	*05/29/2016 01:00*
1	**user-371682** I keep my opinion that an ampler analyses of toxic effect on cells should consider labeling of apoptosis, autophagy and ROS markers in situ of different liver areas. The electron microscopy approach is also important to observe the ultrastructure of hepatocytes, such as RER, SER, mitochondria	*05/29/2016 11:41*
0	**user-200863** The animal model used is optimized for CV conditions with increase hypercholesterolemia and spontaneous atherosclerosis and not for liver effects. It is appropriate to examine liver of the animals used in CV studies; however, may need a comparative study in other animal model optimized for the liver effects.	*06/06/2016 11:10*
0	**user-609692** I agree that ultrastructural studies would add value for all the reasons noted above and add correlative information to the biochemical studies on the same groups of animals.	*06/05/2016 12:49*
1	**user-922124** A measure of cell proliferation using BrdU mini-pumps would be useful to assess cell proliferation in liver and other tissue.s	*05/31/2016 11:14*

ID #1796 (https://scipinion.com/surveys/67/custom_results/1796)

How suitable is the Apoe-/- mouse model to detect additional exposure effects in the already challenged liver?

Not an excellent model to replicate the human condition but certainly among the best we currently have available.

suitable: In[de Leon et al J Liver 2:132, 2013] clear effect of cigarette smoke over control can be observed. This indicates that effects are measurable above those determined in the already challenged liver and therefore it provides a model to examine relative effects of 3R4F and THS2.2.

As previously mentioned, I believe that this is a very poor model, especially to assess hepatic insult. Observing what are likely small hepatic effects resulting from smoking are likely being missed due to the high basal hepatic injury. This study is seriously lacking due to the absence of appropriate controls.

The model may indeed be more difficult to assess hepatotoxicity than others, although past studies point to a lack of hepatotoxicity by CS. Even so, the xenobiotic response to CS in the Apoe-/- mouse was readily apparent as judged by the transcriptomics data.

It is my opinion that leptin deficient mouse (ob/ob) or leptin-resistant (db/db) mouse model might have been a better choice for this study.

First of all, based on the literature, Apoe deficiency did not alter the cholesterol balance across the liver though it affected the hepatic cholesterol metabolism (Kuipers et al, Hepatology. 1996 Jul;24(1):241-7). Interestingly, Apoe deficient mice do not become obese or develop insulin resistance. However, when these mice fed with typical high calory diet such as western diet, these mice develop (Dis Model Mech. 2010 MarApr; 3(34): 156–166) some liver function alteration. So with normal fed condition Apoe deficient mice may not lead to challenged liver condition. Evidently this study also did not find any hepatotoxic effects. In addition, smoke exposure in these mice did not lead to hepatotoxicity though there was significant gene and protein expression these indicate that the observed alteration in gene and protein expression may be necessary to maintain liver homeostasis or amount of smoke or its metabolites did not reach Liver sufficiently. So first cotinine concentrations should be checked in Liver. Depends on the results, one can plan accordingly. In the cotinine concentrations are very low in Liver, it is wise to think twice before using Apoe KO mice model for smoke induced liver toxicity. Evidently smokers are not having significant liver failure. In any event, the knowledge obtained from this study would be helpful in a subset of smokers who also have altered cholesterol metabolism like that of Apoe deficiency. In summary, additional smoke exposure to Apoe-/- mouse model may not be suitable. This may be suitable when these mice fed high calorie diet/

Not valuable unless the study is concerned with the <5% of the population that are equivalent to the ApoE-/- mouse.

Apoe -/- animal model is suitable to detect addtiional expsoure effectes based on several published papers

Again, The Apoe-/- mouse model is useful but suggest including male mice in the studies for the reasons noted above.

Apoe-/- mouse model is very specific to develop CVD even spontaneously when feed basal diet and therefore best suited for CVD. It is logical to use the same animals to evaluate liver effects along with CVD; however, it will provide almost the same information which can be gleaned from normal mice. I am not sure about the extent of impact/sensitivity of the lipids in liver as this knockout mouse model as it affect lipids. Bottomline, Apod-/-knockout mice is to better understand the inflammatory background of atherosclerosis.

According to the literature, Apoe KO is a good model to observe the toxic effects of CS on the lungs, cardiovascular system and liver; however, WT animals cannot be neglected.

Comments

SCORE		
0	**user-382348**	*06/03/2016 18:52*
	This is the biggest problem with this study, the baseline insult is just too high to see what are likely minimal effects on the liver. This appears to be a consensus among most others.	
1	**user-550063**	*05/29/2016 01:06*
	Once the authors can prove that ApoE deficiency alone or smoke alone causes the sufficient toxicity either by adding fat diet or increasing the smoke duration, then they can confidently take this model for future studies. Otherwise the authors are required to demonstrate the ultra-structural changes in liver upon ApoE deficiency and/smoke exposure,.	
0	**user-200863**	*06/06/2016 11:13*
	Agree with 371682	
1	**user-371682**	*05/29/2016 11:48*
	Considering the reviewer's opinions, there is no consensus in the use of APOE -/- mouse exposed to CS to observe liver conditions. So, it is imperative that the authors bring more discussion and more controls for this issue.	
0	**user-922124**	*05/31/2016 11:20*
	Using a diseased model like this may have limited value beyond what they show in this series of papers. The lack of a formal validation of this animal model, sparse historical controls compared to WT mice and again the PMI group needs to consider how will they be able to convince a regulatory person who has never seen the use of this model for this application .	
	user-609692	*06/05/2016 13:01*

0	Agree with all the above comments and suggest that perhaps ultrastructural / ultrastructural morphometric studies on the livers may be the most sensitive and specific way to determine early effects to help validate the model.

ID #1797 (https://scipinion.com/surveys/67/custom_results/1797)

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?

Yes. Not at this time.

Both methodologies are appropriate according to current standards. For proteomics a SILAC approach (SILAC mouse: [Cell. 2008 Jul 25;134(2):353-64] and subsequent papers from the M Mann group) could be used to facilitate quantification of global proteomic changes but this is highly expensive and the approach taken here is sufficiently informative.

The proteomics platform is well done. The use of a QExactive is considered "state-of-the-art". The lipidomics platform, while complete, is a bit unclear. The IS should have been spiked into the homogenization buffer (i.e. 70% MeOH +BHT) prior to performing the extraction. The only other possible issue is the limit of detection on the QTRAP 5500. The sensitivity on the 6500 is orders of magnitude greater and thus, potentially important lipids may be missed using their "Shotgun" platform.

The LC-MS methods appeared in line with most studies referenced. There are alternative methods and instruments that might be used, such as UHPLC coupled with ICR-FT/MS.

Yes, the approaches are appropriate

Yes the lipidomics and proteomics used states of the art technology. I have n o other recommendations. The flaw in this study is not the methodology rather is the lack of a dose-response to make causal links between exposures and disease outcomes.

I believe that the mass spectrometry instruments and approaches for evaluating lipidomics and proteomics are presently state -of -the- art. One might consider RIA approaches for specific proteins once target molecules of interest have been identified by mass-spectral screening.

The methods reflects the current state of the art in the field

Yes. These methods and instruments are reflecting the current state-of-the-art in the field. The authors have done lipid related proteome in Lungs (Titz et al, 2016). This is the best biological validation. In future ,this combined approach can be used for every lipidomic study.

Yes, it does. I don't have any additional comment.

As far as I know about the instruments, the ones used are definitively up-to-date. I think it is the best available and precise method for the omics approach.

Comments

SCORE		
0	**user-382348** Agreed, a consensus exists.	*06/03/2016 18:58*
2	**user-550063** As many reviewers suggested, the methods and instruments used in performing lipidomics and proteomics analyses reflect the current state-of-the-art in the field.	*05/29/2016 01:09*
2	**user-371682** There is a consensus in this question.	*05/29/2016 11:49*
0	**user-200863** Agree with all.	*06/06/2016 11:14*
0	**user-922124** The genomics portion does not reflect state of the art and does not consider the epigenetic components of this disease.	*05/31/2016 11:21*
0	**user-609692** Agreed a consensus here.	*06/05/2016 13:02*

ID #1798 (https://scipinion.com/surveys/67/custom_results/1798)

Having inspected the study data in the various repositories provided, as well as in the Supplementary materials provided with the publications, and 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	Total
In-life observations, mortality, body weight, gross pathology	*2* **9.09%** 1 *3* **90.91%** 10	*2* **9.09%** 1 *3* **90.91%** 10	11
Biomarkers of exposure, urine	*2* **18.18%** 2 *3* **81.82%** 9	*2* **18.18%** 2 *3* **81.82%** 9	11
Biomarkers of exposure, blood, plasma	*2* **9.09%** 1 *3* **90.91%** 10	*2* **9.09%** 1 *3* **90.91%** 10	11
Clinical chemistry, including blood lipoproteins	*2* **27.27%** 3 *3* **72.73%** 8	*2* **18.18%** 2 *3* **81.82%** 9	11
Hematology	*3* **100.00%** 11	*3* **100.00%** 11	11
Respiratory physiology	*1* **9.09%** 1 *2* **18.18%** 2 *3* **72.73%** 8	*1* **9.09%** 1 *2* **18.18%** 2 *3* **72.73%** 8	11
BAL fluid - inflammatory mediators	*2* **18.18%** 2 *3* **81.82%** 9	*2* **18.18%** 2 *3* **81.82%** 9	11
BAL cells (free ling cells) - identification and activation markers	*2* **18.18%** 2 *3* **81.82%** 9	*2* **18.18%** 2 *3* **81.82%** 9	11
Histopathology, upper respiratory tract	*2* **27.27%** 3 *3* **72.73%** 8	*2* **9.09%** 1 *3* **90.91%** 10	11
Histopathology, lung	*2* **30.00%** 3 *3* **70.00%** 7	*2* **18.18%** 2 *3* **81.82%** 9	11
	1	*1*	

Histopathology, liver	9.09% 1 2 18.18% 2 3 72.73% 8	9.09% 1 2 18.18% 2 3 72.73% 8	11
Histopathology, other organs/tissues	2 27.27% 3 3 72.73% 8	2 18.18% 2 3 81.82% 9	11
Histomorphometry, lung	3 100.00% 11	2 9.09% 1 3 90.91% 10	11
Plaque size measurement, planimetry	1 9.09% 1 2 9.09% 1 3 81.82% 9	2 18.18% 2 3 81.82% 9	11
Plaque size measurement, micro-CT	2 9.09% 1 3 90.91% 10	3 100.00% 11	11
Dataset, transcriptomics	2 18.18% 2 3 81.82% 9	1 9.09% 1 2 18.18% 2 3 72.73% 8	11
Dataset, lipidomics	2 18.18% 2 3 81.82% 9	2 18.18% 2 3 81.82% 9	11
Dataset, proteomics	3 100.00% 11	2 9.09% 1 3 90.91% 10	11

Comments

SCORE 0 — **user-550063** — *05/29/2016 01:14*
I feel most of the parameters are sufficient except Respiratory physiology and Plaque size measurement, planimetry. 1. Expected time and smoke induced change was not reflected in respiratory physiology. For example, pressure volume loop is very similar for both month 2 and month 3 (3R4f) and same scene form month 6 and month 8 (Fig. 6 from Philips et al, 2016). 2. Insufficient explanation and improper illustration in Plaque size measurement, planimetry.

SCORE 0 — **user-382348** — *06/03/2016 19:00*
As previously stated, liver histology was largely non-existent. So yes, this could be improved by simply including it. Along with immunohistochemical analyses to validate the other data sets.

SCORE 0 — **user-200863** — *06/06/2016 11:16*
Agree with 371682

SCORE
2

user-371682 *05/29/2016 12:13*

Observing the reviewers opinion for this question, I realize that Respiratory physiology, liver histopatology and planimetry were the weaker points of the paper.
It doesn't mean the data are not reliable, just means that there would be room for improvements.

SCORE
-1

user-609692 *06/05/2016 13:04*

Agree with all the above comments.

ID #1799 (https://scipinion.com/surveys/67/custom_results/1799)

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.

	Importance and fitness for purpose (1-5)	Confidence (1-5)	Total
Availability of raw data	*4* **27.27%** 3 *5* **72.73%** 8	*3* **9.09%** 1 *4* **36.36%** 4 *5* **54.55%** 6	11
Annotation of the metadata	*4* **36.36%** 4 *5* **63.64%** 7	*3* **18.18%** 2 *4* **54.55%** 6 *5* **27.27%** 3	11
Detailed enough text description of the methods	*2* **9.09%** 1 *4* **18.18%** 2 *5* **72.73%** 8	*2* **9.09%** 1 *3* **9.09%** 1 *4* **27.27%** 3 *5* **54.55%** 6	11
Availability of the source code and/or software	*2* **9.09%** 1 *3* **18.18%** 2 *4* **18.18%** 2 *5* **54.55%** 6	*2* **9.09%** 1 *3* **18.18%** 2 *4* **27.27%** 3 *5* **45.45%** 5	11
Possibility to run the code and/or software on own datasets to judge of the quality of the tool	*3* **18.18%** 2 *4* **36.36%** 4 *5* **45.45%** 5	*2* **9.09%** 1 *3* **27.27%** 3 *4* **18.18%** 2 *5* **45.45%** 5	11
	3 **9.09%** 1	*1* **9.09%** 1 *2* **9.09%** 1	

Use of multiple methods on the same data leading to similar conclusions	*4* **45.45%** 5 *5* **45.45%** 5	*3* **18.18%** 2 *4* **27.27%** 3 *5* **36.36%** 4	11
Other (please specify)	*4* **100.00%** 1	*4* **100.00%** 1	1

Comments

SCORE 0	**user-200863** I have high confidence. agree with 55063	*06/06/2016 11:17*
SCORE 3	**user-550063** I agree with many reviewers who suggested the high confidence. However, the deep validation related to proteome is still missing. In the lipidomics, it seems the authors have selected the portion of further analysis based on their comfort.	*05/31/2016 03:45*

ID #1800 (https://scipinion.com/surveys/67/custom_results/1800)

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	*Insufficient* **9.09%** 1 *Partially sufficient* **18.18%** 2 *sufficient* **72.73%** 8	*Insufficient* **9.09%** 1 *Partially sufficient* **9.09%** 1 *sufficient* **81.82%** 9	11
Biomarkers of exposure, urine	*Partially sufficient* **18.18%** 2 *sufficient* **81.82%** 9	*Partially sufficient* **9.09%** 1 *sufficient* **90.91%** 10	11
Biomarkers of exposure, blood, plasma	*Partially sufficient* **9.09%** 1 *sufficient* **90.91%** 10	*sufficient* **100.00%** 11	11
Clinical chemistry, including blood lipoproteins	*Insufficient* **9.09%** 1 *Partially sufficient* **9.09%** 1 *sufficient* **81.82%** 9	*Insufficient* **9.09%** 1 *Partially sufficient* **9.09%** 1 *sufficient* **81.82%** 9	11
Hematology	*Insufficient* **9.09%** 1 *sufficient* **90.91%** 10	*Insufficient* **9.09%** 1 *Partially sufficient* **9.09%** 1 *sufficient* **81.82%** 9	11
Respiratory physiology	*Partially sufficient* **18.18%** 2 *sufficient* **81.82%** 9	*Partially sufficient* **18.18%** 2 *sufficient* **81.82%** 9	11
BAL fluid - inflammatory mediators	*Partially sufficient* **18.18%** 2 *sufficient* **81.82%** 9	*sufficient* **100.00%** 11	11
BAL cells (free lung cells) - identification and activation markers	*Partially sufficient* **9.09%** 1 *sufficient* **90.91%** 10	*sufficient* **100.00%** 11	11
Histopathology, upper respiratory tract	*Insufficient* **18.18%** 2 *Partially sufficient* **9.09%** 1 *sufficient*	*Insufficient* **9.09%** 1 *Partially sufficient* **18.18%** 2 *sufficient*	11

	72.73% 8	**72.73%** 8	
Histopathology, lung	*Insufficient* **9.09%** 1 *Partially sufficient* **18.18%** 2 *sufficient* **72.73%** 8	*Insufficient* **9.09%** 1 *Partially sufficient* **18.18%** 2 *sufficient* **72.73%** 8	11
Histopathology, liver	*Insufficient* **18.18%** 2 *Partially sufficient* **9.09%** 1 *sufficient* **72.73%** 8	*Insufficient* **18.18%** 2 *Partially sufficient* **9.09%** 1 *sufficient* **72.73%** 8	11
Histopathology, other organs/tissues	*Insufficient* **27.27%** 3 *Partially sufficient* **27.27%** 3 *sufficient* **45.45%** 5	*Insufficient* **18.18%** 2 *Partially sufficient* **27.27%** 3 *sufficient* **54.55%** 6	11
Histomorphometry, lung	*Partially sufficient* **9.09%** 1 *sufficient* **90.91%** 10	*Partially sufficient* **9.09%** 1 *sufficient* **90.91%** 10	11
Plaque size measurement, planimetry	*Partially sufficient* **9.09%** 1 *sufficient* **90.91%** 10	*Partially sufficient* **9.09%** 1 *sufficient* **90.91%** 10	11
Plaque size measurement, micro-CT	*sufficient* **100.00%** 11	*sufficient* **100.00%** 11	11
Dataset, transcriptomics	*Partially sufficient* **18.18%** 2 *sufficient* **81.82%** 9	*Insufficient* **9.09%** 1 *Partially sufficient* **18.18%** 2 *sufficient* **72.73%** 8	11
Dataset, lipidomics	*Partially sufficient* **18.18%** 2 *sufficient* **81.82%** 9	*Insufficient* **9.09%** 1 *Partially sufficient* **18.18%** 2 *sufficient* **72.73%** 8	11
Dataset, proteomics	*Partially sufficient* **9.09%** 1 *sufficient* **90.91%** 10	*Partially sufficient* **9.09%** 1 *sufficient* **90.91%** 10	11

Comments

SCORE		
0	**user-550063**	*05/29/2016 01:27*
	Most of the reviewers indicated the sufficiency in the results presentation. I also agree with this. However, I would like to point out few minor pitfalls in some results. In-life observations: gross pathology parameters like color of the lung (black due to tar), firmness, granularity, haemorrhagic lesion, raised or shrinked appearance of lung or liver should have been presented. Respiratory physiology in Figure 6 of Philps et al, 2016 did not describe about the finding "similar appearance of pressure/volume loop" both in Month 6 and Month 8 and also both in Month 2 and Month 3. All Histopathology data: The representative data of emphysema (H @ E staining). Liver pathology and upper respiratory tract should have been shown rather then giving bar diagrams. Histopathology, other organs/tissues: Aorta histopathology should have been shown and Heart histopahology should have been done. Plaque size measurement, planimetry: Figure 3 of Philps et al, 2016 The obvious finding related to THS2.2, 6 months and 8 months difference is not highlighted or explained.	
SCORE		
0	**user-200863**	*06/06/2016 11:18*
	Yes, they are in sufficient detail.	
SCORE		
1	**user-922124**	*05/31/2016 11:24*
	There was sufficient detail in the published data.	
SCORE		
1	**user-609692**	*06/05/2016 13:06*
	Sufficient detail presented in the papers.	

ID #1801 (https://scipinion.com/surveys/67/custom_results/1801)

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	**90.91%** 10	**9.09%** 1	**0.00%** 0	11
Lung / respiratory effects in Phillips et al. 2016	**81.82%** 9	**9.09%** 1	**9.09%** 1	11
Vascular / atherosclerotic effects in Phillips et al. 2016	**81.82%** 9	**9.09%** 1	**9.09%** 1	11
Cardiac effects in Szostak et al. 2016	**63.64%** 7	**36.36%** 4	**0.00%** 0	11
Clinical chemistry and systemic effects in Phillips et al. 2016	**81.82%** 9	**18.18%** 2	**0.00%** 0	11
Hepatic effects in Lo Sasso et al. 2016	**72.73%** 8	**18.18%** 2	**9.09%** 1	11

Comments

SCORE **0** — **scipi-admin** — *05/25/2016 10:12*
Question form Sponsor: Please clarify which interpretation/conclusion go beyond what the data supports.

SCORE **0** — **ehjd** — *05/27/2016 23:46*
I did not find conclusions going beyond what data supports

SCORE **0** — **user-550063** — *05/29/2016 01:41*
Lung / respiratory effects in Phillips et al. 2016: 1. Figure 6: Pressure Volume loops for THS are very similar for both month 2 and month 3 and also for month 6 and month 8. This was not discussed. 2. Figure 7: A) Why the age induced change of lung volume was not altered in Switch ? Cessation at 3 months (1 month cessation) had shown significant increase in lung volume compared to Sham and this was not mentioned ! It seems that THS 3 months had shown increased lung volume ! Was it significant ? This trend was not mentioned ! What was the necessity of Lung volume normalization to body weight provided body weight is altered most due to systemic effect like loss of anti-gravity muscles. 3. Figure 11: The reduction of many cell types like neutrophils, lymphocytes and macrophages was not observed in the alveolar lumen compared to BAL Fluid data (Figure 8). Do the authors have used same mice for both free lung cells in BALF and cells in alveolar lumen, Explanation is required. 4. Figure 12: A) It seems that Cessation (8 months) significantly increase Mean chord length. But at this time point, there was no inflammation, no neutrophil, sham like pressure volume loop and no significant alteration in BAL fluid proinflammatory mediators. How to explain these discrepancies ? Do the authors think of the involvement of structural cells ? Is it due to autoimmune phenomena ? 5. Figure 14 and Figure 15 What was the necessity of calculating BIF and functional protein clustering provided THS did not show any change in gene or protein expression ? Vascular / atherosclerotic effects in Phillips et al. 2016: 1. The authors had seen the up-regulation of most of the lipid and its derived metabolites with smoke exposure compared to Sham, MRTPs. What is the status of anti-inflammatory lipid mediators like lipoxins, resolvins etc. At least they should have measured in Switch and cessation groups where there is a resolution of inflammation. 2. Figure 3: The effect of 6 months THS on % of plaque not explained properly. The authors mentioned about the effects of THS on switching groups. It seems that switching had shown significant increase. They talked about the 2 months background. Why they are not talking about the cumulative toxicity of THS ? Why there was a inconsistency in this group while other group had shown gradual increasing trend and this sudden increase was not observed in 7 months data of microCT in Figure 4. Cardiac effects in Szostak et al. 2016: While the authors did not find any change in expression until 3 months of exposure, the selection of 2 months switching or cessation is not justified for this study. This indicates that cigarette smoke has different time dependent effects on different organs. This could be either due to differential tolerability of different organs or due to differential accessibility of smoke and its mediators to different organs. So the authors should have performed preliminary studies with cardiac tissue in different time points to see the effects on cardiac gene expression before selecting 2 month cessation or switching. Further, blindly selecting 2 months cessation or switching may not be suitable for all organs. It is really surprising that the authors did not do either validation of transcriptomics data by western or RT-PCR or H & E staining of the hearts to see the possibility of cardiac hypertrophy, inflammation or other pathological lesions before interpretation of data. Since the determination of the effects of smoke on cardiac muscle is relatively new and controversial, the authors should have done the cardiac morphology and cardiac functional parameters before doing transcriptomics to get meaningful conclusions. Earlier studies have demonstrated the increased cardiac muscle mass with decreased cardiac function. These studies have been performed in C57 mice but the different study design with THS and Apoe is new to literature. In that case, the observed literature findings cannot be used for extrapolation of the transcriptomics of this study. Clinical chemistry and systemic effects in Phillips et al. 2016: 1. Figure 2: Effects of THS2.2 seems to be underestimated. It seems that THS2.2 exposure had shown some good changes like reduction in glucose, elevation in LDL (3 months). How all of a sudden there was a change in HDL in THS2.2 ? this data reflected in Total cholesterol as well. Though the authors have written about the inconsistency they did not discussed about the reasons for the discrepancy and solutions for the same. For example, different pooling of samples were not discussed and solution like the repeatability of entire experiment was not discussed. Rather the authors wanted to find a solution by applying other alternative methodologies. Hepatic effects in Lo Sasso et al. 2016: 1. While the histopathological data revealed that CS did not cause apparent hepatotoxicity, there was an impairment of specific biological networks. It is not clear the changes in gene expression is due to mild hepatotoxicity or homeostasis. 2. They should have done cotinine concentrations in Liver to see smoke reached to Liver. 3. Figure 2 of Lo Sasso et al, Inhalation toxicology: Periportal interstitial increase of collagen fibers , THS2.2 at 3 month had shown steep decrease compared to its own 2 month. And this decrease was followed by steep increase that was significant compared to Sham. This was not discussed properly. 4. Similarly, BIle duct hyperplasia and mononuclear inflammation (Figure 2B) were also higher in THS2.2 at 6 month compared to Sham. This was not discussed properly.

SCORE **1** — **user-382348** — *06/03/2016 19:06*
I agree with user-922124. The lack of a dose response, which I had not previously considered, does present more of a "casual linkage". Also, again agreeing, the real question is whether or not the THS2.2 is a major hazard (especially compared to CS), which I think the data largely supports, and would support even with a different model and correct statistics.

SCORE **0** — **user-200863** — *06/06/2016 11:19*
Agree, they are well supported.

SCORE
0

user-609692 *06/05/2016 13:1(*

I agree with all the above comments and the need for inclusion of dose response studies and perhaps more detailed time-course studies depending on available resources to address the issue of causality.

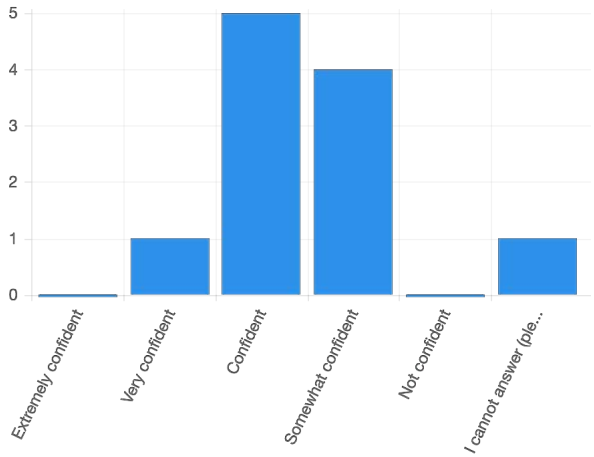
SCORE
3

user-922124 *05/31/2016 11:3(*

As stated earlier these data are qualitative since there is no dose response to make causal linkages. The chemistry data would have predicted a far lesser effect than CS so the results from these studies are not surprising. The main question that these studies do little to resolve is a quantitative risk assessment. There are a number of genomic tools available to conduct risk assessment. They real question is how much of a hazard is THS2.2 compared to air and to nicotine vapors alone and these studies.

ID #1802 (https://scipinion.com/surveys/67/custom_results/1802)

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?



Legend

answers: 11

skips: 0

Comments

SCORE 3 — **user-550063** — *05/29/2016 01:47*
Like many reviewers, I am also confident. However, I would like to point out some drawbacks that prevented me to give the status of very or extremely confident. They are, a) the dynamicity of the biological events, b) numerous positive and negative feedbacks at every molecular levels and c) all transcripts will not lead to biological activities due to PTMs. So these are the major hindrances in assessing the BIF.

SCORE 0 — **user-382348** — *06/03/2016 19:10*
550063 raises great points, it is a model and without validation, that is all that it will remain. There far too much that regulates an entire biological network to solely rely on a computational analysis.

SCORE 0 — **user-609692** — *06/05/2016 13:11*
Agree with all the above comments. Nothing more to add.

SCORE 0 — **user-200863** — *06/06/2016 11:23*
Agree with above comments.

SCORE 0 — **user-922124** — *05/31/2016 11:43*
I agree with 550063. The dynamic nature of genomic networks is revealed by dose-response and modeling. Cell responds to stressors in a hierarchal fashion with initial adaptive changes in response to xenobiotic exposure (e.g., CYP459 induction) at lower dose progressing to adverse changes (e.g., induction of p53 DDR) . The data generated here are limited.

ID #1803 (https://scipinion.com/surveys/67/custom_results/1803)

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* **45.45%** 5 *Low* **27.27%** 3 *Medium* **27.27%** 3	*High* **45.45%** 5 *Low* **27.27%** 3 *Medium* **27.27%** 3	11
Aortic arch planimetric plaque measurements (Figure 3)	*High* **63.64%** 7 *Low* **9.09%** 1 *Medium* **27.27%** 3	*High* **63.64%** 7 *Low* **18.18%** 2 *Medium* **18.18%** 2	11
microCT-based aortic arch plaque measurements (Figure 4, Suppl. Fig. 9, and supplementary files)	*High* **63.64%** 7 *Low* **9.09%** 1 *Medium* **27.27%** 3	*High* **63.64%** 7 *Low* **18.18%** 2 *Medium* **18.18%** 2	11
Plasma and aortic arch lipidomics (Figure 5)	*High* **45.45%** 5 *Low* **9.09%** 1 *Medium* **45.45%** 5	*Cannot Answer* **9.09%** 1 *High* **27.27%** 3 *Low* **18.18%** 2 *Medium* **45.45%** 5	11
Respiratory physiology (Figure 6)	*Cannot Answer* **9.09%** 1 *High* **72.73%** 8 *Medium* **18.18%** 2	*Cannot Answer* **9.09%** 1 *High* **72.73%** 8 *Low* **9.09%** 1 *Medium* **9.09%** 1	11
Lung volume measurements (Figure 7)	*High* **36.36%** 4 *Low* **27.27%** 3 *Medium* **36.36%** 4	*High* **36.36%** 4 *Low* **27.27%** 3 *Medium* **36.36%** 4	11
Bronchoalveolar lavage fluid – FACS analysis of free lung cells (Figure 8)	*High* **100.00%** 11	*High* **90.91%** 10 *Low*	11

		9.09% 1	
Bronchoalveolar lavage fluid – Multianalyte Profiling (Figure 9, supplementary Fig. 12)	*High* **63.64%** 7 *Medium* **36.36%** 4	*High* **54.55%** 6 *Low* **18.18%** 2 *Medium* **27.27%** 3	11
Nasal epithelium histopathological evaluation (Figure 10)	*High* **72.73%** 8 *Low* **18.18%** 2 *Medium* **9.09%** 1	*High* **72.73%** 8 *Low* **18.18%** 2 *Medium* **9.09%** 1	11
Lung histology (Figure 11, Supplementary Results File 2)	*High* **90.91%** 10 *Medium* **9.09%** 1	*High* **81.82%** 9 *Low* **9.09%** 1 *Medium* **9.09%** 1	11
Lung histomorphometry (Figure 12)	*High* **72.73%** 8 *Medium* **27.27%** 3	*High* **72.73%** 8 *Low* **9.09%** 1 *Medium* **18.18%** 2	11
Lung transcriptomics results – Differentially expressed genes (Figure 13, Supplementary Fig. 13)	*High* **54.55%** 6 *Low* **9.09%** 1 *Medium* **36.36%** 4	*High* **54.55%** 6 *Low* **9.09%** 1 *Medium* **36.36%** 4	11
Lung transcriptomics results – NPA/BIF analysis (Figure 14)	*High* **54.55%** 6 *Medium* **45.45%** 5	*High* **36.36%** 4 *Low* **9.09%** 1 *Medium* **54.55%** 6	11
Lung proteomics analysis (Figure 15, Supplementary Figs. 14 to 16)	*High* **72.73%** 8 *Medium* **27.27%** 3	*High* **54.55%** 6 *Low* **9.09%** 1 *Medium* **36.36%** 4	11
The totality of the data, collectively (All figures)	*High* **90.91%** 10 *Medium*	*High* **90.91%** 10 *Low*	11

	9.09% 1	9.09% 1	

Comments

SCORE		
0	**user-550063**	*05/29/2016 01:54*
	Like many, I also agree withe the criticalness of the collected data. In any event, I have few small concerns: 1. Clinical chemistry: If the aortic arch plaque measurements are available, the need of this clinical chemistry is not required and also there are inconsistencies of the results with increasing the exposure. 2. Plasma and aortic arch lipidomics (Figure 5): Plasma lipidomics may not valuable in future studies compared to aortic arch as there was a minimal change. In addition, in the context of complex biological involvement it is difficult to come to conclusion about the pathogenic role of individual lipid class. 3. Bronchoalveolar lavage fluid; Multianalyte profiling is having less importance as these profiling more or less towards the inflammatory cells recruitment. In any event, MMP activity,status of MMP and TIMP are having crucial importance in the context of emphysema. 4. Lung transcriptomics results – Differentially expressed genes and NPA/BIF analysis: In the presence of proteomics data, transcrptomics is having less importance as all transcripts are not leading towards biological activities as there are numerous biologial events between transcripts and meaningful biology like transcript life span, posttranslational modifications, involvement of microRNAs, protein folding and so on.	
SCORE		
1	**user-509661**	*05/31/2016 12:49*
	The authors have looked at the right end points as far as the BALf analysis and its clinical utility is concerned. Undoubtedly, there are significant differences observed between the tobacco products among the parameters examined both at cellular and proteomic level. Inclusion of additional analytes such as albumin levels: an index of air-blood barrier permeability, hemoglobin: for alveolar hemorrhage , LDH for cytotoxicity etc may provide meaningful insights regarding the molecular and biochemical mechanisms underlying the early pathological events.	
SCORE		
0	**user-200863**	*06/06/2016 11:25*
	No additional comments.	
SCORE		
2	**user-922124**	*05/31/2016 11:47*
	Overall the systems toxicology approach and these endpoints in the study were appropriate. How to decide which endpoints to go forward with in WT animals is uncertain. The lack of considering epigenomic effects remains a concern	
SCORE		
0	**user-609692**	*06/05/2016 13:17*
	I agree with the comments regarding the need for inclusion of epigenetic data to strengthen the systems toxicology approach that seems to be evolving here.	

ID #1804 (https://scipinion.com/surveys/67/custom_results/1804)

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.

Age is an important variable that needs to be carefully integrated into the design of studies of this nature. It is reasonable that age was not integrated given the complexity of the experimental design.

Because the ApoE-/- background results in such stark pathologies simply with age, I would expect nearly all of the timepoints to look different should the correct animal model be utilized. This is especially evident in the aortic arch plaque formation, where the sham group displays "25% plaque formation" by month 8. I do not believe that the age used for these mice is the issue, but rather the model as a whole.

For the endpoints investigated, the age group is appropriate.

Certainly in many commonly used mouse strains there will be an increased incidence of cancer, as in humans. There are likely to be age-related changes in metabolic rate and animal behavior. The increased morbidity may not affect specific endpoints but rather the study as a whole.

The age of the animals could impact the results since a number of biological patterns change in senescent animals due to attenuated sexual hormonal influences. This particularly true for females when they go out of estrus since this life event will undoubtedly influence all the omics data endpoints.

For the ApoE-/- mouse the exposure length was sufficient however, studies done with WT C57/Bl6 a chronic inhalation study will likely be needed for safety assessment of THS2.2 compared to air.

This is a very sensitive part of the study. Mouse metabolism is different from humans and the extrapolation should be conducted very carefully. I would try to make a parallel of human age and mouse age in WT animals that don't suffer the problems of Apoe -/- mouse.

Age in this model will affect cardiovascular aspects. E.g. plaques will already be bigger at later age in Apoe- mice and this could render the animals no longer a good model to analyse cardiovascular effects of CS versus THS2.2.

1. Respiratory physiology: Though aging as such cannot directly cause COPD, but it increases the susceptibility of the lung to develop COPD (Proceedings of the American Thoracic Society, Vol. 6, No. 7 (2009), pp. 570-572) 2. Atheosclerosis: This is very obvious to any strain of mice including Apoe KO mice.

Since age affects athreosclerosis development, if animals were older, it may present some difficulties in distingusing test article effect from age effect. ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. Y Nakashima, A S Plump, E W Raines, J L Breslow, R Ross Arteriosclerosis, Thrombosis, and Vascular Biology. 1994; 14: 133-140

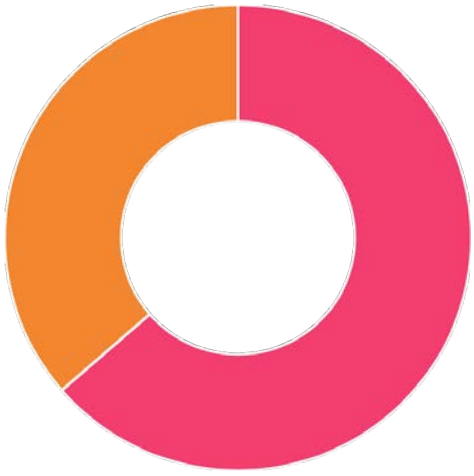
I will be most interested in knowing longer-term exposure effects and not starting the exposure in older animals. The reason for this is to see the long-term exposure followed by cessation in older humans and also the combined effects of exposure and age when many biochemical and physiological changes occur.

Comments

SCORE		
2	**user-550063** As many reviewers suggested I also agree that age could affect the plaque pathology and respiratory physiology.	*05/29/2016 01:5*
0	**user-382348** 922124 expresses my exact concern, as previous addressed.	*06/03/2016 19:5*
1	**user-922124** The age and length of study in this diseased models is limited by the spontaneous disease	*05/31/2016 11:4*
0	**user-200863** Age will increase the systemic exposure as numerous physiological parameters (e.g., metabolism, clearance) will be changing with age. Increase in the systemic dose will intensify the effects at lower doses to that observe at higher doses.	*06/06/2016 11:2*
1	**user-609692** Age of the animals in relation to length of the study are key factors in determing the final results and should be carefully controlled.	*06/05/2016 13:1*

ID #1805 (https://scipinion.com/surveys/67/custom_results/1805)

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



Legend

Yes: 7 *(64%)*

No: 0 *(0%)*

I do not see any indication of airspace enlargement in this animal model: 0 *(0%)*

I cannot answer : 4 *(36%)*

answers: 11

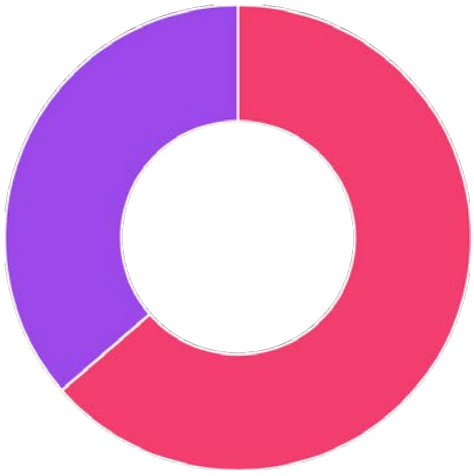
skips: 0

Comments

SCORE		
2	**user-550063** It is very clear that lung function results mentioned in the study are indicative of emphysematous changes in 3R4F exposed animals.	*05/29/2016 02:0*
0	**user-200863** Agree with the comments.	*06/06/2016 11:2*
1	**user-509661** The results are supportive of emphysematous changes in the 3R4F exposure group	*05/29/2016 22:0*
0	**user-609692** The results suggest emphysematous changes in the 3R4F treatment group.	*06/05/2016 13:2*

ID #1806 (https://scipinion.com/surveys/67/custom_results/1806)

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?



Legend

Less airspace enlargement: 7 *(64%)*

More airspace enlargement: 0 *(0%)*

No airspace enlargement seen in any of the two exposed groups: 0 *(0%)*

Similar airspace enlargement seen in both exposed groups: 0 *(0%)*

I cannot answer the question: 4 *(36%)*

answers: 11

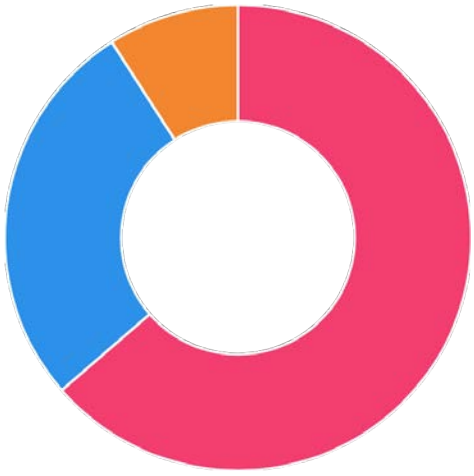
skips: 0

Comments

SCORE		
4	**user-550063** It is clear from lung function results that THS aerosol exposed animals have less airspace enlargement compared to 3R4F exposed animals.	*05/29/2016 02:02*
0	**user-200863** Agree with 550063	*06/06/2016 11:30*

ID #1807 (https://scipinion.com/surveys/67/custom_results/1807)

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)?



Legend

To a large extent: 7 *(64%)*

To a small extent: 3 *(27%)*

Not at all: 0 *(0%)*

I cannot answer the question: 1 *(9%)*

answers: 11

skips: 0

Comments

SCORE 1 — **user-550063** — *05/29/2016 02:08*

Overall, the physiological changes are dynamic and these are due to the net result of variety of cellular involvement, numerous adoptive and dynamic processes in every single cell type. On the other hand, lung transcriptomics data interpretation (NPA/BIF) can capture just a snapshot of dynamic bigger level events. So this snapshot cannot explain the complex physiological changes. 1. Lung is a complex organ with at least 40 different cell types. In this context, just upregulation or downregulation of some cellular parameters like apoptosis, autophagy, DNA damage, cell proliferation, cell stress and so on, cannot directly reflect the biological events like inflammation or emphysema in the context of multicellularity of the lungs. If cell death increased, in which cell type it is increased ? Is epithelia dying ? Is immune cell dying ? This kind of questions might be applicable to each and every biological event that has been listed in BIF analysis. 2. In addition every biological event is balanced by other event like MMP activity by TIMP activity. So just observing ECM degradation as tissue damage, we cannot come to straight away conclusion of emphysema because the same tissue had also shown increase of balancing events like fibrosis, wound healing and immune regulation of tissue repair. 3. Further, some of the biological events like cellular stress, DNA damage, ER stress, autophagy are not always detrimental. Indeed these homeostatic mechanisms are necessary for cell survival. If the severity of insults is severe beyond the capacity of these homeostatic mechanisms, it could lead to detrimental effects.

SCORE 0 — **user-382348** — *06/04/2016 11:02*

Again, in agreement with 550063 and 922124

SCORE 0 — **user-200863** — *06/06/2016 11:31*

Agree with other comments.

SCORE 0 — **user-609692** — *06/05/2016 13:23*

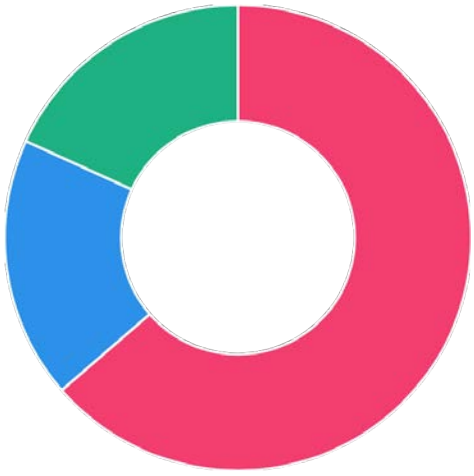
I agree with all the above reviewers.

SCORE 2 — **user-922124** — *05/31/2016 11:50*

I agree with 550063. A number of these concerns could be addressed in a proper dose-response study in WT and ApoE mice

ID #1808 (https://scipinion.com/surveys/67/custom_results/1808)

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: 7 *(64%)*

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 *(18%)*

No, the only reliable approach to understand pathomechanisms is to use separate, mechanism-specific investigations in various dedicated model systems: 2 *(18%)*

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

answers: 11

skips: 0

Comments

SCORE 0 — **user-382348** — *06/04/2016 15:10*

As 550063 points out, the context is extremely important, rather than the broad, sweeping term "inflammation" or "oxidative stress". I am sure this is getting redundant, but rather than using another transgenic/knockout model as 550063 proposes, a simple WT control mouse would greatly assist in dissecting which is due to the insult, rather than the genetic background of the mouse.

SCORE 0 — **user-200863** — *06/06/2016 11:36*

Agree with 550063 and 922124. A dose-response study is needed to make sure the effects are not at nonlinear systemic dose.

SCORE 1 — **user-922124** — *05/31/2016 11:52*

Yes this systems approach is the right approach to assess this issues however again I agree with 550063, and again this needs dose-response data.

SCORE 0 — **user-609692** — *06/05/2016 13:33*

I think there is agreement on the value of the systems toxicology approach and the need for dose -response studies. Not sure that using a (simple?) WT control mouse will help move things down the field. It might further obfuscate matters. Perhaps a good time course study on the Apoe mice to look at when the lung disease begins would be useful.

SCORE 1 — **user-550063** — *05/29/2016 02:21*

I see the responses are surprising. Because the resultant net inflammation and oxidative stress would be different depends on the type of insult, types of cellular involvement and so on. For example, eosinophilic inflammation leads to bronchoconstriction rather than cavity in the lung whereas tuberculous inflammation leads to cavity in the lungs. So ultimately, types of inflammatory mediator decide the pathophysiology rather than just inflammation. Theoretically, Apoe deficiency could increase the inflammation and oxidative stress. However, the existing literature have controversial information. For example, Apoe has been shown to have a protective role as its deficiency promotes oxidative stress (Biochem Biophys Res Commun. 1994 Jun 30;201(3):1567-74 and Free Radic Biol Med. 2002 Oct 15;33(8):1115-20). 2. It is difficult to come to conclusion that the resultant inflammation and oxidative stress in this study design is due to oxidants present in smoke or host response. Depends on the severity of insult, host response mediated events are compensatory. So to dissect this, one need to have anti-inflammatory or anti-oxidant therapy along with cigarette smoke or using NRF-2 KO mice . Using these approaches, one can come to conclusion that event that has happened is harmful or not. Cessation may serve as similar control, but unfortunately this group had also shown the little but significant persistence of inflammation (might be due to epigenetic or autoimmune phenomena). The caution is also required when using Nrf2 disruption as Nrf-2 itself is having pro-atherogenic effect along with its anti-oxidant function (PLoS One. 2008;3(11):e3791). Figure 6B from Titz et al, 2016, revealed that NRF2 perturbation was significant in C57 compared to Apoe KO mice. These evidences indicate the Apoe design may be suitable for transversal evaluation of oxidative stress. In summary, in my opinion separate mechanism specific investigations that use either pharmacological or genetic modulatory strategies are required.

ID #1809 (https://scipinion.com/surveys/67/custom_results/1809)

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?

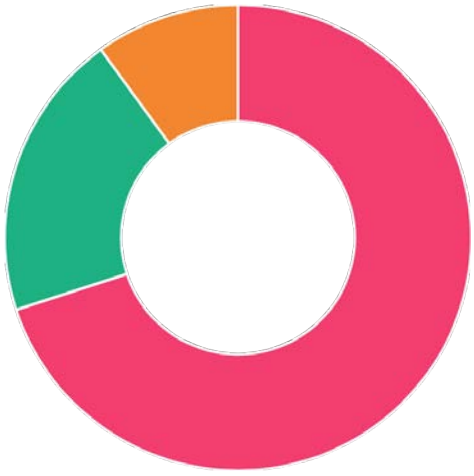
Clear.	The question states "How clear were the changes". Addressing this question, the authors did not display the changes in a way that is easy to discern. Using the heat maps, it seems that the authors again are flooding the reader with numbers/figures, rather than highlighting the actual changes. It is evident if you inspect the data closely that there are clear differences between the ApoE-/- and C57 mice. This can be seen "clearly" in the panel E of the Lung Lipidome, where significant (according to their inappropriately done statistics) differences can be seen in the 3R4F groups in the C57 compared to the ApoE-/-. This is especially evident with the Cer and Glc/GalCer classes. This furthers my concern that this is an inappropriate model to use for this study, although there may be some differences due to the inconsistent treatment times between the C57 and ApoE-/- mice (5 vs 6 and 7 vs 8).
The data in Titz et al. suggested very consistent changes in the lung lipidome in response to CS in these strains. A few genes/proteins were differentially affected, but overall the vast majority of expression differences were preserved across wild-type and apoE-deficient mice.	It was evident that the changes in lipid profiles was more pronounced in C57BL/6 mice than in Apoe-/- mice between 3R4F and pMRTP/THS2.2 The differences in the perturbations of lipid homeostasis between ApoE-deficient mice and wild-type C57BL/6 mice (among tested lipids both in C57 and ApoE KO), is minimum. The difference was related to cholesteryl ester. This was also reflected the Steroid biosynthesis and Steroid hormone biosynthesis strikingly as these two events were significantly higher in Apoe KO mice compared to C57 mice (Figure 2B). Interestingly, this striking difference was also observe in liver also (Figure 4D from La Sasso er al, 2016) indicates the importance both Liver and Lung. This difference can make major difference in inflammation as mild difference in steroid can have major effect in body metabolism.
There are some clear differences between Apoe and WT mice based on KEGG pathways particularly lipid metabolism	The data presented in Figure 3C would indicate general similarities between the 2 strains and that the observed lipidomic responses appear to be related to a more general physiological process held in common between the 2 strains.
C57BL/6 mice seem to be more responsive to lung lipid metabolism biomarkers even the Apoe-/- was also responsive to the CS.	Modest difference with more pronounced effects in APoE- than in C57b6 mice. This is obvious for instance in fig 2a,b where 3R4F causes somewhat more effects. In fig 3c it is clear that effects largely overlap.
Little bit clear	THS2.2 - ApoE -/- 1,2,3,6,7 months pMRTP - C57BL/6 1,2,3,6,8 months Comparing with Sham group, the lipid metabolism was very much clear disturbed in both mouse strains. Comparing both strains, Fig. 1: Lung lipidome showed colesterol and PCO/PCP were higher on Apoe-/-.

Comments

SCORE		
0	**user-382348** no comments, addressed in Stage 1.	*06/04/2016 15:15*
0	**user-200863** no additional comments.	*06/06/2016 11:37*
0	**user-550063** Kind of mixed responses. In my opinion, the differences are minimum. Steroid biosynthesis and Steroid hormone biosynthesis are very different between two strains.	*05/31/2016 03:14*
0	**user-609692** No comments	*06/05/2016 13:34*
0	**user-922124** no further comments	*05/31/2016 11:53*

ID #1810 (https://scipinion.com/surveys/67/custom_results/1810)

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: 7 *(70%)*

Only the 2D measurements confirm the statement: 0 *(0%)*

Only the CT imaging confirm the statement: 2 *(20%)*

No, the statement is not supported by these data: 1 *(10%)*

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

answers: 10

skips: 1

Comments

SCORE 2 — **user-550063** — *05/29/2016 02:25*

Based on the 2D measurements, the authors had claimed that "Following either cessation or switching to THS2.2 aerosol, the plaque area was significantly smaller than in the 3R4F CS exposure group at the months 3 and 8 timepoints-----". They have not considered 6 months effects as both cessation and switching at 6 months time had shown clear increase of the plaque percentage and were comparable to 3R4F 6 months.

SCORE 0 — **user-200863** — *06/06/2016 11:37*

Yes.

ID #1811 (https://scipinion.com/surveys/67/custom_results/1811)

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?

Yes, adequate qualitative information on protein/gene pathways.

I think the gene/protein level profiling was critical in the assessment. The analysis of changes in the lung in Titz et al. required molecular characterization and beautifully tied together the morphological changes related to emphysema to underlying mechanistic responses such as the ECM network.

Yes.

Yes, GSEA and PSEA results of Titz et al., and Lo Sasso et al. effectively contribute to the assessment and they have given adequate information on the protein/gene pathways involvement. However, it seems that the authors have not given the importance to striking pathways like steroid hormone synthesis in Apoe mice. Maybe the aim of the study is not to compare the C57 with ApoE KO mice. But the deep exploration of Steroid biosynthesis and Steroid hormone biosynthesis pathway might give crucial information as most of COPD patients are relatively resistant to steroid.

Yes

The gene /protein set analyses presented by Titz etal and Lo Sasso etal. do contribute to the assessment but provide only limited information on the protein/gene pathways involved. In other words, not a comprehensive snapshot of all the protein / gene pathways potentially involved.

I am not sure about this.

Somewhat. Of course, many of these effects have been observed previously in inhalation studies (i.e. the inflammatory markers). The comparison between gene and protein is not particularly helpful, however, and the correlation between mRNA and protein expression is typically about 40%. The protein expression does provide some useful information as to what pathways are activated using this model, but I do not find the transriptomics that useful.

Omits data in Titz et al do not add much to the obvious conclusion that cessation or switching to THS2.2 attenuates effects caused by 3R4F but they provide qualitative info on potential involvement of SREBP-regulated changes in lipid metabolism. The same goes for data in LoSasso et al 2016 where also qualitative info on lipid and xenobiotic metabolism and iron homeostasis is added.

They give some informaiton on the protein/gene pathways involvement

Both papers presented extensive data on BALF, lung and liver transcriptomics and proteomics that are adequate to the goals proposed by the authors.

Comments

SCORE		
1	**user-550063** Like many, I also agree that GSEA and PSEA results effectively contribute to the assessment.	*05/29/2016 02:27*
0	**user-922124** Yes I agree with comments	*05/31/2016 11:54*
0	**user-200863** yes they do.	*06/06/2016 11:39*

ID #1812 (https://scipinion.com/surveys/67/custom_results/1812)

The analysis of lung transcriptomics data using the causal network model (Philips et al. 2016) predicted a robust CS-induced increase in extracellular matrix (ECM) degradation that disappears upon cessation.

	Yes	No	I cannot answer	Total
Are the quantified histopathological changes in the lung tissue that occur with CS exposure consistent with ECM degradation throughout CS exposure?	**72.73%** 8	**9.09%** 1	**18.18%** 2	11
Are the inflammatory mediators in BALF reflective of observed increase in ECM degradation during CS exposure?	**90.91%** 10	**0.00%** 0	**9.09%** 1	11
Are any of the lung functional parameters likely reflective of ECM/tissue degradation?	**54.55%** 6	**0.00%** 0	**45.45%** 5	11

Comments

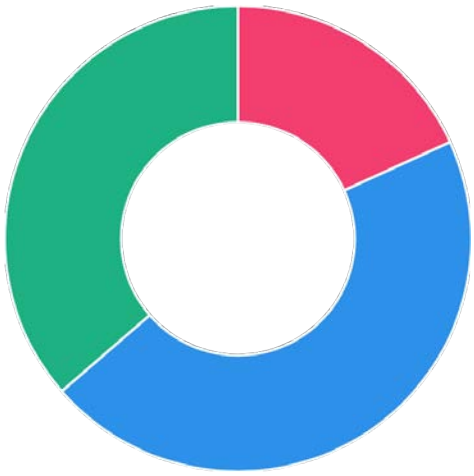
SCORE **0** — **user-382348** — *06/04/2016 15:22*
There seems to be a large consensus. No comments

SCORE **2** — **user-550063** — *05/30/2016 02:40*
It seems unequivocal response except occasional No. But considering these one may have a doubt for first question. 1. Mean chord length (Figure 12A, Philips et al, 2016): 8 months cessation still showed significant increase of Mean chord length (and thus more ECM degradation). In contrast to causal network prediction, it did not come to baseline. 2. Pulmonary emphysema Score (Figure 12D, Philips et al, 2016): All time points in cessation group showed significant increase of pulmonary emphysema Score. In contrast to causal network prediction, it did not come to baseline.

SCORE **0** — **user-609692** — *06/05/2016 13:35*
No comments

ID #1813 (https://scipinion.com/surveys/67/custom_results/1813)

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?



Legend

CS constituents are too diluted to induce CYPs when they reach the liver: 2 *(18%)*

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: 5 *(45%)*

Other (please explain): 4 *(36%)*

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

answers: 11

skips: 0

Comments

SCORE		
2	**user-550063** This question would not have been here if the authors would have measured nicotine and cotinine levels in liver.	*05/30/2016 02:35*

ID #1814 (https://scipinion.com/surveys/67/custom_results/1814)

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 test items: 1) an early prototype MRTP (pMRTP) and 2) the Tobacco Heating System version 2.2 (see Titz et al. 2016)	0.00% 0	9.09% 1	36.36% 4	27.27% 3	27.27% 3	11
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	18.18% 2	54.55% 6	27.27% 3	11
Analysis of the effects of the products on multiple systems (respiratory, cardiovascular) in a single model	0.00% 0	9.09% 1	9.09% 1	36.36% 4	45.45% 5	11
Concordance of results for specific mechanisms (e.g. oxidative stress or inflammation) across diverse studies and model systems (Apoe-/- mouse and in vitro assays)	0.00% 0	0.00% 0	18.18% 2	54.55% 6	27.27% 3	11

Comments

SCORE 0 — **user-382348** — *06/04/2016 15:5(*
Both 550063 and 922124 raise my concerns as well.

SCORE 0 — **user-922124** — *05/31/2016 12:0(*
I agree with 550063. This work will all need to be repeated in a proper chronic study in WT mice examining 40+ tissues

SCORE 1 — **user-550063** — *05/31/2016 03:2(*
Most of the reviewers have given moderate to strong weight. I also agree for questions 1 and 4. But using single model for both diseases are not having strong weight as each organ may need different duration of smoke exposure for the manifestation of pathological changes. For example, there was no cardiac changes even after 6-8 months smoke exposure; Liver did not show any toxicity even after 6-8 months; Lungs show good ECM degradation after 5-6 months. These indicate that one cannot take a single model for checking pathology in multiple organs. Pilot studies are required with different duration of smoke exposure. Since both RRPs did not cause any significant gene expression there is real usefulness of side-by-side analysis .

SCORE 0 — **user-200863** — *06/06/2016 11:4(*
This may be due to the animal model used. A short-term comparative study or data analysis from existing literature in another model (e.g., WT) will be helpful.

ID #1815 (https://scipinion.com/surveys/67/custom_results/1815)

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?

Yes. 1. The return to control levels upon cessation or switching to THS2.2 or pMRTP. 2. Consistency between proteome and lipidome profiles.

This study is like a first step for long way to go for a concept of giving complementary mechanistic information as this is really superficial but good study to scan the alteartion of lipid and its related proteome. I could not see any alteration that can explain the pathogenesis of COPD. What should be changed ? 1. Validation of proteomic data is required (western, ELISA, IHC) 2. One best particular pathway can be focussed. 3. Interference studies are required to confirm the mechanistic role of that pathway in the pathogenesis. 4. Cessation and switch can be planned after 5/6 months 3R4F exposure as 2/3 months 3R4F exposure hardly affected the proteome. 5. Steroid biosynthesis and steroid hormone biosynthesis is a novel direction compared to other known directions. This direction can be emphasized after the protein validation experiments.

Yes, they provide complementary information in the sense of perhaps early and late toxicological mechanisms. The proteomic effects may participate in the initiating events while the lipidomic data may more be more a reflection of cell death and replacement events. Both are important to the overall pathological outcomes. This idea could be evaluated by looking for correlations with morphological events and changes in cell populations over time.

inflammation, antioxidant enzymes, pulmonary funciton

Yes. Arachidonic acid metabolism/ sphingolipid metabolism and proteins implicated in reactive oxygen species have changed.

Yes they examined and had biological overlap in 3 key areas surfactant lipd/protein correspondence alterations in eicosanoids/metabolizing enzyme correspondence sphingolipid alterations and enzyme changes.

Yes. 1. Figure 1 E and F are clearly showing differential profiles in different group. 2. Figure 2 is clearly showing the difference between the two mouse species and among different groups for lipid-related lung proteome. 3. Other figures and plots are showing the same results and very obvious on the effects of CS on all the biomarkers whereas, similar effects are much lower in the other groups including non-traditional (heat-not-burn) smoking devices.

I do not believe so. Without an actual readout as to the functional activity of the changes observed, it is these data do not provide any mechanistic information. The increase in FASN is suggestive of an increase in total 16:0 species; however this is not universal across the board. The biggest question is, what is the consequence of the increases/decreases in any altered lipid species? What is the functional role of an increase in total 16:0? No mechanistic information is really displayed, but rather correlations are drawn between measured alterations in the proteome and lipidome.

yes: -- Induction of surfactant proteins Sftpa1 and Sftpd combined with increased PC 16:0/16:0, PC 16:0/16:1, and PC 16:0/14:0. -- Increase in Ptgs1(COX1) combined with increased prostaglandins D2/E2

Both approaches indicated that many lung molecules were affected by the exposure of CS. Most of the molecules increased its concentrations. Increase in several lipids were correlated with changes in the surfactant metabolism, phospholipases activity. Phosphatidylcholine was the most affected by far, followed by SM, phosphatidylserine, ether-linked phospholipids (PE O), PG and GD1.

Comments

SCORE		
0	**user-382348** I do not think they are, as evidenced by my answer. Yet, like 550063 mentions, this is a crazy question to ask. Without a mechanistic investigation, we can't really know...	*06/04/2016 15:55*
0	**user-550063** This is really tough at the same time crazy question in the context of very scarce literature in this direction. Very careful depth experiments are required to answer this question.	*05/30/2016 01:59*
0	**user-200863** no comment.	*06/06/2016 11:46*

ID #1816 (https://scipinion.com/surveys/67/custom_results/1816)

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?

Several of the networks can explain the profiles observed.	Inflammatory infiltration under CS exposure involved neutrophils > lymphocytes > dendritic cells > macrophages. The numbers of all cell types returned to the sham/THS2.2 group level upon cessation or switching. Likewise, BIF analysis points to 14 inflamation-related subnetwork NPAs. In agreement with inflammatory cells detected, these include Treg Signaling, Th... signaling, Neutrophil Signaling, Macrophage Signaling, Dendritic Cell Signaling, Cytotoxic T −cell Signaling. All these NPAs return to THS2.2 levels after switching or cessation. So indeed, BIF analysis points to inflammatory networks that can explain the type of inflammation.
GM-CSF levels and differences in alveolar dendritic cells between the two groups (this would be one network).	Secreted inflammatory mediators: 1. Lymphotactin (found increased in cessation at 8 months) is known T-cell chemoattractant chemokine (Am J Pathol. 2001 Nov;159(5):1751-61.) and this can explain the increased lymphocytes in cessation. 2. MIP 1 alpha (CCL3) attracts macrophages, monocytes and neutrophils (J Leukoc Biol. 2005 Jul;78(1):167-77). This could explain the presence of neutrophils (also in BAL) and macrophages in cessation ans switch groups. 3. Monocyte chemoattractant protein-1 (MCP-1/CCL2) that regulate migration and infiltration of monocytes and macrophages (J Interferon Cytokine Res. 2009 Jun; 29(6): 313–326./macrophages) could explain the presence of macrophages in both cessation and switch. 4. MPO indicate the presence of active neutrophil (in BAL of cessation and switch groups) 5. M-CSF, chemokine, attract macrophages to the site of inflammation. (Mol. Cell. Biol. May 2005 vol. 25no. 10 4211-4220). This could explain the presence of macrophages in both cessation and switch. 7. IL-1beta induces neutrophil migration (Inflammation. 2008 Feb;31(1):36-46.). This could explain the presence of neutrophil (in BAL of cessation and switch groups). 8. GM-CSF known for its role in recruitment of neutrophil to site of insult (J Leukoc Biol. 2011 Jun;89(6):945-53.), This could explain the presence of neutrophil (in BAL of cessation and switch groups) inflammatory networks from the BIF analysis 1. Th1-Th2 signaling and Th17 signaling could explain the presence of neutrophils and macrophages (Th17 is well known for its neutrophilia and Th2 can induce arginase in macrophages). 2. Neutrophil signaling can explain the presence of neutrophils in BAL of cessation and switching. 3. Macrophages signaling for the presence of macrophges in cessation and switching. 4. Epithelial innate immune activation can explain the presence of variety of inflammatory cells as the epithelial secreted mediators recruits variety of immune cells depends on severity of insult (Nat Immunol. 2010 Aug;11(8):656-65). 5. Cytotoxic T cell and B cell signaling could explain the presence of Lymphocytes in cessation and switching. 6. Epigenetics could indicate the amplification of inflammation or relative persistence of inflammation as smoke induced cytokines may cause the epigenetic changes such a way that there will be inflammation even after quitting (Multidisciplinary Respiratory Medicine 2011, 6:210). The relative persistence of inflammation even after quitting smoking is the major concern (Thorax. 2006 Feb; 61(2): 96–97 and Multidisciplinary Respiratory Medicine 2011, 6:210). This leads to thinking of initiation of autoimmune phenomena that can amplify the inflammation even without fresh insult.
yes, There remain increases in IgA, ILs, monocyte attractants, and MMP-9 and the BALF shows increased neutrophils/macrophages.	Perhaps depending upon when in the inflammatory process samples are collected. Consider looking at changes in BIF and BALF and correlating with histopathology data from early, middle and late phases of the experimental protocol. A time course study of these parameters in relation to histopathology data.
For BIF or RBIF shown in Figure 14 and BALF in Figure 8 show that both in the cessation and switch groups the biological impact never reached above 10% of the biological impact observed in 3R4F CS groups. Similary, the FLC counts almost reached to the sham level within one month and completely returned to the sham levels by 8 months. A similar reversal of the metaplasia and hyperplasia were observed in the nose and of inflamatory changes in the lungs in the switch and cessation groups reaching to sham levels after 6 months. Such effects were never observed in the THS groups. From Figure 11, I can discern mostly unpigmented and pigmented macrophages were responsible for the inflamation.	I do not believe the BIF analysis to be overly useful in this situation. The BALF does assist in determining the type of inflammatory cell infiltration, however standard immunohistochemistry would be a much more useful direct measurement of the type of inflammatory cells that are playing a role . Even after a careful read of the suggested literature, I didn't accumulate enough knowledge during this review to correlate the results of BIF analysis and type of cell infiltration. However, BALF cell counts showed that alveolar dendritic cells were the majoritarian cell type in the cessation and switch groups comparing to the Sham. Authors could determine the amounts of dendritic subtypes, plasmacytoid and myeloid. Dendritic cells are vital for both the initiation and the maintenance of allergic airway inflammation in asthma.
noe	

Comments

SCORE		
2	**user-859026** I agree with the comments of one of the reviewers that for BIF or RBIF shown in Figure 14 and BALF in Figure 8 show that both in the cessation and switch groups the biological impact never reached above 10% of the biological impact observed in 3R4F CS groups. It is unclear if this is even a significant observation.	*06/05/2016 19:23*
2	**user-550063** There are number of inflammatory networks from the BIF analysis or secreted inflammatory mediators in BALF explained the type of infiltrated inflammatory cell observed during the histopathological evaluation in the cessation and switch group.	*05/30/2016 01:51*
0	**user-922124** The demonstration of inflammatory networks combined with cellular assessment of BALF support	*05/31/2016 13:00*
0	**user-200863** agree with 859026	*06/06/2016 11:49*

ID #1817 (https://scipinion.com/surveys/67/custom_results/1817)

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?

Despite the 20X differences reported for THS2.2, it is intriguing that profiles between 3RF4 and THS2.2 can be comparable. This homology should be explored further.

These studies using single exposure levels of CS compared to THS2.2 qualitatively shows a reduction in risk.

The data presented are adequate for the studies conducted. However, I would have liked to see several scenarios of exposure, e.g., longer exposure to all, switching and cessation groups are exposed for longer period of time before switching, use of humanized animal models.

no

none

None that I can determine.

The comparison between the two groups has been satisfactory

No- the story seems rather complete. My only concern with the use of only female mice in these studies. The rationale for this seems tenuous and not really supported by the cited literature.

None of particular importance, aside from the differing timepoints in Titz et al.

1. Philips et al, 2016: Aortic arch plaque area measurements: Switching groups (6 months)also had shown the significant (it seems) increase of percentage of Plaque compared to Sham 6 months. Statistical significance was not calculated and it was discussed. 2. Liver histopathology data from La Sasso et al, 2016: The 6 months THS had shown similar harmful effects compared to 3R4F towards bile duct hyperplasia, and inflammatory cell recruitment. These are not discussed properly. 3. Proteomics data from La Sasso et al, 2016: THS has shown the clear upregulation of some Steatosis and Phospholipidosis proteins especially at 8 months. 4. sPLS analysis from La Sasso et al, 2016: 8 months THS exposure and Switch group altered almost of 90 % genes. These data were not given emphasis and statistical tests were not tested. The cumulative effects of these mild to moderate grade gene alteration might have cumulative toxicity. 5. Parenchymal amunt of fat from La Sasso et al, 2016: In contrast to the authors claim (Despite the time-dependent increase of parenchyma fat accumulation in the liver tissue observed in all groups, no statistically significant histopathological differences among exposure groups were observed.), THS both 8 months and Switch 8 months clearly shown the accumulation of parenchymal fat. This is well correlated with Figure 3 where THS had shown the increase of steatosis. Indeed, the authors tested the significant against its own 1 month group. However, they failed to test such a drastic change with Sham groups. 6. Titz et al, 2016: Protein set enrichment analysis: "Synthesis and degradation of ketone bodies" got significantly (seems) altered with Switch and THS groups and this was not discussed.

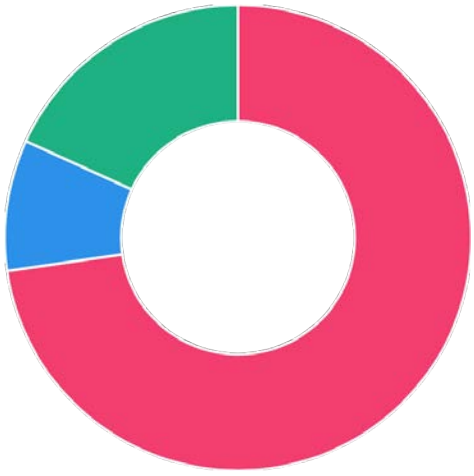
The discussion sections are quite ample in Phillips, Titz, Lo Sasso and Szostak papers. However, I would include few sentences discussing the possible allergens response, asthma development and dendritic cells subtypes in Titz. Phillips, Titz, Lo Sasso and Szostak papers are of excellent quality.

Comments

SCORE		
0	**user-200863** lack of dose-response and determination of systemic dose for kinetic linearity.	*06/06/2016 11:5*
0	**user-859026** I would agree with the concern that only one exposure dose is examined in the study design.	*06/05/2016 19:2*
0	**user-550063** One may see the number of listed concerns as shown above and then possibly relook this.	*05/30/2016 01:4*
3	**user-922124** The lack of a dose-response is concerning since much of the reported studies will need to be repeated again in WT rodents.	*05/31/2016 13:0*

ID #1845 (https://scipinion.com/surveys/67/custom_results/1845)

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



Legend

Yes: 8 *(73%)*

No: 1 *(9%)*

I cannot answer: 2 *(18%)*

answers: 11

skips: 0

Comments

SCORE
2

user-550063 *05/30/2016 01:42*

Tough question as one has to consider both statistical and biological significance. Though the authors claim that 2 months 3R4F smoke exposure develop emphysema based on some statistical significance for some parameters, overall only 6 months had shown good emphysema in my opinion. See Figure 12D (Philips et al, 2016), Pulmonary emphysema at 2 months is having borderline significance. Interestingly, one month also had shown the statistical significance. They why the authors did not select one month. See Figure 12E (Philips et al, 2016), alveolar density showed marginal change at 2 months. See Figure 12F (Philips et al, 2016), alveolar surface density showed a similar effects at 2 months compared to Sham 2 months. See Figure 12A (Philips et al, 2016), mean chord length, a crucial parameter of emphysema, showed marginal change at 2 and 3 months. See Figure 12B (Philips et al, 2016), bronchiolar attachment showed marginal change at 2 and 3 months.

ID #1819 (https://scipinion.com/surveys/67/custom_results/1819)

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

No.

In phillips et al 2016 liver histology does not reveal any exposure-related changes above the age-related effects in apoe- mice. In LoSasso et al 2016, Apoe- mice also show exposure unrelated transcriptomic changes in the liver over time. On top of this, there appears to be a specific downregulation of pyrimidine/purine metabolism in the liver of THS2.2. exposed APoe- mice (LoSasso et al 2016 Figure 4D, proteomics) but besides that effects are negligible.

In my opinion, both 3R4F and THS exposures behaved similarly based on the histopathological analysis. In fact, both THS and 3R4F increased the accumulation of collagen fibers, bile duct hyperplasia and infiltration of mononuclear inflammatory cells in periportal and interstitial areas at 6 months time point. Evidently, both THS and 3R4F leads to reduce the gene expression related necrosis (less necrosis may be related more bile duct hyperplasia). However, the authors could not find the clear time-consistent differences in histopathology endpoints. This could be due to the different studies for different time point (different pooling) rather than exposing all mice together and then sacrifice at different time points. Figure 2 from La Sasso et al, 2016: The 6 months harmful effects of both THS and 3R4F is clearly visible in transcriptomics as THS, 3R4F, and switch data all clustered together at 6 and 8 months time points. Figure 3: Proteomics: THS has shown the clear upregulation of some Steatosis and Phospholipidosis proteins especially t 8 months and these upregulations were not visible in Switch groups. This could be due to accumulative toxicity of THS which needs at least 8 months exposure. Figure 4D: It is really strange that though 8 months THS exposure and Switch group altered almost of 90 % genes proteomics could not find them. This indicates the possibility of alteration of set of microRNAs by THS. Figure 6: Similar strange results in Metallothionein 1 and 2 (mt 1 and mt 2). 8 months THS had shown upregulation of these genes (both Switch and THS 8 months) without change in the respective protein. This indicates the possibility of alteration of set of microRNAs by THS to cause such discrepancy. Otherwise THS may have effect on protein degradation machinery such a way that it degrades these proteins. Figure 5B: In contrast to the authors claim, THS both 8 months and Switch 8 months clearly shown the accumulation of parenchymal fat. This is well correlated with Figure 3 where THS had shown the increase of steatosis. Overall, it seems that THS affected the liver lipid metabolism at moderate level compared to 3R4F.

Yes there appears to be some slight induction GSTs (e.g., mu class) that is involved in detoxication of electrophiliic compounds. The lack of Cyp1a2 in the THS2.2 group, known to be induced by nicotine is a concern. Some follow up the microarray data using a second methods for specific suspect genes qRT-PCR would eliminate this concern

I do not; however, as indicated multiple times, I believe the model chosen is masking any effects that may be present.

I find essentially no evidence based on the data provided and linked to THS2.2.

The study is based on using Apoe-/- strain, so cannot make conclusive inferences.

The finding of periportal interstitial fibrosis in the CS, THS2.2 and cessation groups relative to the sham controls at later (6 momth) time point is suggestive of some mild cell injury process from the THS2.2 aerosol (Lo Sesso etal., 2106). Suggest considering the effects of cadmium which is toxic to vascular endothelial cells.

Yes. I can see the difference of traditional CS on the induction of molecular response in Figure 4A, induction of protein expression profile related to lipid-metabolism (Figure 5C), induction of oxidative stress related proteins (Figure 6), increased hematocrit to compensate for low oxygen carrying capability from smoking of traditional cigarette (3R4F).

No, I didn't notice any substantial effect. I do see changes in 3 months porphyrin and fatty acid elongation, 8 months pyrimidine and purine metabolism, and retinal metabolism. But according to the authors, there were no statistical difference comparing to Sham.

no

Comments

SCORE		
1	**user-859026** I think the lack of ascending doses with higher THS2.2 particulates leads to uncertainty with the liver evaluation.	*06/05/2016 19:29*
0	**user-550063** Many reviewers have suggested that THS aerosol did not have substantial effect in the liver tissue. In my opinion, the authors had written in a biased way. But careful scrutinization of the findings without considering the authors description may reveal many flaws in their description. I have listed many as shown above.	*05/31/2016 03:38*
0	**user-200863** Only marginal, effects may be more pronounced at higher doses in dose-response study.	*06/06/2016 11:53*
0	**user-922124** There appear to be marginal effects of THS2.2 in liver that needed follow up by a second method. Microarrays are not considered to be highly quantitative platform.	*05/31/2016 13:08*

ID #1820 (https://scipinion.com/surveys/67/custom_results/1820)

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)?

	Good model	Poor model	Other (please explain below)	Total
Inflammatory diseases	**72.73%** 8	**18.18%** 2	**9.09%** 1	11
Respiratory diseases	**72.73%** 8	**18.18%** 2	**9.09%** 1	11
Cardiovascular diseases	**90.91%** 10	**0.00%** 0	**9.09%** 1	11
Neurological diseases	**18.18%** 2	**36.36%** 4	**45.45%** 5	11
Hepatotoxicity	**45.45%** 5	**27.27%** 3	**27.27%** 3	11
Metabolic syndrome / diabetes	**36.36%** 4	**27.27%** 3	**36.36%** 4	11

Comments

SCORE **1** — **user-550063** — *05/30/2016 01:25*
Interestingly, many reviewers indicated the usefulness of Apoe-/- mice in Inflammatory, Respiratory and Cardiovascular diseases. In my opinion, Apoe-/- mice may be good for restricted inflammatory diseases like Th1 or TNF-alpha dominant diseases as these mice inherently showed the up regulation Th1 response with higher levels of TNF alpha. Similarly, Apoe-/- mice seems to be the good model in restricted neurological diseases in which brain cholesterol homoeostasis is essential like Alzheimer disease, and demyelinating diseases.

SCORE **0** — **user-922124** — *05/31/2016 13:08*
I agree with 550063

SCORE **0** — **user-609692** — *06/05/2016 13:44*
Agree

SCORE **0** — **user-200863** — *06/06/2016 11:55*
Agree with 859026

SCORE **1** — **user-859026** — *06/05/2016 19:34*
There is plenty of inbred mouse strain-dependent variability in disease susceptibility. Widely studied C57BL/6 mice are resistant to atherosclerosis, and thus the use of the apoE knockout to increase susceptibility. For Alzheimer's disease, C57s do not develop the disease upon aging like humans, and a transgene greatly overexpressing beta amyloid is required for a disease model. ApoE knockouts on their own are not used for this type of neurological disease.

ID #1821 (https://scipinion.com/surveys/67/custom_results/1821)

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.

	Not appropriate	Moderately appropriate	Very appropriate	Total
Dose	**18.18%** 2	**54.55%** 6	**27.27%** 3	11
Regimen	**9.09%** 1	**63.64%** 7	**27.27%** 3	11
Duration	**27.27%** 3	**63.64%** 7	**9.09%** 1	11
Biomarkers of exposure	**9.09%** 1	**27.27%** 3	**63.64%** 7	11

Comments

SCORE **0** — **user-859026** — *06/05/2016 19:38*
The aerosol exposure mimics second hand smoke inhalation in humans. I would imagine direct inhalation induces more damage in airway epithelia and the lung.

SCORE **0** — **user-550063** — *05/30/2016 01:18*
As majority of reviewers accepted the appropriateness of biomarkers, I also point out the same. Similarly, as many suggested the moderate appropriateness of dose, regiment and duration, I also point out the same due to following reasons. Dose and Regimen: Overall total dose is fine. However, the two days missing doses and crowding the cigarette exposure (compared to widespread periodical exposure in human) precludes proper extrapolation to humans. The missing doses and days might allow some time for repair and homeostasis.

SCORE **0** — **user-609692** — *06/05/2016 13:46*
Good points from 550063 and 922124- I agree

SCORE **1** — **user-922124** — *05/31/2016 13:10*
The data are qualitative in nature and not easily extrapolated to humans unless the individual are ApoE variants.

SCORE **0** — **user-200863** — *06/06/2016 11:57*
The data are qualitative, need dose-response and knowledge of systemic exposure for better extrapolation to humans.

ID #1822 (https://scipinion.com/surveys/67/custom_results/1822)

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?

None using this model. The measurement of nicotine is likely accurate, however I do not trust the model utilized, so it is hard to extrapolate this to "which mechanisms are relevant for human disease".

From the data presented in this collection of murine studies, I feel that the CS exposure paradigm revealed both atherogenic mechanisms as well as COPD mechanisms that would be initiated in humans following a period of smoking. Oxidative stress responses, inflammation resulting in plaques in CVD and specific induction of pathways leading to ECM damage in COPD.

oxidative stress, emphesematous changes, atherosclerosis are all major disease mechanisms

1. ECM degradation 2. Oxidative stress 3. Inflammation 4. Aortic arch lipid deposition

Pathobiological mechanisms are translatable. The inherent resistance of mice to disease complications needs to be investigated as it can serve to better define human response to injury.

Initiation of inflammation in the lung followed by pulmonary scarring and development of COPD.

Mechanism of emphysema and chronic bronchitis and to some extent cardiac response.

Potential CVD effects of CS in individuals with ApoE deficiency. Not sure how this will apply to the general population since this "short cuts" the long term repeated daily inhalation of CS.

Oxidative stress and UPR together with inflammatory responses contributing to lung emphysema and vascular pathologies (e.g. plaque formation)

In my opinion, the animal model exposed to CS provides a fairly comparable panorama of the CS effects on humans. Despite the obvious differences in physiology of mouse and humans, mouse biological responses of the lung and liver can be somehow extrapolated to humans. The data presented in all cited papers provided a strong background to move to the research next level: human studies.

chronic inflammatory reponse

Comments

SCORE		
0	**user-609692** Agree with both	*06/05/2016 13:46*
3	**user-550063** As many suggested inflammation, oxidative stress are the major mechanisms that can be translatable to humans.	*05/30/2016 23:41*
0	**user-922124** Common effects like inflammation/ROS could be translatable to people.	*05/31/2016 13:11*
0	**user-200863** Agree with the comments.	*06/06/2016 11:58*

ID #1823 (https://scipinion.com/surveys/67/custom_results/1823)

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?

No. I think these studies have captured most of the aspect of CS of concern and showing the lack of hallmark responses including biomarkers of toxicity when used the modified heat-not-burn tobacco delivery methods. I would like to see if such products can also be made available for "Sheha" and "Hukka" or water-pipe users.

Studies that are compliant with OECD test guidance and studies that include dose response to conduct quantitative risk assessment for THS2.2 compared to air. Addition of the informative biomarkers shown here to a guidance study. Unfortunately the rats in the preferred species for inhalation toxicology all of these studies were done in mice that have limited historical controls.

I think that the majority of the measurements conducted are reliable. The only major issues are the statistical comparisons and the model utilized.

In my opinion the methodologies used in these studies are appropriate

No suggested alternatives

1. Micro CT thorax for the best assessment of emphysema 2. Transthoracic echocardiography, 24 hr BP for cardiovascular function 3. Micro CT for anti-gravity skeletal muscle for skeltal muscle loss 4. fMRI for brain for cognitive dysfunction

It would be helpful to see a more comprehensive concentration-response analysis.

No - The methodologies seem very comprehensive and should capture the effects of 3R4F and THS2.2 on the respiratory tract. Suggest focusing on which cell types in the lung are responding to the CS and THS2,2 vapors to more accuarately develop a mode of action risk assessment for CS and THS2.2.

No additional methodologies suggested - move to clinical studies

An interesting experiment would be testing the effects of nicotine alone (and byproducts). I think in the future the nicotine will be the only chemical available for the lung. I think the other chemical products derived from tobacco heating will be meaningless.

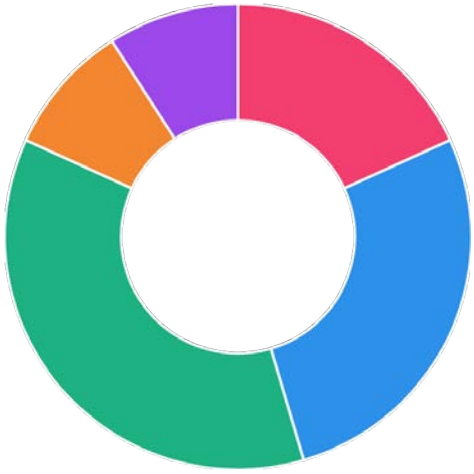
none suggested

Comments

SCORE		
0	**user-200863** Agree with 550063	*06/06/2016 11:5*
1	**user-509661** The presented results examine the monocyte adhesion rate, transendothelial migration etc. Have the authors considered evaluating the malignant transformation potential of human bronchoepithelial cells (BEAS 2B cells) and tumorogenesis after long-term exposure to aerosols from CS and other tobacco products?	*05/30/2016 12:4*
3	**user-550063** I agree with many reviewers who suggested that the existing methods are sufficient to prove the point. In any event one can use Micro CT thorax for the best assessment of emphysema and skeletal muscle loss.	*05/30/2016 01:0*
0	**user-609692** No Comments	*06/05/2016 13:4*
0	**user-922124** no further comments	*05/31/2016 13:1*

ID #1824 (https://scipinion.com/surveys/67/custom_results/1824)

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. 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?



Legend

Extremely confident: 2 *(18%)*

Very confident: 3 *(27%)*

Confident: 4 *(36%)*

Somewhat confident: 1 *(9%)*

Not confident: 1 *(9%)*

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

answers: 11

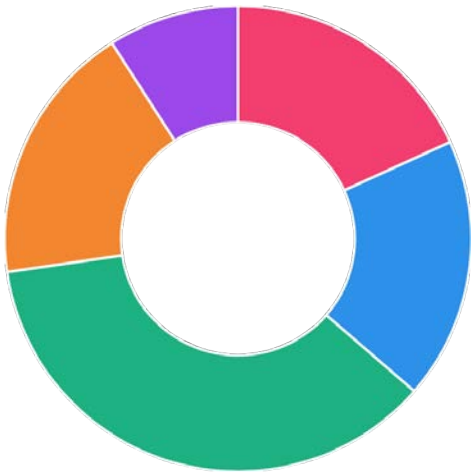
skips: 0

Comments

SCORE		
0	**user-609692**	*06/05/2016 13:5(*
	Good points and I wonder if a more detailed time course study with more closely timed interim sacrifices would be helpful to address these issues.	
3	**user-550063**	*05/30/2016 01:0(*
	The biological impact changes between smoking cessation and continuing smoke exposure is really drastic. These changes suggest that cessation had restored number of pathological features except BAL inflammatory cells. However, I am not confident as cessation has been done before the development of fullest disease.	
1	**user-922124**	*05/31/2016 13:1*
	The limitation of this model developing spontaneous disease seem to be a primary driver for length of exposure, Regulatory agencies like FDA will likely want to see chronic effects beyond 6 mths	
0	**user-200863**	*06/06/2016 12:0*
	I agree with 550063 and mentioned in many of my answers/comments that the outcome may have been different if the exposure had been extended beyond 2 months.	
0	**user-609692**	*06/05/2016 13:5(*
	Good points and I wonder if a more detailed time course study with more closely timed interim sacrifices would be helpful to address these issues.	

ID #1825 (https://scipinion.com/surveys/67/custom_results/1825)

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. 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?



Legend

Extremely confident: 2 *(18%)*

Very confident: 2 *(18%)*

Confident: 4 *(36%)*

Somewhat confident: 2 *(18%)*

Not confident: 1 *(9%)*

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

answers: 11

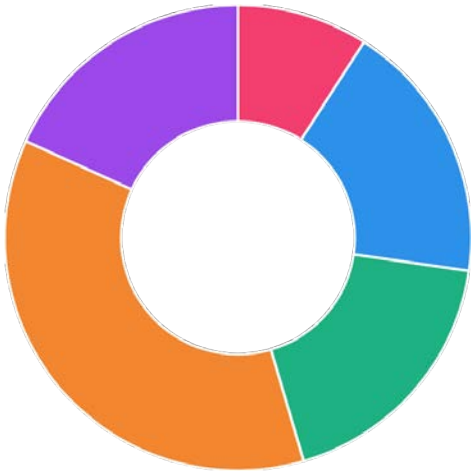
skips: 0

Comments

SCORE		
2	**user-550063** The biological impact changes between switching to THS2.2 and smoking cessation are very minimum. These changes suggest that THS2.2 switching is as better as cessation. However, I am not confident as switching has been done before the development of fullest disease.	*05/30/2016 00:5*
0	**user-200863** I agree with 550063 and mentioned in many of my answers/comments that the outcome may have been different if the exposure had been extended beyond 2 months.	*06/06/2016 12:0*
0	**user-609692** I agree with the above comment.	*06/05/2016 13:5*
0	**user-859026** In these particular studies, the difference was minimal between cessation and switching. I have doubts that this will translate as well to humans, as a result of dose and length of exposure and other factors related to assay sensitivities.	*06/05/2016 20:4*

ID #1826 (https://scipinion.com/surveys/67/custom_results/1826)

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?



Legend

Extremely confident: 1 *(9%)*

Very confident: 2 *(18%)*

Confident: 2 *(18%)*

Somewhat confident: 4 *(36%)*

Not confident: 2 *(18%)*

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

answers: 11

skips: 0

Comments

SCORE		
0	**user-609692** No comment	*06/05/2016 13:53*
1	**user-550063** This is really tough question it seems as one can see equally mixed responses. In my opinion, to identify COPD or CVD drug targets, one might also need Systems Medicine approach rather than merely Systems Toxicology approach for following reasons: 1. These studies are pathophysiology oriented study rather than therapeutic oriented. So biological events during restoration were not captured. 2. Though the cessation can be taken as restoration point of view, the cessation has been done even before the full development of the disease. As a result, it is difficult to conclude whether the observed biological perturbations are due to harmful effects of smoke or host compensatory mechanisms to maintain homeostasis. 3. Though the study had looked numerous pathways superficially through OMICs strategies, not a single pathway or its kinetic details is investigated in depth. 3. Treatment control (smoke exposed with treatment leads to restore the disease features) are necessary to pick the drug target.	*05/30/2016 00:56*
0	**user-200863** These may provide some guidance; however, pathways may be different.	*06/06/2016 12:04*
0	**user-922124** Point 2 and 3 from 550063 are major concerns for this reviewer	*05/31/2016 13:16*
1	**user-859026** I am somewhat skeptical that the most targetable pathways or critical elements would be identified in CS-induced COPD. Without identifying modifiers of disease risk in humans, it will be hard make reasonable guesses for screening.	*06/05/2016 21:03*

ID #1827 (https://scipinion.com/surveys/67/custom_results/1827)

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.

Yes, the findings are translatable to other environmental exposures. For instance, oxidative stress is a common pathway for environmental injury (Miller and Ramos, 2004).

They could be translated to risk assessment of other toxicants. For instance in case of chronic air pollution changes in lipid metabolism and activation of inflammatory responses likewise appears to contribute to aspects of atherosclerosis identified using the same Apoe- model (Circ Res. 2014 Oct 10;115(9):770-80). Likewise, in studies examining "Beijng ambient particle exposure using the Apoe- model, again inflammation, oxidative stress and altered lipid metabolism were identified in parallel with enhanced signs of atherosclerosis (Cell Stress Chaperones. 2014 Sep;19(5):715-24). This points to common mechanisms that can be leveraged for risk assessment purposes.

In my opinion, only for comparison as different environmental air pollutants may be effecting through different mechanism. For example, what to expect if these biomarkers are observed.

Yes, because the cigarette smoke effect findings can be leveraged for risk assessments of the same or other inhaled toxicants found in workplace and environmental exposures. Examples would included fine particulates, organic and inorganic chemical mixtures such as emissions from coal-fired power plant which contain similar types of chemicals. Persons occupationally exposed to PAHs as cooks in the restaurant industry might be another group of concern.

None that I am aware of.

I do not believe so. Each study should be treated uniquely, as there is too much variability in sample preparation, data analysis etc.

Not, not without a dose response

Certainly yes. I would include the particulate matter (PM). 1. Teng TH, Williams TA, Bremner A, Tohira H, Franklin P, Tonkin A, Jacobs I, Finn J. A systematic review of air pollution and incidence of out-of-hospital cardiac arrest. J Epidemiol Community Health. 2014 68(1):37-43 2. Bhatt SP, Dransfield MT. Chronic obstructive pulmonary disease and cardiovascular disease. Transl Res. 2013;162(4):237-51 3. Weichenthal SA, Godri-Pollitt K, Villeneuve PJ. PM2.5, oxidant defence and cardiorespiratory health: a review. Environ Health. 2013, 4;12:40

cigarett smoking may not e comparable with other ihaled toxicants in terms of dose and frequency.

1. Cigarette contains more than 5000 chemicals and its toxicity is further amplified by its burning. If there is any considerable overlap between active chemicals and the burning process, one can confidently leverage the findings of cigarette smoke to risk assessment of other inhaled toxicants, such as environmental air pollution. 2. So kitchen smoke and industrial smoke and some level vehicular smoke may get such constituent and process overlap, indeed, Indian women who exposed to biomass fuel used kitchen smoke develop cigarette smoke exposure like COPD (Koul, Lung India. 2013 Jul-Sep; 30(3): 175–177). 3. The workplace exposure mostly has single dominant chemicalwithout burning process like silica, cotton, asbestas etc. Mostly these exposures leads to decline in lung function but in a different way like developing lung injury followed by fibrosis. The site of fibrosis is different in emphysema and these diseases. There is small airway remodeling in COPD whereas parenchymal fibrosis is observed in most of the work exposure or occupational lung diseases. So the translation is difficult in some conditions and partially relevant in some conditions. There is a necessity of comparison and side by side chemical analysis of the components smoke and other toxic inhalants before attempting to such leveraging.

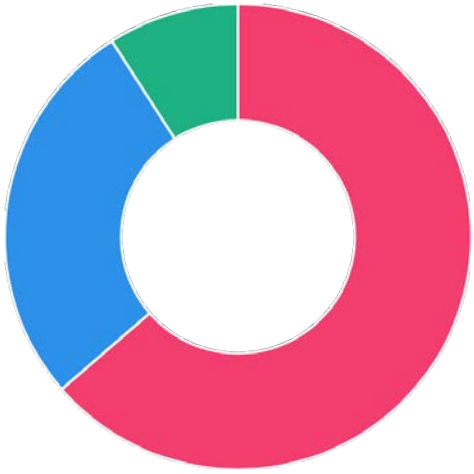
Yes, particularly for air-pollution related effects.

Comments

SCORE		
0	**user-200863** I agree with 550063 and would like to add the need of dose-response study for better understanding.	*06/06/2016 12:07*
0	**user-609692** I really agree with 550063 here since mixtures toxicology is just taking off and CS in combination with other common inhaled agents such as formaldehyde or solvents is a pressing public health issue.	*06/05/2016 13:55*
2	**user-550063** Almost equal mixed responses. In my opinion, before leveraging or translating to other toxicants, side by side chemical analysis of the components of smoke and other toxic inhalants should be done.	*05/30/2016 00:52*

ID #1828 (https://scipinion.com/surveys/67/custom_results/1828)

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:



Legend

The correlation between human and murine plaque-enriched lipids is suggestive of related pathomechanisms in plaque formation in this mouse model of atherosclerosis.: 7 *(64%)*

The three unchanged lipids represent the ApoE deficiency-related imbalance in lipid metabolism, particularly the disrupted reverse cholesterol transport.: 3 *(27%)*

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: 1 *(9%)*

Other (please explain): 0 *(0%)*

answers: 11

skips: 0

Comments

SCORE		
0	**user-609692** Agree with both of the above	*06/05/2016 13:5*
2	**user-550063** I agree with many reviewers suggestions of related pathomechanisms in plaque formation in this mouse model of atherosclerosis.	*05/30/2016 00:4*
0	**user-922124** I agree with the majority of the reviewers comments	*05/31/2016 13:1*
0	**user-200863** Agree.	*06/06/2016 12:0*

ID #1829 (https://scipinion.com/surveys/67/custom_results/1829)

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?

Yes, the more comprehensive the better, especially when considering the introduction of a "safer" alternative.

No, I believe examination of nose/lung (histopathol, omics, and functional) combined with analysis of blood samples and cardiovascular system is in fact sufficient to capture the critical impacts.

I do not think that there are any other tissues/samples that need to be analyzed. Had WT mice been used, I think this study would be much more "translatable". In addition, correct statistical comparisons should have been conducted (previously discussed).

I consider these studies to be quite comprehensive and cannot think of any additional aspects for future studies. However, may like to add or subtract depending upon the nature of the study and underlying questions/issues needing answers.

no other suggestions

Reproductive organ systems are worthy of study since a number of hormones are similar among mammals. These organ systems could respond on either a primary or secondary basis.

Again OECD inhalation study (90 day or chronic) in wild type rats using THS2.2 will likely be needed at some point with examination of 40 tissues.

CS exposure has being implicated in many toxic effects in other organs, tissues and reproduction: eyes, brain, skin, join, muscles, stomach, bladder, intestines, reproductive organs, bone marrow cell production, embryo development problems. Some conditions such as Rheumatoid Arthritis, diabetes, Inflammatory Bowel Disease, Immune Function and Autoimmune Disease, Ectopic Pregnancy, Breast Cancer and others have also been implicated with CS exposure.

1. Skeletal muscle as skeletal muscle loss has been observed in most of the severe COPD patients. In addition, GOLD stage severity has been shown to correlated with loss of particular skeletal muscle like anti-gravity muscle like erector spinae (Tanimura K et al, Ann Am Thorac Soc. 2016 Mar;13(3):334-41). In mice soleus, anti-gravity muscle can be tested and the methods like fMRI or micro CT for assessing cross sectional area, muscle electrophysiology, walking tests along with other omics. 2. Brain: The cognitive dysfunction has been observed in most of COPD patients. The possible reasons could be hypoxia and hypercapnia. Micro CT and fMRI brain and EEG along with other omics approach can be tried.

Tissues examined for the end points are appropriate

Comments

SCORE 0 — **user-200863** — *06/06/2016 12:11*
It is always nice to analyse everything one can think of; however, in my opinion, the tissues examined are the major ones that are primary targets and should be given priority. Other tissues can be evaluated when one has unlimited resources.

SCORE -1 — **user-922124** — *05/31/2016 13:19*
I agree with 550063. These disease are affected by most of the major organs and clearly sampling shortened list of tissues is not adequate. These studies need to be comprehensive and look at the 40tissues as per OECD

SCORE 2 — **user-550063** — *05/30/2016 00:47*
Number of reviewers have suggested various organs as COPD is a systemic disease and it affects almost every organ in our body. In any event, weightage should be given to organs by which morbidity and mortality are happening to COPD patients. But caution is required when one use multiple organs. Because single exposure model may not be suitable for all organs as smoke may not reach all organs equally. For example, these studies did not see any significant change in liver toxicity, and cardiac muscle gene expression till 5-6 months.

ID #1830 (https://scipinion.com/surveys/67/custom_results/1830)

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% 6	30.00% 3	10.00% 1	0.00% 0	0.00% 0	0.00% 0	10
Clinical, short term	27.27% 3	45.45% 5	27.27% 3	0.00% 0	0.00% 0	0.00% 0	11
In vivo	54.55% 6	36.36% 4	9.09% 1	0.00% 0	0.00% 0	0.00% 0	11
In vitro	0.00% 0	30.00% 3	40.00% 4	30.00% 3	0.00% 0	0.00% 0	10

Comments

SCORE **0** — **user-609692** — *06/05/2016 13:57*
No comments here.

SCORE **1** — **user-550063** — *05/30/2016 00:41*
As many agree, clinical long term is required to see the effects of THS2.2 to get a complete picture as many non-modified commercial cigarettes will have long term effects due to the possibility of autoimmune destruction. Short term switching also can be tried to see its effects on acute inflammation parameters. Since mice are naturally resistant to smoke induced pathology, one can try minipigs as the airway anatomy and function of mini-pigs are closer to humans compared to mice biologically. Best in vitro model is required rather than simple adhesion and TEM and the improved model should consider foam cell lesion induction, 3D vascuar wall, Co-Culture model with monocyte, smooth muscle cells and endothelial cells (these three cell types act together to create the complex picture)

SCORE **0** — **user-922124** — *05/31/2016 13:21*
Clinical studies although useful will not permit the assessment of chronic THS2.2 exposure. This requires both clinical and "pre-clinical" work as used in drug development

SCORE **0** — **user-200863** — *06/06/2016 12:13*
Some kind of targeted study in human volunteers who use these products.

ID #1831 (https://scipinion.com/surveys/67/custom_results/1831)

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 (Scale of 1-5, 5 being the highest)?

	Importance	Strength	Total
Inflammation	*4* **9.09%** 1 *5* **90.91%** 10	*2* **9.09%** 1 *4* **36.36%** 4 *5* **54.55%** 6	11
Oxidative stress	*2* **9.09%** 1 *3* **9.09%** 1 *4* **36.36%** 4 *5* **45.45%** 5	*1* **9.09%** 1 *2* **9.09%** 1 *4* **45.45%** 5 *5* **36.36%** 4	11
Extracellular matrix (ECM) production, deposition and degradation	*3* **18.18%** 2 *4* **27.27%** 3 *5* **54.55%** 6	*3* **27.27%** 3 *4* **54.55%** 6 *5* **18.18%** 2	11
Metabolic alterations	*2* **9.09%** 1 *3* **45.45%** 5 *4* **36.36%** 4 *5* **9.09%** 1	*3* **36.36%** 4 *4* **54.55%** 6 *5* **9.09%** 1	11
Other (please specify)	*1* **25.00%** 1 *3* **25.00%** 1 *4* **25.00%** 1 *5* **25.00%** 1	*1* **33.33%** 1 *3* **33.33%** 1 *5* **33.33%** 1	4

Comments

SCORE **0** — **user-382348** — *06/03/2016 18:56*
Inflammation, certainly, is the most important determinant. The data suggest that the THS2.2 is a better option, compared to CS, in reducing the inflammatory processes. That said, without the proper controls (WT MICE) it is hard to definitively state this.

SCORE **0** — **user-609692** — *06/05/2016 13:55*
Agree that inflammation is the key driver for COPD.

SCORE
0

user-550063 *05/30/2016 00:3*

I agree with the majority of reviewers who indicated that inflammation is the major parameter that is relevant to human disease as it alone can lead to all other parameters. In addition to inflammation, oxidative stress and ECM imbalance, epithelial dis-integrity is also an emerging parameter that participates in COPD pathogenesis. THS sufficiently reduced the inflammation except BAL fluid inflammatory mediators upon switching. THS sufficiently reduced the many oxidative parameters except BAL fluid oxidant markers like MPO upon switching. THS sufficiently reduced the many ECM deposition parameters.

SCORE
1

user-922124 *05/31/2016 13:2*

Inflammation is a key determinant and there is less impact of THS2.2 compared to CS

SCORE
0

user-200863 *06/06/2016 12:1*

Inflammation

ID #1832 (https://scipinion.com/surveys/67/custom_results/1832)

Specific disease mechanisms can be appropriately studied in in vitro models. PMI tested RRPs with assays that recapitulate two mechanisms relevant to atherosclerosis, namely adhesion of monocyte to endothelial cells (Poussin et al. 2016 Toxicology 339(2):73-86) and transendothelial migration (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.

THS2.2 appears to be less atherogenic than 3R4F. In vitro models recapitulate in vivo biology if used properly and if data is interpreted with caution.	(i). I agree with the authors' conclusions that aqueous aerosol extract from THS2.2 reduced the effects of the monocytic cells adhesion to human coronary endothelial cells comparing with 3R4F. The authors measured 9 different analytes in both tested aqueous extractions, and with exception for nicotine, all chemicals were lower in THS2.2 fluid. (ii). I think it is an elegant experiment especially using human cells to test the atherogenic effects of THS2.2 and 3R4F aerosol exposure.
From Poussin et al 2016 is is clear that 3R4F promotes adhesion of monocytic cells to coronary arterial endothelial cells and that this is strongly reduced for THS2.2 exposure (some effect at higher concentrations). From van der Toom et al 2015, studying chemotactic migration of monocytic cells through a monolayer of coronary arterial endothelial cells is is clear that treatment with 3R4F induces monocyte production of cytotoxic and inflammatory cytokines and decreased transendothelial migration. Evidence is shown that THS2.2 has significantly reduced effects over 3R4F on these parameters. It can be concluded that THS2.2 exposure shows reduced atherogenic-like effects as compared to 3R4F in vitro. These in vitro studies provide some additional insight to the in vivo atherogenesis studies.	As indicated, the ApoE background is providing a significant level of background plaque formation in these mice. Overall, this type of assay would be extremely helpful and it is my belief, based on the other data (i.e. the other manuscripts provided) that the effects on plaque formation would be much more drastic in the THS2.2-treated mice if they had been used in a different model. I think that something that is getting lost here is that this is simply a better alternative to the 3R4F cigarettes and should not be considered as "healthy" and completely devoid of the effects typically observed in "3R4F-smoking patients.
	(i) THS2.2 aerosol contains a much lower concentration of atherogenic components than found in CS aerosol, based on the adhesion and migration assays. (ii) The in vitro assays allow for carefully controlled conditions involving specific cell types and experimental designs to investigate exogenous administration of complex mixtures (aerosols). These studies can unambiguously point to chemicals that alter functions or capabilities of specific cell types (e.g. monocyte adhesion to endothelial cells). One of the caveats is that the in vitro 'environment' is not a good reflection of the in vivo complexity and temporal dynamics cannot be modeled easily.
Gene expression changes in vitro are dose and time course dependent and to mimic an inhalation exposure is problematic. In vitro exposure using the aqueous extracts in this assay is a useful tool to allow dose-response assessment. How this can be quantitatively related to in vivo effects is uncertain.	This is a complex question since physically cells are cultured in aqueous or semisolid media to maintain cellular viability and chemicals in CS or THS2.2 are in a vapor phase which creates a problem with regard to how to create an appropriate exposure system that is relevant to in vivo exposures. One would ideally like to know the degree to which chemicals in a vapor phase partition into an in vitro culture media aqueous phase .
As expected traditional cigarette (3R4F) caused an array of effects for both lung and heart and use of the THS significantly reduced, but not eliminated, these effects. Use of in vivo methods is the gold standard; however, a trend could be observed in vitro allowing to further improve the in vivo design before starting the study in order to conserve the use of animals and save resources.	The method of analyzng adhesion of monocyte to endothelical for transendothelial migraion could be very powerful tool to analyze response to THS2.2 and 3R4F
	First of all, I would like to mention some drawbacks of the two main in vitro studies mentioned in this review, Poussin C et al, 2016,Toxicology article: 1. Table 2: The concentrations of all 8 carbonyl molecules mentioned in Aerosol Chemistry Page in SysToX Portal (I am not sure how these concentrations were estimated) and Table 2 were different. In any event there will be batch to batch and lot to lot variations. But interestingly concentrations for THS2.2 abPBS of these 8 molecules are more or less similar in Table 2 (except Buyraldehyde) whereas 3R4F concentrations are always higher (Please see comparison Table). I do not know the reason. One needs to consider this discrepancy to avoid unhealthy comparisons. 2. In vitro Adhesion Assay: As per the Materials and Methods, the authors should have used fresh uninduced monocytes for the adhesion assay rather than smoke treated monocytes. In the section 3.2. subtitled “No significant increase of MM6 cell-HCAEC adhesion was observed following fresh direct and indirect exposures to THS2.2 aqueous extract at concentrations exhibiting maximal adhesion with 3R4F aqueous extract“, the authors have interpreted “In the I treatment, the increase of 3R4F aqueous extract concentrations showed a biphasic response curve indicative of cytotoxic effects on MM6 cells beyond a certain concentration range (Supplementary Fig. 2A).” The Supplementary Fig. 2A, had shown increased cell death with increasing the concentrations of 34RF aqueous extract. As per the Materials and Methods the authors have used supernatant of cultured monocytes that were induced with 34RF aqueous extract. But it seems from their statement that they may have used smoke extract induced used monocytes for the adhesion assay because supernatant may not have huge concentrations of TNF-alpha to kill the cells (high concentrations of recombinant TNF–alpha did not cause a reduction in adhesion

rate. [Please forgive me if I misunderstood]. 3. The authors have claimed "To achieve similar increases of MM6 cell-HCAEC adhesion with THS2.2 aqueous extract as observed for 3R4F, 10-fold higher concentrations (2.25 puffs/ml) were necessary for FD treatment, and 20–25-fold higher concentrations (1.125 puffs/ml) were required for I treatment (Fig. 1 and Supplementary Fig. 3)." Through this sentence, it seems that the authors had compared 3R4F with THS2.2 inconsistently. Former claim is OK for two reasons: a) no biphasic effect and b) they found similar increase with 2.25 puffs/ml (THS2.2) and 0.225 puffs/ml (3R4F). But latter claim is not perfect for two reasons: a) there was no similar increase between 1.125 with 0.045 and b) there is a biphasic effect/ no dose dependent effect. In this context, Why they did not compare 1.125 with 0.225 ? It seems to be the poor comparison. 4. It is not clear and confusive why the authors had selected 0.06 and 0.225 puffs/ml in I treatment even after the observation of low adhesion rates in these conditions. Because 0.225 dose had shown very less adhesion rate and the same dose caused more cell death. It seems that the expression analysis (figure 2) was performed with dying and less adhesive cells. This could be strongest reason why these two treatments (0.06 and 0.225 puffs/ml in I treatment) has shown less differential expression when compared to 0.225 dose at FD treatment which had shown very high adhesion rate. Only 0.225 puffs/ml in FD treatment seems to be perfect as this treatment had shown more adhesion rate along with more differential gene expression. All these indicate that there is a poor correlation between the adhesion rate and the gene expression. In this context, there is a less meaning of further analysis. 5. It seems they have not measured any apoptosis parameters and they just relied on the counting of the HCAECs Van der Toorn et al, Food and Chemical Toxicology article: It is not clearly understood why the authors have selected the doses which showed more apoptosis for the chemotaxis studies. It would be very obvious that dying cells move slowly. There is a good correlation between 7-AAD positivity and chemotaxis. For example, 3R4F showed more 7-AAD positive from 0.05 puffs and THS2.2 it is from 1 puffs/ml (Figure 2) and on the other hand the reduction in chemotaxis and TEM starts from 0.05 in case of 3R4F and from 1 in case of THS2.2 (Figure 3). So it seems that the authors had used very high concentration that leads to apoptosis and it did not affect or reduce chemotaxis. The authors had missed an observation of borderline increased TEM with 0.01 dose of THS2.2 (Figure 3B). This indicates that the experiment can be done with lesser concentrations also. In this context, the authors have pointed other hypothesis for substantiate their observations. Question 1: The relative atherogenic effect of THS2.2 and 3R4F aerosol exposure: THS had shown the reduction of both adhesion of monocyte to endothelial cells and TEM. These indirectly suggests that THS may not promote atherogenesis. Question 2: The usefulness of in vitro assays to recapitulate in vivo atherogenic effects: It depends on which in vitro assay has been used for recapitulating in vivo atherogenic effects. For example, the assays like adhesion and chemotaxis of monocytes are very simpler and highly reductionist approach. This approach may be really mimic the atherogenesis. A better model is required to good recapitulation of human atherogenesis. 1. 3D vascular wall model (Robert J et al, PLoS One. 2013 Nov 14;8(11):e79821) 2. Co-culture of arterial wall cells and macrophagemodel Wada Y et al, Yonsei Med J. 2000 Dec;41(6):740-55 3. Coculture model of transmigrant monocytes and foam cell formation (Takaku M et al, Arterioscler Thromb Vasc Biol. 1999 Oct;19(10):2330-9). 4. Co-culture of endothelial cells and smooth muscle cells in modified fibrin gel scaffold to mimic human plaque (Dorweiler B et al, Thromb Haemost. 2006 Jan;95(1):182-9).

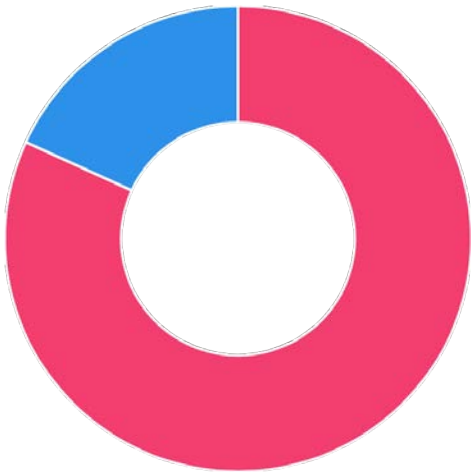
The results conclude that the atherogenic potential of 3R4F is much higher than THS2.2. The results also demonstrate the chemotaxis and transendothelial migration to be much lower in THS2.2 treatment group than in 3R4F treatment group. Definitely, the in vitro model system is a surrogate to study atherogenesis.

Comments

SCORE		
1	**user-550063** The authors have used very simple reductionist models. A better model is required for good recapitulation of human atherogenesis like 3D vascular wall , Co-culture models. and so on .	*05/29/2016 02:44*
0	**user-922124** Lung models either 3D or lung slices that retain the critical resident macrophage component are likely to be better models	*05/31/2016 12:57*
0	**user-200863** Agree with 550063	*06/06/2016 12:17*
0	**user-609692** No comment	*06/05/2016 14:00*

ID #1833 (https://scipinion.com/surveys/67/custom_results/1833)

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: 9 *(82%)*

Some compromises in design (which ones?) are not weighed out by the benefits, and hence separate models would be more efficient: 2 *(18%)*

Further reduction of animals could be envisioned by the following accomplishments: (please specify): 0 *(0%)*

answers: 11

skips: 0

Comments

SCORE **0** — **user-200863** — *06/06/2016 12:19*

Agree with all three comments.

SCORE **1** — **user-550063** — *05/30/2016 03:03*

Considering 3Rs, the authors have used the animals economically like the single model for two diseases. This is economically and ethically fine. But scientifically speaking it has some flaws in my opinion. 1. Because it has been shown that Apoe is essential for the alveologenesis, one cannot distinguish smoke mediated alveolar disruption and Apoe deficiency mediated alveolar pathology. 2. Similarly, same smoke exposure designs have been used for various different studies like cardiac, liver, lung lipidome etc though 6-8 months did not affect cardic gene expression significantly. So this approach did not fulfil the aims of each study. 3. For example, TitZ et al study aim is to see the effects of pMRTP and THS as such or switching on lung lipidome. However, the lung lipidome related proteome did not show any significant gene expression till 5/6 months. Then what is the point of switching at 2 months ? 4. Similar trend has been seen in cardiac study (Szostak). Indeed, the authors have taken 6 and 8 months for further analysis (Figure 2) indicates that till 5 months, there was no significant effect. 5. Similarly, they should have done the hepatotoxicity parameters first before performing all OMICs approaches. Since there was no visible hepatotoxicity, there was no real use of OMICs studies because the data cannot differentiate the changes from toxic materials or compensatory mechanisms. I feel that the observed changes in OMICs may be host beneficial compensatory mechanisms to maintain liver homeostasis. In short, careful preliminary experiments are required before bigger projects. So separate models with separate designs are required to answer specific questions. At the same time, one should not hesitate to repeat the entire experiments as biological experiments always have a tendency to give inconsistencies and variations. Considering the reduction in the animals, one should not compromise the quality of study and data.

SCORE **0** — **user-922124** — *05/31/2016 13:27*

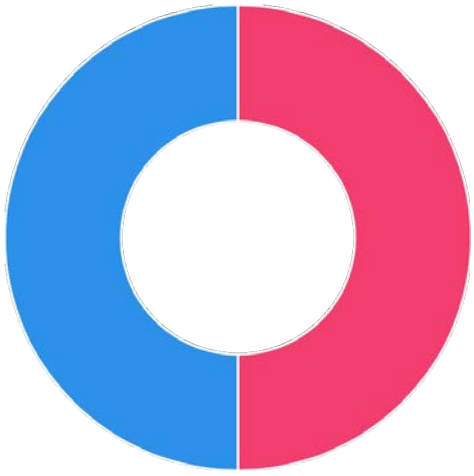
In my opinion this does not contribute dramatically to the 3Rs since the PMI group will likely need to do standard OECD compliant inhalation using rodents anyway. So the savings in animals lives may not be a factor since this data will not likely supplant an OECD guidance study. The sophisticated studies here and animals used in these studies will likely not be the final studies in the safety assessment of THS2.2

SCORE **1** — **user-609692** — *06/05/2016 14:06*

I agree with both sets of comments above but would suggest that these data (even negative ones) are instructive towards the goal of MOA risk assessments which should, in the long -run, reduce animal use and provide a better scientific basis for regulatory decision making.

ID #1969 (https://scipinion.com/surveys/67/custom_results/1969)

In your opinion, does the absence of an inflammatory reaction in THS2.2 exposed animals, inferred from the proteomic or transcriptomic analyses, indicate that lung function is normal in these mice?



Legend

Yes: 5 *(50%)*

No: 5 *(50%)*

answers: 10

skips: 0

Comments

SCORE		
0	**user-200863** This is consistent in all in vivo and in vitro studies; therefore, I agree.	*06/06/2016 12:2(*

ID #1970 (https://scipinion.com/surveys/67/custom_results/1970)

To what extent are the mouse studies on the effect of switching, which are necessarily short term as compared to the human situation, predictive for the effect of switching in smokers? If not, what would be the better alternative?

Animal models are always limited in reproducing the human condition.	The chemistry of the THS2.2 product and the effect of switching are clear under the conditions used here in this human model of disease. Some of my response to question 1 applies here. Although the authors and the design of the study makes this clear, convincing regulators is entirely different. No DR, and a shortened time frame using a diseased animal model with a high spontaneous disease necessitating shortened timeframes of study is problematic assuming FDA will regulate these product like a small molecule for non-life threatening disease indication. A clinical trial in human would be useful here with some dose-responsive biomarkers.
For recovery of lung function and absence of signs of COPD-related markers the short term study is sufficient. For mutagenesis-related effects it is not.	This was one sensitive point I raised during the survey. Since I study aging, the age equivalence between mouse and human is part of my experimental discussion. They are of course different organisms but is it well accepted in the field an age equivalency. So I think the experiment should had considered longer exposure times for CS to become more comparable to human situation.
a study where animals are exposed for half of their natural expected life and observation up to their death rather than sacrificing at specific study-day	The answer here is complicated by intrinsic differences in longevity and biology of the 2 species. These factors will complicate predictions since one would need to relate mouse years to human years in a defensible way and provide assurances on the similarities between mouse and human lung cells for the selected parameters to be measured. That said, the pulmonary systems between the 2 species are clearly analogous and some useful predictive information should be generated in a broad biological sense. One just must acknowledge the limitations of the model. Any other non-human model will also have similar limitations.
I would be confident that the switching effects on inflammatory responses would carry over to humans. I am less confident about how the vascular changes would be modified.	In my opinion, this is a very illogical comparison. Firstly, we don't have available data or at least data presented to the reviewers on the parameters examined as a comparison between rodent and humans. This is more of subjective question. What is important and significant from the animal studies is the unbiased, controlled exposure treatments between the animal groups and the differences in the results observed among the treatment groups.
Short term switching in mouse models would not be able to predict the real switching effects in humans as these short time switching cannot reflect exact human pathology. So long term switching would be the better alternative.	This is a truly challenging question. Short-term in a mouse is not necessarily short-term in a human. I think the real challenge is interpreting these data in the KO mouse model with out a control. It is hard to say what the true "end-points" should be.

Comments

SCORE 2 **user-200863** *06/06/2016 12:28*

These studies are quite predictive in separating the real effects of the active ingredients in cigarettes from those of pyrolisis. Scientifically, it is very convincing that most of the effects related to CS are not from the tobacco rather from the combustion. Whether e-cigarettes is regulated as drug or not is up to FDA and not under discussion here, it is clear that using new approaches to deliver the active ingredients are much safe.

ID #1971 (https://scipinion.com/surveys/67/custom_results/1971)

Both CS and THS2.2 contain some cadmium which can both cause production of reactive oxygen species (ROS) and act as a potent inducer of metallothionein (MT) and a number of other stress proteins. Both oxidative stress and MT induction were observed in the reported studies. How could one separate these 2 types of effects and use the information to support Mode of Action(MOA) based risk assessment to further quantify differences in risk between CS and THS2.2?

By inclusion of additional controls in the experimental design. Also, use of proxy indicators of non-specific responses.

Both CS and THS2.2 share in common an number of items that are listed in the FDA HPHC list, e.g., the neurotoxin acrylamide and the heat not burn emissions has not been comprehensively examined rather the focus was on the HPHC list. This needs to be addressed using proper DR study to identify shape of dose-response curve for these effects at the genomic and protein level to identify the TTC based on either oxidative stress of MT induction.

The correct experiment here would be to expose the mice to Cd-enriched aerosol without tobacco products and revisit all the parameters. Also, estimate the Cd concentrations in blood, kidneys, lung, bone, brain and liver.

Based the data presented these two effects can not be separated. In vitro studies using lung epithelial cell cultures or a lung-on-a-chip model could be combined with ROS scavengers to dampen ROS production and assess to what extent effects are caused by ROS production.

Cadmium is a major contaminant of tobacco products (~1-5ug/cigarette) which gets into this crop via the use of cadmium containing phosphate fertilizers and active uptake of this metal by tobacco plants. Other metals such such as mercury are sometimes present at much lower or non-dectectable concentrations. The result of this phenomenon is that smokers are known to have renal cadmium concentrations about 2 times those of non-smokers with females about 2 times higher than males(See CDC NHANES). Cadmium also induces MT and produces oxidative stress. Separating these 2 concomitant types of effects should help support MOA risk assessments in delineating differences in risk between CS and THS2.2 by providing basic information on the roles of MT in mediating the sources of oxidative stress(eg. cadmium versus ROS generated by other CCS/THS2.2 chemicals) in this model system. The use of MT knockout mice, as suggested by another reviewer, should yield some useful information in this regard.

I think overall it would be hard to discriminate the chemical constituents that drive these events, whether cadmium or others. Testing of each, one by one in ROS assays and then looking at protein expression or MT activity in vitro is one way to obtain MOA.

The basic principle of toxicology is -Dose makes the poison. It is the relative dose of these metallic constituents in the aerosol that would determine the extent of ROS production or induction of metallothionein. Cells have antioxidant reserves such as glutathione to check and balance ROS. However, how rapidly they are exhausted and replenished would depend on the overall dose of the toxicant (which could be cadmium or others). When considering the toxicity from aerosol exposure from CS or THS2.2, one needs to consider the relative doses and bioavailabilities of these toxicants in the organ systems examined.

First of all I am not sure why biasedness has been given to cadmium in the question as both CS and THS2.2 contain almost equal quantities of mercury (compared to cadmium) which can cause production of reactive oxygen species (Olivieri G et al, J Neurochem. 2000;7:231-6) and induce metallothionein even more potently than cadmium (Kramer KK et al, Toxicol Appl Pharmacol. 1996;136:94-100). However, the CS or THS exposures to metallothionein knockout mice would separate these two types effects at modest levels though not at complete levels as oxidative stress and MT induction are having feedback controls.

Well, I am not sure that this is that imperative in this sense. While Cd is a potent inducer of both oxidative stress and MT, the amount detected in the THS2.2 is > 2 orders of magnitude lower than that of CS (CS = 63.7 ± 3.3ng and THS2.2 = 0.4 ± 0.01ng). With this massive difference in Cd concentrations between the two groups, there should be a massive difference in both ROS and MT induction. Again, I think that the biggest thing to assess here is that the THS2.2 system is designed to be a modified risk tobacco product, and it seems to me that decreasing the Cd concentrations so drastically should undoubtedly reduce the risk.

Comments

SCORE		
0	**user-200863**	*06/06/2016 12:31*
	I think a dose-response study will be helpful in understanding these effects and path forward for MoA risk assessment approach.	

ID #1972 (https://scipinion.com/surveys/67/custom_results/1972)

Two reviewers expressed concern over the lack of a dose-response for CS and THS2.2. They stated: "The difference in concentration-response profiles between 3R4F and TSH2.2 need to be explored further". "The lack of dose-response in all of these studies is a significant concern". In your opinion, does the lack of testing for dose-response for both CS and THS2.2 limit the conclusions about the effects of THS2.2 and/or switching in a disease model? Would a 28/90-day inhalation study in rats following OECD guideline 412/413 be sufficient to address this concern?

Dose-response is a tenant of toxicology. Such data always adds credibility and validation. The suggested study does not replace dose response study.

Qualitative single dose, single time point , shortened study length using diseased animals data as shown in all of these, unfortunately does very little to support the types of quantitative questions that appear to be a significant part of the post-debate. Significant resources used here could have been used to do the proper regulatory studies first then development of appropriate disease models. I still have not seen support based on the genomics data for use of this animal model beyond this narrow application here. There was no down regulation of ApoE in WT mice in the omics data and ApoE mice represent a small fraction of the human population that are smokers. Again, the THS2.2 as regulated by FDA may likely require a full tox package as used for IND up to and beyond phase III clinical trials including a chronic cancer bioassay and a multi-generation repro tox bioassay.

Dose-response it definitively an important physiological parameter to strengthen the experiment. However, I don't think it limited the conclusions. This new suggested experiment would be great to address this point.

The suggested inhalation study in rats would indeed address this concern. However, I do not feel that this is in fact a real concern given the strong significant differences between CS and THS2.2 at dosages matched for nicotine exposure.

qualitatively, no. quantitatively, yes. A dose-response study is needed in order to make stronger argument.

The lack of dose-response data for CS and THS2.2 is a problem since, in its absence, one not know the shape of the dose-response curve or where data from the present studies might be placed on the curve. As noted by the 2 reviewers, this limits the conclusions which may be drawn for both the effects of THS2,2 and/or switching. I support the addition of dose-response studies for this reason. The OECD 412/413 inhalation studies in rats would not address the concern since such studies would be in another species and the issue here is about dose-response in this special strain of mouse. It would be hard to make comparisons. On the other hand, the guidelines specify rodents which could also include mice so why not use mice for the dose response studies?

Yes, I believe the lack of additional exposure across a range of particulate concentrations leaves one less confident that THS2.2 has dramatically less biological impact than CS and weakens the argument that switching is as useful as cessation.

In my opinion, testing for dose-response for both CS and THS2.2 does not limit the conclusions of the study about the effects of THS2.2 and/or switching in a disease model. However, the inclusion of does-response would potentially address some of the concerns regarding the toxicant levels and also provide mechanistic insights with regard to the observed physiological/pathological differences between the two groups.

The problem would persist even one does 28/90-day inhalation study in rats following OECD guideline 412/413 if the authors are not changing the study design. I have expressed my concern in the question "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?". Summary: From the data, it seems that the authors have planned 5 small studies (5 different time points) and each one has multiple groups and multiple endpoints. This may be potent reason for the lack of dose-response relationship. Rather than this approach, with a reduction of some endpoints, common pool experiments like All (all time exposure) mice should be given similar exposure and every time point particular groups will be sacrificed.

I agree very much with this sentiment. A dose-response would be very informative.

Comments

SCORE
0

user-200863 *06/06/2016 12:38*

The validity of these studies are not jeopardized by not having dose-response. These studies have clearly shown the extent of safety in using these products when compared with traditional smoking. These studies are, however, of qualitative nature and dose-response studies such as 412/413 will be of great value. One thing that may need further evaluation is exposure for longer period of time to CS, e.g., 1 year followed by evaluation until the natural death. In the past I was involve in such studies for dioxin where we noticed that almost all death in exposed animals was from lung cancer which appeared very late, much later than signature liver tumors, however progressed very aggressively causing death. This would have been missed if not let the animal live to their death.

ID #1973 (https://scipinion.com/surveys/67/custom_results/1973)

Several experts expressed concerns over the duration of exposure. They stated: “Do you expect similar datasets for cessation or switching after 6 months 3R4F exposure?”. “Would the animal study reflect with more fidelity the comparison with the human CS exposure with longer exposure time?”. QUESTION: Would you expect to see similar responses to CS, THS2.2 and/or switching after longer durations of exposures?

As stated earlier, the data may vary as a function of duration of exposure. Needs to be tested.	The length of exposure can have a profound impact on a number of endpoints. In cancer bioassay with DNA reactive carcinogens like ethylene oxide tumors do not begin to arise until after 1 yr of exposure, this is not unique, a number of compound exposure related effects occur later in rodent. Again a proper dose-response and time course study will likely be needed to convince regulators. In the case of humans it is uncertain when smokers have reached a "point of no return", i.e., no matter what they do progression of disease is irreversible; so at some point switching may have no measurable beneficial effect. Shortened studies like the one reported here leave this unanswered.
I expect to see a worsening of the physiological parameters and more comparable to the human exposure scenario. Lung cancer is one of the most dramatic consequences of tobacco smoke for humans and this was by far not seen in any animal here with CS exposure only.	Indeed, as was my concern longer exposures may be needed to more realistically model the effect of switching in humans. However, I do not think moving from 3 to 6 months exposure before switching is useful. The model as it it stands has been used optimally in that respect. The short time nature of these studies in mice will always limit the representativeness for a human situation where switching would occur after many years of smoking.
It is difficult to predict effects of longer exposure and yes, longer exposure will be more relevant for comparison with humans.	Yes, I would expect some similar responses to CS and THS2.2 but varying by degree of severity but with cell death and replacement phenomena and scarring superimposed on top. These predictable effects may complicate interpretation of the molecular datasets but provide more information on ultimate biological outcomes. Again, correlative morphological studies should help interpretation by identifying which cell types are involved over time.
I think you would begin to see permanent tissue- and system function level changes that are difficult to reverse. My guess is that the gene/protein expression changes would reverse even after 6 month exposure.	To the specific question, would animal study reflect with more fidelity the comparison with human CS exposure, in my opinion, it is an appropriate comparison. Human's smoke intermittently. The breathing rate, tidal volume of a mouse and other physiological parameters of respiration are different and cannot be equated with Human's. What is important and significant from the animal studies is the unbiased exposure treatments between the animal groups and the differences in the results observed among the treatment groups. After reading Jobse B.N. et al's work “Impact of inflammation, emphysema, and smoking cessation on V/Q in mouse models of lung obstruction” Respir Res. 2014; 15(1): 42, I am convinced that even a longer exposure would not have made a significant differences in the end points observed, unless the researcher consider looking at different end points such as expression of tumor specific genes etc. I am citing this as a reference study from a different group to justify my stand.
I do not expect the similar responses if switching is being done after longer (6 months) CS exposure as there would be severe and irreparable cellular damaging events would have occurred with long term exposure.	This is very similar to my response for question 2. I think this is a hard thing to predict. In the APOE knockout mouse, I don't believe any differences would be observed as the underlying pathogenesis from the genetic manipulation would become overwhelming. The WT mice may provide more information here.

Comments

SCORE 0 — **user-200863** — *06/06/2016 12:41*

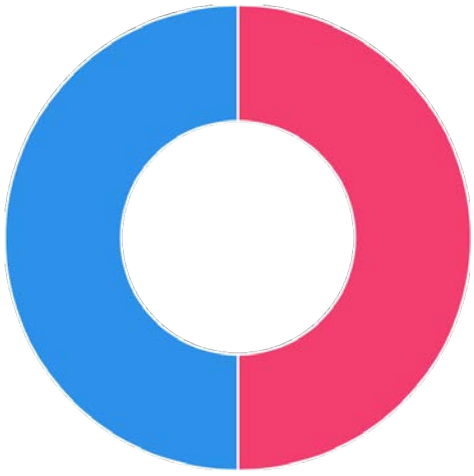
Which is: The validity of these studies are not jeopardized by not having dose-response. These studies have clearly shown the extent of safety in using these products when compared with traditional smoking. These studies are, however, of qualitative nature and dose-response studies such as 412/413 will be of great value. One thing that may need further evaluation is exposure for longer period of time to CS, e.g., 1 year followed by evaluation until the natural death. In the past I was involve in such studies for dioxin where we noticed that almost all death in exposed animals was from lung cancer which appeared very late, much later than signature liver tumors, however progressed very aggressively causing death. This would have been missed if not let the animal live to their death.

SCORE 0 — **user-200863** — *06/06/2016 12:40*

May be not in the CS and switching groups. I don't see any additional effects in THS2.2 group. See my comments to the earlier question.

ID #2015 (https://scipinion.com/surveys/67/custom_results/2015)

In your opinion, does the absence of an inflammatory reaction in THS2.2 exposed animals, inferred from the proteomic or transcriptomic analyses, indicate that lung function is normal in these mice?



Legend

Yes: 5 *(50%)*

No: 5 *(50%)*

answers: 10

skips: 0

ID #2016 (https://scipinion.com/surveys/67/custom_results/2016)

To what extent are the mouse studies on the effect of switching, which are necessarily short term as compared to the human situation, predictive for the effect of switching in smokers? If not, what would be the better alternative?

Animal models are always limited in reproducing the human condition.	The chemistry of the THS2.2 product and the effect of switching are clear under the conditions used here in this human model of disease. Some of my response to question 1 applies here. Although the authors and the design of the study makes this clear, convincing regulators is entirely different. No DR, and a shortened time frame using a diseased animal model with a high spontaneous disease necessitating shortened timeframes of study is problematic assuming FDA will regulate these product like a small molecule for non-life threatening disease indication. A clinical trial in human would be useful here with some dose-responsive biomarkers.
For recovery of lung function and absence of signs of COPD-related markers the short term study is sufficient. For mutagenesis-related effects it is not.	This was one sensitive point I raised during the survey. Since I study aging, the age equivalence between mouse and human is part of my experimental discussion. They are of course different organisms but is it well accepted in the field an age equivalency. So I think the experiment should had considered longer exposure times for CS to become more comparable to human situation.
a study where animals are exposed for half of their natural expected life and observation up to their death rather than sacrificing at specific study-day	The answer here is complicated by intrinsic differences in longevity and biology of the 2 species. These factors will complicate predictions since one would need to relate mouse years to human years in a defensible way and provide assurances on the similarities between mouse and human lung cells for the selected parameters to be measured. That said, the pulmonary systems between the 2 species are clearly analogous and some useful predictive information should be generated in a broad biological sense. One just must acknowledge the limitations of the model. Any other non-human model will also have similar limitations.
I would be confident that the switching effects on inflammatory responses would carry over to humans. I am less confident about how the vascular changes would be modified.	In my opinion, this is a very illogical comparison. Firstly, we don't have available data or at least data presented to the reviewers on the parameters examined as a comparison between rodent and humans. This is more of subjective question. What is important and significant from the animal studies is the unbiased, controlled exposure treatments between the animal groups and the differences in the results observed among the treatment groups.
Short term switching in mouse models would not be able to predict the real switching effects in humans as these short time switching cannot reflect exact human pathology. So long term switching would be the better alternative.	This is a truly challenging question. Short-term in a mouse is not necessarily short-term in a human. I think the real challenge is interpreting these data in the KO mouse model with out a control. It is hard to say what the true "end-points" should be.

ID #2017 (https://scipinion.com/surveys/67/custom_results/2017)

Both CS and THS2.2 contain some cadmium which can both cause production of reactive oxygen species (ROS) and act as a potent inducer of metallothionein (MT) and a number of other stress proteins. Both oxidative stress and MT induction were observed in the reported studies. How could one separate these 2 types of effects and use the information to support Mode of Action(MOA) based risk assessment to further quantify differences in risk between CS and THS2.2?

By inclusion of additional controls in the experimental design. Also, use of proxy indicators of non-specific responses.

Both CS and THS2.2 share in common an number of items that are listed in the FDA HPHC list, e.g., the neurotoxin acrylamide and the heat not burn emissions has not been comprehensively examined rather the focus was on the HPHC list. This needs to be addressed using proper DR study to identify shape of dose-response curve for these effects at the genomic and protein level to identify the TTC based on either oxidative stress of MT induction.

The correct experiment here would be to expose the mice to Cd-enriched aerosol without tobacco products and revisit all the parameters. Also, estimate the Cd concentrations in blood, kidneys, lung, bone, brain and liver.

Based the data presented these two effects can not be separated. In vitro studies using lung epithelial cell cultures or a lung-on-a-chip model could be combined with ROS scavengers to dampen ROS production and assess to what extent effects are caused by ROS production.

Cadmium is a major contaminant of tobacco products (~1-5ug/cigarette) which gets into this crop via the use of cadmium containing phosphate fertilizers and active uptake of this metal by tobacco plants. Other metals such such as mercury are sometimes present at much lower or non-dectectable concentrations. The result of this phenomenon is that smokers are known to have renal cadmium concentrations about 2 times those of non-smokers with females about 2 times higher than males(See CDC NHANES). Cadmium also induces MT and produces oxidative stress. Separating these 2 concomitant types of effects should help support MOA risk assessments in delineating differences in risk between CS and THS2.2 by providing basic information on the roles of MT in mediating the sources of oxidative stress(eg. cadmium versus ROS generated by other CCS/THS2.2 chemicals) in this model system. The use of MT knockout mice, as suggested by another reviewer, should yield some useful information in this regard.

I think overall it would be hard to discriminate the chemical constituents that drive these events, whether cadmium or others. Testing of each, one by one in ROS assays and then looking at protein expression or MT activity in vitro is one way to obtain MOA.

The basic principle of toxicology is -Dose makes the poison. It is the relative dose of these metallic constituents in the aerosol that would determine the extent of ROS production or induction of metallothionein. Cells have antioxidant reserves such as glutathione to check and balance ROS. However, how rapidly they are exhausted and replenished would depend on the overall dose of the toxicant (which could be cadmium or others). When considering the toxicity from aerosol exposure from CS or THS2.2, one needs to consider the relative doses and bioavailabilities of these toxicants in the organ systems examined.

First of all I am not sure why biasedness has been given to cadmium in the question as both CS and THS2.2 contain almost equal quantities of mercury (compared to cadmium) which can cause production of reactive oxygen species (Olivieri G et al, J Neurochem. 2000;7:231-6) and induce metallothionein even more potently than cadmium (Kramer KK et al, Toxicol Appl Pharmacol. 1996;136:94-100). However, the CS or THS exposures to metallothionein knockout mice would separate these two types effects at modest levels though not at complete levels as oxidative stress and MT induction are having feedback controls.

Well, I am not sure that this is that imperative in this sense. While Cd is a potent inducer of both oxidative stress and MT, the amount detected in the THS2.2 is > 2 orders of magnitude lower than that of CS (CS = 63.7 ± 3.3ng and THS2.2 = 0.4 ± 0.01ng). With this massive difference in Cd concentrations between the two groups, there should be a massive difference in both ROS and MT induction. Again, I think that the biggest thing to assess here is that the THS2.2 system is designed to be a modified risk tobacco product, and it seems to me that decreasing the Cd concentrations so drastically should undoubtedly reduce the risk.

ID #2018 (https://scipinion.com/surveys/67/custom_results/2018)

Two reviewers expressed concern over the lack of a dose-response for CS and THS2.2. They stated: “The difference in concentration-response profiles between 3R4F and TSH2.2 need to be explored further”. “The lack of dose-response in all of these studies is a significant concern”. In your opinion, does the lack of testing for dose-response for both CS and THS2.2 limit the conclusions about the effects of THS2.2 and/or switching in a disease model? Would a 28/90-day inhalation study in rats following OECD guideline 412/413 be sufficient to address this concern?

Dose-response is a tenant of toxicology. Such data always adds credibility and validation. The suggested study does not replace dose response study.

Qualitative single dose, single time point , shortened study length using diseased animals data as shown in all of these, unfortunately does very little to support the types of quantitative questions that appear to be a significant part of the post-debate. Significant resources used here could have been used to do the proper regulatory studies first then development of appropriate disease models. I still have not seen support based on the genomics data for use of this animal model beyond this narrow application here. There was no down regulation of ApoE in WT mice in the omics data and ApoE mice represent a small fraction of the human population that are smokers. Again, the THS2.2 as regulated by FDA may likely require a full tox package as used for IND up to and beyond phase III clinical trials including a chronic cancer bioassay and a multi-generation repro tox bioassay.

Dose-response it definitively an important physiological parameter to strengthen the experiment. However, I don't think it limited the conclusions. This new suggested experiment would be great to address this point.

The suggested inhalation study in rats would indeed address this concern. However, I do not feel that this is in fact a real concern given the strong significant differences between CS and THS2.2 at dosages matched for nicotine exposure.

qualitatively, no. quantitatively, yes. A dose-response study is needed in order to make stronger argument.

The lack of dose-response data for CS and THS2.2 is a problem since, in its absence, one not know the shape of the dose-response curve or where data from the present studies might be placed on the curve. As noted by the 2 reviewers, this limits the conclusions which may be drawn for both the effects of THS2,2 and/or switching. I support the addition of dose-response studies for this reason. The OECD 412/413 inhalation studies in rats would not address the concern since such studies would be in another species and the issue here is about dose-response in this special strain of mouse. It would be hard to make comparisons. On the other hand, the guidelines specify rodents which could also include mice so why not use mice for the dose response studies?

Yes, I believe the lack of additional exposure across a range of particulate concentrations leaves one less confident that THS2.2 has dramatically less biological impact than CS and weakens the argument that switching is as useful as cessation.

In my opinion, testing for dose-response for both CS and THS2.2 does not limit the conclusions of the study about the effects of THS2.2 and/or switching in a disease model. However, the inclusion of does-response would potentially address some of the concerns regarding the toxicant levels and also provide mechanistic insights with regard to the observed physiological/pathological differences between the two groups.

The problem would persist even one does 28/90-day inhalation study in rats following OECD guideline 412/413 if the authors are not changing the study design. I have expressed my concern in the question “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?”. Summary: From the data, it seems that the authors have planned 5 small studies (5 different time points) and each one has multiple groups and multiple endpoints. This may be potent reason for the lack of dose-response relationship. Rather than this approach, with a reduction of some endpoints, common pool experiments like All (all time exposure) mice should be given similar exposure and every time point particular groups will be sacrificed.

I agree very much with this sentiment. A dose-response would be very informative.

ID #2019 (https://scipinion.com/surveys/67/custom_results/2019)

Several experts expressed concerns over the duration of exposure. They stated: “Do you expect similar datasets for cessation or switching after 6 months 3R4F exposure?”. “Would the animal study reflect with more fidelity the comparison with the human CS exposure with longer exposure time?”. QUESTION: Would you expect to see similar responses to CS, THS2.2 and/or switching after longer durations of exposures?

As stated earlier, the data may vary as a function of duration of exposure. Needs to be tested.	The length of exposure can have a profound impact on a number of endpoints. In cancer bioassay with DNA reactive carcinogens like ethylene oxide tumors do not begin to arise until after 1 yr of exposure, this is not unique, a number of compound exposure related effects occur later in rodent. Again a proper dose-response and time course study will likely be needed to convince regulators. In the case of humans it is uncertain when smokers have reached a "point of no return", i.e., no matter what they do progression of disease is irreversible; so at some point switching may have no measurable beneficial effect. Shortened studies like the one reported here leave this unanswered.
I expect to see a worsening of the physiological parameters and more comparable to the human exposure scenario. Lung cancer is one of the most dramatic consequences of tobacco smoke for humans and this was by far not seen in any animal here with CS exposure only.	Indeed, as was my concern longer exposures may be needed to more realistically model the effect of switching in humans. However, I do not think moving from 3 to 6 months exposure before switching is useful. The model as it it stands has been used optimally in that respect. The short time nature of these studies in mice will always limit the representativeness for a human situation where switching would occur after many years of smoking.
It is difficult to predict effects of longer exposure and yes, longer exposure will be more relevant for comparison with humans.	Yes, I would expect some similar responses to CS and THS2.2 but varying by degree of severity but with cell death and replacement phenomena and scarring superimposed on top. These predictable effects may complicate interpretation of the molecular datasets but provide more information on ultimate biological outcomes. Again, correlative morphological studies should help interpretation by identifying which cell types are involved over time.
I think you would begin to see permanent tissue- and system function level changes that are difficult to reverse. My guess is that the gene/protein expression changes would reverse even after 6 month exposure.	To the specific question, would animal study reflect with more fidelity the comparison with human CS exposure, in my opinion, it is an appropriate comparison. Human's smoke intermittently. The breathing rate, tidal volume of a mouse and other physiological parameters of respiration are different and cannot be equated with Human's. What is important and significant from the animal studies is the unbiased exposure treatments between the animal groups and the differences in the results observed among the treatment groups. After reading Jobse B.N. et al's work “Impact of inflammation, emphysema, and smoking cessation on V/Q in mouse models of lung obstruction” Respir Res. 2014; 15(1): 42, I am convinced that even a longer exposure would not have made a significant differences in the end points observed, unless the researcher consider looking at different end points such as expression of tumor specific genes etc. I am citing this as a reference study from a different group to justify my stand.
I do not expect the similar responses if switching is being done after longer (6 months) CS exposure as there would be severe and irreparable cellular damaging events would have occurred with long term exposure.	This is very similar to my response for question 2. I think this is a hard thing to predict. In the APOE knockout mouse, I don't believe any differences would be observed as the underlying pathogenesis from the genetic manipulation would become overwhelming. The WT mice may provide more information here.

Appendix D: Auditor's Report
NeutralScience Audit 2 (NSA2) for SciPinion Expert Panel

March 23, 2016

Documents received for review

Audit package documents were received from SciPinion on March 3, 2016 and transmitted to the Auditors on March 4, 2106. The complete package as received from SciPinion is included the March 16, 2016 email transmittal with this report to SciPinion, including Table 1 as referenced in the Auditor reports. The Auditors also reviewed the general topic of the panel as presented in the Audit package. Table 1 is also printed in Appendix 1. Only those candidates that SciPinion identified as having met (or "maybe" met) the minimum criteria were audited.

Auditors selected

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

Christopher Portier, PhD (link provided to LinkedIn profile)

Audit findings

The Audit package delivered to SciPinion on March 16 included correct values for each auditor in the detailed reviews of their signed reports. However, the overall summary table and composite scores derived from the values for one Auditor were incorrectly entered into the composite table below. The corrected summary table below provides the values as intended by the Auditors in their detailed review reports. The individual audits are provided in Appendix 2 with supporting tables that provide comments on each candidate review. A ranking system was used by the Auditors where 0 indicates that the candidate did not meet the minimum expertise criteria, 1 indicates that the candidate met the criteria, and 2 indicates that in addition to meeting the criteria the candidate was well qualified for the described task. A summary of findings is presented below.

Summary table

Portier	Farland	Composite	Candidate ID #	Name
2	2	4	1002	[redacted]
2	2	4	518	[redacted]
2	2	4	815	[redacted]
2	2	4	665	[redacted]
2	2	4	604	[redacted]
2	1	3	1022	[redacted]
2	1	3	1029	[redacted]
2	1	3	48	[redacted]
2	1	3	1044	[redacted]
2	1	3	1047	[redacted]
2	1	3	1053	[redacted]
2	1	3	326	[redacted]
1	2	3	1010	[redacted]
1	2	3	1013	[redacted]
1	1	2	1006	[redacted]
1	1	2	1009	[redacted]

Portier	Farland	Composite	Candidate ID #	Name
1	1	2	571	[redacted]
1	1	2	1039	[redacted]
1	1	2	1041	[redacted]
1	1	2	576	[redacted]
1	1	2	1051	[redacted]
1	1	2	1054	[redacted]
1	1	2	514	[redacted]
0	1	1	1023	[redacted]
0	1	1	1099	[redacted]
1	0	1	1016	[redacted]

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



Richard Canady, PhD
NeutralScience L3C
rcanady@neutralscience.org

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

[To ensure anonymity, Table 1 has been deleted]

Appendix 2 – Individual Auditor Reports with reference to associated files in email transmittal to SciPinion March 16, 2016

Auditor Report: SciPinion Scientific Review Package NSA2

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. Candidate Experts for Peer Review Panel (supplementary Excel spreadsheet provided March 4, 2016) 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)
1002	[redacted]
518	
815	
1010	
1013	
665	
604	

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

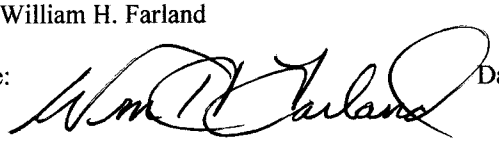
Candidate#	Name (Last, First)
1006	[redacted]
1009	
571	
1022	
1023	
1029	
1039	
1041	
48	
1044	
576	
1047	
1051	
1053	
1054	
1099	
326	
514	

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

Candidate#	Name (Last, First)
1016	[redacted]

Notes on each candidate appear in Appendix 1, NSA2Table1 to this report.

Auditor: William H. Farland

Signature:  Date: 3/9/16

Appendix 1

Table NSA2Table1

Candidate#	Name (Last, First)	Audit outcome *	Comment
1002	[redacted]	2	Well trained and experienced toxicologist with focus on toxicokinetics and mode of action of chemicals. Excellent publication record with strong hits in relevant categories. Seems well qualified for the task at hand. Meets minimal criteria.
518		2	Respiratory toxicologist with focus on genomics approaches to understand lung disease. Excellent training and experience. Seems well qualified for the task at hand. Meets the minimal criteria.
815		2	Toxicologist and exposure assessor with experience in inhalation toxicity of particles and fumes. Good publication record and relevant review experience. Meets minimal criteria and seems well qualified for this task.
1006		1	Toxicologist with Veterinary background and focus on exposure to nanoparticles. Few hits in CV search which are not particularly relevant to the task at hand. Meets the minimal criteria.
1009		1	Cell biologist with experience in genomics and transcriptomics. Little experience in mouse models and no tissue specific key word hits. Meets minimal criteria.
1010		2	Toxicologist with mouse lung and liver experience. Genomics and lipidomics experience. Good publication record and review experience. Meet minimal criteria and seems well qualified for the task.
1013		2	Pharmacologist with experience and interest in atherosclerosis and cardiovascular disease. Use of ApoE (-/-) mouse model for studies. Appears well suited for the task at hand. Meets minimal criteria.
571		1	Trained pathologist with toxicologic testing experience. Few hits in key word search. Fitness to the task at hand hard to assess. Meets the minimal criteria.
1022		1	Experienced genetic toxicologist. Background in toxicogenomics and work with transgenic mice. Appears to have mostly cell-based experience. Meets minimal criteria.

Candidate#	Name (Last, First)	Audit outcome *	Comment
1023	[redacted]	1	Molecular geneticist with experience in proteomics and functional genomics. Few hits in key word search. Meets minimal criteria.
1029		1	Cell biologist and biochemist with a relatively strong key word score card. Experience in mouse models and with lipidomics. Meets minimal criteria.
1039		1	Toxicologist with focus on risk assessment. Several certifications (DABT, CChem, QP_{RA}) but little evidence of experimental work or expertise for the review task at hand. Very Few publications in peer reviewed journals or with apparent technical details. Only two key word hits. Meets minimal criteria.
1041		1	Molecular biologist with strong transcriptomics and proteomics background. Appears to have limited experimental experience. Few relevant hits in key word search. Good review experience but unclear in what areas. Meets minimal criteria.
48		1	Experienced toxicologist with focus on dioxins and related compounds. Work with mice and dioxins. Not much evidence for work in the lung or other areas related to the charge. Meets minimal criteria.
1044		1	Toxicologic pathologist and microscopist. Few hits in key word search, but not particularly relevant to the task at hand. Meets minimal criteria.
576		1	Cell biologist with focus on metals toxicity in marine plants and mammals. Journal review experience primarily in plant journals. Few key word hits for mice and lipids. Meets minimal criteria.
1047		1	Strong background in toxicology, and genomics and some systems work. Specializes in liver. No lung or respiratory in CV search. Some cardiovascular. Only 5 published papers since 2006. Meets minimal criteria.
1051		1(?)	Toxicology consulting background, focused on risk assessment. No indication in CV of skills specific to this task other than general knowledge. Recent work experience outside of research and testing field. Publications mostly not relevant to this task. Meets minimal criterial but suitability as reviewer is questionable.

Candidate#	Name (Last, First)	Audit outcome *	Comment
665	[redacted]	2	Highly experienced molecular toxicologist. Strong response to key word search and publications review to suggest an excellent fit for the task at hand. Meets minimal criteria.
1053		1	Veterinary pathologist and biochemist with experience with mouse models and gene expression in tissues. No indication of respiratory track focus; a few hits for the liver. Meets minimal criteria.
1054		1	Molecular biologist with genomics and bioinformatics experience. Focus on genomics with a few key word hits for mouse and liver. Hard to see a toxicological perspective from the CV. Meets minimal qualifications,
604		2	Strong toxicologist with experience in lung and liver impacts in mouse systems. Experimental focus on metals but publications with other compounds. Excellent review experience. Well suited for the task at hand. Meets minimal criteria.
1099		1	Physical chemist with what appears to be limited computational biology experience. No indication of experience with toxicology studies or animal testing and assessment. Meets minimal criteria, but questionable experience for this task.
326		1	Toxicologist with experience in toxicogenomics and systems biology. No hits in key word search other than mention of genomics. Strong publication review experience cited. Meets minimal criteria.
514		1	Cell physiologist with experience in mouse models and bases for respiratory disease. While the focus appears to be on asthma and immune responses, he has experience with molecular aspects of respiratory disease. Genomics and lipids also show up in key word search. Meets minimal criteria.
1016		0	Biochemist with lipidomics background related mostly to neurosciences. Does not meet the criterion of 5 first or last authored papers and the 4 that are cited are not very relevant to the topic at hand. Does not look like a particularly good fit for this review panel so would score as not meeting the minimal criteria although it, technically, is a close call

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

Auditor Report: SciPinion Scientific Review Package NSA2

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. Candidate Experts for Peer Review Panel (supplementary Excel spreadsheet provided March 4, 2016) 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
1006	[redacted]
1009	
1010	
1013	
571	
1039	
1041	
576	
1051	
1054	
514	
1016	

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

Candidate#	Name
1002	[redacted]
518	
815	
1022	
1029	
48	
1044	
1047	
665	
1053	
604	
326	

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

Candidate#	Name
1023	[redacted]
1099	[redacted]

Notes on each candidate appear in Appendix 1, NSA2Table1 to this report.

Auditor: Christopher J. Portier

Signature:  Date: March 15, 2016

Appendix 1

Table NSA2Table1

Candidate#	Name	Audit outcome *	Comment
1002	[redacted]	2	Broad experience in relevant areas, strong toxicology background.
518	[redacted]	2	Strong credentials in lung physiology and molecular biology with experience in multiple toxicants. Limited systems toxicology experience or with other organ systems.
815	[redacted]	2	Considerable experience in all areas and a history of doing literature reviews for assessing toxicity.
1006	[redacted]	1	Currently post-doc with considerable experience in the response of the lung to nanomaterials. No indications of omics experience but experience on ADME that would be useful.
1009	[redacted]	1	clear expertise in omics and bioinformatics. Limited in working with environmental agents, inhalation exposures and work with direct toxicity in rodent models.
1010	[redacted]	1	Recent doctorate, still in post-doc, impressive pub record for his time since PhD, relevant experience in all areas except cardiovascular.
1013	[redacted]	1	Late post-doc with extensive bibliography. Expertise in genetics, systems biology and cardiovascular disease.
571	[redacted]	1	Classic regulatory toxicologist with almost all work focused on oral toxicity, no inhalation experience. No indication of systems toxicology or in depth mechanistic experience or work with transgenics.
1022	[redacted]	2	Highly qualified.
1023	[redacted]	0	Geneticist who appears to have some bioinformatics skills but most of this work he appears to be not a major author. The works with him as a major author, the work focuses on phylogenetics. No apparent experience in toxicology.
1029	[redacted]	2	Excellent credentials for the peer-review with experience in most areas of interest to the peer-review. Very hard to judge the Russian papers, but the English ones are clearly relevant.
1039	[redacted]	1	Most of the publications here are reports from or to an agency dealing with specific compounds or with methods in risk assessment. No indication of technical experience with modern toxicological methods, genomics and other areas of interest.
1041	[redacted]	1	A bioinformatics specialist with limited experience in any specific area associated with this peer review. Good systems skills but very limited toxicology skills. Would serve as a good support on methods for the peer-review.

48	[redacted]	2	Highly qualified, years of experience in toxicology and risk assessment. Moved with the field into molecular biology tools using "omics". Focused mostly on dioxin-like compounds may be a bit limiting.
1044	[redacted]	2	Very qualified general toxicologist with experience in systems toxicology. Experience with toxicogenomics for different agents, especially nanomaterials.
576	[redacted]	1	Limited experience in mammalian systems looking at toxicants. Good molecular biology experience in non-mammalian species.
1047	[redacted]	2	Extensive experience and highly qualified in the areas needed.
1051	[redacted]	1	Some experience in this area, but looks more like an administrative type person who got his degree in toxicology and then moved into risk assessment areas. Not clear what his contributions would be here.
665	[redacted]	2	Highly qualified, National Academy member.
1053	[redacted]	2	An experienced veterinary pathologist with some work also in molecular biology.
1054	[redacted]	1	Extensive experience in genomics, especially in the area of analytical tools. Focus has been predominantly in the neuro are which does not necessarily transport well to the topics under study here.
604	[redacted]	2	Broad experience in toxicology and systems toxicology, all relevant to the peer review. Knowledge of human and animal toxicity.
1099	[redacted]	0	This candidate is an excellent computational chemist with an impressive set of research articles of using computational chemistry to solve questions about structure and excellent articles on improving these algorithms. None of this experience is applicable to the problem the panel appears to be addressing.
326	[redacted]	2	Well-published toxicologist in areas directly relevant to the task of the peer-review committee. Has experience in multiple toxic endpoints and hazards and has worked and taught in the area of systems biology.
514	[redacted]	1	Good experience in pulmonary issues and molecular biology, but limited in systems toxicology, omics and chemical harm to cells.
1016	[redacted]	1	Clearly the most experienced in lipidomics and lipids. Limited experience with toxic chemicals and their impact on health.

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

NEUTRALSCIENCE

Verification of Expert Participation

Furthermore, upon review of the information provided to me for the SciPinion user accounts and correspondence for this peer review, I attest that to the best of my knowledge the review comments and judgments of the peer reviewers provided in "Peer Review of Data for a Modified Risk Tobacco Product (THS 2.2, PMI Study Number 15015): Part 1: In Vivo and In Vitro Studies " (signed by Sean M. Hayes and Christopher R. Kirman and dated August 30, 2016) belong to the identities indicated in **Table 2**.

Table 2.

Portier	Farland	Composite	Candidate ID #	Name
2	2	4	1002	[redacted]
2	2	4	815	
2	2	4	665	
2	2	4	604	
2	1	3	1022	
2	1	3	326	
1	2	3	1010	
1	1	2	1006	
1	1	2	576	
1	1	2	1054	
1	1	2	514	

Signature: **Date:**

8/30/2016

Print:

Richard Canady, PhD

Affiliation:

Managing Director,
NeutralScience, L3C

Appendix E

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

- 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

Deleted:
Deleted: to
Formatted: Font:Not Bold
Formatted: Font:Not Bold, Italic
Formatted: Font:Not Bold
Formatted: Font:Italic
Formatted: Font:Italic
Formatted: Font:Not Bold
Deleted: It is
Deleted: ed from the panel
Deleted: of
Deleted: that
Deleted: they
Deleted: they
Deleted: they
Deleted: they
Deleted: /justify
Deleted: s (also
Deleted: answers and also
Deleted: they
Deleted:) at least briefly in the associated comment boxes

- 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.

Deleted: they

Deleted: they

Deleted: for the histopathology panel: they

Deleted: .

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.

Deleted: or

Deleted: for

Deleted: understand

Deleted: RELATIVE t

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.*

Formatted

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)

Deleted: Amongst

Deleted: used in similar studies

Deleted: , and given challenges around measuring markers in urine in animal experiments,

Deleted: which biomarkers of exposure would you recommend using for future studies?

- 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?

Deleted: I

Deleted: tuitive

Deleted: formative

Deleted: histo

Deleted: evaluation

Deleted: in line

Deleted: complement this with

Deleted: a more specific analysis (e.g., immunohistochemistry)?

- 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?

Deleted: Would you suggest a better/more suitable approach if any?

- 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"

Deleted: adequately

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?**

Formatted: Font:Italic

Deleted: - based gene expression analysis

- 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*

Deleted: do you see an interference between
Deleted: ed
Deleted: i
Deleted: (compared with wild-type) and the effect of CS exposure
Deleted: area
Deleted: network model-based
Deleted: disappears
Deleted: Which
Deleted: morphological
Deleted: are
Deleted: for the duration of CS exposure
Deleted: Which of
Deleted: the increased
Deleted: Which of
Deleted: are
Comment [BS9]: If possible, free text box for each question, but keep the 3 "subquestions" together.
Deleted: ing
Deleted: (a major CS constituents-inducible CYP isozyme)

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

Deleted: R

Deleted: p

Deleted: adding

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)?

Deleted: Id

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?

Deleted: to be substantial

- 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.

Deleted: in the liver tissue

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
- 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

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?

Deleted: C
Deleted: switching to THS2.2 aerosol
Deleted: ous
Deleted: smoke
Deleted: , benchmarked against smoking cessation?

- 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)

Deleted: ation

Deleted: to study
Deleted: significantly
Deleted: level
Deleted: of similar studies

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

Deleted: ed
Deleted: effects

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)

Deleted: o

Formatted: Font:Italic

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*