Author’s response to reviews

Title: A single-blinded, single-centre, controlled study in healthy adult smokers to identify the effects of a reduced toxicant prototype cigarette on biomarkers of exposure and of biological effect versus commercial cigarettes

Authors:

Christopher J Shepperd (jim_shepperd@bat.com)
Nik Newland (nik_newland@bat.com)
Alison Eldridge (alison_eldridge@bat.com)
Don Graff (donald.graff@CELERION.com)
Ingo Meyer (Ingo.Meyer@mps-hamburg.com)

Version: 3 Date: 14 May 2013

Author’s response to reviews: see over
Dear Professor Liu,

We thank you for a very thorough review of our recently submitted protocol manuscript (MS: 4924676358765939)

**Title:** A single-blinded, single-centre, controlled study in healthy adult smokers to identify the effects of a reduced toxicant prototype cigarette on biomarkers of exposure and of biological effect versus commercial cigarettes

Please see our responses to your report below.

We believe that, in a number of cases (particularly with regard to analytical and statistical methodologies), the level of detail requested is not in keeping with typical study protocols. This view was confirmed by reference to protocols already published in *BMC Public Health*. We would expect to publish this information in post-study papers and hope that the revision, with some additional detail, will be acceptable.

Yours Sincerely,

Jim Shepperd
Reviewer's report
Title: A single-blinded, single-centre, controlled study in healthy adult smokers to identify the effects of a reduced toxicant prototype cigarette on biomarkers of exposure and of biological effect versus commercial cigarettes

Version: 2 Date: 7 March 2013

Reviewer: Youcheng Liu

Reviewer's report:
General comments:
1. This study tries to evaluate if reduced-toxicant prototype (RTP) cigarettes actually reduce smokers’ exposures to toxicants in tobacco smoke as well as to reduce the risks of some health effects related to smoking. It contributes to the knowledge and understanding of the effectiveness of such RTP products in reducing exposures and smoking-related diseases. Therefore it is an important study.
2. In this manuscript the authors intend to publish the study protocol which falls in the journal scope of publication types and it is also relevant to publish the protocol so it will be useful to other investigators in the research community.
3. In the study design, the authors were creative in that they not only used smokers as a control group, but also used ex-smokers and never-smokers as control groups. This provides a better way of a controlled trial to compare the difference in exposure levels as well as indicators of risks of health effects.
4. The study is very ambitious with many exposure markers and effect markers to be evaluated. It is hard to know which markers are the main ones or the most important ones used for exposure and health effects.
5. Since this is a single-blinded study in which investigators and staff are not blinded, bias from researchers is possible.
6. The manuscript needs more details and clarity.
7. Although overall the English is OK, there is a need to polish the writing and correct some defects.

Major Compulsory Revisions
1. In the Abstract, instead of having objectives placed in the method section, the authors would provide more details on the study design, subject selection, measurements of various assessment indicators such as biomarkers, physiological measures, etc.

The abstract has been reworked, based on reviewers suggestions

2. In the Background (Page 5, Para. 1), biomarkers of effective dose were introduced, but its meaning was not explained; more details on their relevance need to be provided as authors also intend to evaluate them; in other words, why these exposure and effect markers are selected; related literature should be cited;
3. Although the primary and secondary objectives were provided, the authors did not state the hypotheses to be tested; it would be good to state clearly what hypotheses to be tested;  

The hypothesis has been added with the objectives (Lines 142-145).

4. It is suggested the authors re-check the recommendations from CONSORT (http://www.consort-statement.org/) for trial-related publications, particularly the item checklist. Although the authors in general followed the recommended items, some parts are missing; example: randomization procedures and mechanisms for the allocation of participants into the study groups and control groups and the random allocation of the RTP to the study group. Additionally the flow diagram recommended by CONSORT could be used to show the study design and sample sizes; this will facilitate the description on the study design, selection of participants, etc.

Words have been added to describe the way in which subjects were allocated to groups. A simple diagram has been added to indicate study design and sample sizes (Figure 2). However, the CONSORT flow diagram would normally be used to show the flow of subject numbers through the study (incl numbers discontinued, excluded from analysis etc.), which is less appropriate for a protocol manuscript.

5. Inclusion/exclusion criteria: the authors indicated the ex- and never-smoker groups provide background levels of biomarkers of environmental exposures, but did not indicate if these participants would come from the same communities as the smokers with similar air pollution levels; occupational exposure to nicotine such as tobacco farmers and farm workers and other chemicals such as pesticides should be excluded or limited; what about employees of restaurants and bars where smoking was not prohibited? these should be considered as well; need clarification;

A sentence has been added to state that all subjects would be recruited from the same communities, i.e. the non-smoking controls are representative of the same environment that the smokers are exposed to (Lines 195-196). We do not exclude any of these occupations as we require to know what the background exposure will be in a typical population recruited from the Hamburg area. Past studies have shown that it can be difficult to recruit participants and we wish to minimise the exclusion criteria whilst still having an appropriate non-smoking control group.

6. More on randomization of participants and the cigarette products: the allocation of participants into the study groups vs. the control groups was based on the ISO tar yield of their usual cigarette brand as the authors claimed; is there a way this allocation of participants into the study vs. control groups can be randomized? Additionally, or in other words, can the allocation of the RTP to the study group be randomized at individual level? Also the description currently on
the cigarette blinding is a little confusing; instead of “control cigarettes” (Methods, Para. 16), “study cigarettes” could be used which include those regular ones for the control group and the RTP ones for the study groups; 

In order to complete all of the events on all subjects, it is necessary to split the groups up into subgroups in the clinic and these are staggered in time, as shown in Figure 1. Also, there is an attempt to match the groups with respect to gender and age. Therefore, to ensure subgroups are filled, subject availability is ensured and groups are matched for age and gender, it has been accepted that full randomisation into control or test group is not possible. Smokers are recruited and subgroups filled based on order of screening, age, gender and availability for study start time. Similarly, the age and gender of non-smokers are taken into account at recruitment in order to attempt a match with the smoking groups.

Words have been added to the text to explain this point (Lines 305-319).

7. The authors mentioned the smoking groups will adhere to a diet sheet. Does that eliminate those food items that have nicotine such as tomatoes and green peppers?

These items were not eliminated from recommended diet. We do not believe it is necessary to exclude tomatoes and green peppers for several reasons. Firstly, the amount of nicotine present in tomatoes and green peppers is very low compared with tobacco smoke (see http://www.nejm.org/doi/full/10.1056/NEJM199308053290619 in particular the letter from Jack Henningfield). Secondly, tomatoes (especially) are so much part of normal diet that asking subjects to avoid them for the duration of the study would present a challenge for compliance. Finally, the non-smoking control groups have been included in the design to account for any environmental exposure to compounds of interest, including nicotine (i.e. via diet or environmental tobacco smoke).

8. Since quitting smoking is not used in this study as an outcome assessment, the smoking-cessation workshops seem irrelevant; in fact if may interfere with the evaluation on the effect of the RTP; Need more clarification;

In a previous smoking study we conducted at the same clinic, inclusion of a smoking cessation workshop was an absolute requirement from the ethics committee before they would grant approval. Therefore, we have included it at the outset. Words have been added to this effect (Lines 405-407).

9. Provide description and details on the collection and analysis of all biomarkers including who will do the analysis, where they will be analyzed, procedures and quality control (QC) and quality assurance (QA) measures.

This study involves the analysis of 26 biomarkers of exposure and 35 biomarkers of biological effect, as listed in Table 1 and Tables 9 and 10. It does not seem
reasonable to add analytical details with QC and QA measures for all of these analytes in a protocol manuscript. It would add many pages and is a level of detail that BMC Public Health protocol manuscripts do not normally contain. The analytical details will be published post study along with the results.

Minor Essential Revisions
Methods:
1. The smoking subgroups were not clearly described and the rationale for the ratio of 3/2 vs. 2/3 were not explained;

Additional words have been added (Lines 198-200)

2. Sample size calculations: the study did not provide the logic and description on the sample size determination; on which effectiveness indicators were the sample sizes determined?

Some additional words have been added to this section (lines 569-578)

3. Methods, Para 27: this should be moved to the data management and analysis section;

This sentence/paragraph has been removed as it is already stated in a previous paragraph.

4. Provide details of the urine collection method (using what device);

Words added (lines 452-453)

5. Provide details on the saliva test assay;

Words added (lines 476-480)

6. Provide details on the blood collection and analytical methods;

We believe that adequate detail regarding blood collection and collected volumes is provided in the text and associated table 10. There are 35 blood biomarkers for analysis in this study. It is not practical to provide analytical details of all of these biomarkers in the protocol manuscript. However, the analytical details will be published, along with the results, post-study.

7. Provide details on the exhaled carbon monoxide analysis;

We state that the exhaled CO will be determined using a commercially available EC50 Micro III Smokerlizer CO meter (Bedfont, Maidstone, UK), or similar device. We do not know what additional details we can provide.

8. Provide details on the MLE to toxicants in the sponsor's lab, such as extraction
method, analytical equipment, QA/QC procedures, etc.;

We have added some more detail (lines 509-512). However, this is a complicated methodology and it is not possible or appropriate to provide the full details in this study protocol. However, a reference is provided (line 500), if more details are required.

9. Provide a reference on the equipment used for puffing, inhalation and exhalation measurements; describe in more detail how they work; what is the relevance of these tests to the study?

A reference to the equipment and its use has been added, along with reasons for these measurements and a more detailed description of the equipment and procedure (Lines 515-526).

10. Provide details on the pulmonary function test such as the equipment to used and measurement procedures;

Measurement of pulmonary function is a standard procedure in the clinical setting and details of equipment and procedure should not be necessary.

11. Provide details on quality-of-life, sensory and diet and lifestyle assessments such as the content of the questionnaires, how they will be administered, who will do them and how the scoring will be done;

Some additional text added to describe how these questionnaires will be administered (i.e. self-administration using a tablet device). The content of each questionnaires is already described in broad terms, but some additional words added (lines 546-563). We believe that the scoring and analysis of each of these questionnaires is outside the scope of this manuscript.

12. Provide details on the sample size calculations, such as the main indicators used, the powers, significant levels, etc. (although aromatic amines were mentioned, no details are provided on calculations with 50 as the size for all exposure markers); also for effect markers, no calculation details are provided for the size of 50;

Additional details and explanation has been provided (Lines 566-578).

13. Statistical analysis description is sketchy and the actual plan is not provided; the authors indicated it will be prepared, but in fact a plan is needed in the protocol and its publication; how data will be collected, transcribed, entered, checked for errors, analyzed using what methods to evaluate the differences within individuals, within groups and between groups and to achieve the study objectives; Celerion was mentioned for the analysis task, but what is Celerion? Where it is located?
The full statistical analysis plan (which amounts to 100+ pages) is not normally required until database lock, and it is conventional to provide a summary of the planned statistical approach in the protocol. This is what we have included in the protocol and manuscript. The level of statistical detail in our manuscript is in-keeping with other protocol manuscripts published by BMC Public Health. However, we agree that this section could be clarified and some additional words have been added (lines 591-593).

14. Need more content in the discussion, such as expected results, potential problems and alternative approaches, strengths and weaknesses of the study design.

We believe that a discussion of expected results, potential problems and strengths and weaknesses are outside the scope of a protocol manuscript. This material will be reported in a post-study paper.

Figures and tables:
1. Table 2: instead of the web link, a reference should be added to the list and the link on the table removed; what do “1A2, 2B6 …” mean? Need a note to explain these;

Reference added and explanation of CYP codes provided with table.

2. Table 3: suggest adding “groups” to the ‘Control’; how were the amount of tar and CO determined for each brand of cigarettes? Are these available based on the brands from manufacturers? Provide a note to explain;

Table 3 revised as requested. Tar, nicotine and CO determined using ISO methods (info added).

3. Tables 4, 5, 6, 7: suggest replacing “Sample” with “sampling”;

Correction made

4. Tables 9 and 10: add notes to explain all short names;

Notes added

5. Figure 1: suggest changing the title to “Study design and scheduled events”; need notes to explain if each time block is a day or a week; in the legend, a red vertical line was used, but it is not seen in the group bars; instead a black line was used; need to change.

Title changed and recommended changes made to the figure.

Discretionary Revisions
Abstract:
1. Para 2: spell the ISO out; *Words added (line 35)*
2. Keywords: spell out PREP. *Words added (lines 61-62)*

**Background:**
1. Para 1: define this smoker population as worldwide or specific regions; *added ‘worldwide’ (line 67)*
2. Para 1: IOM is usually used instead of IoM; *Corrected throughout manuscript*
3. Para 1: provide references for the IOM statement; *Quote corrected and reference added (lines 69-70)*
4. Para 2, subheading: replace “reduced exposure” with “reduced-exposure” in the subheading; *Alteration made (line 72)*
5. Para 2: add an “s” to “toxicant”; *Correction made (Line 76)*
6. Para 2: change “is” to “are”. *Correction made (line 79)*

**Level of interest:** An article of importance in its field

**Quality of written English:** Needs some language corrections before being Published

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**
I declare that I have no competing interests.