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.

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;

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.

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;

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;

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?

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;

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.

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;

2. Sample size calculations: the study did not provide the logic and description on the sample size determination; on which effectiveness indicators were the
3. Methods, Para 27: this should be moved to the data management and analysis section;

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

5. Provide details on the saliva test assay;

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

7. Provide details on the exhaled carbon monoxide analysis;

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

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?

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

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;

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;

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?

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

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;

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;

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

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

Discretionary Revisions

Abstract:
1. Para 2: spell the ISO out;
2. Keywords: spell out PREP.

Background:
1. Para 1: define this smoker population as worldwide or specific regions;
2. Para 1: IOM is usually used instead of IoM;
3. Para 1: provide references for the IOM statement;
4. Para 2, subheading: replace “reduced exposure” with “reduced-exposure” in the subheading;
5. Para 2: add an “s” to “toxicant”;
6. Para 2: change “is” to “are”.

**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.