Reduction in Harmful or Potentially Harmful Constituents Following Partial or Complete Substitution of Cigarettes with Electronic Cigarettes: Clinical Evidence

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1. Introduction

E-cigarettes are becoming an increasingly popular alternative to conventional tobacco cigarettes among smokers worldwide.

The concentration of toxicants (including many Harmful and Potentially Harmful Constituents [HPHCs]) in e-cigarette aerosols has been found to be generally tens to thousands of times lower than in tobacco smoke; many toxicants are simply not present in e-cigarette aerosols at detectable levels (e.g. Fig. 1). As a result, e-cigarette aerosols elicit minimal biological responses in conventional regulatory *in vitro* toxicology assays compared to conventional cigarettes (e.g. Fig. 2). While such data does not prove if switching to an e-cigarette is "less harmful" than continued cigarette use in isolation it does give a very good indication of the potential and the confidence to move into clinical studies.

There is relatively little information available on consumer exposure to HPHCs resulting from the use of e-cigarettes compared to conventional cigarettes. To that end, the objective of this study was to compare changes in selected urine, blood and exhaled breath biomarkers of exposure to HPHCs among different user groups following a 5 day forced-switch from usual conventional cigarette brand to:

(i) exclusive use of a commercially available blu™ e-cigarette;
(ii) dual use of a commercially available blu™ e-cigarette and the subject's usual conventional cigarette brand; or
(iii) discontinued use of all tobacco or nicotine products.

The biomarkers of exposure to the HPHCs selected included a number of cigarette smoke constituents representing classes of compounds believed to be the most significant contributors to smoking-associated disease risks as reported by the FDA.

Reduced formation of toxicants in blu™ e-cigarette aerosol vs. conventional cigarette smoke…

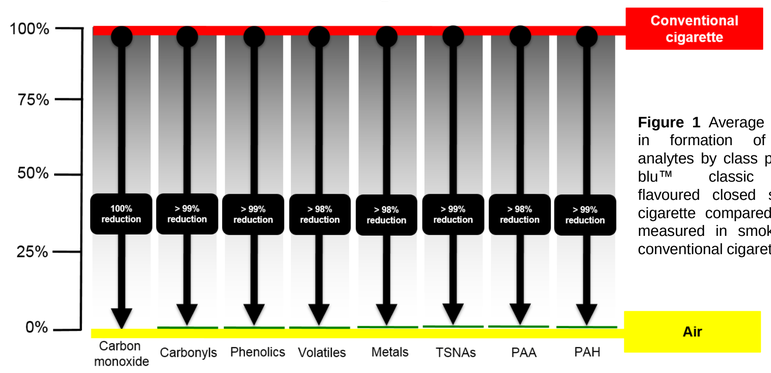

Figure 1 Average reductions in formation of selected analytes by class per puff for blu™ classic tobacco flavoured closed system e-cigarette compared to levels measured in smoke from a conventional cigarette [1].

…leads to reduced toxicity *in vitro*

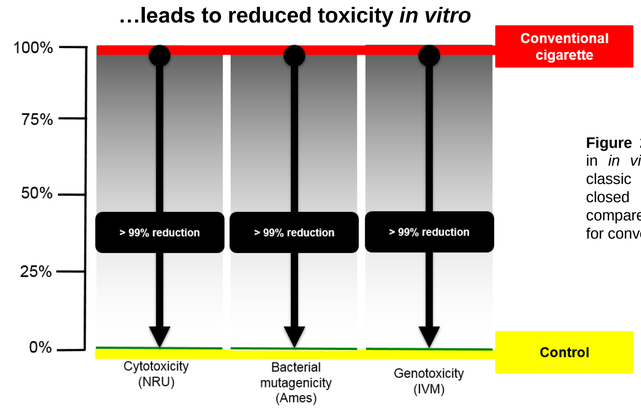

Figure 2 Average reductions in *in vitro* toxicity for blu™ classic tobacco flavoured closed system e-cigarette compared to levels measured for conventional cigarette [2].

2. Study Design

ClinicalTrials.gov Identifier: NCT02385227

Subject inclusion criteria: healthy adult male and female smoker, 21 to 65 years of age; conventional cigarette smoker for at least 12 months prior to study and smoked an average of 10 full flavour cigarettes per day; smoker status confirmed by positive urine cotinine (≥ 500 ng/mL) and exhaled CO > 12 ppm at screening; prior use of an e-cigarette was not an exclusion criteria, provided all other criteria were met.

Clinically-confined smokers (*n*=45) were randomized into groups that partially (*n*=15) or completely (*n*=15) substituted their usual conventional cigarette brand with a commercially available classic tobacco rechargeable closed system blu™ e-cigarette (2.4% nicotine), or discontinued all tobacco or nicotine products (*n*=15), for 5 days (Fig. 3). Dual users could smoke no more than 50% of the number of cigarettes per day reported during screening (hence 'partial substitution'). Subjects were familiarised with the e-cigarette device during enrolment. Products were used *ad libitum* throughout and the study was conducted in the USA.

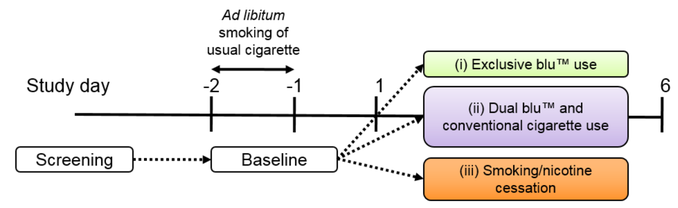

Figure 3 Study overview

Urinary and blood biomarkers of exposure were chosen to represent major classes of HPHCs (see *Section 3* for biomarkers measured), including those for carbonyls and tobacco-specific nitrosamines, and were assessed at baseline and again after 5 days of e-cigarette product use or cessation (see *Section 3*). Each biomarker was measured using validated methods [3,4,5]. Physiologic effects as measured by exhaled carbon monoxide (CO) and nitric oxide (NO) were assessed at baseline and during the 5 day forced-switch to determine changes associated with product use or cessation, and differences between cohorts.

3. Reductions in Blood and Urine Biomarkers of HPHC Exposure from Day -1 to Day 5

- Reducing consumption of conventional cigarettes over 5 days according to the requirements of the study resulted in sizeable reductions in exposure to a number HPHCs (Figs. 4 and 5).
- Smoking cessation lead to a 66% to 98% reduction in excretion of the urine biomarkers of exposure evaluated in this study (Fig. 5). The smallest reduction was seen in NNAL, which has the longest half-life of the individual biomarkers listed [6]. Predictably, significant decreases were also observed in the COHb, nicotine, and the nicotine metabolite cotinine, as the cessation subjects had no exposure to CO or nicotine (Fig. 4).
- The reductions in biomarkers of exposure to HPHCs observed in the exclusive e-cigarette use group were mostly comparable to those seen in the cessation group (Fig. 5), with the notable exceptions of the nicotine and nicotine metabolite biomarker as these subjects continued to consume nicotine from the e-cigarette (Fig. 4).
- Dual users smoked 52% fewer conventional cigarettes compared to screening and 33% fewer compared to Day -1. Dual users who had substituted half of their self-reported daily cigarette consumption with the e-cigarette exhibited reduced biomarker levels that appear broadly proportional to the reduced numbers of cigarettes smoked. Reductions in the urine biomarkers of exposure for this group ranged from 22% to 37% (Fig. 5). With the exception of the nicotine metabolite cotinine, which showed a non-statistically significant increase in blood at the end of the exposure study (Fig. 4).
- Overall, measurable nicotine and cotinine were present in the samples from exclusive e-cigarette users, but levels of biomarkers for HPHCs were significantly lower, and many were indistinguishable, from those of subjects who had ceased to use any nicotine product (Figs. 4 and 5). The excretion and concentration of all exposure biomarkers evaluated in this study were higher in the dual use group at Day 5 compared to the cessation group (Figs. 4 and 5).

% change in blood biomarkers of exposure after 5 days

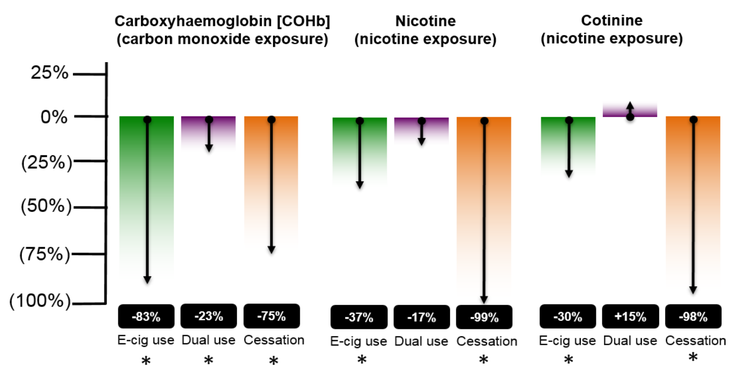

Figure 4 % change from baseline (Day -1) to end of exposure study (Day 5) per study group for blood biomarkers of HPHC exposure. E-cig use, exclusive use of blu™ classic tobacco 2.4% e-cigarette; dual use, dual use of blu™ classic tobacco 2.4% e-cigarette and own brand of conventional full flavour cigarette; cessation, smoking and nicotine product cessation. * denotes differences from baseline (Day -1) to Day 5 were statistically significant at an alpha level of 5%.

% change in urine biomarkers of exposure after 5 days

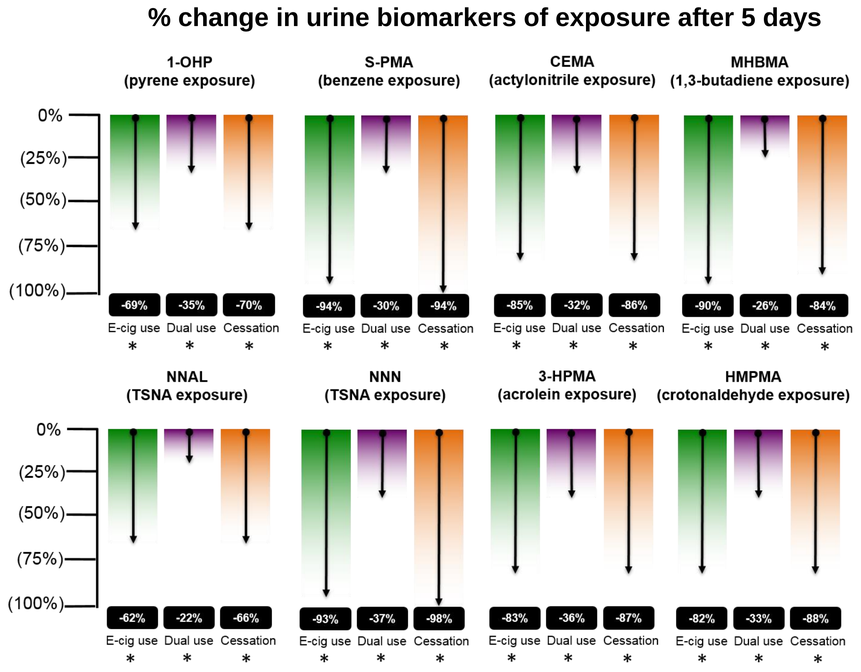

Figure 5 % change from baseline (Day -1) to end of exposure study (Day 5) per study group for urine biomarkers of HPHC exposure. E-cig use, exclusive use of blu™ classic tobacco 2.4% e-cigarette; dual use, dual use of blu™ classic tobacco 2.4% e-cigarette and own brand of conventional full flavour cigarette; cessation, smoking and nicotine product cessation. * denotes differences from baseline (Day -1) to Day 5 were statistically significant at an alpha level of 5%.

4. Changes in Exhaled CO and NO Levels

- Physiological changes associated with smoking reduction were noted in both exhaled carbon monoxide (CO) and nitric oxide (NO) endpoints. CO exposure is often estimated by either CO concentrations in exhaled breath or from CO bound to haemoglobin (Fig. 4).
- All groups experienced decreases in exhaled CO at Day 5 compared to Day -1, with decreases in the cessation and exclusive e-cigarette use groups around 90%, and 31% in the dual use group (Fig. 6). Further, there were no differences between the cessation and exclusive use group measurements on Day 5 whereas the dual use group had higher exhaled CO compared to cessation; as expected since this group still consumed conventional cigarettes.
- Smoking has been reported to decrease NO production but the mechanism remains incompletely understood. Exhaled NO is used as a noninvasive biomarker of inflammation in the airways, and can be detected in expired breath. In this study, exhaled NO was observed to increase from Day -1 to Day 5 in the cessation (49%) and exclusive e-cigarette use groups (56%), whereas the dual use groups experienced minimal changes (Fig. 6).
- The findings associated with exhaled breath biomarkers in the cessation and exclusive e-cigarette use groups are consistent with other research findings associated with reductions in exhaled CO and increases in exhaled NO following smoking cessation [e.g. 6,7,8].

Changes in exhaled breath endpoints over study period

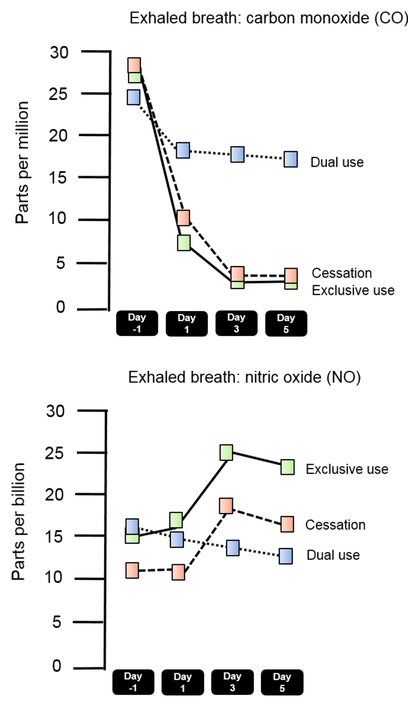

Figure 6 Changes in exhaled breath CO and NO endpoints over study period per study group. E-cig use, exclusive use of blu™ classic tobacco 2.4% e-cigarette; dual use, dual use of blu™ classic tobacco 2.4% e-cigarette and own brand of conventional full flavour cigarette; cessation, smoking and nicotine product cessation.

5. Conclusions

- The data presented here demonstrate that smokers who completely substituted conventional cigarettes with e-cigarettes over a short period of time (5 days) experienced reductions in exposure to a number of HPHCs as measured by urine, blood and exhaled breath biomarkers of exposure. Moreover, the data show that subjects who switched to dual use also experienced significantly reduced HPHC exposures after partially replacing cigarettes with the blu™ e-cigarette product, albeit to a lesser extent. The data are consistent with the findings of other investigations which have demonstrated that e-cigarette use results in a decrease in biomarkers of tobacco exposure [e.g. 9,10,11].
- The present study extends the findings of [1] (summarised in Fig. 1), which showed the e-cigarette aerosol levels of HPHCs such as carbonyl compounds, tobacco-specific nitrosamines, polycyclic aromatic hydrocarbons and other constituents are in the order of 1500 times lower than those found in the smoke of conventional tobacco cigarettes (*i.e.* <2 µg/puff vs. ~3,000 µg/puff), with the observation that the blu™ closed system e-cigarette produced markedly lower levels of exposure biomarkers when used by smokers in lieu of their usual cigarette brand style for a period of 5 days.
- It has been suggested that dual use may be a public health concern because of a possibility that it exposes smokers to greater risks than those encountered by smoking conventional cigarettes alone [12]. Furthermore, a recent study reported that dual use of e-cigarettes whilst continuing to smoke did not result in reduced exposure to known carcinogens and toxicants [13]. The study presented here enforced a reduction in daily cigarettes smoked on the dual use group as an initial examination of the responsiveness of the measured HPHC exposure biomarkers to moderately-reduced smoking combined with unlimited *ad libitum* usage of e-cigarettes. Under these conditions, the data show that dual users experienced significant reductions in all of the urine biomarkers assessed. It appears there may also be a relationship between the magnitude of reduction in biomarkers of exposure to HPHCs in this group and the reduction in conventional cigarettes smoked. The impact of longer term exclusive and dual e-cigarette use on biomarker of exposures to HPHCs are planned.
- Whether the reductions in toxic and carcinogenic constituent exposures such as those observed here may have the potential to reduce risks for chronic, smoking-caused diseases for long-term e-cigarette users who have partially or completely abandoned cigarette smoking warrants further investigation.
- Overall, the present study shows the great potential that the blu™ closed system e-cigarette may provide for smokers seeking an alternative to tobacco products; the role that biomarkers of exposure may play a role in assessing and comparing exposure to HPHCs across different product categories; and supports the case for regulating e-cigarettes differently from tobacco-containing products.

References

[1] Tayyarah, R., and G. A. Long. 2014. 'Comparison of select analytes in aerosol from e-cigarettes with smoke from conventional cigarettes and with ambient air', *Regul Toxicol Pharmacol*, 70: 704-10.
[2] Misra, M. *et al.* 2014. 'Comparative in vitro toxicity profile of electronic and tobacco cigarettes, smokeless tobacco and nicotine replacement therapy products: e-liquids, extracts and collected aerosols', *Int J Environ Res Public Health*, 11: 11325-47.
[3] FDA Guidance to Industry: Bioanalytical Method Validation 2001.
[4] Good Laboratory Practices (GLP) for Non-Clinical Laboratory Studies 21 CFR Part 58 21 CFR 58.
[5] European Medicines Agency: Guideline on Bioanalytical Method Validation (EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr.2).
[6] Ripoll, J. *et al.* 2012. 'Clinical trial on the efficacy of exhaled carbon monoxide measurement in smoking cessation in primary health care', *BMC Public Health*, 12: 322.
[7] Kharitonov, S.A. *et al.* 1995. 'Acute and chronic effects of cigarette smoking on exhaled nitric oxide' *Amer J Resp Crit Care Med*, 152(2): 609-612.
[8] Hogman, M. *et al.* 2002. 'Increased nitric oxide elimination from the airways after smoking cessation', *Clin Sci (Lond)* 103(1): 15-9.
[9] Caponnetto, P. *et al.* 2013. 'EffiCiency and Safety of an eLectronic cigAreTte (ECLAT) as tobacco cigarettes substitute: a prospective 12-month randomized control design study', *PLoS One*, 8: e66317.
[10] McRobbie, H. *et al.* 2015. 'Effects of Switching to Electronic Cigarettes with and without Concurrent Smoking on Exposure to Nicotine, Carbon Monoxide, and Acrolein', *Cancer Prev Res (Phila)*, 8: 873-8.
[11] Polosa, R. *et al.* 2011. 'Effect of an electronic nicotine delivery device (e-Cigarette) on smoking reduction and cessation: a prospective 6-month pilot study', *BMC Public Health*, 11: 786.
[12] Grana, R. *et al.* 2014. 'E-cigarettes: a scientific review', *Circulation*, 129: 1972-86.
[13] Shahab, L. *et al.* 2015. 'Exposure to selected toxicants and carcinogens as a function of smoking status and long-term use of nicotine replacement therapy or electronic cigarettes' Abstract presented at the 21st annual meeting of the Society for Research on Nicotine and Tobacco, Philadelphia, PA. 2015.