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Environmental health hazards of e-cigarettes and their components: Oxidants and copper in e-cigarette aerosols

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ABSTRACT

To narrow the gap in our understanding of potential oxidative properties associated with Electronic Nicotine Delivery Systems (ENDS) i.e. e-cigarettes, we employed semi-quantitative methods to detect oxidant reactivity in disposable components of ENDS/e-cigarettes (batteries and cartomizers) using a fluorescein indicator. These components exhibit oxidants/reactive oxygen species reactivity similar to used conventional cigarette filters. Oxidants/reactive oxygen species reactivity in e-cigarette aerosols was also similar to oxidant reactivity in cigarette smoke. A cascade particle impactor allowed sieving of a range of particle size distributions between 0.450 and 2.02 μm in aerosols from an e-cigarette. Copper, being among these particles, is 6.1 times higher per puff than reported previously for conventional cigarette smoke. The detection of a potentially cytotoxic metal as well as oxidants from e-cigarette and its components raises concern regarding the safety of e-cigarettes use and the disposal of e-cigarette waste products into the environment.

1. Introduction

Investigation of the safety of electronic cigarettes (e-cigarettes) and related products, or Electronic Nicotine Delivery Systems (ENDS), is currently increasing (Printz, 2014). Classes of ENDS include disposable/non-refillable e-cigarettes, refillable e-cigarettes, refillable e-pens, e-hookah pens, e-cigars, and other fast-emerging products. While there is a growing body of research on the direct health hazards of e-cigarette use, there is a dearth of research on the potential environmental health hazards posed by improper disposal of these devices (Chang, 2014). Major components comprising a typical ENDS e-cigarette include a lithium-ion battery (LIB), light-emitting diode (LED) lights, microprocessor, metal casings, wires, plastics, and other absorbent polymers that stabilize components and secure vaporizable liquids to retain them inside the device. Most of these parts may come into contact with toxic contaminants detected in e-cigarettes, and should be disposed of properly (Goniewicz et al., 2014a, 2014b). Disposal procedures and guidelines for ENDS are currently not subjected to regulatory/government oversight which may be necessary to avoid potential public health risks that have yet to be fully assessed and made available to consumers. This potential problem is of particular concern given the continuing increase in the number of ENDS consumers (CDC, 2013).

Many ENDS replicate the size and appearance of conventional tobacco cigarettes while others involve "chambers" that are able to be easily refilled with larger volumes of vaporizable solvents. Each proprietor of ENDS may include variable ingredients, such as flavors, nicotine, and other additives to enhance the ENDS experience, as well as glycerin, propylene glycol, and polyethylene glycol that are predominantly used in the e-cigarette industry to produce

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Abbreviations: DCFH-DA, 2′-7′-dichlorodihydrofluorescein diacetate; DCFH, 2′-7′-dichlorodihydrofluorescein; e-cigarette, electronic cigarette; ENDS, electronic nicotine delivery systems; EPR, electron paramagnetic resonance; LIB, lithium-ion battery; ROS, reactive oxygen species; MMAD, mass median aerodynamic diameter.

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aerosols (Drummond and Upson, 2014). The potential toxicities of these substances and other unregulated additives that could absorb into the skin or be vaporized and subsequently be inhaled are incompletely understood (Orr, 2014).

With conventional cigarettes, the majority of known health related hazards are due directly to smoking and second-hand exposure. Many of the dangers of tobacco use are well established, and proper tobacco cigarette disposal is an ongoing environmental concern (Novotny et al., 2009). The emergence of ENDS presents new challenges for public health officials both for individual users' health and those near exposure ranges, and for how ENDS components are disposed of. Therefore, there is both a health risk as well as an environmental hazard concern. ENDS/e-cigarettes typically employ a battery power source, and both rechargeable and non-rechargeable varieties are readily available. It is difficult to forecast future consumption rates of both reusable (rechargeable) and disposable (non-rechargeable) ENDS or the technological evolution of vaporization/nicotine delivery methods and power sources.

Lithium-ion batteries (LIB) are the primary power source for ENDS as well as many other popular electronic devices. It is not clear which LIB technology is typically used in ENDS or if different manufacturers employ preferred LIB types. Some of the components found in LIBs include heavy metals that are known to have toxic effects on living organisms (Kang et al., 2013). The projected amount of disposal for LIBs may increase substantially with growing ENDS consumerism.

The second component that is liable to high rates of disposal comprises the replaceable cartridges (cartomizers) that contain the vaporizable liquid. The cartomizer combined with the battery in many instances is designed to resemble a conventional cigarette. Cartomizers would be expected to be discarded more frequently in regular waste or as litter relative to batteries. This would only add to ongoing cigarette filters (butts) disposal and potentially introduce new chemicals that accumulate alongside those in cigarette butts which are harmful to animals and flora (Moriwaki et al., 2009; Slaughter et al., 2011). Unfortunately, there is no information for ENDS litter to make comparisons to conventional cigarettes and their toxic impact on environments and habitats.

The present study investigated using a semi-quantitative measurement of oxidants/reactive oxygen species (ROS) from e-cigarette components (cartomizers and batteries) as compared to conventional cigarette butts to assess if there is a potential for environmental exposure to these materials which are expected to contribute to tobacco waste and environmental pollution. We extended our studies to detect the presence of oxidants/ROS associated with e-cigarette aerosols and measured aerosol particle size distribution and copper levels to assess if there is a potential concern for oxidants/ROS induced toxicity when inhaling ENDS/e-cigarette aerosols.

2. Materials and methods

2.1. *Cigarette products assessed*

The following types of electronic vaporizing devices and conventional cigarettes, such as Blu® electronic cigarettes (Lorillard Technologies, Inc.), eGO Vision® Spinner (Vision High-Tech Electronics Limited, Shenzhen, Guangdong, China), Marlboro® 100s (Philip Morris USA Inc.), and Kentucky 3R4F reference cigarettes (Tobacco Research Institute, University of Kentucky, Lexington, KY) were used in this study.

2.2. *E-cigarette components and detection of their oxidant reactivity*

We obtained used cartomizers from e-cigarette users involved in a larger study examining e-cigarette emissions as a function of consumer behavior (Dr. Risa Robinson, Rochester Institute of Technology, Rochester, NY). The products were rechargeable Blu e-cigarettes (batteries, $n = 7$ and cartomizer, $n = 17$) used over a 24 h period and then returned to the laboratory. E-cigarette cartomizers were disassembled and metal casings separated. All internal materials removed from the cartomizer metal housing, including polyfill absorbent material, wicking material, heating elements and electrical wires, silicone caps, and residual e-cigarette fluid absorbed were submerged in 2′-7′-dichlorodihydrofluorescein (DCFH) solution for 5 h. For filter oxidant reactivity, conventional cigarette filters were removed from cigarettes either unused or following tobacco smoke filtering. A laboratory vacuum line was used to replicate puffs every 30 s for 4–5 s until the cigarette was consumed. Both unused and smoke exposed filters were placed in DCFH solution for 5 h. Non-functional LIBs were placed in DCFH solution for 5 h. All incubations were carried out at room temperature in darkness. For cartomizers and LIBs, equal volumes of DCFH solution placed in test tubes alone for 5 h were considered controls. The DCFH solution was measured for fluorescence as for e-cigarette vapor and conventional cigarette smoke (see section 2.3).

2.3. *Cell-free oxidants/reactive oxygen species (ROS) assay*

The relative levels of ROS produced from electronic cigarette vapor or smoke from filtered tobacco cigarettes was determined using semi-quantitative measurements of oxidative/reactive oxygen species (ROS) by 2′,7′dichlorofluorescein diacetate (H2 DCF-DA) fluorogenic probe (EMD Bioscience, CA). Oxidation of DCFH (derived from H2 DCF-DA) converts it into fluorescent molecule (DCF) indicating the presence of free radicals such as ROS (i.e. H_2O_2) or potentially other reactive oxidants (Black and Brandt, 1974; Myhre et al., 2003). For each exposure, 5 ml of dichlorofluorescein-horse radish peroxidase (DCFH-HRP) solution developed to assess oxidant reactivity in cell free systems (Hung and Wang, 2001; Jiang et al., 2008), was loaded into a clean glass bubbler (prism research). A laboratory pump (FMI, Syosset, NY) with a flow range of 0–1296 ml/min was switch activated using an FMI stroke rate controller set at 60% flow to draw a steady stream of e-cigarette vapor/tobacco cigarette smoke directly through the DCFH solution (Fig. 1). E-cigarette vapor was puffed through DCFH solution in the bubbler at room temperature for 4–5 s at 30 s intervals for a total of 10 min (Hua et al., 2013). For filtered tobacco cigarettes, 4–5 s of smoke was drawn through DCFH at 30 s intervals for 5 min (approximately 2 cigarettes to minimize tar and particulate build up). All tobacco cigarettes (3R4F, Marlboro 100s) were combusted within an approved chemical flow hood and the samples protected from direct and ultraviolet light to prevent photo-autooxidation of DCFH. Following exposures, sample tubes were placed on ice and protected from light sources until analysis. A spectrofluorometer was used to measure oxidized dichlorofluorescein (DCF) fluorescence at an absorbance/emission maximum of 485 nm/535 nm. Hydrogen peroxide standards between 0 and 50 μM were created from 1 M stock and reacted at room temperature for 10 min with prepared DCFH solution in a total of 5 ml. These standards were then used to calibrate spectrofluorometer fluorescence intensity units to numerically match respective hydrogen peroxide concentrations that produce increasing amounts of DCF fluorescence in the presence of horseradish peroxidase (HRP).

All ENDS/e-cigarette DCFH reactions were carried out in a dark room at room temperature for 10 min which was adequate to