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Highly Reactive Free Radicals in Electronic Cigarette Aerosols

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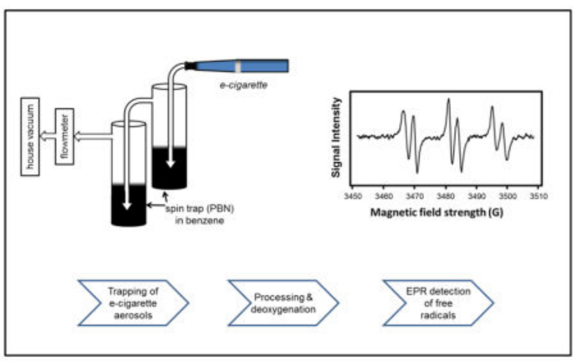
Abstract

Electronic cigarette (EC) usage has increased exponentially, but limited data is available on its potential harmful effects. We tested for the presence of reactive, short-lived free radicals in EC aerosols by electron paramagnetic resonance spectroscopy (EPR) using the spin-trap phenyl-N-tert-butylnitrone (PBN). Radicals were detected in aerosols from all ECs and eliquids tested (2.5×10^{13} to 10.3×10^{13} radicals per puff at 3.3V) and from eliquid solvents propylene glycol and glycerol and from “dry puffing”. These results demonstrate, for the first time, the production of highly oxidizing free radicals from ECs which may present a potential toxicological risk to EC users.

Graphical abstract



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Author Contributions
All authors have given approval to the final version of the manuscript.

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Supporting Information. A detailed description of the materials and methods is provided. This material is available free of charge via the Internet at http://pubs.acs.org.

Electronic cigarettes (EC) are battery-powered devices that deliver nicotine without burning tobacco.[1–3] Although often marketed and perceived as a less harmful alternative to conventional cigarettes, there is currently a lack of clear, comprehensive, quantitative evidence on the toxicants in EC aerosols.

Cigarette smoke contains high concentrations of toxic free radicals ($>10^{16}$ molecules/puff) including reactive oxygen species (ROS) and reactive nitrogen species (RNS).[4, 5] Oxidative stress due to exposure to cigarette smoke free radicals has widespread impact on many critical cellular pathways including cell proliferation, metabolism, survival and inflammation. Oxidative stress/damage resulting from exposure to these free radicals is likely an important mechanism by which smoking induces diseases such as cancer,[6] cardiovascular disease [7] and chronic obstructive pulmonary disease (COPD).[8–10] While recent studies have pointed to the possible production of stable, long-lived radicals by EC;[11–13] there is no data on the production of short-lived, highly reactive radicals. Our current aim was to determine if such radicals are present in EC aerosols by using electron paramagnetic resonance (EPR) with spin trapping, the only direct method for detecting such radicals.[14, 15]

Aerosols were generated (Figure S1, described in detail in the Supporting Information) using commercially available EC batteries (eGo-ce4 3.3 V and Tesla 3.0–6.0 V), cartomizer (SmokTech: XXL, 1.5 ohms resistance, dual heating coils) and eliquids as well as their solvents propylene glycol and glycerol. Puffs were simulated based on human usage conditions[16] (puff duration, 5 sec; puff interval, 20 sec; flow rate, 500 ml/min and number of puffs, 40 per experiment). EC aerosols were passed through two impingers (approx. 20 cm downstream) containing a benzene solution with the spin-reagent trap phenyl-N-tert-butylnitrone (PBN). PBN-radical adducts were then analyzed using EPR.[14, 15, 17, 18]

EPR spectra clearly indicate the presence of PBN spin adducts in aerosols generated from all eliquids and voltages tested indicating the presence of short-lived free radicals (Figure 1). Both eliquid 2 and eliquid 3 yielded characteristic 6-line EPR spectra (a_N = 14.05 G, a_H = 2.21 G, and a_N = 14.17 G, a_H = 2.47 G, respectively), suggesting the presence of a sole, dominant PBN radical adduct at the time of analysis. This does not preclude the possibility that multiple radical species were generated during the experiment. In contrast, eliquid 1 yielded a PBN-adduct spectrum characteristic of multiple trapped radical species. At a potential of 3.3 V, radical production was estimated to be 10.3, 4.0 and 2.5×10^{13} radicals per puff (calculated from 40 cumulative puffs) for eliquids 1, 2 and 3, respectively. These values are 100- to 1000-fold lower than those measured previously in mainstream smoke from conventional cigarettes.[4, 5] A direct comparison is difficult since puff profiles differ greatly between the two products, with EC users tending to take larger, longer puffs with a slower flow rate than conventional cigarette smokers.[16, 19] Extrapolating from the results, an EC user taking 200 puff per day[20] or vaping 25 times per day (from data in Kosmider et al[16]) would result in 2×10^{15} radials per day. Free radical exposure from air pollution (PM2.5) has been estimated to be about 2×10^{14} per day.[21] This would indicate that exposure from E-cig use can be 10-fold greater than from air pollution. When aerosols were collected in the absence of PBN (i.e., benzene only) and subjected to EPR in order to determine the presence of less-reactive, long-lived radials, no EPR signals were observed (data not shown). This finding suggests that detectible levels of stable radicals were not present in the e-liquid aerosols.

Most eliquids contain the solvents propylene glycol (PG) and/or glycerol, with nicotine and flavorings at levels that vary considerably.[22–24] We examined if these EC solvents, themselves, may be a source of free radicals in EC aerosols. In aerosols generated from both propylene glycol and glycerol, distinct PBN radical adducts were observed (Figure 2). The EPR profiles differed between the two solvents, suggesting two discrete types of radicals. At a potential of 3.3 V, radical production was estimated to be 3.9×10^{13} and 2.2×10^{13} radicals per puff for PG and glycerol, respectively. No EPR spectra were observed in the absence of PBN suggesting the absence of long-lived radicals. It is not known to what extent radicals generated from these solvents correspond to the radical profiles of the eliquids tested.

The EC cartomizer chamber contains absorbent and wicking materials that deliver eliquid to the heating element. “Dry puffing” can occur where the wick is not sufficiently supplied with eliquid prior to puff initiation as a result of an empty cartomizer or overheating of the coil in the process of normal usage. When dry puffing conditions were simulated using an empty cartomizer PBN spin adducts were observed suggesting the presence of trapped radicals (Figure 3). At a potential of 3.3 V, radical production was estimated to be 10.2×10^{13} radicals per puff. These radicals were not observed when the heating element was severed, battery was turned off, or absorbent and wicking materials were removed from the cartomizer prior to the experiment. These findings suggest the production of radicals from the heating/burning of the dry wicking material by the heating element.

There is limited data available on the nature of thermal degradation products and the toxicity of inhaled aerosols of compounds present in EC. This study adds reactive free radicals to the list of potentially toxic products which have been found in EC aerosols. As described above, damage from these radicals has been implicated as an important causal factor in a variety of tobacco related diseases and disorders. However, there is currently insufficient data to determine the potential harm from exposure to EC-generated radicals. Additional data on the levels of exposure and the types of radicals would be required. Since the overall levels of radicals are significantly lower than those observed in conventional cigarette smoke, it might be expected that the degree of damage might be less, but this depends on the identity and reactivity of the specific radicals produced.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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ABBREVIATIONS

EC	electronic cigarettes
EPR	electron paramagnetic resonance
PBN	phenyl-N-tert-butylnitrone
ROS	reactive oxygen species
RNS	reactive nitrogen species
COPD	chronic obstructive pulmonary disease

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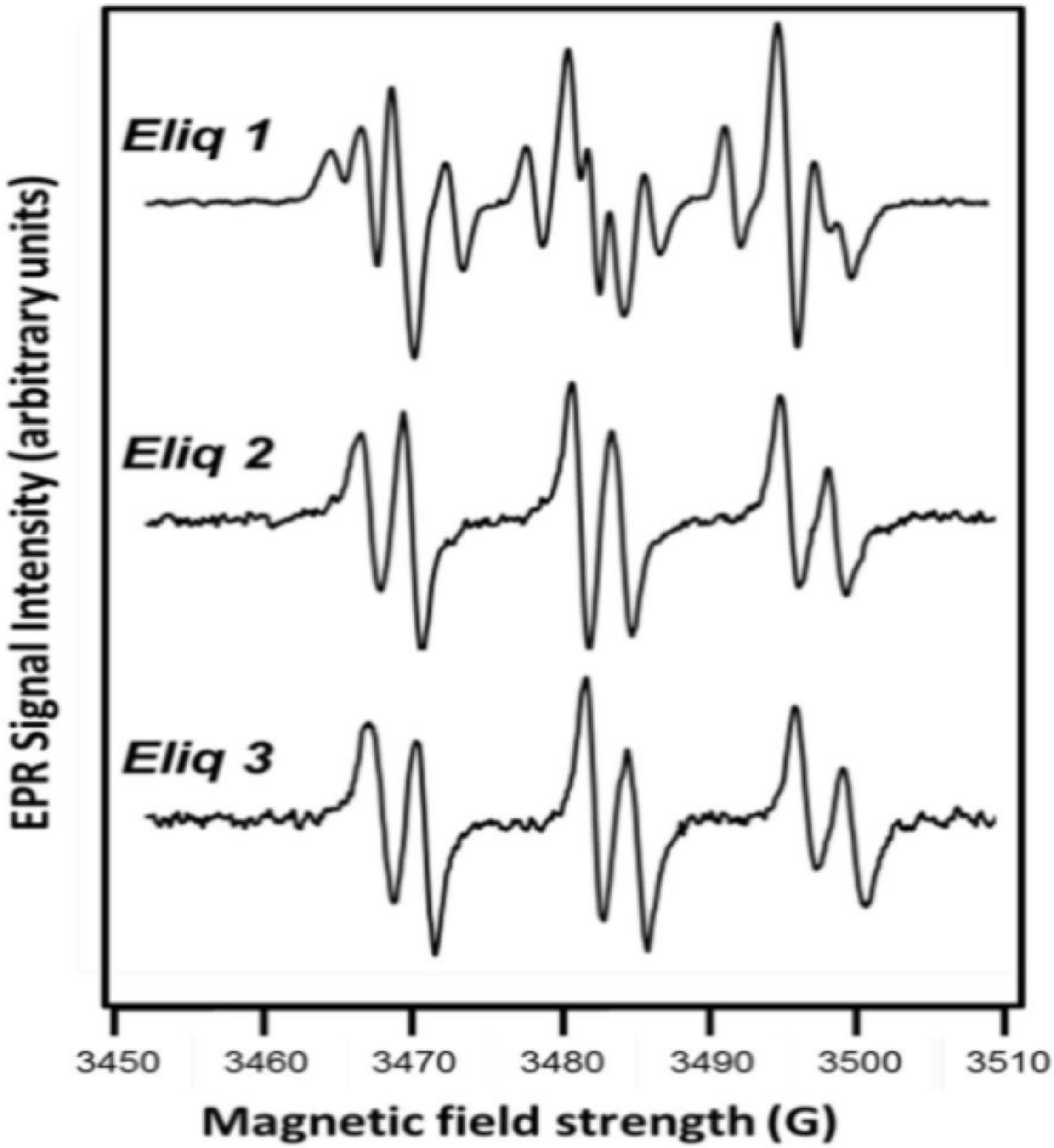

Figure 1.

Highly reactive free radicals in e-cigarette (EC) aerosols. Representative EPR spectra obtained from commercially available eliquids at 3.3 V.

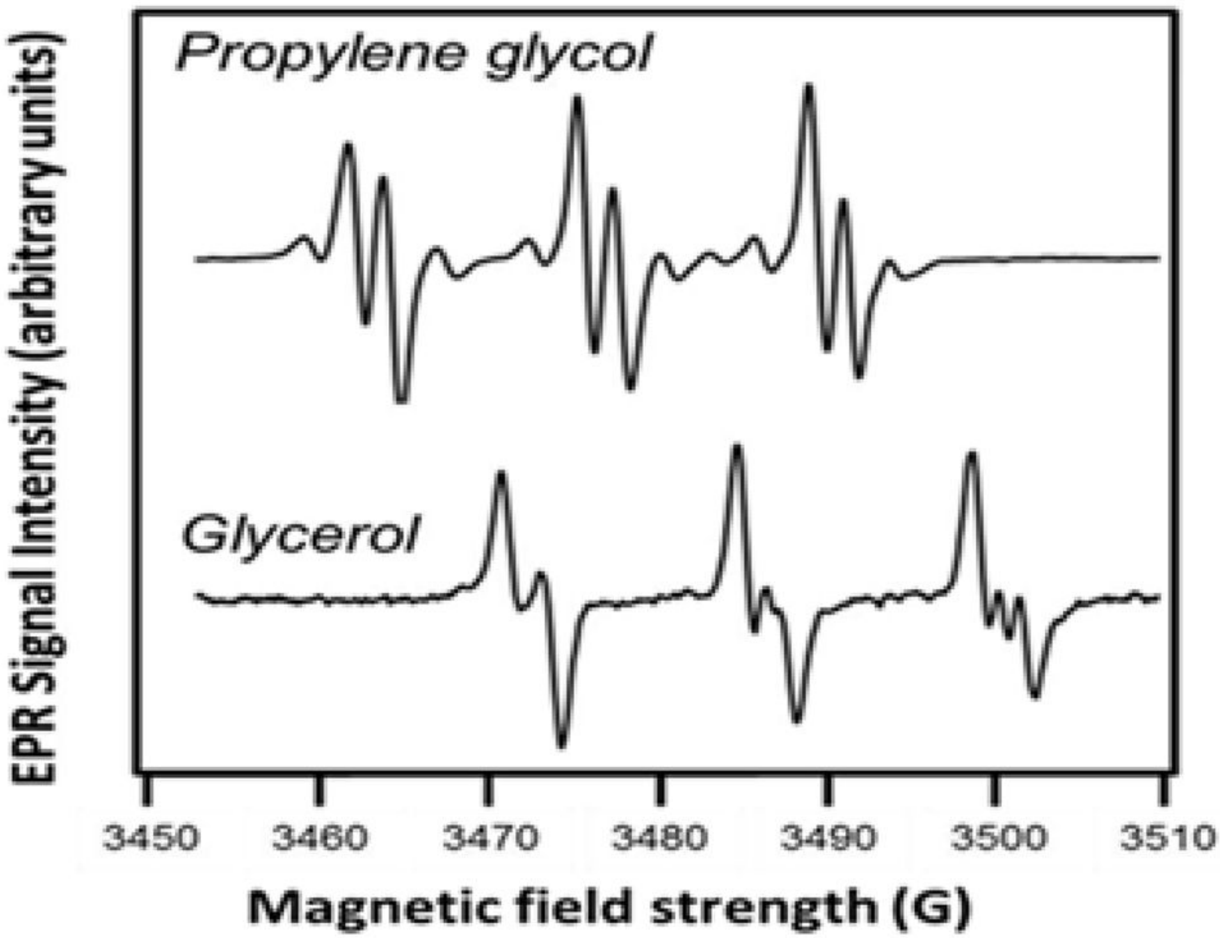

Figure 2.
Highly reactive free radicals obtained from e-cigarettes using eliquid solvents. Representative EPR spectra from propylene glycol or glycerin at 3.3 V.

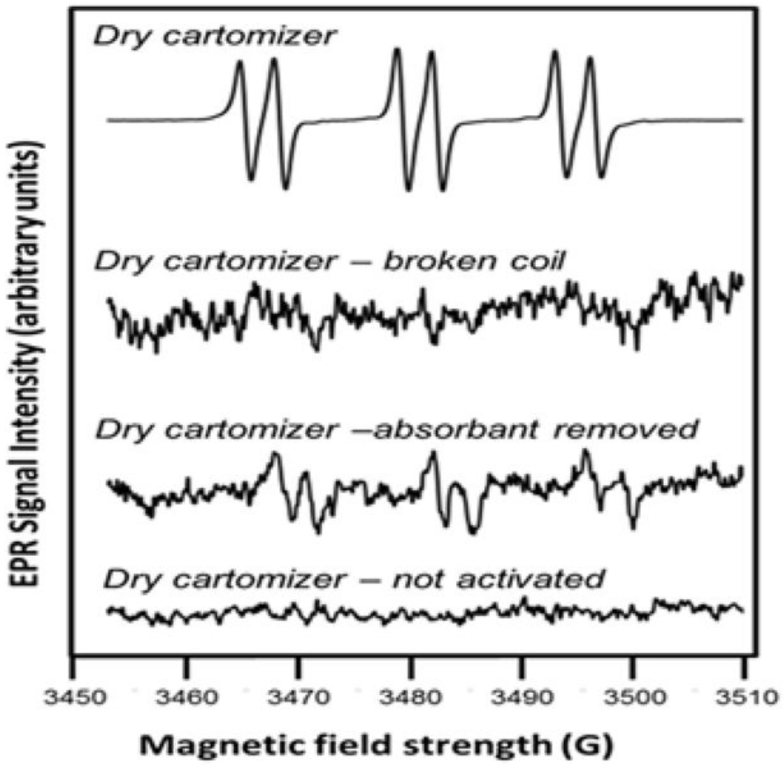

Figure 3.

Free radicals derived from from "dry puffing". Representative EPR spectra obtained under "dry puffing" conditions with an intact cartomizer or with cartomizers that had the heating mechanism disabled, absorbent material removed or not activated.