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Chemical and Physiological Interactions Between E-Liquid Constituents: Cause for Concern?

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Chemical and physiological interactions between e-liquid constituents: cause for concern?

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ABSTRACT

Studies of Electronic Nicotine Delivery Systems (ENDS) toxicity have largely focused on individual components such as flavour additives, base e-liquid ingredients (propylene glycol, glycerol), device characteristics (eg, model, components, wattage), use behaviour, etc. However, vaping involves inhalation of chemical mixtures and interactions between compounds can occur that can lead to different toxicities than toxicity of the individual components. Methods based on the additive toxicity of individual chemical components to estimate the health risks of complex mixtures can result in the overestimation or underestimation of exposure risks, since interactions between components are under-investigated. In the case of ENDS, the potential of elevated toxicity resulting from chemical reactions and interactions is enhanced due to high operating temperatures and the metallic surface of the heating element. With the recent availability of a wide range of e-liquid constituents and popularity of do-it-yourself creation of e-liquid mixtures, the need to understand chemical and physiological impacts of chemical combinations in ENDS e-liquids and aerosols is immediate. There is a significant current knowledge gap concerning how specific combinations of ENDS chemical ingredients result in synergistic or antagonistic interactions. This commentary aims to review the current understanding of chemical reactions between e-liquid components, interactions between additives, chemical reactions that occur during vaping and aerosol properties and biomolecular interactions, all of which may impact physiological health.

WHAT THIS PAPER ADDS

⇒ Electronic Nicotine Delivery Systems (ENDS) usage results in exposure to complex chemical mixtures.
⇒ ENDS risk assessments for regulatory purposes mainly rely on assessments of individual chemicals or of composite commercial mixtures of e-liquids and/or their corresponding aerosols. There is a significant knowledge gap concerning the health risks from the combined exposure to multiple chemicals from ENDS usage.
⇒ This brief report highlights recent studies showing that specific combinations of compounds associated with ENDS can lead to altered chemical emission profiles or non-additive biological effects.

INTRODUCTION

The impact of Electronic Nicotine Delivery Systems (ENDS) toxicity on physiological health is an ongoing concern and a better understanding is needed to guide research themes and regulations. The toxicity of ENDS depends on factors including vaping patterns of an individual, device design and operation, power and resulting heat output, as well as the chemical makeup of the refill liquid ('e-liquid').[1] Vaping involves aerosolising mixtures of nicotine, solvents, flavorants and various other additives. As of 2017, there were approximately 15 500 available e-liquid formulations[2] comprised of >200 flavorant chemicals.[3] The number of e-liquid formulations appears to have trended upwards to the present time since a 2017 study, which is the most thorough recent report to date. For example, a study published in 2021 reported close to 20 000 different commercial e-liquids with 250 flavour descriptors.[4] Furthermore, do-it-yourself (DIY) e-liquid preparation, wherein people who use ENDS create their own e-liquid mixtures, has become increasingly popular, in part as a reaction to an enforcement policy of flavoured cartridge-based ENDS.[5 6] The popularity of mixing e-liquid flavours in the USA was reported in Wave 2 (2014–2015) of the Population Assessment of Tobacco and Health Study, a nationally representative study of youth (12–17 years) and adults (18+ years), where 38.7% youth and 21.8% adults who used ENDS in the past 30 days reported using more than one flavour in their e-liquid.[7]

There has been extensive effort in the ENDS field towards the analysis of commercial e-liquid and aerosol chemical components. Gas chromatography and liquid chromatography, incorporating a variety of detection systems, particularly mass spectrometry, are the major analytical methods used to date.[1] However, a wide variety of specific sampling and analytical techniques have been developed for ENDS research[1] to address the challenging complexity and dynamics of the ENDS product landscape.[8]

ENDS risk assessments for regulatory purposes largely rely on assessments of individual chemicals or of composite commercial mixtures of e-liquids and/or their corresponding aerosols. There is a significant knowledge gap concerning the health risks from the combined exposure to multiple chemicals from ENDS usage. For example, chemical studies have shown that ENDS solvents and other molecular components can undergo incomplete combustion (thermal oxidation) reactions to a varying degree, depending on the device power, e-liquid makeup and puffing behaviour.[1] Indeed, e-cigarettes have also been described as 'chemical reactors' in literature.[9 10] Other reactions besides

incomplete combustion may also occur between specific e-liquid ingredients, such as flavorant acetal formation during storage, thereby further altering molecular structures and properties, which contributes to the complex chemical mixtures of ENDS aerosols.[11] Moreover, studies of the in vitro or in vivo biological effects of vaping have focused on either a specific e-liquid chemical ingredient, such as a single additive, or on exposure to commercial, composite e-liquids or aerosols thereof.[12] However, it is well-known in the pharmaceutical field that different drug molecules may produce non-additive synergistic or antagonistic physiological effects when used in combination.[13]

Herein, this paper aims to describe the current understanding of chemical and biological properties of specific chemical combinations found in ENDS e-liquids and aerosols. This issue is relatively under-investigated,[14 15] but is timely and significant considering the extensive availability of e-liquid formulations and the popularity of DIY vaping. The examples described below provide evidence that interactions between certain e-liquid ingredients can lead to unique, non-additive toxicological effects.

Chemical reactions between e-liquid components

A major difference between e-cigarettes and cigarettes is the fact that e-cigarette chemical formulations are liquids: it is well-known that organic molecules are typically more reactive in solution since dissolution enhances intermolecular interactions. For example, a striking illustration of changes to e-liquid compositions on storage was discovered by Erythropel *et al*. Their findings suggested that common aldehyde flavorants such as benzaldehyde, cinnamaldehyde, citral, ethylvanillin and vanillin readily react with the e-liquid solvents propylene glycol (PG) and glycerol (GL) to form substantial amounts of new compounds (acetals, conversions of 50%–95% depending on the aldehyde), within a timeframe of days to weeks, depending on the aldehyde in question. As expected, the resulting acetals exhibited differential physiological properties from either the flavorant or solvent components that form the respective acetal under the exclusion of a water molecule. PG-derived acetals diminished cellular energy metabolic functions, including basal respiration, ATP production and spare respiratory capacity.[16] PG/GL acetals induced cultured bronchial epithelial cell death at lower concentrations than the parent aldehydes.[17]

Interestingly, a study from the Netherlands (NL) did not detect benzaldehyde in a representative sample of 320 NL-marketed e-liquids.[18] However, benzaldehyde was reported by industry in the EU-CEG dataset to be present in 12.4% of >16 k NL-marketed e-liquids[19] It is quite possible that this inconsistency between reported data and analytical measurements is a result of benzaldehyde reactivity, such as with PG or GL to form acetals, or that the sample of commercial e-liquids did not contain benzaldehyde. Kerber *et al* later demonstrated that acetal formation could be inhibited by nicotine or water, but was promoted by the presence of benzoic acid.[20] Gschwend *et al* identified additional flavorant adducts, including acetals derived from ketones. They also found acetals in 32% (n=142) of the sampled commercial e-liquids.[21] Overall, it is concerning that several frequently used aldehyde flavorants are transformed to different species through reactions with the e-liquid solvents under storage conditions. This leads to new compounds that also exhibit unique toxicological properties, and occurs in potentially one-third, or more,[20] of all commercial products.

Toxicological interactions between flavorant/additive molecules

Muthumalage *et al* were the first to determine that mixtures of e-liquid components can exhibit synergistic interactions.[22] They discovered that flavorants trigger reactive oxygen species (ROS) production and an associated inflammatory response in monocytes. Higher production of ROS (and cytotoxicity) was found in a 10 flavorant e-liquid mixture than was anticipated from the sum of the contributions of each specific flavorant.[22] The authors concluded that mixing multiple e-liquid flavorants caused the greatest cytotoxicity, implying a bigger health risk when mixtures of flavorants are present ve a single flavorant.

Marescotti *et al* found that, of 28 flavorants studied, 2-acetylthiazole, allyl hexanoate, α-pinene, citronellol, guaiacol, linalool, methyl anthranilate, 3-methyl-2,4-nonanedione, 3-(methylthiol) propionaldehyde and phenethyl alcohol each resulted in human bronchial epithelial cell cytotoxicity.[23 24] When mixtures were investigated, cytotoxic properties deviated from the cytotoxicity observed using one specific flavorant. Citronellol had the greatest impact on mixture toxicity, whereas other chemicals resulted in synergistic effects.[23]

A study by Baldovinos *et al* analysed the cytotoxic effects of individual compounds and binary mixtures of a representative terpene (*R*-limonene) and an additive (triethyl citrate) on human lung cell models.[25] Data were analysed to determine the effects of 97:3 and 80:20% v/v (triethyl citrate/limonene) binary mixtures on BEAS-2B and A549 cells. LC_{50} values and isobolograms were used to assess toxicity and chemical interactions. The data showed that limonene were more cytotoxic than triethyl citrate. Isobolographic analyses confirmed that the mixtures resulted in an antagonistic chemical interaction (ie, reduced toxicity). Further testing of different ratios of binary mixtures is needed for chemical interaction screening to inform safety assessments.

Larcombe *et al* investigated potential synergistic toxicity between nicotine and e-liquid solvents.[26] Using a mouse model, they showed that inhalation of 0/100% PG/GL aerosols, with or without 12 mg/mL of nicotine, impaired lung function (airway resistance, tissue damping and elastance) in a more pronounced manner than similar ENDS aerosols composed of 100/0% PG/GL. In this study, the presence of nicotine did not significantly impact the altered lung physiological responses, indicating that the inhalation of ENDS aerosols can impair lung function independently of nicotine content. Although an interaction between nicotine and e-liquid solvents leading to impaired lung function was not observed, this study revealed that ENDS solvents alone can induce adverse pulmonary effects in vivo.[26] However, molecular interactions between e-liquid solvents and nicotine impact nicotine exposure. For example, e-liquids composed of >70% PG have been found to aerosolise faster and lead to greater nicotine aerosol concentrations than GL-rich e-liquids.[27] This was further confirmed in people who used ENDS wherein PG-rich e-liquids was associated with increased nicotine delivery.[28 29]

Taken together, these in vitro and in vivo studies show toxicological interactions following exposures to e-liquid constituents, since non-additive toxicological effects of individual e-liquid components were noted. Despite these observations, no mechanisms or chemical reactions/interactions underlying these effects were evaluated. Thus, the chemical interactions between the e-liquid constituents leading to synergetic toxicological effects are still mainly unknown. A recent study by Pappas *et al*,[30] however, showed that interaction between acids in nicotine salt with coil metals resulted in enhanced transfer of metal oxide, highlighting a mechanism by which e-liquid constituents and

ENDS design characteristics interact, and by this mean, may lead to increased toxicity.

Chemical reactions that occur during vaping

Molecular changes can be promoted during vaping due to catalytic reactions on the metal surface of ENDS heating filaments. Saliba *et al*[31] reported that the metal wires (eg, the iron/chromium/aluminium alloy Kanthal, stainless steel and the nickel/chromium alloy black nichrome) promoted the breakdown of PG to toxic carbonyls such as formaldehyde, methyl glyoxal and acetaldehyde, at temperatures <250°C, and as low as <80°C.[31] Importantly, this is further evident that dry coils and overheating are not the sole reasons[32] for elevated carbonyl toxicant emissions in vaped aerosols.

Formaldehyde derived from partial combustion of PG and GL during vaping has been shown to react with PG and GL to form new formaldehyde derivatives (hemiacetals).[33] Unlike gaseous formaldehyde, which is water soluble and deposits largely in the upper respiratory tract, the hemiacetals are less water soluble and preferentially partition into the aerosol particulate phase. They can thus be delivered more deeply into the airways/lungs than gaseous formaldehyde, which is concerning.[34]

CONCLUSION

The examples described above embody strong evidence that interactions between certain e-liquid ingredients can lead to chemical reactions as well as cause unique, non-additive toxicological effects. Awareness and understanding of the health-related impact of specific combinations of ingredients is thus a necessary and important step to better understand the complex mixtures that e-cigarette liquids and importantly, the aerosols that users are actually exposed to, are. In turn, regulators and researchers should build on the existing although limited knowledge of the toxicity of mixtures as one important tool of many to better understand the toxicity of e-cigarette aerosol mixtures if the goal is to offer safer, truly lower risk alternatives to cigarettes, and to effectively protect public health in light of thousands of existing flavour combinations.

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