National Institute for Occupational Safety and Health
NIOSH

Comments to FDA

Comments of the National Institute for Occupational Safety and Health to the Food and Drug Administration (FDA) in response to *Establishment of a Public Docket; Electronic Cigarettes and the Public Health Workshop*

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The National Institute for Occupational Safety and Health (NIOSH) thanks the Food and Drug Administration (FDA) for the opportunity to submit comments to the FDA docket established for submission of written comments regarding three FDA workshops. The establishment of the public docket was published in the *Federal Register* (FR) on December 2, 2014 [79 FR 71437]. Our comments relate mainly to toxicological considerations of flavorings and health effects in users.

Questions (italicized) are copied from the FDA Tobacco Products News & Events page as of February 20, 2015: http://www.fda.gov/TobaccoProducts/NewsEvents/ucm428317.htm.

Toxicological considerations

13. What are the toxicological concerns associated with long-term inhalation of aerosols containing propylene glycol, glycerin and flavorings?

The inhalation toxicity of most flavorings is a major concern that has not been investigated. In addition, a combination of volatility and reactivity is believed to contribute to the toxicity of some flavorings [FEMA 2012; Hubbs et al. 2012]. Volatility is a concern because a volatile chemical can become a vapor and reach the respiratory tract. E-cigarettes produce a complex mixture that is inhaled, and risk assessment of complex mixtures is difficult [Cassee et al. 1998]. Since e-cigarettes are intended for inhalation, volatility is a likely characteristic of flavorings used in e-cigarettes. Many volatile flavorings in e-cigarettes have uninvestigated inhalation toxicity; current data are not sufficient to accurately predict the risk from the combined effects of many flavorings. In addition, studies have observed a potentially disabling and life-threatening flavorings-related lung disease in workers who make or use flavorings [California Department of Public Health 2012; Kanwal et al. 2006; Kreiss 2012; Kreiss et al. 2012; NIOSH 2014]. The butter flavoring chemical, diacetyl, is associated with flavorings-related lung disease [Kreiss et al. 2002; NIOSH 2011]. Diacetyl and related flavoring chemicals, such as 2,3 pentanedione, can be hazardous to inhale, even though they have long been considered safe to eat. Consistent with their effect in humans, studies in rodents demonstrate that these compounds are toxic to the lining of airways, affect sensory nerves, and alter lung function [Goravanahally et al. 2013; Hubbs et al. 2012; Hubbs et al. 2008; Larsen et al. 2009; Morgan et al. 2008; Morgan et al. 2012; Palmer et al. 2011; Zaccone et al. 2013]. Therefore, safety data from consumption of flavorings does not necessarily translate into safety when inhaled.

Based upon a model of diacetyl vapor absorption in humans and rats, diacetyl inhaled through the human mouth was predicted to increase the dose to the deep lung when compared with inhalation through the nose. This finding is particularly relevant to exposures during vaping [Gloede et al. 2011]. Toxicity depends upon dose and the deep lung is the most affected tissue in workers with flavorings-related lung disease [Akpinar-Elci et al. 2004; CDC 2013; Morris and Hubbs 2009].

Regarding the combination of flavorings with propylene glycol and glycerin in aerosols, specific concerns exist for lung and cardiovascular function: 1) propylene glycol or glycerin alone may elicit pathophysiological and/or pathological changes in lung function; and 2) interactions between the effects of the individual agents may heighten toxicity. Thus, it is very important to discern whether any ingredients in vapor potentiate the pharmacological effects of nicotine, a

potent drug that affects many organs [Schroeder and Hoffman 2014]. A recent study indicated that nicotine did not modify the cytotoxic or oxygen stress-initiating effects of vapor from e-cigarettes when tested using human cultured airway epithelial cells [Scheffler et al. 2015]. The physiological effects of the vapors with and without nicotine co-exposure were not examined in that study.

14. What is known about the toxicities of inhaled flavorings? Are some inhaled flavorings more toxic than others?

To date, NIOSH research on flavoring chemicals has found that two α-dicarbonyl flavorings, diacetyl and 2,3-pentanedione, each damage the airway lining [Hubbs et al. 2012; Hubbs et al. 2008; Morgan et al. 2008; Morgan et al. 2012; Palmer et al. 2011]. These compounds are present in aerosols from many e-cigarettes and traditional cigarettes [Farsalinos et al. 2015a,b; Hubbs et al. 2015]. In addition, lung disease in multiple workplaces that make or use flavorings was associated with exposure to diacetyl, which is considered to be a safe component of food [Kreiss 2007; Kreiss et al. 2002]. These findings indicate that lung disease can occur in workers exposed to flavorings long believed to be safe components of food when eaten.

Direct comparisons of the potential inhalation toxicity of various flavorings are rare. However, recent publicly available data on the short term (13-week) toxicity of acetoin (online at http://tools.niehs.nih.gov/cebs3/ntpViews/?investigationNumber=002-01593-0000-0000-0), can be compared with the publicly available data on the short term (13-week) toxicity of diacetyl (http://tools.niehs.nih.gov/cebs3/ntpViews/?investigationNumber=002-01180-0000-0000-2) and published data. Acetoin is a flavoring found in many workplaces that use diacetyl and has a similar chemical structure, with an alpha-hydroxyketone in acetoin replacing the reactive alpha-diketone of diacetyl. In the 13-week inhalation studies, significant changes were seen in the respiratory tract of rodents inhaling 25 parts per million (ppm) diacetyl but not in the respiratory tract of rodents inhaling 800 ppm acetoin. The data indicate strongly that some inhaled flavorings are more toxic than others.

15. What strategies can be used to evaluate the potential toxicity of inhaled flavorings in humans?

While the greatest toxicity will occur in the lungs, the lungs are not an insulated compartment and other organs may be affected by inhaled agents. Inhaled agents can cause systemic effects.

The potential toxicity of inhaled flavorings on important lung functions can be assessed by in vivo and in vitro models. In vivo models include measurement of pulmonary function and pathological assessment of damage to the upper and lower airways (i.e., lining of nasal passages, large conducting airways of the lung, small bronchioles, and alveoli). Assessment of cellular and acellular samples of lavage fluid provides indices of acute inflammatory responses. Long-term inhalation toxicity testing is needed to evaluate chronic changes, including potential carcinogenicity.

In vitro studies of tissues removed from exposed animals, as well as examination of the effects of flavorings administered to isolated tissues from unexposed animals, would reveal: (1) whether

the ability of airway smooth muscle to contract and relax is changed; (2) whether ion transport by epithelium (which maintains lung fluid balance), or its pro-inflammatory function, is compromised; and (3) whether control of breathing is impacted by damage to nerves innervating the lung. Short-term in vitro testing can also indicate cytotoxicity and reactivity with biologic molecules including RNA, DNA, and protein. Important additional tests include mutagenicity and cell transformation which can be early predictors of potential carcinogenicity.

For workers, NIOSH recommends that substituting one flavoring for another should only be used for hazard control "after carefully evaluating potential substitutes for toxicity" [NIOSH 2015].

16. What strategies can be used to demonstrate that an individual flavor ingredient additive does not increase the inherent toxicity of the e-liquid and aerosol?

The strategies noted above would help predict toxicity. The ability to enhance toxicity would generally require inhalation toxicity studies of toxicity potentiation.

Health Effects in Users

1. What are the known short and long-term health effects of e-cigarettes in experienced users? What are potential other short and long-term health effects of e-cigarettes in users that should be evaluated?

Insufficient data are available to accurately predict the risk associated with use of e-cigarettes or secondhand exposure to the particulate aerosols and gases they emit. Nonetheless, the FDA and others have noted that e-cigarette cartridges and aerosols may contain harmful chemicals and substances such as diethylene glycol, nicotine, formaldehyde, acetaldehyde, toxic metals, and others [FDA 2014; Kosmider et al. 2014; Jensen et al. 2015]. In addition, several organizations have expressed concern about potential long-term health effects of using or secondhand exposure to electronic nicotine delivery systems (ENDS), such as e-cigarettes, while recognizing that current data are insufficient to quantify the risks [Wagener et al. 2012; BMA 2013; Kamerow 2013; AIHA 2014; Bhatnagar et al. 2014; Drummond and Upson 2014; Schraufnagel et al. 2014]. Thus, evaluating potential long-term health effects of ENDS use is an important research priority [Andrade and Hastings 2013; AIHA 2014].

Preliminary, limited reports are available about adverse short-term effects, such as significantly increased airways resistance [Gennimata et al. 2012] and respiratory irritation and cough, particularly among individuals with asthma [Tsikrika et al. 2014].

2. What are the potential short and long-term health effects of inhaling humectants (e.g., propylene glycol, glycerin), flavorings and other e-liquid additives?

A recent review of the safety of ENDS use concluded that more research is needed to determine risks from components of the liquids used in ENDS, including flavoring components in particular "because the effects of inhaling flavoring substances approved for food use are largely unknown" [Farsalinos and Polosa 2014]. NIOSH research of occupational exposure to flavoring chemicals has shown that some flavorings intended for ingestion, such as diacetyl and related

chemicals, can cause serious lung disease when inhaled. The best known type of lung disease caused by diacetyl is obliterative (constrictive) bronchiolitis, but it is possible that exposure also causes other types of lung disease [NIOSH 2004, 2011; CDC 2013]. Thus, flavorings known to be safe for ingestion cannot be assumed to be safe for inhalation without specific testing for toxicity via the inhaled route.

The American Industrial Hygiene Association (AIHA) recently published a white paper addressing specific constituents of e-cigarettes [AIHA 2014]. They noted that (1) glycerin heating or pyrolysis could result in the formation of acrolein, formaldehyde, and acetaldehyde in vapor emissions; (2) exposures to propylene glycol-containing theatrical fogs may contribute to acute and chronic diseases and health issues, including asthma, wheezing, chest tightness, decreased lung function, respiratory irritation, and airway obstruction; and (3) diethylene glycol, a potential impurity of propylene glycol, is a respiratory irritant that can cause serious systemic toxicity if ingested in sufficient quantities.

3. What strategies can be used to evaluate the short and long-term health effects of e-cigarettes in users?

Studies to evaluate the basic toxicology of ENDS emissions and their components would be useful and have been discussed above. Studies of short- and long-term health effects in direct users and those with secondhand exposures would also be valuable. Regarding short-term effects, surveillance efforts can continue to track calls to poison centers via the National Poison Data System for poisonings related to e-cigarettes (e.g., number of nicotine poisoning events related to e-cigarettes) (http://www.cdc.gov/nceh/hsb/chemicals/ncrs.htm). Another short-term surveillance effort would be use of the National Electronic Injury Surveillance System to track acute injuries or illnesses related to use of e-cigarettes or exposure to their emissions that resulted in admissions to emergency departments (http://www.cpsc.gov/en/Research--Statistics/NEISS-Injury-Data/). Ongoing surveillance would help in tracking the population burden of ENDS use over time and could provide an opportunity to document any associations between personal use or secondhand exposure and long-term adverse health effects.

4. What biomarkers and clinical endpoints can be used to assess the impact of e-cigarettes on user health?

Many traditional markers used to assess respiratory health (e.g., respiratory symptoms evaluated by questionnaire, pulmonary function response, including airways hyper-reactivity, measures of airways inflammation, measures of respiratory tract permeability, measures of mucociliary function), cardiovascular health (e.g., heart rate variability, dysrhythmias, peripheral vascular function), and carcinogenesis (e.g., genetic changes in target cells such as airways epithelial cells) could be applied to evaluating the short- and long-term effects of exposures to e-cigarettes. Longitudinal spirometry and, in some cases, inspiratory and expiratory chest computed tomography would be useful in studies evaluating flavoring-related lung disease [NIOSH 2011]. Because the main purpose of ENDS use is nicotine delivery, measures such as cotinine concentrations in biological fluids of exposed individuals can be used as a biomarker of exposure [Ballbè et. al. 2014].

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