



# High nicotine exposure in rodents is unlikely to inform about its toxicity in humans

*To the Editor:*

We read with interest the research letter by REINIKOVAITE *et al.* [1], in which the authors reported same level of damage in the lungs of Sprague Dawley rats subjected to whole body chronic exposure of e-cigarette vapour emissions and cigarette smoke, as well as to subcutaneous injection of nicotine. Their conclusion is that e-cigarette use and long-time consumption of nicotine are just as toxic as tobacco cigarettes. We appreciate the authors' intention to address concerns related to the potential long-term health effects of e-cigarette use and nicotine exposure, but there are a number of methodological considerations that lessen the impact of their findings.

First, in spite of the authors' effort to establish a meaningful puff/puff comparison between e-cigarette vapour and cigarette smoke, rats weighting about 200 g were being exposed to 600 puffs of vapour or smoke in just over 4 h in small gas chambers of <1 m<sup>3</sup> volume. Humans weigh, on average, 400 times more than Sprague Dawley rats and yet they usually inhale the same 600 puffs in just over 3 days. Under these specific experimental conditions, animals' overexposure to aerosol mass and its contents is highly likely. This is not uncommon, as there is proliferation of toxicological studies that are often performed with high-dose exposure protocols that are not applicable to normal human consumption [2]. When calculating the dose to be used, particularly if this is based upon human data, allometric scaling should be used, to take into account the large differences in metabolic rate between animals of different body mass [3, 4]. Moreover, in the work of REINIKOVAITE *et al.* [1], rats were exposed daily for 4 h for a total of 5 weeks; such exposure protocol is designed to maximise harm for the induction of pathological status (*e.g.* emphysema or chronic obstructive pulmonary disease (COPD)), which is used to study the characteristics of the disease in animal models (inflammation, basic mechanisms, response to therapies, *etc.*) [5]. Also, we noted that the authors did not mention animal health during the study, and standard toxic endpoints detailed in Organisation for Economic Cooperation and Development test number 413 (organ weights, gross pathology, *etc.*) were not assessed [6].

Second, in trying to equate similar level of plasma nicotine of adult smokers in the much smaller animal model (weighing approximately 400 times less than humans), the authors end up injecting a highly toxic dose. In histopathological studies of rats, it has been found that subcutaneous administration of nicotine doses similar to those used by REINIKOVAITE *et al.* [1] can cause extensive tissue damage, not only in the lung, but also in other organs including the liver, kidneys and brain [7, 8]. Thus, the dose used in the study is toxic and far from being informative of the nicotine effects in humans under the normal condition of consumption. It would have been ideal to include a rat group exposed to e-cigarette without nicotine to reach more revealing conclusions. Moreover, given that it is well established that the protease/anti-protease ratio is the central component of our understanding of emphysema, it is incongruous to advance the alternative hypothesis that it is nicotine causing lung destruction in smokers. This hypothesis is also at variance with the epidemiological evidence; for example, biomass fuel combustion is a well-known causative factor for COPD not dependent on nicotine [9, 10].

Third, in stark contrast with the concerns raised by REINIKOVAITE *et al.* [1] that e-cigarettes are as toxic as tobacco cigarettes and can cause lung damage, there is emerging evidence from real-life surveys [11] and the prospective evaluation of lung function and high-resolution computed tomography of regular vapers



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**More realistic exposures protocols are required to provide reliable toxicological information about electronic cigarette use in the context of smoking harm reduction** <http://bit.ly/2YkZHWG>

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who have never smoked in their life [12], indicating that long term e-cigarette use is unlikely to raise significant lung health concerns. Moreover, despite their being millions of regular e-cigarette users worldwide, there has been no evidence of emerging respiratory disease outbreaks in recent years.

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# Lessons learned from animal models: adverse effects of electronic cigarettes

*From the author:*

We thank R. Emma and colleagues for their interest in our preclinical study to investigate the effects of e-cigarettes on the lung. Our study was initiated from clinical observations that children exposed to second-hand e-cigarette vapour at home and undergoing surgery have a longer recovery and healing time

than children from a non-smoking environment. For this reason, our animals underwent whole body (or so called second-hand smoke) exposure to e-cigarette vapour.

We must clarify that the whole body exposure is very different from direct inhalation and the number of puffs delivered into the 32-m<sup>3</sup> exposure chamber in experimental conditions were comparable to the use of electronic cigarettes and tobacco cigarettes by people.

The amount of nicotine used for injections was based on previous studies to produce stable plasma nicotine levels of approximately 25 ng·mL<sup>-1</sup>, which is comparable with plasma levels (in the 10–50 ng·mL<sup>-1</sup> range) of habitual smokers [1, 2]. In our study nicotine was injected as nicotine di-tartrate salt. Thus, the actual dose used was below high toxicity levels [2] and we believe that our data are highly informative of the nicotine effects in humans under normal conditions of consumption.

We closely monitored animal health during the 35-day exposure period and, as anticipated, animals exposed to cigarette smoke lost close to 15% of the body weight when compared to room air exposed controls. The same body weight loss was observed in animals exposed to e-cigarette vapour or subjected to nicotine injections. We documented the adverse effects of e-cigarette exposure by lung pathology [3] and by skin necrosis in a skin flap survival model *in vivo* [4]. In a skin flap survival model, we showed that exposure to e-cigarette vapour produced damaging effect on skin microcirculation and interfered with the healing process. Our findings suggest that e-cigarette exposure is harmful not only to the lung, but also to the skin microcirculation.

We believe that our study on the adverse effects of e-cigarettes merely touches on the tip of the iceberg. New emerging studies in animal models and cell cultures show that flavourings alone used in e-juices can trigger an inflammatory response in monocytes, mediated by ROS production, endothelial dysfunction, DNA damage and induction of apoptosis in endothelial and epithelial cells. These studies provide insights into potential pulmonary toxicity and tissue damage in e-cigarette users [5–10].

It is premature to draw longitudinal conclusions from a prospective 3.5-year study of non-smoking daily e-cigarette users [11]. More studies are urgently needed regarding the public health consequences of e-cigarettes; so far, the scientific body of evidence on the health effects of e-cigarettes is very limited [12, 13]. Alarming, e-cigarette use from 2017 to 2018 increased 78% among high school students (11.7% to 20.8%) and 48% among middle school students (3.3% to 4.9%) from 2017 to 2018 [14], and poisoning events leading to emergency room visits related to liquid nicotine in electronic nicotine delivery systems in young children have been on the rise [15].

We strongly believe that considering recent discoveries the regulatory policy recommendations for humans based on preclinical studies are highly valuable. Due to the much longer life span, humans experience toxic effects after decades of smoking, while preclinical animal models, due to a much shorter time scale, usually demonstrate the first warning signs of possible adverse effects of e-cigarette vapour to human health.

Our data show that e-cigarettes have a signature of harm in the lung that challenges the concept that switching from cigarettes to e-cigarettes is a healthier alternative.



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**Electronic cigarettes have a signature of harm in the lung that challenges the concept that switching from cigarettes to e-cigarettes is a healthier alternative** <http://bit.ly/2Z3AhdU>

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