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REGULATION AND NONCLINICAL SAFETY ASSESSMENT OF E-CIGARETTES

Zhang Haifei, Joinn (Suzhou) Safety Pharmacology Department

In China, as many as 1 million people die from smoking-related diseases every year and the smoking rate of adolescents has reached 18.6%[1]. New tobacco products such as e-cigarette is increasingly popular worldwide, and have become an emerging way of tobacco use among young people [2].

Herbert A Gilbert first raised the concept of e-cigarettes in 1963, turning liquid nicotine into steam with heat, imitating the feeling of smoking, but his idea did not come to fruition eventually. In 2003, Han Li, a Chinese pharmacist, put this concept into practice, and registered patents for his own electronic cigarettes in China, the United States, and the European Union, and became a veritable inventor and patent holder of electronic cigarettes[3]. Same year, Ecigarettes went on market and quickly sold more than 100 million yuan.

Mechanism of E-Cigarettes

Based on the smoke-generating mechanism, electronic cigarettes (e-cigarettes) can be divided into two types: heated-not-combusted e-cigarette and oil-nebulizing e-cigarette. The main mechanism of heated-not-combusted e-cigarette is low-temperature heating, which releases smoke by heating a new type of tobacco (similar to cigarette) that is inserted in the cigarette holder. As to oil-nebulizing e-cigarette, cigarette oil is electrically heated to nebulize and produce smoke. The battery in the cigarette holder is rechargeable and reusable, and the cigarette oil is refillable for continued use. Currently in China, e-cigarettes are mostly the oil-nebulizing type. Differentiated by the oil storage and guiding system, the oil-nebulizing e-cigarettes can be further divided into resistor oil-nebulizing e-cigarette and porous ceramic oil-nebulizing ecigarette. The resistor type uses a cotton or fiber wick to absorb and guide cigarette oil in the cartridge to the heating coil. When the coil is heated, smoke is produced. For the porous ceramic type, a heat resistor is used to guide and heat the cigarette oil. The ceramic resistor is powered with electricity to heat the cigarette oil to produce smoke. In order to produce better smoke taste, the porous ceramic nebulization core has evolved to the third generation thick film printing technology.

Harms of E-Cigarettes

Highly-concentrated e-cigarette aerosols will irritate the respiratory tract to varying degrees, and can trigger an inflammatory reaction leading to diseases such as pharyngitis, bronchitis, pneumonia and so on, especially for patients with asthma and other respiratory diseases [4]. Studies have shown that the aerosols released by e-cigarettes contain carbonyl compounds (formaldehyde, acetaldehyde, acrolein,

glyoxal), volatile compounds (propylene glycol, glycerol), tobacco-specific nitrosamines and polycyclic aromatic hydrocarbons, lead, cadmium, nickel and other heavy metals[5]. According to some tests, 40-60mg of nicotine can cause death in adults[6]. Nicotine is listed in China's 2015 "Catalogue of Highly Toxic Chemicals".

In 2019, New England Magazine reported for the first time 142 cases of e-cigarette-related lung diseases in Illinois and Wisconsin in the United States[7]. These cases were named "EVALI" (Ecigarette, or Vaping, Product Use-Associated Lung Injury). As of February 2020, the US CDC had received 2,668 EVALI case reports[8]. The patient's typical imaging findings were bilateral diffuse ground-glass lesions. Lipid-rich macrophages (lipid-laden macrophages) were common in patients' lung lavage fluid, and vitamin E acetate was found in lung lavage fluid of many patients[9]. There is no definitive conclusion regarding the cause of the disease. However, it is believed that the vitamin E acetate used in e-cigarettes containing THC is closely related to the outbreak of lung injury related to e-cigarettes or nebulized products. The US CDC recommends avoiding the use of e-cigarettes containing THC[10]. In February 2020, the FDA began to ban the sale of e-cigarette products with added flavors other than mint.

Market Regulation of E-Cigarettes

In China, e-cigarettes are neither medicines nor health products, medical devices, or tobacco, and therefore in a state of "three noes", which refers to "no product standards", "no quality supervision", and "no safety assessment". Only in a few cities are e-cigarettes regulated by tobacco control regulations.

In April 2014, the European Union issued a newly revised Tobacco Product Directive – 2014/40/EU which clearly included e-cigarettes in the scope of control. In 2016, the European Union issued (EU) 2016/586 as the technical standards of 2014/40/EU to provide technical support for e-cigarette liquid filling[11]. In addition, relevant standards should include, but are not limited to, e-cigarette production raw materials, nicotine concentration, heavy metal content, smoke liquid flavor and additives.

In July 2017, the U.S. Food and Drug Administration (FDA) announced PMTA (Premarket Tobacco Application) for the first time, and required that all e-cigarette brands and manufacturers submit applications before August 2022. PMTA requires that legal promotion of any new tobacco product after February 15, 2007 must be reviewed and approved by the FDA. The FDA needs to determine whether the product is beneficial to public health (taking smokers and non-smokers as a whole). Once determined eligible, the product is issued "Premarket Tobacco Product Marketing Order" Certificate. The PMTA review includes nine items: application qualifications, basic information, descriptive information, product samples, product labels, scientific research results, product attributes and production processes, in vivo toxicology studies, and PHCS (hazardous and potentially harmful ingredients) . E-cigarettes can only be sold in the name of tobacco products through PMTA, and must also pass RMTP (Modified Risk Tobacco Product) before they can be recognized as reduced-harm products. In 2019, the United States extended the PMTA application deadline to September 9, 2020. Rseynolds American Inc., Fontem US LLC, Altria Group Inc., JUUL Labs, etc. have already submitted PMTA applications. Chinese e-cigarette manufacturer, Bode Electronic Cigarette, formally submitted 9 PMTA applications to the FDA. IQOS, a heated-not-combusted e-cigarettes by Philip Morris Companies Inc., a global tobacco giant, passed the PMTA application in April 2019.

Toxicological Assessment Requirements of E-Cigarettes

In June 2019, the FDA issued guidelines for e-cigarette pre-marketing applications[12]. FDA's Center for Tobacco Products is responsible for review and approval of e-cigarettes, and is guided by APPH, Appropriate for the Protection of the Public Health). The FDA's guidelines provide principles of the nonclinical assessment of e-cigarettes: nonclinical research alone is not enough to support the marketing of tobacco products; the FDA also does not recommend long-term studies such as carcinogenic trials to support applications; applicants should compare the health risks of its own products with similar and different types of tobacco products; applicants should conduct a comprehensive literature search and collect relevant public toxicity data; applicants should analyze each component in great details. In terms of toxicological assessment, in vitro toxicity tests such as genotoxicity and cytotoxicity should be conducted; in vivo toxicological tests should be conducted to verify safety. Toxicological tests should be conducted on suitable animal models, and the dose design should be based on the potential human exposure level, with high dose simulating the maximum smoking exposure of human beings. In the experiment, a scientific basis for assessment should be provided for human exposure levels, which serves as the basis for comparison with other tobacco products in terms of ingredients, flavors, metals and other additives. The aerosol characteristics of each component, including particle size, should be described in the test. FDA encourages applicants to communicate with CTP as early as possible to discuss whether the experiment is appropriate and acceptable.

The guidelines for PMTA applications point the direction of toxicological tests, but did not set specific test requirements. This poses certain challenges for e-cigarette manufacturers to apply for PMTA. Applicants must have a comprehensive understanding of relevant literature and materials, especially in terms of toxicity of product components, and have a thorough understanding

of the PMTA toxicity assessment principles. In the actual assessment, it is difficult for CRO to design a 100% suitable nonclinical assessment strategy for the client due to limited understanding of product composition and toxicity data, as well as lack of experience communicating with CTP.

IQOS' nonclinical data is currently the only review data that can be used for reference. In terms of nonclinical toxicology, IQOS conducted 5 strains of Ames test and mouse lymphoma cell gene mutation test on the aerosol produced by tobacco and mint-flavored e-cigarettes and control cigarette 3R4F. The results of the control cigarette 3R4F were positive and mutagenic, and the results of the two IQOS products were negative. In addition, in the neutral red uptake test, the cytotoxicity of the aerosol produced by the IQOS e-cigarette was compared with that of the control cigarette 3R4F, which proved that in the in vitro test, the cytotoxicity of the IQOS product was significantly lower than that of the control cigarette. In the in vivo toxicity test, following the OECD413 guidelines, a 90-day inhalation toxicity test on tobacco-flavored and mint-flavored e-cigarettes was conducted. Three dose groups were created for the test product and the control product respectively, and a Sham group was also created. In the accompanying toxicology analysis, the urine metabolites of carboxyhemoglobin, nicotine and other aerosol components were tested. In the accompanying aerosol characteristics study, total particulate matter, CO, nicotine, formaldehyde, acetaldehyde, acrolein, and particle size were analyzed. In addition to the conventional toxicological endpoints, the animal's right lung was lavaged to determine the number of white blood cells in the lavage fluid. The lung tissue was sampled for RNA extraction, and the changes in genomic expression were tested and analyzed. Moreover, an 18-month inhalation carcinogenesis test on A/J mice was conducted. Three dose groups, a 3R4F control cigarette group, and an air control group were set up, with inhalation for 6 hours/day, 5 days a week. The results showed that the degree of inflammation and adaptive changes caused by IQOS was much lower than that of control cigarettes.

In addition to the conventional nonclinical toxicology studies, IQOS also conducted a series of in vitro and in vivo toxicology tests in terms of toxicity mechanisms and systemic toxicology for the application of RMTP. By integrating traditional toxicology results with multi-level biological information, especially transgenic, metabolomics, and proteomics technologies, and with the help of model software, IQOS analyzed the changes in the molecular level and the final pathological results after IQOS is given. Systemic toxicology has not yet been carried out in China.

The history of e-cigarettes is still very short, and toxicity data is relatively limited. The assessment of toxicity and risk control are the foundation of regulation and development of ecigarettes. The development of the e-cigarette industry should be conducive to public health. With increasing toxicity data, it can be foreseen that the perception of the toxicity of e-cigarettes will be more complete and fair. Based on current data, e-cigarettes is expected to gradually replace traditional tobacco in the future with its advantages of low toxicity and convenience.

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