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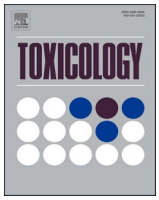

Research progress and prospect on the safety of heated tobacco products

Yi Liu, JiXue Cao, Jing Zhang, Guang Chen, ChengHao Luo *, Long Huang *

China Tobacco Hubei Industrial Co., Ltd, Wuhan 430040, China

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ABSTRACT

In recent years, Heated tobacco products (HTP) have gradually entered the market and become more and more popular with consumers because of their low risk (compared with traditional cigarette). With the increasing popularity and proportion of HTP in the international market, people pay more and more attention to the safety evaluation of HTP, but there is still a lack of systematic review of HTP safety research. In this review, the harmful components of HTP, multi-organ functional programming effects (including respiratory system, cardiovascular system, etc.), and mechanism of the effect generation (including oxidative stress, inflammatory response, etc.) were systematically reviewed, the safety effects of HTP and traditional cigarettes were compared in detail, and the shortcomings and future research directions in the field of HTP safety were discussed. In summary, this review conforms to the general trend of contemporary "tobacco and health", helps people to understand and evaluate HTP more systematically, and provides a strong theoretical support and literature basis for the tobacco industry to carry out HTP risk assessment and exposure improvement.

1. Introduction

As a special consumer product, tobacco has a history of more than 500 years since its birth. Tobacco originated in Central and South America, was first discovered by Indians, spread from the American continent to the rest of the world by Christopher Columbus in 1492, and spread from Europe to China around 1581 (Hatsukami and Carroll, 2020). Since the late 1970 s, the number of smokers in China has ranked first in the world, becoming a veritable tobacco country. Tobacco products include traditional cigarettes, snuff, chewing tobacco, etc. Traditional tobacco products have been proved to be harmful to human health and can induce diseases such as lung cancer, emphysema and atherosclerosis, so "smoking is harmful to health" has been widely recognized (Chan et al., 2022). However, due to the increase of social pressure, the acceleration of the life pace and the addictive nature of tobacco products, some people need to rely on tobacco products to relieve work pressure and relax their spirit, so it is urgent to develop "safe and effective" tobacco products (Perez-Milena et al., 2006).

Under the above context, in the past decade, tobacco companies in various countries have successfully developed Heated tobacco products (HTP), which can fully release the active substances in tobacco by heating tobacco instead of burning tobacco. Thus, the damage of tobacco products to human body can be reduced (Bar-Zeev et al., 2023), which not only meets people's demand for tobacco products, but also helps to reduce the risk of tobacco products to body health. In addition, the statistical survey analysis found that the age distribution of HTP-related publicity and HTP use was the highest among adults aged 18–24 years, followed by adults aged 25–44 years, 45–64 years, and then the elderly aged 65 years and above; the people who try to use HTP have a certain relationship with their educational background. Generally speaking, the higher the educational background, the greater the probability of trying HTP; Men are more likely to use HTP than women. Residents in urban areas are more likely to use HTP than those in rural areas (Grilo et al., 2021). With the increasing popularity of HTP market and the increasing investment in HTP, the safety research of researchers on HTP has also stepped into a new stage, and a new "discussion" has been born (Ratajczak, 2020). Therefore, this paper summarizes the latest research progress of HTP safety evaluation, which helps people to understand HTP deeply, and lays a certain theoretical and literature foundation for improving the HTP safety.

2. Basic overview of HTP

HTP is a new type of tobacco product that uses an external heat source (that is a smoking device, the heating temperature is generally below 350 °C) to heat the tobacco in the cigarette to create a flavor. HTP have the property of heating tobacco rather than burning it, and the tobacco is in a non-burning state during the smoke or interval (Bar-Zeev

* Corresponding authors.
E-mail addresses: luoch@hbtobacco.cn (C. Luo), huanglong@hbtobacco.cn (L. Huang).

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Table 1
Differences between HTP, E-cigarette and traditional cigarettes.

Basic characteristic	HTP	E-cigarettes	Traditional cigarette
Tobacco substrate	Most of the tobacco added atomizing agent	Vape juice	Mostly traditional tobacco
Tobacco source	Tobacco leaves, tobacco fragments, tobacco powder, etc	No	Main parts of tobacco
Taste	Various flavors (coffee, mint, etc.)	Various flavors (coffee, mint, etc.)	Mainly tobacco flavor
Cigarette length	Usually shorter, Generally 40–60 mm long	No ccigarettes	It is usually longer, with the most Common length being 84 mm
Smoking device	Needed	Needed	No needed
Side flow smoke	Basically no side flow smoke	Basically no side flow smoke	Exist no side flow smoke
Heating temperature	Usually < 350 ℃	Usually < 200 ℃	Usually > 650 ℃

HTP, Heated tobacco products.

et al., 2023). E-cigarettes, also called electronic cigarettes, e-vapor products, or vapes, are a popular smoke-free category of products without tobacco. These battery-powered devices vaporize a liquid solution, also known as an e-liquid, containing nicotine and/or flavors. In the past decade, the international traditional tobacco market has begun to shrink, and relevant statistics show that the sales volume of traditional tobacco in various countries has declined at a rate of more than 2% per year, and the global tobacco industry has entered a new period of seeking changes and breakthroughs (Brett et al., 2021). In 2022, global E-cigarette sales were 18.85 billion US dollars, and HTP sales were 32.38 billion US dollars. 2023 global E-cigarette sales of 19.34 billion US dollars, HTP sales of 38.30 billion US dollars, an increase of 2.6%, 18.3%. It shows that the market share of HTP increases more significantly than that of E-cigarettes.

HTP, E-cigarettes and traditional cigarettes have obvious differences in raw materials, materials and use methods, as shown in Table 1. For example, HTP heat real tobacco within a specific temperature range, using an electronic heat-control system to prevent it from burning. E-cigarettes do not use tobacco. Instead, they vaporize an e-liquid solution containing nicotine and flavors when a user draws on it. In recent years, HTP has successfully entered the market with its advantages of safety, hygiene and efficiency, and has been favored by consumers. Its representative products include IQOS (Philip Morris International), GLO (British American Tobacco), MOK&COO (China Tobacco), etc. (Duan et al., 2023). According to the survey, HTP's market competition is becoming more and more fierce, and sales continue to rise. Philip Morris dominates the international market, accounting for about 79% of the total HTP market. Global HTP sales are expected to reach US $68 billion by 2027, an increase of about 7 times compared to 2020 (Duan et al., 2023). It can be seen that HTP is the "new star" of the tobacco market. However, enterprises in various countries still need to deepen HTP research and development, including HTP equipment research and development, process optimization and so on. Among them, the supervision organization and consumers are more concerned about the toxicology research and safety evaluation of HTP, which is also one of the key points for HTP to further develop the market. Based on the comprehensive research around the world, this paper summarizes the research progress of HTP safety evaluation, and puts forward the corresponding shortcomings and future research directions, which will help tobacco companies in various countries to strengthen the safety research of HTP.

3. Harmful ingredients and classification of HTP

Mainstream smoke and side-flow smoke of tobacco products contain many harmful components, which is the main reason affecting the safety of tobacco products. Because HTP has basically no side-flow smoke, the release of harmful components and the impact on ambient air are significantly reduced compared with traditional cigarettes. Based on the detection and analysis results of HTP aerosol components and relevant key concerned components. This section summarizes the main harmful components of HTP products, and compares the contents of relevant harmful components in HTP aerosols with those in traditional cigarettes.

3.1. Main harmful components

By synthesizing various key concern lists and literature reports, and combining the characteristics of HTP, the main harmful components of HTP aerosol can be roughly summarized into Inhalable particulate matter, carbonyl compounds, tobacco specific nitrosamines (TSNAs), polycyclic aromatic hydrocarbons (PAHs), phenolic compounds, carbon monoxide (CO) and nitrogen oxides, NH3, hydrocyanic acid (HCN), volatile organic compounds (VOCs), heavy metals and other categories:

(1) Inhalable particulate matter: the toxic effect of inhalable particulate matter is related to its particle size and carrying charge, and the smaller the particle size, the easier it is to enter the lung; The more positive charge it carries, the more toxic it is. Studies found that traditional cigarette smoke particle size is greater than the HTP aerosols, release of HTP aerosol particle size small and easy to spread, the HTP aerosol particle size less than 1 microns in higher levels of particulate matter (HTP aerosol 44 mg/cig, traditional cigarette smoke 36 mg/cig) (Yu et al., 2022). According to relevant research reports, inhalable particles in traditional cigarette aerosols contain many components, such as nitrate, vinylpyridine, indene, guaiacol and so on. The inhalable particles in the aerosol of HTP mainly include glycerol, propylene glycol and so on. And studies have confirmed that the increased content of nicotine contributes to the absorption of these components, so the higher nicotine content of traditional cigarettes will cause inhalable particles to enter the human body more easily than HTP (Zhang et al., 2021). In addition, through computer simulation, Clement Kleinstreuer found that the entry of these inhalable particles into the body is affected by particle-particle interaction, ambient gas temperature, and ambient gas density (Kleinstreuer and Feng, 2013), in view of the fact that most of the particles in traditional cigarette smoke exist in solid form and are easy to polymerize, the particles in HTP aerosol mostly exist in gaseous phase and liquid form, and finally concluded that traditional cigarette inhalable particles have a greater impact on the body than HTP.

(2) Carbonyl compounds: Previous detections have reported that the content of aldehydes and other carbonyl compounds in traditional cigarette smoke is relatively high. Studies have shown that compared with normal population, HTP aerosol exposure can also lead to a significant increase in the inhalation amount of formaldehyde, acetaldehyde and other carbonyl compounds (Baaranet al. 2019). However, after testing, it was found that compared with traditional cigarettes, the proportion of carbonyl compounds (such as formaldehyde, etc.) in HTP aerosols was significantly reduced to 50%-70%, and the safety of aerosols was significantly improved (Bekki et al., 2017).

(3) TSNAs: During the process of TSNAs from filter tip to mainstream smoke, the retention rate of TSNAs in HTP aerosol is 25%, while the retention rate of TSNAs in traditional cigarette smoke is 10%. And the data indicate that the content of TSNAs in HTP aerosol is one-tenth of that in traditional cigarette smoke, which decreased significantly (Solomou et al., 2023).

(4) PAHs: Recent studies have found that in traditional cigarettes, the content of PAHs in tobacco leaves before burning is low, and the highest concentration of PAHs in tobacco butts after burning is 1449 ±113 ng (Nordlund, 2020). Compared with traditional cigarette smoke, the PAHs content in HTP aerosols decreased by about 20 times, but the

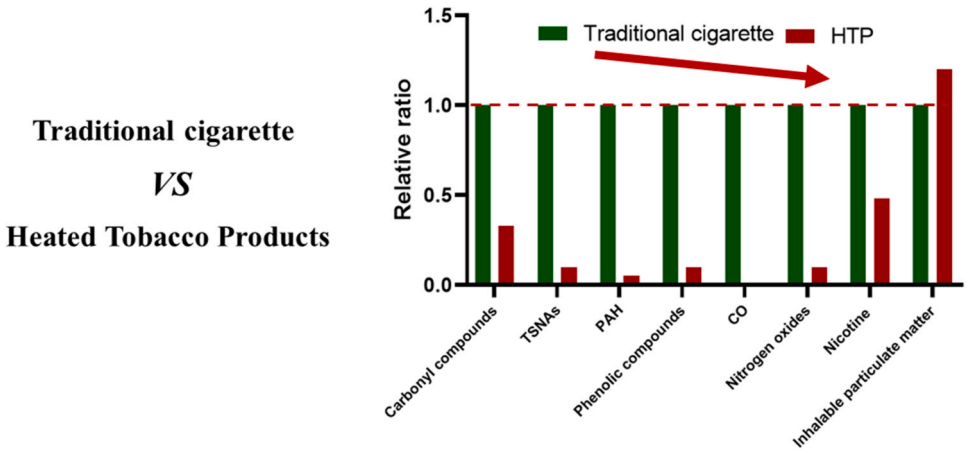

Fig. 1. Schematic diagram of the proportion about harmful components in HTP and traditional cigarettes. HTP, Heated tobacco products. TSNAs, nitrosamines; PAHs, polycyclic aromatic hydrocarbons.

PAHs concentration in HTP aerosols increased before and after HTP use (from 43.37 ng to 69.36 ng). In general, the content of PAHs in HTP aerosol is significantly lower than that in traditional cigarette smoke (Solomou et al., 2023).

(5) Phenolic compounds: Combined with multi-party detection data (including PMI), the content of phenolic compounds in HTP aerosol is significantly lower than that in traditional cigarette smoke (Uguna and Snape, 2022). Examples include catechol (14.3–14.7 μg/cig in HTP, 84.2–98.1 μg/cig in traditional cigarettes), phloroglycinol (0.016–0.055 μg/cig in HTP, 1.75–2.0 μg/cig in traditional cigarettes, phenol (1.3–1.4 μg/cig in HTP, 12.8–14.4 μg/cig in traditional cigarettes) (Nordlund et al., 2020).

(6) CO and nitrogen oxides: CO is produced by the reaction of carbon and oxygen, especially when the temperature is greater than 350 °C, CO is also one of the main compounds in the gas phase of the smoke, and a series of studies have shown that the concentration of CO in traditional cigarette smoke is high, which will have an adverse effect on the lung and heart. Recent studies have tested the CO concentration of HTP aerosol and traditional cigarette smoke and found (St Helen et al., 2017) that there is no significant difference in the CO conversion rate of HTP aerosol, but the CO concentration in HTP aerosol is about 100 times lower than that of traditional cigarette smoke. That is, the HTP is 0.44 ± 0.04 mg/cig and 33.0 ± 1.8 mg/cig for traditional cigarettes). In addition, nitrous oxide content is about 17.3 μg/cig in HTP aerosols and about 400 μg/cig in traditional cigarette smoke, which is also significantly reduced by about 90–95% (Bekki et al., 2017). This indicates that the concentration of CO and nitrogen oxides in HTP aerosol is significantly reduced, and its safety is significantly improved than that of traditional cigarettes.

In addition to the above major harmful components, the content of NH3, HCN, volatile organic compounds and other related substances in HTP aerosol is also lower than that in traditional cigarette smoke (Nordlund, 2020). In addition, nicotine is the main component of tobacco product aerosols, and it is also recognized as the main component of tobacco product effects. Based on the nicotine detection data, in tobacco filler (concentration per cigarette): it was 15.0 ± 0.1 mg/cig for traditional cigarette and 4.7 ± 0.1 mg/cig for HTP; in mainstream cigarette smoke: it was 1.9 ± 0.1 mg/cig for traditional cigarette and 1.1 ± 0.1 mg/cig for HTP. This indicates that the nicotine concentration of HTP is significantly lower than that of traditional cigarettes (Bekki et al., 2017). Most studies have shown that the nicotine content in HTP aerosols is reduced by 50–80% compared to traditional cigarettes (Upadhyay et al., 2023). However, considering the smoke bomb length and smoking time of HTP, the nicotine content in HTP aerosol and traditional cigarette smoke still needs further comparative calibration study and evaluation (Upadhyay et al., 2023). Based on the above studies, we found that the content of major harmful components in HTP aerosols is much lower than that in traditional cigarettes (see Fig. 1), which will contribute to the "effective transformation" of traditional cigarette consumption to HTP users and promote the development of tobacco health.

3.2. Other harmful ingredients

In addition to the above main harmful components, there are still some components in HTP aerosols that have not been identified or their toxic effects have not been clarified. For example, HTP usually contains a high content of glycerin and various flavors and other additives, the research confirms that aerosolised propylene glycol and glycerol produce mouth and throat irritation and dry cough (Callahan-Lyon et al. 2014), and some data shows that it may increase oxidative stress/inflammation and are likely to be involved in effecting to lung damage (Prasad and Bondy, 2022), but whether it can be produced or transformed into harmful ingredients under heating is unclear (Li et al. 2019). Compared with traditional cigarettes, test results showed that the content of acenaphthenene in HTP aerosols increased by about 3 times, but whether acenaphthenene is harmful to health is unclear (Basiaran et al.2019). In addition, some researchers have proposed that there are residues of metals and compounds in the filter rods and aluminum foil rolls used by HTP, including phthalates, chlorophenols, polychlorinated biphenyls, aluminum, etc. (Li et al., 2019), whether these substances will have an impact on human health after continuous heating is unknown. This also puts forward higher quality requirements for further development of safe and healthy HTP tobacco materials. At the same time, some researchers pointed out that if the heating device of HTP is not guaranteed to be clean in time, the residual particles in the heating device will be heated repeatedly, which is likely to lead to the release of new harmful substances (Bentley et al., 2020). Based on the above studies, we propose that the harmful ingredients and effects of HTP need to be further confirmed, including HTP aerosols, smoking device, etc., which will promote the development and safety of HTP.

4. Effects of HTP on multi-organ function

Although the damage effect of HTP on the body is significantly reduced compared with traditional cigarettes, its impact on multi-tissue function is still the focus of the tobacco companies. Based on the relevant literature reports, we comprehensively reviewed the effects of HTP on multi-organ function from the multi-system dimension such as respiratory system and cardiovascular system, including the effects on tissue/organ morphology and key functional gene expression.

4.1. Respiratory system effects

The respiratory system consists of the airways and lungs. As an important part of the respiratory system, lung is the main place of gas exchange in human body and the main target orga by the harmful components of smoke and aerosol (Confalonieri et al., 2017). Lung development goes through five stages: embryonic stage, pseudoglandular stage, tubular stage, cystic stage and alveolar stage, and lung function is coordinated by the interaction of epithelial tissue and mesenchymal tissue (Mullassery and Smith, 2015). Previous studies have confirmed that traditional cigarettes can cause lung dysfunction, lung cancer, emphysema and other diseases, indicating that traditional cigarettes have a strong toxic effect on the lung (Benowitz, 2009). In cellular research experiments on the effects of HTP on lung function, transcriptome enrichment analysis found that when lung epithelial cells were exposed to HTP aerosols, oxidative stress, DNA damage, lipid peroxidation and other signaling pathways changed, leading to apoptosis of lung epithelial cells (Bravo-Gutierrez et al., 2021). In animal studies of the effects of HTP on lung function, the researchers found that when female rats were exposed to HTP aerosols (6 hours a day for 90 days), an increase in lung weight, a significantly enhanced inflammatory response in the lungs, and impaired lung function were observed, lung function was significantly impaired. But when compared to traditional cigarettes, the negative effects of HTP on lung function are significantly reduced (Oviedo et al., 2016). In a clinical population study of the effects of HTP on lung function, when people without lung-related diseases switched from smoking traditional cigarettes to HTP smoking, a decrease in the number of white blood cells, an increase in the maximum lung inspirations per second, and a significant improvement in lung function were observed (Pataka et al., 2020). Similarly, when patients with chronic obstructive pulmonary disease switch from traditional cigarettes to HTP, significant improvements are observed in measures related to lung function, including maximum lung inspiratory volume and respiratory resistance (Pataka et al., 2020). The above studies have confirmed that the impact of HTP on lung function and the occurrence probability of lung-related diseases caused by HTP are significantly reduced compared with traditional cigarettes, which also provides a more adequate safety basis for the transformation of traditional cigarette consumers to HTP users.

4.2. Cardiovascular system effects

The cardiovascular system consists of the heart, arteries, capillaries and veins, which are responsible for supplying oxygen, nutrients and other functions to the body. In recent years, with the significant increase in the incidence of cardiovascular disease, whether tobacco products can cause cardiovascular disease has also attracted more attention. Whereas the U.S. Food and Drug Administration (FDA) has approved Philip Morris' IQOS products for the market, which indicates that HTP products provide assurance for cardiovascular health, However, further research is needed to address consumer concerns (Yaman et al., 2021). A population cohort study (including 22 HTP users with an average age of 33±5 years without cardiovascular disease) showed that HTP can increase heart rate, blood pressure, pulse and other indicators, and is prone to induce atherosclerosis (Ioakeimidis et al., 2020). In addition, blood Nox2,levels, a marker of oxidative stress that causes vascular endothelial dysfunction, were significantly higher when the subjects were exposed to traditional cigarettes and HTP, but the HTP had a much smaller effect on relevant indicators than traditional cigarettes. It is suggested that the effect of HTP on cardiovascular system is much less than that of traditional cigarettes (Nabavizadeh et al., 2018). Animal and cellular studies have shown that exposure to HTP aerosols can dilate blood vessels in rats. However, when mononuclear macrophages were exposed to HTP aerosols, oxidative stress-related indicators, including elevated intracellular reactive oxygen species levels and glutathione depletion, increased in a time and dose-dependent way (Wang et al., 2020). Recent studies have also confirmed that HTP may cause vasoconstriction through the sympathetic nerve, which raises blood pressure and increases heart rate. In addition, some literature suggests that HTP may also lead to atherosclerosis by affecting lipid metabolism (Biondi-Zoccai et al., 2019). Combined with the current research around the world, the negative impact of HTP on cardiovascular system function is less than that of traditional cigarettes, and the relevant animal and population studies are still needed for detailed statistical analysis.

4.3. Nervous system effects

The nervous system is the main system that regulates the body environmental balance in the body. Like traditional cigarettes, the effect of HTP on the nervous system is mainly mediated by nicotine, and it is addictive. Because nicotine is chemically similar to the endogenous neurotransmitter acetylcholine, it acts by binding to nicotinic acetylcholine receptors (nAChRs) (Gray et al., 2005). The effect of nicotine and acetylcholine in the body is basically the same, but the clearance rate of the two in the body is different. Nicotine does not act in the same way as endogenous acetylcholine. After endogenous acetylcholine is released from axon endings, it has a short survival time and is broken down by acetylcholinesterase within a few milliseconds, while nicotine is not broken down by acetylcholinesterase and can exist for a long time and exert long-term effects on nicotinic receptors (Dani, 2015). When nicotine binds to nAChR, sodium and potassium channels open and receptors are activated, which promotes the release of a variety of neurotransmitters (including dopamine, norepinephrine, acetylcholine, serotonin, beta-endorphins, and gamma-aminobutyric acid), producing pleasure, arousal, and excitement. And reduce anxiety and control emotional stability (Benowitz, 2010). After long-term use of HTP, brain nerves will undergo adaptive changes (including changes in nicotine-related synaptic connections, changes in the number of receptors, etc.). If you stop using HTP at this time, withdrawal effects will be induced, including irritability, anxiety, lack of concentration, hunger, and increased appetite (Benowitz, 2001). However, given that the nicotine content in HTP is significantly lower than in traditional cigarettes, the magnitude of the associated withdrawal effect is weaker than in traditional cigarettes. In addition to the above effects, HTP causes a conditioned reflex or "compensation effect" that forces people to consume nicotine as a reward effect, leading to a significant increase in desire for tobacco products (Barbeau et al., 2013). Therefore, the neural effect of HTP products is similar to that of traditional tobacco products (that is, addiction and withdrawal reaction), but the degree of addiction and withdrawal reaction of consumers is weaker than that of traditional cigarettes, which is beneficial to consumers' health.

4.4. Reproductive system effects

The reproductive system is the key to maintain the secondary sexual characteristics of the body and ensure fertility. The male reproductive system consists of testicles and vas deferens, which produce sperm and secrete androgens. The female reproductive system is composed of ovaries and fallopian tubes, which have the functions of ovum production and secreting estrogen (Sansone et al., 2022). Previous studies have systematically confirmed the reproductive toxicity of traditional cigarettes, which can inhibit male testosterone synthesis and spermatogenic function, reduce sexual desire, and have negative effects on female reproductive function, but the literature on reproductive toxicity of HTP is still limited. A recent study from Oita University in Japan (Yoshida et al., 2020) showed that when pregnant mice were exposed to HTP aerosols and traditional cigarette smoke (exposure on days 7 and 14 of pregnancy, each exposure was 55 mL and inhalation duration was 2 s, when the interval of inhalation was 30 min, the duration was 20 min), and the testicles of the offspring mice were obtained at 14 weeks after birth for detection. The results confirmed that the morphology of spermatogenic tubules, sperm count and serum testosterone levels were

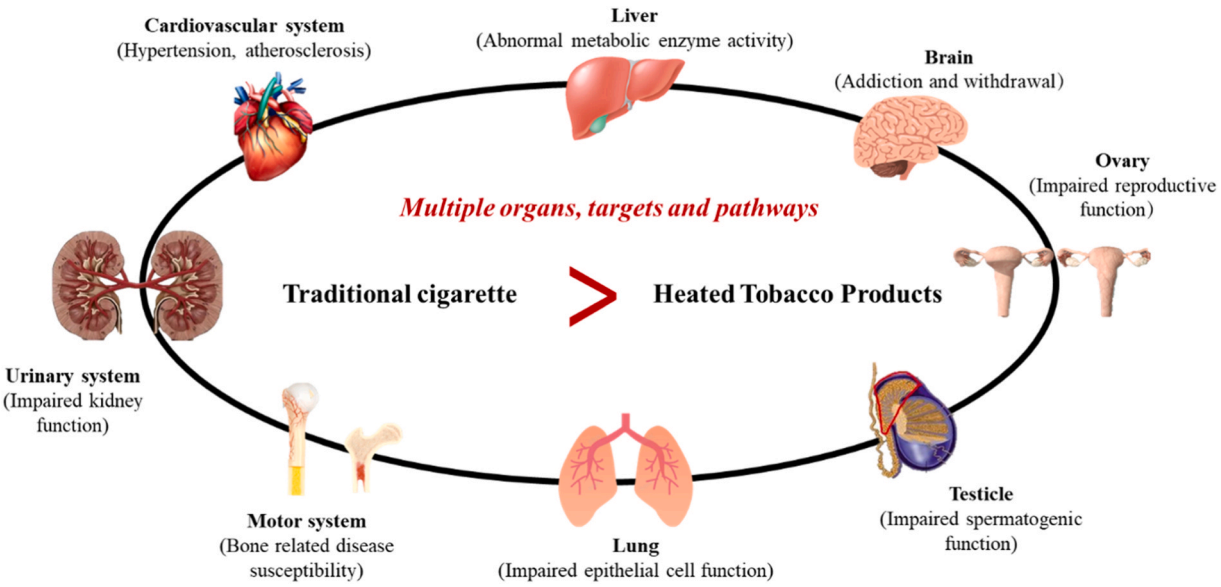

Fig. 2. Effects of HTP on multi-organ function. HTP, Heated tobacco products.

decreased in the prenatal HTP aerosol exposure group. However, the above pathological changes were not observed in the traditional cigarette exposure group. In addition, prenatal exposure to HTP aerosols delayed the sexual maturity in male offspring mice (Yoshida et al., 2020), so the research team proposed that HTP aerosols were more reproductive toxic to offspring than traditional cigarettes. This contradicts the current mainstream view that HTP aerosol is less toxic than traditional cigarette smoke. In addition, a population cohort study in Italy collected and analyzed information from normal and smoking women (including traditional cigarettes and HTP). The results showed that compared with the normal group, the blood levels of Anti-Mullerian hormone (AMH) were significantly reduced in the smoking group, the number of ovum collected was significantly reduced, the success rate of fertilization was lower, and the ovarian reserve and quality were decreased (Galanti et al., 2023). Unfortunately, this cohort study did not group HTP users with traditional cigarette users and therefore could not effectively compare the toxic effects of HTP and traditional cigarettes on the ovaries. In summary, HTP may have a negative effect on the function of human reproductive system, but is the change of reproductive function caused by HTP more powerful than that of traditional cigarettes? There is still controversy.

4.5. Other effects

In addition to the above effects, HTP also has some effects on other systems. For example, in the liver (van der Plas et al., 2020), where nicotine can be metabolized to cotinine, no significant changes in liver weight and coefficient were observed when rats were exposed to HTP aerosols (5 days/week for a total of four weeks, with a nicotine exposure of 113 μg/cig. At present, some studies have detected the nicotine level and cotinine level in the human body after exposure to traditional cigarettes or HTPs. The results show that the nicotine concentration in the urine of HTP users is 1200 ng/mg, and the concentration of cotinine is about 2000 ng/mg. In people who use traditional cigarettes, the urine nicotine concentration is 1600 ng/mg, and the concentration of cotinine is about 2900 ng/mg (Kawasaki et al., 2021). However, the level of local oxidative stress in the liver increased, the expression of antioxidant enzymes such as glutathione S-transferase decreased, the metabolic expression of uridine diphosphate glycoside transferase increased, and the expression of liver functional enzymes such as aspartate aminotransferase was abnormal. In addition, HTP aerosols have been shown to have an effect on the immune system (Heluany et al., 2022). When mice were exposed to HTP aerosols (twice a day for 1 hour each time, for 1 week), serum nicotine levels increased significantly, and the number of lymphoid organ cells decreased significantly via nAChR mediated, spleen cell proliferation was inhibited, and interleukin 2 (IL-2) secretion was reduced. Which worsens the symptoms of rheumatoid arthritis. Based on the above research status, we speculated that the effects of HTP are characterized by multiple organs, multiple targets and multiple pathways (see Fig. 2), and different organs have different sensitivities to the effects of HTP. However, the effects of HTP on multiple organs in the body (including the establishment of animal models of HTP aerosol exposure, comprehensive evaluation of the effects on various organs, and comparative studies on the effects between HTP and traditional cigarettes, etc.) still need to be further explored.

5. Mechanism of function changes in multiple organs caused by HTP

HTP can change the functional programming of various organs in the body through various ways. Based on the research status around the world, it can be summarized as the following main mechanisms, including oxidative stress mechanism, cell proliferation and apoptosis mechanism, and inflammatory response mechanism.

5.1. Mechanism of oxidative stress

Oxidative Stress (OS) is a physiological state where the oxidative reaction and antioxidant reaction are unbalanced, in which free radicals play an important role in the process of diseases and aging caused by oxidative stress (Baek and Lee, 2016). Smoking traditional cigarettes can directly induce more severe oxidative stress response, and may activate proto-oncogenes, inactivate tumor suppressor genes, and induce mutations in DNA repair genes (Caliri et al., 2021). Lung is the main target organ of smoke exposure, and a number of studies have confirmed that exposure to HTP aerosol can affect lung oxidative stress response (Wang et al., 2024), thus affecting lung function.

When the lung fibroblasts were exposed to the HTP aerosol, it was observed that the cell viability showed concentration-dependent decrease. In addition, it is known that H_2O_2 is a key effector molecule of oxidative stress, after labeling the H_2O_2 encoding gene, it was observed that the intracellular fluorescence intensity was enhanced after HTP exposure, and the intracellular H_2O_2 content was significantly increased. It has been confirmed that HTP can increase the oxidative

stress level of lung fibroblasts, but the oxidative stress response of lung fibroblasts induced by HTP aerosol is significantly lower than that of traditional cigarettes (Lyu et al., 2022). When bronchial epithelial cells were exposed to HTP aerosols, it increases the binding activity of anti-oxidative stress factor-nuclear factor 2-related factor 2 (Nrf2) reaction element and promotes the Nrf2 expression, while inducing the expression of downstream targets HMOX1 and NQO1, showing an enhanced antioxidant effect, which is consistent with the reactivity of traditional cigarettes (Giebe et al., 2021). However, the abnormal increase of Nrf2 expression mediated by traditional cigarette smoke can cause DNA damage in cells, but no abnormal levels of epigenetic markers and DNA damage were observed in cells after HTP exposure (Khalil et al., 2021). In addition, a number of studies have also suggested that harmful components such as polycyclic aromatic hydrocarbons and the particulate matter in HTP aerosols may be the factors inducing OS (Zarcone et al., 2023). In summary, oxidative stress is the main mechanism of organ function changes caused by tobacco products, but HTP significantly reduces the oxidative stress level compared with traditional cigarettes, which partly explains that HTP is healthier than traditional cigarettes. At the same time, the effective control of oxidative stress effect caused by HTP may be one of the methods to decrease the damage effect of HTP.

5.2. Mechanism of cell proliferation and apoptosis

Orderly cell proliferation and apoptosis is the basic premise to ensure the number and vitality of cells, and is one of the necessary conditions for the homeostasis and normal function of tissues and organs. Some studies have pointed out that abnormal cell proliferation and apoptosis is one of the inducements in the damage effect caused by traditional cigarettes. In a study of HTP on cell proliferation and apoptosis, when human gingival fibroblasts and human keratinocytes were exposed to HTP aerosol extract, enhanced cell viability was observed, but no apoptotic changes were observed, and the cell number in S and G2 phase increased. At the same time, P53 gene expression was increased and Bcl2 gene expression was inhibited in fibroblasts and keratinocytes after exposure, suggesting that HTP may induce oral cell proliferation, which seems to have a beneficial effect on the oral microenvironment (Zarcone et al., 2023; Pagano et al., 2021). In addition, when comparing the effects of HTP and traditional cigarettes on apoptosis, it was found that traditional cigarette smoke could enhance apoptosis by changing the histone phosphorylation level of apoptosis-related genes (such as apoptosis regulator EGR1) (Hattori et al., 2021). However, HTP aerosol has no significant effect on histone modification and expression of apoptosis genes, and the mechanism of inducing apoptosis enhancement remains to be clarified. Pablo Scharf et al. exposed lymphocytes to traditional cigarettes, HTP aerosols or air (1 hour/time, twice a day for 5 days), and found through flow cytometry analysis that compared with the air exposure group, both traditional cigarettes and HTP aerosol exposure could lead to enhanced apoptosis of T cells and increased number of dead cells. However, the HTP effect is significantly weaker than that of traditional cigarettes (Scharf et al., 2021). In summary, HTP usually affects cell proliferation but has no obvious effect on apoptosis, and may has a positive effect on cell number.

5.3. Mechanism of inflammatory response

Inflammation is a basic pathological process in which the living tissue with the vascular system reacts against various damage factors. Exogenous physical, chemical and biological factors can induce inflammation in the body, and HTP is no exception (Bosilkovska et al., 2020). Analysis of HTP components confirmed that the related additives in HTP (including maltol, vanillin, coumarin and cinnamaldehyde, etc.) can cause human bronchial epithelial cells and lung fibroblasts to release pro-inflammatory cytokines, increase IL-8 levels, and enhance inflammatory response (Gerloff et al., 2017). When human coronary endothelial cells were exposed to HTP aerosol and traditional cigarette smoke, TNF signaling pathway and inflammatory response were activated, and then mediated the activation of downstream nuclear factor kappa B (NF-κB). Finally, the abundance of adhesion proteins (such as e-selectin and intercellular adhesion molecule 1) is up-regulated, but the concentration of HTP aerosol needs to be increased by 15 times to achieve the same inflammatory effect of traditional cigarettes, indicating that the inflammatory side effects caused by traditional cigarettes are much stronger than HTP (Poussin et al., 2018). In animal models, when female and male rats were exposed to HTP aerosol for 90 days, the number of neutrophils and lymphocytes in blood decreased in a concentration-dependent way, while the number of monocytes, eosinophils and basophils changed inconsistently or did not show a significant change trend between female and male rats. Showing some gender differences (Phillips et al., 2018). In addition, HTP exposure increases levels of local lung inflammatory mediators, such as matrix metalloproteinases (3.6-fold increase) and interferon beta (5-fold increase), which may contribute to impaired lung function (Phillips et al., 2019). The above results suggest that the abnormal inflammatory signal is a non-negligible mechanism in the functional changes of various organs caused by HTP.

5.4. Others

In addition to the above recognized mechanisms, recent studies have shown that HTP may also alter organ function by affecting the abundance of intestinal flora and altering epigenetic modifications of target genes. In some studies, Apo-E mice were first exposed to traditional cigarette smoke and then changed to HTP aerosol for six months to build the animal model. The results of second-generation sequencing of intestinal flora showed that intestinal microbial composition and gene expression were significantly altered after exposure to traditional cigarette smoke. The abundance of Akkermansiaceae (Akkermansiacceae, a gram-negative bacterium, was successfully isolated and identified in 2004. Akkermansiacceae is the first species of verruca microbacteria. Due to its unique effects in metabolic diseases, cancer and immunotherapy, Akkermansiacceae has attracted a lot of attention and research, and is a rising star in probiotics) was increased (Battey et al., 2021). However, after exposure to HTP aerosol, the abundance of Akkemansiaceae decreased significantly, but that of Lactobacillaceae (Lactbacillacceae is a general term of various bacteria that can degrade glucose and other sugars into lactic acid. Lactbacillacceae has strong ability to metabolize carbohydrates and produce acid, and can synthesize glucan and heteropolysaccharide. Lactbacillacceae is widely distributed in the oral cavity and digestive tract, and plays an important role in maintaining the health of human and higher animals) increased (Battey et al., 2021). The study suggests that these gut microbiome changes may be important in the development of disease caused by HTP. Epigenetic modification is a type of change that affects gene expression without altering gene sequence, including DNA methylation, histone modification, and non-coding RNA. After exposure to traditional cigarette smoke, the expression levels of miR-146a/b and miR-182 and other miRNAs in local lungs are elevated, and they are involved in mediating lung inflammation. However, whether miR-146a/b and miR-182 play a role in lung function impairment induced by HTP aerosol exposure has not been reported (Sewer et al., 2016). When oral and gingival organoid is exposed to HTP aerosol, multiple miRNAs expression upregulation can be detected in its culture. Through mRNA-miRNA interaction, GO and KEGG pathway enrichment analysis, the signal pathways such as "organism growth and development/p53 signal transduction/inflammation response" are highly enriched. It is suggested that HTP aerosols may induce enhanced inflammatory response through miRNA, thereby leading to oral and gum damage (Zanetti et al., 2018). Therefore, in order to effectively reduce the risk of HTP exposure, researchers should further explore the mechanism of its effects comprehensively and systematically.

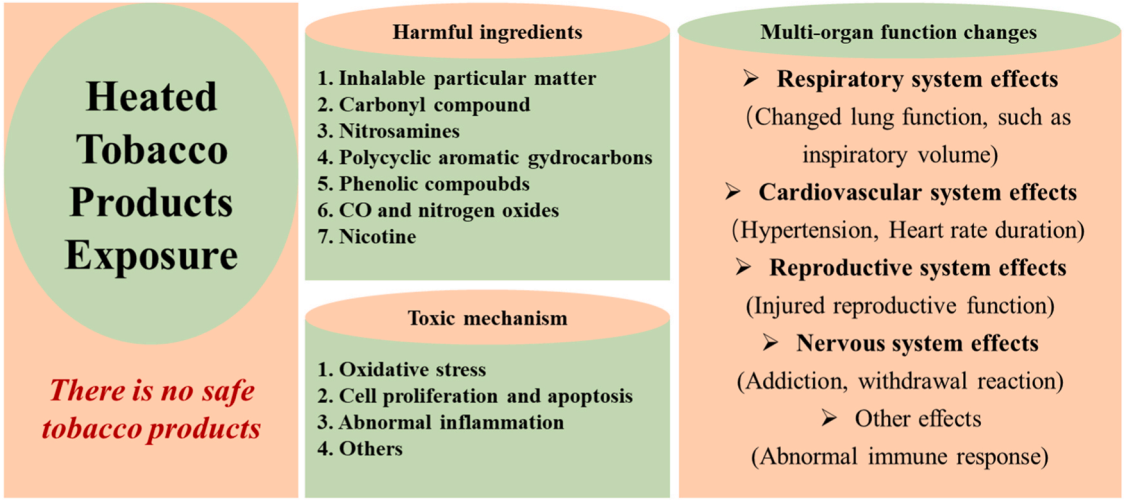

Fig. 3. The effect of HTP on body function and its mechanism. HTP, Heated tobacco products.

6. Future outlook

In view of people's increasing emphasis on life and health, from the perspective of "tobacco and health", the effective transformation of traditional cigarette consumers to HTP users is in line with the general trend of the tobacco market. Based on the relevant literature, we propose that the exposure risk and safety evaluation of HTP still need further research in the following aspects:

1. HTP aerosol: There are still some components in HTP aerosol that have not been clearly qualitatively and quantitatively, which depends on the development of relevant detection technology and the improvement in the accuracy of detection equipment;

2, HTP smoking device: A number of studies have pointed out that repeated heating and uneven heating of HTP smoking device may lead to the release of new harmful components. And the plastic in HTP smoking device can also produce new harmful ingredients after heating. In addition, due to the low frequency of replacement and metal heating sheet, HTP smoking device are easy to produce particles and metal component residues, whether these residues will produce more serious negative effects after multiple heating is not clear;

3. Research object of HTP effect: Previous research object of HTP effect basically focused on adult rats/mice, but there were no relevant studies about the effects of HTP on different races, such as pregnant women, the elderly, people with basic diseases, adolescents, etc. At the same time, there are still some difficulties in the long-term tracking research of HTP consumer health, for example high tracking cost, consumers' uncooperation, and difficulty in tracking, which cannot effectively assess the exposure risk of HTP to population health. Therefore, it is particularly important to construct different animal models to simulate the adverse effect caused by HTP in reality.

4. Early warning of HTP effect: At present, there are few population studies on HTP effect. Volunteers should be widely recruited for cohort study, and samples such as blood, saliva and peripheral blood mononuclear cells of subjects should be collected for detection, so as to observe changes in relevant indicators and obtain toxicity early warning markers (for example, the expression and epigenetic modification of the target genes) of HTP. Such as the detection of cotinine (a main metabolite of nicotine) level in body fluids can be used as one of the early warning effect substances.

Based on the latest research status of HTP, this paper summarizes the main harmful components contained in HTP (including nitrosamines, polycyclic aromatic hydrocarbons, inhalable particles, CO, etc.). It was also demonstrated that HTP can induce functional changes of multiple organs (including respiratory system and cardiovascular system) through enhancing oxidative stress, inhibiting cell proliferation and inducing apoptosis, and interfering with inflammatory response (see Fig. 3). In summary, this paper is helpful to further study the impact of HTP on body function, provide a theoretical basis for improving the safety of HTP, and create a new idea for further development and optimization of HTP.

Author contributions

Yi Liu collected literatures, designed the work, wrote and revised the paper, JiXue Cao, Jing Zhang, Guang Chen collected literatures, revised the paper; ChengHao Luo, Long Huang wrote and revised the paper; all authors approved the final manuscript.

CRediT authorship contribution statement

Long Huang: Writing – review & editing, Writing – original draft, Supervision. **jing zhang:** Methodology, Investigation. **JiXue Cao:** Methodology, Investigation. **ChengHao Luo:** Writing – original draft, Supervision. **Guang Chen:** Methodology, Investigation. **Yi Liu:** Writing – review & editing, Writing – original draft, Methodology, Investigation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

References

Baek, J., Lee, M.G., 2016. Oxidative stress and antioxidant strategies in dermatology. Redox Rep. 21 (4), 164–169 (Jul).

Barbeau, A.M., Burda, J., Siegel, M., 2013. Perceived efficacy of e-cigarettes versus nicotine replacement therapy among successful e-cigarette users: a qualitative approach. Addict. Sci. Clin. Pr. 8, 5.

Bar-Zeev, Y., Berg, C.J., Khayat, A., Romm, K.F., Wysota, C.N., Abroms, L.C., Elbaz, D., Levine, H., 2023. IQOS marketing strategies at point-of-sales: a cross-sectional survey with retailers. Tob. Control 32 (e2), e198–e204 (Aug).

Battey, J.N.D., Szostak, J., Phillips, B., Teng, C., Tung, C.K., Lim, W.T., Yeo, Y.S., Ouadi, S., Baumer, K., Thomas, J., Martinis, J., Sierro, N., Ivanov, N.V., Vanscheeuwijck, P., Peitsch, M.C., Hoeng, J., 2021. Impact of 6-month exposure to aerosols from potential modified risk tobacco products relative to cigarette smoke on the rodent gastrointestinal tract. Front Microbiol 12, 587745. Jul 2.

Bekki, K., Inaba, Y., Uchiyama, S., Kunugita, N., 2017. Comparison of chemicals in mainstream smoke in heat-not-burn tobacco and combustion cigarettes. J. UOEH 39 (3), 201–207.
Benowitz, N.L., 2001. Compensatory smoking of low-yield cigarettes. Smok. Tob. Control Monogr. Vol 13, 39–63.
Benowitz, N.L., 2009. Pharmacology of nicotine: addiction, smoking-induced disease, and therapeutics. Annu Rev. Pharm. Toxicol. 49, 57–71.
Benowitz, N.L., 2010. Nicotine addiction. N. Engl. J. Med 362 (24), 2295–2303.
Bentley, M.C., Almstetter, M., Arndt, D., Knorr, A., Martin, E., Pospisil, P., Maeder, S., 2020. Comprehensive chemical characterization of the aerosol generated by a heated tobacco product by untargeted screening. Anal. Bioanal. Chem. 412 (11), 2675–2685 (Apr).
Biondi-Zoccai, G., Sciarretta, S., Bullen, C., Nocella, C., Violi, F., Loffredo, L., Pignatelli, P., Perri, L., Peruzzi, M., Marullo AGM, De, Falco, E., Chimenti, I., Cammisotto, V., Valenti, V., Coluzzi, F., Cavarretta, E., Carrizzo, A., Prati, F., Carnevale, R., Frati, G., 2019. Acute effects of heat-not-burn, electronic vaping, and traditional tobacco combustion cigarettes: the Sapienza University of Rome-Vascular Assessment of Proatherosclerotic Effects of Smoking (SUR-VAPES) 2 Randomized Trial. J. Am. Heart Assoc. 8, e010455.
Bosilkovska, M., Tran, C.T., de La Bourdonnaye, G., Taranu, B., Benzimra, M., Haziza, C., 2020. Exposure to harmful and potentially harmful constituents decreased in smokers switching to Carbon-Heated Tobacco Product. Toxicol. Lett. 330, 30–40. May 5.
Bravo-Gutiérrez, O.A., Falfán-Valencia, R., Ramírez-Venegas, A., Sansores, R.H., Ponciano-Rodríguez, G., Pérez-Rubio, G., 2021. Lung damage caused by heated tobacco products and electronic nicotine delivery systems: a systematic review. Int. J. Environ. Res Public Health 18 (8), 4079. Apr 13.
Brett, E.I., Miloslavich, K., Vena, A., Didier, N., King, A.C., 2021. Effects of visual exposure to IQOS use on smoking urge and behavior. Tob. Regul. Sci. 7 (1), 31–45 (Jan).
Caliri, A.W., Tommasi, S., Besaratinia, A., 2021. Relationships among smoking, oxidative stress, inflammation, macromolecular damage, and cancer. Mutat. Res. 787, 108365.
Callahan-Lyon, P., 2014. Electronic cigarettes: human health effects. Tob. Control Suppl 2 (Suppl 2), ii36–ii40. May; 23.
Chan, K.H., Wright, N., Xiao, D., Guo, Y., Chen, Y., Du, H., Yang, L., Millwood, I.Y., Pei, P., Wang, J., Turnbull, I., Gilbert, S., Avery, D., Kartsonaki, C., Yu, C., Chen, J., Lv, J., Clarke, R., Collins, R., Peto, R., Li, L., Wang, C., Chen, Z., 2022. China Kadoorie Biobank collaborative group. Tobacco smoking and risks of more than 470 diseases in China: a prospective cohort study. Lancet Public Health 7 (12), e1014–e1026 (Dec).
Confalonieri, M., Salton, F., Fabiano, F., 2017. Acute respiratory distress syndrome. Eur. Respir. Rev. 26 (144), 160116. Apr 26.
Dani, J.A., 2015. Neuronal nicotinic acetylcholine receptor structure and function and response to nicotine. Int. Rev. Neurobiol. 124, 3–19.
van der Plas, A., Pouly, S., Blanc, N., Haziza, C., de La Bourdonnaye, G., Titz, B., Hoeng, J., Ivanov, N.V., Taranu, B., Heremans, A., 2020. Impact of switching to a heat-not-burn tobacco product on CYP1A2 activity. Toxicol. Rep. 7, 1480–1486. Oct 29.
Duan, Z., Levine, H., Romm, K.F., Bar-Zeev, Y., Abroms, L.C., Griffith, L., Wang, Y., Khayat, A., Cui, Y., Berg, C.J., 2023. IQOS marketing strategies and expenditures in the United States from market entrance in 2019 to withdrawal in 2021. Nicotine Tob. Res. 25 (11), 1798–1803. Sep 4.
Galanti, F., Licata, E., Paciotti, G., Gallo, M., Riccio, S., Miriello, D., Dal Lago, A., Meneghini, C., Fabiani, C., Antonaci, D., Schiavi, M.C., Scudo, M., Salacone, P., Sebastianelli, A., Battaglia, F.A., Rago, R., 2023. Impact of different typologies of smoking on ovarian reserve and oocyte quality in women performing ICSI cycles: an observational prospective study. Eur. Rev. Med Pharm. Sci. 27 (11), 5190–5199 (Jun).
Gerloff, J., Sundar, I.K., Freter, R., Sekera, E.R., Friedman, A.E., Robinson, R., Pagano, T., Rahman, I., 2017. Inflammatory response and barrier dysfunction by different e-cigarette flavoring chemicals identified by gas chromatography-mass spectrometry in e-liquids and e-vapors on human lung epithelial cells and fibroblasts. Appl. Vitr. Toxicol. 3, 28–40.
Giebe, S., Hofmann, A., Brux, M., Lowe, F., Breheny, D., Morawietz, H., Brunssen, C., 2021. Comparative study of the effects of cigarette smoke versus next generation tobacco and nicotine product extracts on endothelial function. Redox Biol. 47, 102150.
Gray, N., Henningfield, J.E., Benowitz, N.L., Connolly, G.N., Dresler, C., Fagerstrom, K., Jarvis, M.J., Boyle, P., 2005. Toward a comprehensive long term nicotine policy. Tob. Control 14 (3), 161–165 (Jun).
Grilo, G., Crespi, E., Cohen, J.E., 2021. A scoping review on disparities in exposure to advertising for e-cigarettes and heated tobacco products and implications for advancing a health equity research agenda. Int. J. Equity Health 20 (1), 238. Oct 30.
Hatsukami, D.K., Carroll, D.M., 2020. Tobacco harm reduction: past history, current controversies and a proposed approach for the future. Prev. Med 140, 106099 (Nov).
Heluany, C.S., Scharf, P., Schneider, A.H., Donate, P.B., Dos Reis Pedreira Filho, W., de Oliveira, T.F., Cunha, F.Q., Farsky, S.H.P., 2022. Toxic mechanisms of cigarette smoke and heat-not-burn tobacco vapor inhalation on rheumatoid arthritis. Sci. Total Environ. 809, 151097. Feb 25.
Ioakeimidis, N., Emmanouil, E., Terentes-Printzios, D., Dima, I., Aznaouridis, K., Tousoulis, D., Vlachopoulos, C., 2020. Acute effect of heat-not-burn versus standard cigarette smoking on arterial stiffness and wave reflections in young smokers. Eur. J. Prev. Cardiol. 2047487320918365.
Kawasaki, Y., Li, Y.S., Watanabe, S., Ootsuyama, Y., Kawai, K., 2021. Urinary biomarkers for secondhand smoke and heated tobacco products exposure. J. Clin. Biochem Nutr. 69 (1), 37–43 (Jul).
Khalil, C., Chahine, J.B., Haykal, T., Al Hageh, C., Rizk, S., Khnayzer, R.S., 2021. E-Cigarette aerosol induced cytotoxicity, DNA damages and late apoptosis in dynamically exposed A549 cells. Chemosphere.
Kleinstreuer, C., Feng, Y., 2013. Lung deposition analyses of inhaled toxic aerosols in conventional and less harmful cigarette smoke: a review. Int J. Environ. Res Public Health 10 (9), 4454–4485. Sep 23.
Li, X., Luo, Y., Jiang, X., Zhang, H., Zhu, F., Hu, S., Hou, H., Hu, Q., Pang, Y., 2019. Chemical Analysis and Simulated Pyrolysis of Tobacco Heating System 2.2 Compared to Conventional Cigarettes. Nicotine Tob. Res. 21 (1), 111–118. Jan 1.
Lyu, Q., Jiang, L., Zheng, H., Hayashi, S., Sato, K., Toyokuni, S., 2022. Diluted aqueous extract of heat-not-burn tobacco product smoke causes less oxidative damage in fibroblasts than conventional cigarette. J. Clin. Biochem Nutr. 71 (1), 55–63 (Jul).
Mullassery, D., Smith, N.P., 2015. Lung development. Semin Pedia Surg. 24 (4), 152–155 (Aug).
Nabavizadeh, P., Liu, J., Havel, C.M., Ibrahim, S., Derakhshandeh, R., Jacob, P., III, Springer, M.L., 2018. Vascular endothelial function is impaired by aerosol from a single IQOS HeatStick to the same extent as by cigarette smoke. Tob. Control 27 (Suppl 1), s13–s19.
Nordlund, M. What are the differences between smoke and aerosol? PMI Science. accessed 2020-11-17.
Oviedo, A., Lebrun, S., Kogel, U., Ho, J., Tan, W.T., Titz, B., Leroy, P., Vuillaume, G., Bera, M., Martin, F., Rodrigo, G., Esposito, M., Dempsey, R., Ivanov, N.V., Hoeng, J., Peitsch, M.C., Vanscheeuwijck, P., 2016. Evaluation of the Tobacco Heating System 2.2. Part 6: 90-day OECD 413 rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects of a mentholated version compared with mentholated and non-mentholated cigarette smoke. Regul. Toxicol. Pharm. 81 (Suppl 2), S93–S122. Nov 30.
Pagano, S., Negri, P., Coniglio, M., Bruscoli, S., Di Michele, A., Marchetti, M.C., Valenti, C., Gambelunghe, A., Fanasca, L., Billi, M., Cianetti, S., Marinucci, L., 2021. Heat-not-burn tobacco (IQOS), oral fibroblasts and keratinocytes: cytotoxicity, morphological analysis, apoptosis and cellular cycle. An in vitro study. J. Periodontal Res 56 (5), 917–928 (Oct).
Pataka, A., Kotoulas, S., Chatzopoulos, E., Grigoriou, I., Sapalidis, K., Kosmidis, C., Vagionas, A., Perdikouri, EI., Drevelegas, K., Zarogoulidis, P., Argyropoulou, P., 2020. Acute effects of a heat-not-burn tobacco product on pulmonary function. Med. (Kaunas.) 56 (6), 292. Jun 12.
Pérez-Milena, A., Martínez-Fernández, M.L., Pérez-Milena, R., Jiménez-Pulido, I., Leal-Helmling, F.J., Mesa-Gallardo, I., 2006. Tabaquismo y adolescentes: 'buen momento para dejar de fumar? Relación con factores sociofamiliares [Tobacco dependency and adolescents: a good time to give up smoking? Relation to social and family factors]. Aten. Prima 37 (8), 452–456. May 15.
Phillips, B.W., Schlage, W.K., Titz, B., Kogel, U., Sciuscio, D., Martin, F., Leroy, P., Vuillaume, G., Krishnan, S., Lee, T., Veljkovic, E., Elamin, A., Merg, C., Ivanov, N.V., Peitsch, M.C., Hoeng, J., Vanscheeuwijck, P., 2018. A 90-day OECD TG 413 rat inhalation study with systems toxicology endpoints demonstrates reduced exposure effects of the aerosol from the carbon heated tobacco product version 1.2 (CHTP1.2) compared with cigarette smoke. I. Inhalation exposure, clinical pathology and histopathology. Food Chem. Toxicol. 116 (Pt B), 388–413 (Jun).
Phillips, B., Szostak, J., Titz, B., Schlage, W.K., Guedj, E., Leroy, P., Vuillaume, G., Martin, F., Buettner, A., Elamin, A., Sewer, A., Sierro, N., Choukrallah, M.A., Schneider, T., Ivanov, N.V., Teng, C., Tung, C.K., Lim, W.T., Yeo, Y.S., Vanscheeuwijck, P., Peitsch, M.C., Hoeng, J., 2019. A six-month systems toxicology inhalation/cessation study in ApoE-/- mice to investigate cardiovascular and respiratory exposure effects of modified risk tobacco products, CHTP 1.2 and THS 2.2, compared with conventional cigarettes. Food Chem. Toxicol. 126, 113–141 (Apr).
Poussin, C., Laurent, A., Kondylis, A., Marescotti, D., van der Toorn, M., Guedj, E., Goedertier, D., Acali, S., Pak, C., Dulize, R., Baumer, K., Peric, D., Maluenda, E., Bornand, D., Suarez, I.G., Schlage, W.K., Ivanov, N.V., Peitsch, M.C., Hoeng, J., 2018. In vitro systems toxicology-based assessment of the potential modified risk tobacco product CHTP 1.2 for vascular inflammation- and cytotoxicity-associated mechanisms promoting adhesion of monocytic cells to human coronary arterial endothelial cells. Food Chem. Toxicol. 120, 390–406 (Oct).
Prasad, K.N., Bondy, S.C., 2022. Electronic cigarette aerosol increases the risk of organ dysfunction by enhancing oxidative stress and inflammation. Drug Chem. Toxicol. 45 (6), 2561–2567 (Nov).
Ratajczak, A., Jankowski, P., Strus, P., Feleszko, W., 2020. Heat not burn tobacco product-a new global trend: impact of heat-not-burn tobacco products on public health, a systematic review. Int. J. Environ. Res. Public Health 17 (2), 409. Jan 8.
Sansone, A., Limoncin, E., Colonnello, E., Mollaioli, D., Ciocca, G., Corona, G., Jannini, E. A., 2022. Harm reduction in sexual medicine. Sex. Med Rev. 10 (1), 3–22 (Jan).
Scharf, P., da Rocha, G.H.O., Sandri, S., Heluany, C.S., Pedreira Filho, W.R., Farsky, S.H. P., 2021. Immunotoxic mechanisms of cigarette smoke and heat-not-burn tobacco vapor on Jurkat T cell functions. Environ. Pollut. 268 (Pt B), 115863. Jan 1.
Sewer, A., Kogel, U., Talikka, M., Wong, E.T., Martin, F., Xiang, Y., Guedj, E., Ivanov, N. V., Hoeng, J., Peitsch, M.C., 2016. Evaluation of the Tobacco Heating System 2.2 (THS2.2). Part 5: microRNA expression from a 90-day rat inhalation study indicates that exposure to THS2.2 aerosol causes reduced effects on lung tissue compared with cigarette smoke. Regul. Toxicol. Pharm. 81 (Suppl 2), S82–S92. Nov 30.
Solomou, N., Fernández, E., Szafnauer, R., Psillakis, E., 2023. Total and bioavailable polycyclic aromatic hydrocarbons in unused and operated heat-not-burn tobacco products and conventional cigarettes. Chemosphere 335, 139050 (Sep).
Uguna, C.N., Snape, C.E., 2022. Should IQOS emissions be considered as smoke and harmful to health? A review of the chemical evidence. ACS Omega 7 (26), 22111–22124. Jun 22.

Upadhyay, S., Rahman, M., Johanson, G., Palmberg, L., Ganguly, K., 2023. Heated Tobacco Products: insights into composition and toxicity. Toxics 11 (8), 667. Aug 2.
Wang, L., Liu, X., Chen, L., Liu, D., Yu, T., Bai, R., Yan, L., Zhou, J., 2020. Harmful chemicals of heat not burn product and its induced oxidative stress of macrophages at air-liquid interface: Comparison with ultra-light cigarette. Toxicol. Lett. 331, 200–207.
Wang, H., Lu, F., Tian, Y., Zhang, S., Han, S., Fu, Y., Li, J., Feng, P., Shi, Z., Chen, H., Hou, H., 2024. Evaluation of toxicity of heated tobacco products aerosol and cigarette smoke to BEAS-2B cells based on 3D biomimetic chip model. Toxicol. Vitr. 94, 105708 (Feb).
Yaman, B., Akpınar, O., Kemal, H.S., Cerit, L., Yüksek, Ü., Söylemez, N., Duygu, H., 2021. Comparison of IQOS (heated tobacco) and cigarette smoking on cardiac functions by two-dimensional speckle tracking echocardiography. Toxicol. Appl. Pharm. 423, 115575. Jul 15.
Yoshida, S., Ichinose, T., Shibamoto, T., 2020. Effects of fetal exposure to heat-not-burn tobacco on testicular function in male offspring. Biol. Pharm. Bull. 43 (11), 1687–1692.
Yu, S.J., Kwon, M.K., Choi, W., Son, Y.S., 2022. Preliminary study on the effect of using heat-not-burn tobacco products on indoor air quality. Environ. Res. 212 (Pt A), 113217 (Sep).
Zanetti, F., Sewer, A., Scotti, E., Titz, B., Schlage, W.K., Leroy, P., Kondylis, A., Vuillaume, G., Iskandar, A.R., Guedj, E., Trivedi, K., Schneider, T., Elamin, A., Martin, F., Frentzel, S., Ivanov, N.V., Peitsch, M.C., Hoeng, J., 2018. Assessment of the impact of aerosol from a potential modified risk tobacco product compared with cigarette smoke on human organotypic oral epithelial cultures under different exposure regimens. Food Chem. Toxicol. 115, 148–169 (May).
Zarcone, G., Lenski, M., Martinez, T., Talahari, S., Simonin, O., Garçon, G., Allorge, D., Nesslany, F., Lo-Guidice, J.M., Platel, A., Anthérieu, S., 2023. Impact of electronic cigarettes, heated tobacco products and conventional cigarettes on the generation of oxidative stress and genetic and epigenetic lesions in human bronchial epithelial BEAS-2B Cells. Toxics 11 (10), 847. Oct 10.
Zhang, S., Wang, Z., Zhang, J., Guo, D., Chen, Y., 2021. Inhalable cigarette-burning particles: size-resolved chemical composition and mixing state. Environ. Res 202, 111790 (Nov).