Institutet för miljömedicin - IMM

IMM report 2/2025

Health effects of new nicotine and tobacco products

A review of the scientific evidence

IMM
Institute of Environmental Medicine
Institutet för miljömedicin

Karolinska Institutet

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Förord

Denna rapport sammanfattar befintlig forskning om sambandet mellan de nya nikotin- och tobaksprodukterna e-cigaretter, vitt snus samt upphettade tobaksvaror och en rad hälsoutfall, inklusive astma och allergi, cancer, hjärt-kärlsjukdomar, diabetes, lungsjukdomar, graviditet och kvinnors hälsa. Rapporten föranleds av en ökad användning av dessa produkter, särskilt bland ungdomar, unga vuxna och kvinnor. Syftet med rapporten är att sammanfatta den aktuella kunskapen inom området samt att peka på kunskapsluckor och behov av ytterligare forskning. Rapporten publiceras av Institutet för miljömedicin (IMM) vid Karolinska Institutet där forskning och undervisning bedrivs om miljöns påverkan på sjukdomsutveckling. IMM tillhandahåller även expertis inom miljörelaterad hälsoriskbedömning åt svenska myndigheter och internationella organisationer, som EU och WHO. Rapporten är skriven av forskare verksamma vid IMM inom områden som rör miljöpåverkan på ovan nämnda sjukdomar. Medan cigarrettrökning och användningen av brunt snus generellt sett minskar, blir nya nikotinprodukter alltmer populära i Sverige och globalt. Att klargöra hälsoeffekterna av dessa produkter är avgörande för riskbedömare, tillsynsmyndigheter som arbetar med reglering av tobak eller nikotin, samt de som är engagerade i hälsofrämjande insatser.

Preface

This report summarizes existing research on the relationship between the new nicotine and tobacco products e-cigarettes, white snus and heated tobacco products and a range of health outcomes, including asthma and allergy, cancer, cardiovascular disease, diabetes, lung diseases, pregnancy and women's health. The report is prompted by an increased use of these products, especially among adolescents, young adults and women. The aim of the report is to summarize current knowledge in the area and to point at knowledge gaps and research needs. The report is produced by the Institute of Environmental Medicine (IMM) at Karolinska Institutet, where research and teaching are conducted on the influence of the environment on disease development. IMM also provides Swedish authorities and international organizations, such as the EU and WHO, with expertise in environmental health risk assessment. The report is authored by researchers active at IMM in the areas of environmental influences on the above-mentioned diseases. While cigarette smoking and brown snus use are generally decreasing, new nicotine products are increasingly popular in Sweden as well as globally. Understanding the health effects of these products is crucial for risk assessors, regulatory authorities involved in tobacco or nicotine control, and those engaged in health promotion.

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Sammanfattning på Svenska

Bakgrund

Den globala rökepidemin har orsakat över 200 miljoner förtida dödsfall under de senaste 30 åren. Samtidigt som rökningen har minskat tack vare ökad medvetenhet, folkhälsoåtgärder och restriktioner, blir andra tobaks- och nikotinprodukter som elektroniska cigaretter (e-cigaretter eller vejp), upphettade tobaksvaror och tobaksfritt snus (vitt snus) allt populärare, särskilt bland ungdomar och unga vuxna. Dessa nya produkter marknadsförs ofta som säkrare, men lite är känt om deras långsiktiga hälsoeffekter. Nikotin, som är en central komponent, är starkt beroendeframkallande och kopplat till allvarliga hälsorisker, bland annat hjärt- och kärlsjukdomar, diabetes och graviditetskomplikationer. Tillsatser och smakämnen i dessa produkter kan eventuellt innebära ytterligare hälsorisker. Syftet med denna rapport är att sammanfatta befintlig kunskap om hälsorisker knutna till dessa nya produkter, med fokus på astma och allergi, cancer, diabetes, hjärt- och kärlsjukdomar, lungsjukdomar, graviditetsutfall och kvinnohälsa, att identifiera kunskapsluckor och belysa forskningsbehov samt metodologiska utmaningar. Den baseras på en systematisk genomgång av den vetenskapliga litteratur som publicerats till och med första halvåret 2024. Resultaten kan ligga till grund för strategier och beslutsfattande inom folkhälsoområdet.

Astma och allergi

Befintlig epidemiologisk forskning tyder på att e-cigaretter ökar risken för astma och nedre luftvägssymtom. Studierna har dock vissa metodologiska svagheter vad gäller exponeringsmätning och möjligheten att skilja effekter av e-cigaretter från effekter av tobaksrökning. Resultaten är i linje med den omfattande forskning som kopplat rökning till astma. Mekanistiska studier visar att e-cigaretter kan framkalla reaktioner som är kännetecknande för astma, inklusive slemproduktion, remodellering av luftvägarna, samt hyperreaktivitet och infiltrering av immunceller i luftvägarna. Dessutom tyder vissa resultat på att det specifika innehållet i e-vätskan kan ha betydelse för

astmarelaterade reaktioner, vilket behöver utredas närmare. När det gäller andra allergiska sjukdomar tyder tvärsnittsdata på samband mellan användning av e-cigaretter och atopisk dermatit samt allergisk rinit, men forskningsunderlaget är begränsat. Forskningen om upphettade tobaksvaror är knapphändig men tyder på liknande hälsorisker som de som förknippas med e-cigaretter. Inga studier har undersökt en eventuell koppling mellan vitt snus och astma eller andra allergiska sjukdomar.

Cancer

Eftersom de nya tobaksprodukterna funnits på marknaden så kort tid saknas prospektiva epidemiologiska studier av cancerrisk. En fall-kontrollstudie visade dock att risken för lungcancer var högre hos dem som både använde e-cigaretter och rökte vanliga cigaretter jämfört med bland dem som bara rökte vanliga cigaretter. Man har också påvisat cancerrelaterade förändringar i munnen hos e-cigarettanvändare, inklusive förändringar i gener och DNA-skador. E-cigaretter utsätter användarna för samma cancerframkallande ämnen som finns i tobaksrök, även om nivåerna är lägre. De flesta studier som titta på hälsoeffekter baseras dock på enstaka mätningar och självrapporterade data, och det är därför oklart hur mycket man har tagit hänsyn till tobaksrökning. Resultaten stöds dock av djurstudier som visar att e-cigaretter kan orsaka ökad DNA-skada och lungcancer och möjligen även cancer i urinblåsan. Mekanistiska studier visar blandade resultat på grund av att olika e-cigarettprodukter och analysmetoder använts, men indikerar att oxidativ stress och DNA-skador är viktiga mekanismer i cancerutvecklingen. Dessutom överensstämmer de kromosomförändringar som observerats i både djur- och mekanistiska studier med markörer som är knutna till ökad cancerrisk hos rökare. Färre studier har undersökt upphettade tobaksvaror, men de visar liknande exponering för cancerframkallande ämnen, ökad DNA-skada och cellförändringar i experimentella modeller. Vi har inte identifierat några studier om vitt snus och cancerrisk.

Hjärt- och kärlsjukdomar

Flera epidemiologiska studier tyder på att e-cigaretter kan öka risken för hjärt-kärlsjukdom, inklusive hjärtinfarkt och stroke. Dock bygger mycket av forskningen på tvärsnittsstudier, och långtidsstudier som undersöker hur e-cigaretter påverkar blodkärl och hjärthälsa på sikt är fortfarande få. Vissa studier visar att e-cigarettanvändning kan leda till kortsiktiga effekter som högre blodtryck och ökad hjärtfrekvens, vilket också stöds av experiment på celler och djur. Resultaten är dock inte helt samstämmiga, troligen på grund av skillnader i hur studierna är gjorda. Sammantaget finns ännu inte tillräckligt starka bevis för att dra definitiva slutsatser om e-cigaretters långsiktiga påverkan på hjärt-kärlhälsan. För upphettade tobaksvaror och vitt snus saknas studier helt, vilket gör det svårt att bedöma deras eventuella effekter på hjärtat och blodkärlen. Eftersom nikotinhalten ofta är hög i dessa nya produkter finns det dock anledning att misstänka att de kan medföra ökad risk för hjärt- och kärlsjukdomar.

Diabetes

Epidemiologiska studier tyder på en högre förekomst av typ 2-diabetes, insulinresistens och metabolt syndrom bland användare av e-cigaretter jämfört med personer som aldrig använt e-cigaretter. Befintliga studier är dock tvärsnittsstudier och baserades på självrapporterade uppgifter, vilket begränsar bevisvärdet. Inga studier undersökte typ 1-diabetes. Vissa djurstudier tyder på att exponering för e-cigaretter kan försämra energimetabolismen, framkalla oxidativ stress och öka insulinresistensen, men resultaten är inte entydiga. Det finns ingen forskning om vitt snus och diabetes, och endast en studie har undersökt upphettade tobaksvaror. Med tanke på den höga nikotinhalten i vitt snus och upphettade tobaksvaror samt nikotinets kända effekter på insulinresistens och glukostolerans är det rimligt att anta att e-cigaretter, vitt snus och upphettade tobaksvaror ökar diabetesrisken, men detta har ännu inte påvisats.

Lungsjukdomar

Nuvarande forskning visar att användning av e-cigaretter avsevärt kan öka risken för både akuta och kroniska lungeffekter, såsom EVALI (E-cigarette or Vaping Product Use-Associated Lung Injury), kronisk bronkit och KOL. Även om epidemiologiska och kliniska studier på upphettade tobaksvaror fortfarande saknas, tyder aerosolens sammansättning och toxikologiska data på att de kan orsaka liknande skadliga effekter. Det finns allt fler bevis för att både e-cigaretter och upphettade tobaksvaror frigör kemikalier, som kan skada lungorna. Långsiktiga studier är dock avgörande för att fullt ut förstå omfattningen av dessa lungeffekter.

Graviditet och kvinnohälsa

Befintlig epidemiologisk forskning talar för att användning av e-cigaretter under graviditeten kan öka risken för negativa födelseutfall, särskilt för tidig födsel och låg födelsevikt. Studier där försöksdjur exponerats för e-cigaretter under graviditeten tyder på att de kan påverka avkommans tillväxt och hjärnans utveckling, men resultaten är inte entydiga. Det finns fortfarande betydande kunskapsluckor om hur upphettade tobaksvaror och vitt snus påverkar graviditeten och barnets hälsa. Eftersom användning av brunt snus och cigarrettrökning har en skadlig inverkan på flertalet graviditetsutfall, är det sannolikt att liknande risker även gäller för vitt snus. Detta är viktigt att klarlägga.

När det gäller kvinnohälsa syns ett samband mellan e-cigarettanvändning och psykisk ohälsa. Resultaten baseras dock på tvärsnittsstudier, vilket innebär att riktningen på sambandet inte kan fastställas. Få studier har undersökt eventuella samband mellan upphettade tobaksvaror, vitt snus och psykisk hälsa eller andra aspekter av kvinnors hälsa.

Slutsats

Befintlig forskning visar på ett samband mellan användning av e-cigaretter och astma, KOL och andra lungsjukdomar. Visst stöd från ett mindre antal epidemiologiska och experimentella studier finns för samband mellan vejping och hjärt-kärlsjukdomar, typ 2-diabetes och

cancer. Ett fåtal epidemiologiska och experimentella studier tyder på att bruk av e-cigaretter under graviditet skulle kunna ha negativa effekter på fosterutvecklingen men studierna är få och resultaten inte helt entydiga.

Det finns vissa belägg för att upphettade tobaksvaror påverkar risken för cancer, KOL och andra lungsjukdomar samt astma, men forskningen om diabetes, hjärt-kärlsjukdomar och graviditetsutfall är knapphändig.

Forskning om hälsoeffekter av vitt snus är nästan obefintlig.

Forskningsbehov

Forskningen om hälsorisker kopplade till bruk av e-cigaretter, vitt snus och upphettade tobaksvaror är begränsad, särskilt när det gäller långtidseffekter. E-cigaretter har studerats i större utsträckning än övriga produkter. De epidemiologiska studierna av e-cigarettbruk har dock ofta metodologiska problem, bland annat otillräcklig justering för tobaksrökning, självrapporterad information om exponering och kort uppföljningstid. De saknar också oftast information om exponeringsdos och duration. I framtida studier är det viktigt att komma till rätta med dessa problem. Studier av andrahandsexponering för e-cigaretter saknas, vilket är viktigt att belysa med tanke på att exponering för miljötobaksrök är en riskfaktor för allergiska sjukdomar och lungcancer. Studier bör även fokusera på exponering under fostertiden och tidigt i livet, till exempel genom moderns användning under graviditeten. Dessutom krävs mer forskning för att förstå hur olika tillsatser och nikotinnivåer påverkar hälsoriskerna.

Upphettade tobaksvaror har många likheter med e-cigaretter vad gäller innehåll och kan därför ha liknande hälsoeffekter, men det krävs ytterligare studier för att fastställa om så är fallet.

Forskning om vitt snus är nästan obefintlig och det finns ett stort behov av studier på området, särskilt med tanke på att användningen är frekvent och ökar i Sverige, inte minst bland ungdomar och unga vuxna. Den höga nikotinhalten innebär att det kan ha liknande

hälsoeffekter som brunt snus, vilket bland annat inkluderar negativa effekter på fosterutveckling och en ökad risk för typ 2-diabetes. Den höga användningen bland unga kvinnor i barnafödande åldrar är oroande i detta sammanhang och det finns ett akut behov av att undersöka potentiella negativa effekter på graviditetsutfall. Det faktum att vitt snus har funnits på marknaden under så kort tid innebär att vi tyvärr måste vänta i flera år innan långsiktiga effekter i form av en potentiellt ökad risk för typ 2-diabetes, hjärt-kärlsjukdom och cancer kan klargöras i detalj.

Sammanfattningsvis finns det ett akut behov av fler storskaliga, högkvalitativa, longitudinella epidemiologiska studier för att belysa hälsoriskerna kopplade till bruk av e-cigaretter, upphettade tobaksvaror och vitt snus, inklusive men inte begränsat till risken för allergiska sjukdomar, cancer, hjärt-och kärlsjukdomar, diabetes, lungsjukdomar och negativa graviditetsutfall. Framtida studier behöver belysa betydelsen av dos och duration samt specifika produktsammansättningar. Experimentella studier behövs också för att klarlägga vilka underliggande mekanismer som kopplar bruk av nya tobaks- och nikotinprodukter till olika hälsorisker.

SAMMANFATTNING AV KUNSKAPSLÄGET AVSEENDE HÄLSORISKER KOPPLADE TILL DE NYA NIKOTIN- OCH TOBAKSPRODUKTERNA.

	E-cigaretter	Vitt snus	Upphettade tobaksvaror
Astma och allergi	Ökad risk, viss evidens	Begränsad eller obefintlig forskning	Ökad risk, viss evidens
Cancer	Ökad risk, viss evidens	Begränsad eller obefintlig forskning	Ökad risk, viss evidens
Diabetes (typ-2)	Ökad risk, viss evidens	Begränsad eller obefintlig forskning	Begränsad eller obefintlig forskning
Hjärt-och kärlsjukdom	Ökad risk, viss evidens	Begränsad eller obefintlig forskning	Begränsad eller obefintlig forskning
Lungsjukdomar	Ökad risk, stark evidens	Begränsad eller obefintlig forskning	Ökad risk, viss evidens
Graviditet	Ökad risk, viss evidens	Begränsad eller obefintlig forskning	Begränsad eller obefintlig forskning
Kvinnors hälsa	Begränsad eller obefintlig forskning	Begränsad eller obefintlig forskning	Begränsad eller obefintlig forskning

Summary in English

Background

The global smoking epidemic has caused over 200 million premature deaths in the past 30 years. Due to increased awareness of adverse health effects, public health measures, and restrictions, smoking rates have declined. However, new tobacco and nicotine products such as electronic cigarettes (e-cigarettes or vapes), heated tobacco products (HTPs), and tobacco free snus (white snus) are gaining popularity, especially among young people. These new products are often marketed as safer, but evidence on their long-term health effects is limited. Nicotine, a key component, is highly addictive and linked to serious health risks, including cardiovascular diseases, metabolic disorders, and reproductive health. Additives and flavourings in these products may also pose additional health risks. This report aims to summarize existing knowledge on the health risks associated with these new products, focusing on asthma and allergic diseases, cancer, diabetes, respiratory diseases, adverse pregnancy outcomes, and women´s health, to identify knowledge gaps and point out research needs and methodological challenges. It is based on a review of the scientific literature published up to the first half of 2024. The results can inform public health strategies and policymaking.

Asthma and allergic diseases

Existing evidence suggests that e-cigarette use increases the risk of asthma and wheezing. However, published studies vary widely in how they classify exposure and control for cigarette smoking. These findings align with those for conventional cigarettes, which have been linked to adult-onset asthma. Epidemiological evidence is supported by mechanistic studies showing that e-cigarettes can induce key asthma-related features, including mucus production, tissue remodelling, airway hyperresponsiveness, and immune cell infiltration in the airways. Notably, *in vivo* data suggests that the relationship may depend on the specific contents of e-liquids, emphasizing the need for future studies to provide more detailed exposure characterization. For

other allergic diseases, cross-sectional data indicates associations between e-cigarette use and atopic dermatitis as well as allergic rhinitis, though the evidence is limited. Research on HTPs is scarce but suggests similar health risks to those associated with e-cigarettes. For nicotine pouches or white snus, no studies have explored their links to asthma and other allergic diseases, highlighting a significant knowledge gap for this emerging nicotine product.

Cancer

Because the new tobacco products have only been on the market for a short time, no prospective epidemiological studies have been published on cancer risk. However, one case-control study showed that the risk of lung cancer was higher among those who both used e-cigarettes and smoked conventional cigarettes compared to those who only smoked conventional cigarettes. Carcinogenic changes have also been found in the mouth of e-cigarette users, including genetic changes and DNA damage. In addition, vaping exposes users to the same carcinogens found in tobacco smoke, although at lower levels. Most studies of health effects are based on single measurements and self-reported data, so it is unclear how much tobacco smoking was accounted for. The findings are supported by animal studies showing that e-cigarettes may cause increased DNA damage and lung cancer and possibly bladder cancer. Mechanistic studies show mixed results due to the different e-cigarette products and analytical methods used but suggest that oxidative stress and DNA damage are important mechanisms in cancer development. In addition, the chromosomal changes observed in both animal and mechanistic studies are consistent with markers that predict cancer risk in smokers. Fewer studies have investigated heated tobacco products, but they show similar exposure to carcinogens, increased DNA damage and cellular changes in experimental models. We did not identify any studies on white snus and cancer risk.

Cardiovascular disease

Several epidemiological studies, mainly cross-sectional, show an association between e-cigarettes and coronary heart disease and

stroke. However, long-term studies examining the relationship between e-cigarette use and subclinical atherosclerotic markers or major cardiovascular outcomes remain limited. Epidemiological studies also suggest there may be short-term adverse effects, such as increased blood pressure and heart rate, supported by experimental studies in human cells and animal models. However, the findings are inconsistent, possibly due to methodological limitations. The available evidence does not allow for definitive conclusions regarding effects of e-cigarette use on the risk of cardiovascular disease. No studies have specifically addressed HTPs or white snus, which hinders drawing conclusions about their cardiovascular effects. However, since the nicotine content is often high in these new products, there is reason to suspect that they may pose an increased risk of cardiovascular disease.

Diabetes

Epidemiological studies suggest a higher prevalence of type 2 diabetes, insulin resistance, and metabolic syndrome among e-cigarette users compared to never users. However, these studies were cross-sectional and relied on self-reported data, raising concerns about reverse causation and recall bias. No studies examined type 1 diabetes. Animal studies indicate that e-cigarette exposure may impair energy metabolism, induce oxidative stress, and increase insulin resistance, but findings are inconsistent. Research on white snus and diabetes is absent, and only one study has examined HTPs, reporting higher rates of type 2 diabetes and prediabetes among users. Given the high nicotine content of white snus and HTPs, along with the known effects of nicotine on insulin resistance and glucose tolerance, it is reasonable to assume that e-cigarettes, white snus, and HTPs increase diabetes risk, although this remains to be confirmed.

Lung diseases

Current research shows that using e-cigarettes can significantly increase the risk of both acute and chronic respiratory effects, such as EVALI (E-cigarette or Vaping Product Use-Associated Lung Injury), chronic bronchitis, and COPD. While epidemiological and clinical

studies on HTPs are still lacking, the composition of their aerosol and emerging toxicological data suggest they might have similar harmful effects. Evidence is growing that both e-cigarettes and HTPs release chemicals, that can harm the lungs. However, long-term studies are essential to fully understand the extent of these respiratory risks.

Pregnancy and women's health

Current evidence suggests that new nicotine products, such as e-cigarettes, may pose risks to pregnancy outcomes, such as preterm birth and low birth weight. Experimental studies on e-cigarettes during pregnancy indicate potential effects on offspring growth and neurodevelopment, though findings are often conflicting. Research gaps remain significant, particularly for HTPs and white snus and their impact on pregnancy. However, given the documented negative effects of brown snus on pregnancy outcomes, which align with those of conventional cigarettes, similar risks would be expected for white snus. For women's health, there is a very limited number of studies available to date, primarily suggesting a link between e-cigarettes and women's mental illness. Data on HTP and white snus as well as other areas of women's health are scarce. Further research is urgently needed to address these gaps on both pregnancy outcomes and women's health.

Conclusions

Emerging evidence links use of e-cigarettes to asthma, COPD, and other lung diseases, while its association with cardiovascular disease, type 2 diabetes, and cancer is supported by limited but concerning epidemiological and experimental data. There is some evidence for adverse effects of HTPs in relation to cancer, COPD, other lung diseases and asthma, but research on diabetes and cardiovascular disease is scarce. For pregnancy and women's health, e-cigarettes and HTPs may lead to adverse birth outcomes, but studies are few and inconclusive; and there are very limited data on women's health. Research on the health effects of white snus is almost non-existent and urgently needed.

Future direction

Research on the health effects of e-cigarettes, white snus, and heated tobacco use is limited, especially their long-term effects. E-cigarettes have been studied more extensively than the other products. However, the epidemiological studies are hampered by methodological problems including inadequate adjustment for conventional smoking, self-reported information on exposure, and short duration of follow-up. Studies conducted to date also lack information on dose and duration of exposure. In future studies, it is important to overcome these problems. Moreover, second-hand exposure has not been adequately studied, which is important given the known contribution of second-hand cigarette smoke exposure to allergic disease and lung cancer. Studies should also focus on exposure during foetal and early life, for example through maternal use during pregnancy. Additionally, more research is required to understand how vaping habits, flavours, and nicotine levels affect health outcomes. HTPs share many features of e-cigarettes and may have similar health effects, but future studies are needed to establish if this is the case.

Research regarding white snus is almost non-existent and this is urgently needed, especially considering that its use is frequent and increasing in Sweden, particularly among adolescents and young adults. Its high nicotine content implies that it may have the same type of health effects as brown snus, *e.g.,* an increased risk of adverse pregnancy outcomes and type 2 diabetes. The high use among females in childbearing ages is concerning in this context and there is an urgent need to investigate potential adverse effects on pregnancy outcomes and offspring health. The fact that white snus has been on the market for such a short time means that we, unfortunately, will have to wait for several years before potential long-term effects in terms of increased risks of type 2 diabetes, cardiovascular disease, and cancer can be quantified in detail.

To conclude, there is an urgent need for more large-scale, high quality, longitudinal epidemiological studies, to elucidate health effects of e-cigarettes, HTPs and white snus, including but not limited to asthma/allergy, cancer, cardiovascular disease, diabetes, lung

diseases, and pregnancy outcomes. Future studies should assess the influence of dose and duration and specific product formulations. Experimental studies are also needed to address underlying mechanisms linking these exposures to adverse health outcomes.

SUMMARY OF CURRENT EVIDENCE ON HEALTH EFFECTS OF THE NEW NICOTINE AND TOBACCO PRODUCTS.

	E-cigarettes	White snus	Heated tobacco products
Asthma and Allergic disease	Increased risk, some evidence	Limited or no research available	Increased risk, some evidence
Cancer	Increased risk, some evidence	Limited or no research available	Increased risk, some evidence
Diabetes (type 2)	Increased risk, some evidence	Limited or no research available	Limited or no research available
Cardiovascular disease	Increased risk, some evidence	Limited or no research available	Limited or no research available
Lung diseases	Increased risk, strong evidence	Limited or no research available	Increased risk, some evidence
Pregnancy	Increased risk, some evidence	Limited or no research available	Limited or no research available
Women´s health	Limited or no research available	Limited or no research available	Limited or no research available

Introduction

Göran Pershagen

The global tobacco smoking epidemic has claimed more than 200 million premature deaths only during the past 30 years[1], as well as many times more cases of acute and chronic disease, leading to immeasurable human suffering and serious public health consequences. Half of the smokers die from smoking-related diseases and long-term smoking will, on average, shorten the life expectancy by about 10 years. Global smoking rates have decreased following increased awareness of the adverse health effects of smoking and various preventive measures[2]. Changes in attitude towards smoking and smoking restrictions in public places, because of the negative health effects of exposure to environmental tobacco smoke, have also contributed to the decline of smoking. Some countries have even set out to eradicate smoking, such as New Zealand, which enacted legislation to ban sales of cigarettes within a generation.

Other tobacco products than those used for smoking have been popular in some countries. For example, moist snuff (snus) has a long tradition in Sweden[3]. During several decades around 20% of Swedish men have been daily users. Snus has more recently become common also in Norway. Snus users generally have higher nicotine levels in body fluids than smokers, pointing to strong addiction. Although less harmful than smoking, health risks associated with snus have been documented and include cardiovascular and metabolic effects, such as high blood pressure and type 2 diabetes, cancer and adverse pregnancy outcomes as well as increased mortality from cardiovascular diseases[4]. Sales of snus are banned in all countries of the EU, except Sweden, to prevent introduction of “non-traditional” tobacco products.

The serious health consequences of smoking and declining sales have led to attempts to develop less hazardous tobacco products, such as electronic cigarettes (e-cigarettes or vapes), heated tobacco products (HTPs, also called heat-not-burn products) and tobacco free snus

(white snus)[5-6]. An e-cigarette vaporizes a liquid solution, which cools into an aerosol, usually containing propylene glycol and/or glycerine, nicotine, and flavouring. HTPs heat tobacco at lower temperatures than conventional cigarettes, generating an aerosol containing nicotine and other chemicals. Additives are often used, such as flavourings. White snus (also referred to as nicotine pouches) is a Swedish invention and consists of flavoured nicotine pouches for oral use. The evidence on health effects of these three products is limited, particularly regarding effects of long-term use, since their use in large population groups is quite recent.

Nicotine is a crucial component of the new tobacco products. They are often marketed as being "tobacco free" although the nicotine in e-cigarettes and white snus is often extracted from tobacco plants. The addictive potential of nicotine is comparable to that of cocaine and heroin[7], and it is very difficult to quit for most users of nicotine containing products. Nicotine withdrawal affects mood, stress, anxiety, cognition and sleep. Long-term nicotine exposure increases the risk of several cardiovascular, respiratory and gastrointestinal disorders, influences the immune system and is harmful for reproductive health[8]. Furthermore, nicotine affects insulin resistance and predisposes to type 2 diabetes. Other components in the new tobacco products than nicotine may also have toxic properties, such as additives and flavourings.

Recently there has been an increased use of new tobacco and nicotine products, especially among young people. In Sweden white snus was used daily or occasionally in 2022 by 18% and 12% among women and men, respectively, in the age group 16-29 years[9]. Corresponding figures for e-cigarettes were 8% and 5%, respectively. The new tobacco and nicotine products are promoted by the tobacco industry as contributing to "harm reduction". This is a misleading and cynical term since marketing and product development are clearly focused on young people. Teenagers, who generally do not smoke, are targeted by candy flavourings, special marketing events and influencers. Furthermore, the efficacy of the new tobacco and nicotine products in smoking cessation is poorly documented. In fact, use of these products, and development of nicotine addiction, may increase the

risk for subsequent cigarette smoking initiation[10-11]. Globally, legislation on marketing and sales of these new products is often not as strict as for traditional tobacco products, facilitating their introduction into new markets.

The purpose of this report is to summarize and evaluate the evidence on health risks associated with use of new tobacco and nicotine products, primarily e-cigarettes, HTPs and white snus. Previously, the Public Health Agency reviewed the literature on the health effects of these products based on review articles published up to September 2022[12]. The present report includes review papers as well as original studies published through July 2024, providing an updated understanding of their health impacts. Initially, the evidence on cigarette smoking and snus is briefly summarized for each health outcome. We have focused on health outcomes of public health significance that has previously been linked to tobacco use, and where expertise is available at IMM. These include asthma and allergic diseases, cardiovascular disease, cancer, diabetes, lung disease, pregnancy outcomes and women's health. Epidemiological evidence on long-term use is most important for the health risk assessment. However, such studies are scarce for the new products and the evaluation is also based on experimental evidence from humans, animals and tissue models. More traditional forms of tobacco use, such as water pipe and chewing tobacco, are not covered. We hope that the report will be useful for scientists, risk assessors and authorities involved in tobacco and/or nicotine control as well as health promotion.

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New nicotine and tobacco products

E-cigarettes

E-cigarettes, or vapes, are battery-powered and contain a reservoir of 'e-liquid' consisting of nicotine, propylene glycol, glycerol, and flavourings. There are both reusable and disposable products available. E-cigarettes do not contain tobacco, but the nicotine is often extracted from tobacco plants. Unlike a conventional cigarette, there is no combustion of tobacco in an electronic cigarette. Instead, the liquid is heated and turned into vapor, which is inhaled into the lungs. Consequently, lower amounts of incomplete combustion products are generated compared to those found in tobacco smoke. Since the composition of e-liquids is not regulated and e-cigarette devices can function differently, the composition of e-cigarette aerosols vary depending on brand and flavouring but have in general been shown to contain carbonyl compounds, volatile organic compounds, and metals, some of which are human carcinogens[1-2]. E-cigarettes were developed in China around the year 2000 and reached the Swedish market in the 2010s. The market is growing rapidly, with hundreds of brands available, and most tobacco companies are now developing their own brands. Initially, e-cigarettes resembled an ordinary tobacco cigarette but now they come in many different shapes and colours. There is also a wide range of e-liquid flavourings available including fruit and candy taste that may specifically appeal to children. The nicotine exposure provided by e-cigarettes is similar to that provided by traditional cigarettes, but the users practice may differ which will also affect exposure levels[3].

The use of e-cigarettes is increasing in adolescents. According to surveys conducted by the Swedish Council for Information on Alcohol and Other Drugs (CAN), 56% of pupils in the second year of gymnasium (age 17-18 years) reported ever using an e-cigarette in 2024 compared to 24% in 2014[5]. Use was slightly higher in females than in males (Figure 1). Similarly, 45% of girls and 32% of boys in the 9th grade (age 15-16 years) reported ever using e-cigarettes in 2024 which is an increase from the 20% of girls and 25% of boys reporting use in 2014.

White Snus

White snus, also known as nicotine pouches or tobacco-free snus, is similar in format to conventional, tobacco-containing portion snus, but instead of tobacco, the pouches contain a white, finely ground powder. The composition of the contents can vary between manufacturers but usually consists of nicotine, flavourings, water, sweeteners, pH-regulating agents, and fillers made from plant-based fibres. The nicotine content in white snus varies between different products and is often significantly higher than in traditional brown snus. Additionally, white snus often has a high pH, which allows for faster nicotine absorption and increases the risk of addiction. There is a wide range of different flavours available for nicotine pouches, with menthol and fruit flavours being particularly common. Since 2016, when white snus was launched in Sweden, the proportion of snus users (white or brown snus) has increased in adolescents, especially in females (Figure 2). CAN reports that 26% of girls in the second year of gymnasium used snus daily or occasionally in 2024 compared to 5% 2016 and among boys, the proportion using snus increased from 20% to 31% between 2016 and 2024[5]. An increase is also seen in younger children, among pupils in grade 9 (last year of high school), the proportion of users increased from 1% to 14% in girls and from 9% to 17% in boys between 2016 and 2023. No distinction was made between white and traditional brown snus in the survey, but the rise is likely a reflection of the increasing popularity of white snus.

Figure 1. Percentage of 2nd-Year Gymnasium Students Reporting Ever Using E-Cigarettes 2014–2024: Results from CAN's National School Survey 2024[5].

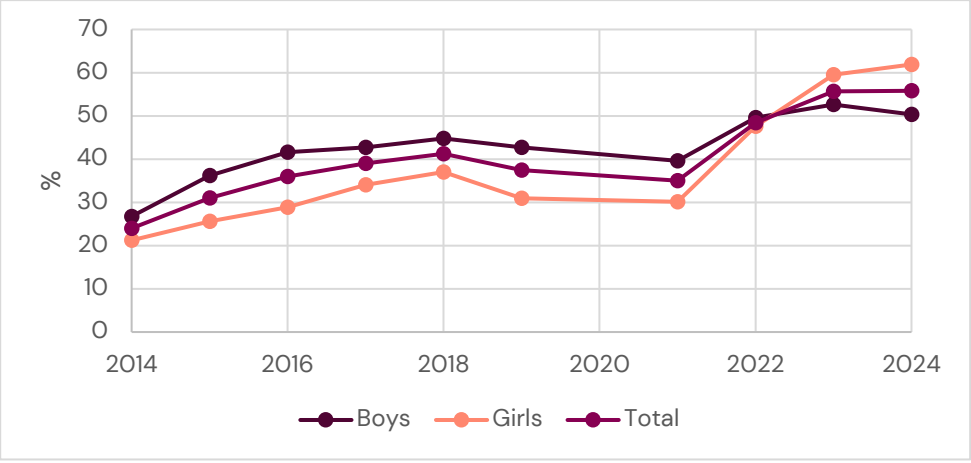
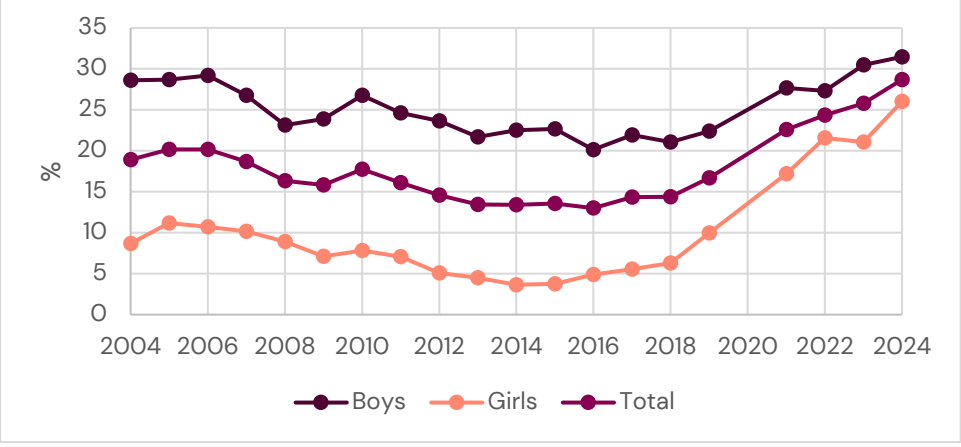


Figure 2. Percentage of 2nd-Year Gymnasium Students Reporting Daily or Occasional Snus Use 2004–2024: Results from CAN's National School Survey 2024[5].



Heated Tobacco Products

A new type of tobacco products that is becoming increasingly popular are heated tobacco products (HTPs) also referred to as heat-not burn products. HTPs heat processed tobacco leaves to around 350°C, producing a nicotine-containing aerosol that the user inhales. This differs from traditional combustible cigarettes, which burn at temperatures as high as 900°C. Since the tobacco is not burned like in a conventional cigarette, HTPs have been marketed as a less harmful alternative. Since May 2024, it is not allowed to flavour HTPs to change the characteristic taste of tobacco[6]. HTPs remain relatively rare in Sweden. In 2023, only 0.3% of the population reported having used heated tobacco at least once in the previous month[7].

Legislation

The Swedish Tobacco Act prohibits the sale of tobacco, including e-cigarettes, to persons under 18 years of age and smoking in, for example, school playgrounds, restaurants, outdoor cafés, and train platforms[8]. The law also regulates marketing, which may not target or depict persons under 25 years of age. For several years, the sale of white snus was not regulated by law. However, in 2022, a new law[9] came into force for tobacco-free nicotine products, with rules such as an 18-year age limit, health warnings and marketing restrictions.

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Methodology

Literature review

Health outcomes
This report focuses on major public health diseases and health outcomes that have been previously linked to tobacco use and are actively researched at the Institute of Environmental Medicine. These include asthma and allergic diseases, cancer, cardiovascular diseases, diabetes, lung diseases, pregnancy outcomes, and women's health.

Smoking and brown snus
Since the literature on health risks associated with smoking and use of brown snus is so extensive we chose to base this part of the review on recently published meta-analyses of epidemiological studies. In addition, we considered evidence from individual studies, Mendelian randomization studies, randomized clinical trials and animal studies.

New nicotine and tobacco products
We set out to identify all studies investigating the new tobacco and nicotine products in relation to asthma and allergic disease, cancer, cardiovascular disease, diabetes, lung diseases, pregnancy and women's health. The searches were conducted individually for each outcome and included both epidemiological studies, investigating the link between the selected exposures and outcomes, and experimental and mechanistic studies (in humans, animals and cell-based) investigating outcome-related endpoints as well as potential underlying mechanisms. In July 2024, an information specialist from the library at Karolinska Institutet conducted the literature searches according to prespecified criteria in Medline, Embase, Cochrane Library and Web of Science without time or language restrictions. The documentation of the search strategies including search terms for each exposure and outcome are available online (https://ki.se/sites/kise/files/2025/03/IMM Report 2_2025 Documentation of literature search.pdf). We do not address harm reduction aspects, such as whether new tobacco and nicotine

products are less harmful than conventional tobacco products. Instead, we focus on assessing the health effects of their use compared to abstaining from all nicotine and tobacco products. Additionally, we have made efforts to exclude studies sponsored by the tobacco industry

Methodological considerations

Epidemiological studies

Most of the human evidence presented in this report is derived from observational data, primarily from **epidemiological studies** that lack an experimental component. However, where possible (primarily when discussing effects of cigarette smoking and brown snus), we have also included findings from randomized clinical trials (RCTs) and Mendelian randomization studies. These study designs typically offer stronger evidence for causality, as they minimize the risk of bias. Conversely, conventional epidemiological studies tend to be more cost-effective and may be the only feasible approach when investigating toxic exposures. The quality of such studies depends on how well exposure (tobacco/nicotine product use) and health is assessed. Self-reported information on exposure is commonly used, and this is a limitation. It is also vital that confounding can be minimized.

The epidemiological study design that provides the strongest evidence is a prospective cohort study, where exposure is measured before the onset of disease, and the incidence of the disease is tracked during follow-up. Sometimes, a case-control design may be the most efficient way to include enough incident cases for meaningful analyses. Apart from the limitations mentioned above, case-control studies often rely on retrospective exposure data, which can be prone to recall bias. Cross-sectional studies, while useful for generating hypotheses, are not suitable for establishing causality, as they cannot determine whether exposure occurred before the outcome.

Meta-analyses and umbrella reviews (reviews of meta-analyses) where results from multiple, often epidemiological, studies are synthesized systematically are considered to provide some of the highest levels of evidence in research. Their validity, however, depends

on the quality of the included studies and the number of studies in a field.

A major advantage of epidemiological, population-based studies is that they allow us to estimate the public health importance of different exposures. This includes quantitative estimation of risk per unit exposure, evaluation of important interaction (including identification of sensitive subgroups of the population) and health impact assessments.

Mendelian randomization is an increasingly popular study design in the medical field. It acts as a type of natural experiment, using genetic variants with known functions to assess whether there is a causal relationship between an exposure and a disease. A key advantage of this approach is that it minimizes confounding, as genetic variants are randomly assigned at conception.

Randomized clinical trials

RCTs are considered the gold standard for determining a causal link between an exposure and disease. This design involves randomly assigning exposure to participants, with both the exposure and the assessment of health effects conducted in a blinded manner.

Human experimental studies

In addition to RCTs, human experimental studies encompass controlled exposure studies that investigate the physiological and biological effects of tobacco and nicotine products under strictly regulated conditions. Exposure modalities in such studies generally use nicotine-naïve subjects and may include inhalation of aerosols from e-cigarettes, heated tobacco products, or other emerging nicotine delivery systems. While these studies offer valuable mechanistic insights and help establish dose-response relationships, their findings are limited to acute and reversible effects, and do not fully capture long-term health consequences. Ethical considerations also restrict the extent of exposure that can be tested in human subjects.

Animal and cell-based mechanistic studies

This report also presents evidence from **animal and mechanistic studies**, including experimental *in vivo* and *in vitro* studies. These studies may be used to assess causality in the absence of human evidence or to investigate the mechanisms responsible for health effects observed in humans or laboratory animals. The quality of such studies and their relevance to humans depend on the experimental design including the choice of model system and exposure regimen. The use of non-human cell models or exposure levels not relevant to human exposure may limit the relevance of any observed positive associations.

Results from animal studies may be considered the strongest evidence because they use an intact organism and can be used to study more complex or chronic health effects such as cancer development. However, due to species differences, the relevance to humans may be questionable depending on the health outcome of interest. An alternative is to use cell models based on human cells, where primary human cells or tissue-like cell models based on human cells are the most relevant *in vitro* models.

In addition, the exposure regimen used in such studies should be relevant to the research question, both in terms of route and level of exposure. For studies on the effects of e-cigarettes and HTPs, this means that inhalation exposure of animals is more relevant than other exposure routes. For *in vitro* studies, this would depend on the target organ. For example, for local effects in the lung, aerosol exposure of an air-liquid interface lung cell model is more relevant than exposure of submerged cells in a traditional cell culture.

Certainty of evidence

Several guidelines have been developed for assessment of the quality of scientific evidence, such as GRADE[1], which focuses on evaluating the quality of risk estimates. The evidence included in this report is generally insufficient to arrive at dose-related quantitative estimates of human health risks resulting from long-term use of e-cigarettes, HTPs or white snus. Therefore, we have settled for qualitative assessments,

i. e. the strength of the evidence for a causal association between the use of a certain nicotine/tobacco product and a specific group of health effects. Epidemiological studies provide evidence directly applicable to humans. The ability to draw causal conclusions from epidemiological data depends on whether there is supporting evidence from other types of studies, such as randomized clinical trials, Mendelian randomization studies, animal studies, or other mechanistic studies. Additionally, the number of studies in each area, their quality and the consistency of their findings play a key role in assessing the overall strength of evidence for a causal link between exposure and disease.

In this report we have used three categories to describe the strength of the evidence: strong, some and limited or no evidence. Strong evidence of a causal association requires that there is strong evidence from epidemiological studies with supporting evidence from mechanistic studies. Some evidence is used when there is at least some epidemiological evidence and supporting evidence from mechanistic studies. The lowest degree of evidence implies that there is little or no evidence from epidemiological studies together with limited or no evidence from mechanistic studies.

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Asthma and allergic diseases

Anna Bergström and Anna Zettergren

Introduction

This chapter covers the current knowledge on new nicotine products and their influence on asthma and allergic diseases, including atopic dermatitis and allergic rhinitis. Allergic disease is common and affects people of all ages but often debuts during childhood. Still, many people experience their first symptoms in adulthood. While asthma prevalence has decreased over the past 30 years the disease burden remains high but varies greatly by region and country[1]. Further, asthma remains the most common chronic disease among children[2]. Atopic dermatitis is the most common chronic inflammatory skin disease globally, with steady prevalence rates over the past 30 years[3]. Allergic rhinitis prevalences have increased over time and affect around 18% of people worldwide[4]. Allergic diseases are systemic diseases with complex pathophysiology caused by an impaired immune system. Allergic diseases are heterogenous in underlying mechanisms and manifestation but have in common that they are triggered by a faulty immune response to a harmless agent, an allergen. Most allergic responses are mediated by immunoglobin E (IgE) antibodies, but the underlying causes are due to a complex combination of genetic, immunological and environmental factors.

Smoking and brown snus

Cigarette smoking have consistently been associated with increased risk of asthma exacerbations and of suboptimal asthma control among individuals with asthma[5]. In addition, observational studies have shown that cigarette smoking increases the risk of adult-onset asthma[6], and this has also been supported by a Mendelian randomization study[7]. Studies have consistently shown an association between cigarette smoking and atopic dermatitis in adults, although there is a lack of prospective studies[81]. Moreover, cigarette smoking may be a risk factor for contact dermatitis[9]. In contrast, studies have

indicated an inverse association between cigarette smoking and rhinitis in adults[7].

Exposure to second-hand smoke (SHS) in foetal life and infancy has been consistently associated with asthma[5]. For example, collaboration between European birth cohorts has shown that exposure to maternal smoking during pregnancy increase the risk of asthma, even among children who were not exposed after birth[10] and that this association persists as the children reach adolescence[11]. SHS exposure in childhood also increases the risk of severe asthma exacerbations and impairs asthma control[5]. Although SHS exposure in early life has also been associated with rhinitis, eczema, and IgE sensitization in some studies, the evidence is not consistent[12-15].

Few studies have reported on the potential effect of snus on asthma and allergy. A Swedish cross-sectional study reported an association between snus use and asthma, also when the analyses were restricted to never-smokers[16]. In addition, a cross-sectional analysis of men and women from Norway, Sweden, Iceland and Estonia, showed an association in individuals who started to use snus in puberty, while no consistent association was found in those who started to use snus after 15 years of age[17]. No studies on the association between snus use and rhinitis or IgE sensitisation were identified. A Swedish cross-sectional study reported no association between snus use and hand eczema[18].

The association between exposure to snus use *in utero* and subsequent asthma has been reported in a few studies, while no studies have reported the association with rhinitis, atopic dermatitis or IgE sensitisation. A Swedish nation-wide register study reported no association between snus use during pregnancy and asthma in the offspring[19]. In contrast, analyses of a birth cohort with participants from Norway and Sweden indicated that *in utero* exposure to snus, as well as cigarettes, may negatively affect infant lung function[20].

Literature review

Among 1861 identified articles, 892 were included for title and abstract screening. Of these, 257 were screened in full-text and 159 articles

were excluded due for reasons including wrong outcomes, wrong study design, wrong comparator, articles funded by tobacco industry, wrong intervention, wrong patient population, wrong setting, article being retracted or unavailable.

Finally, 98 articles remained, including 6 systematic reviews and meta-analyses on e-cigarette exposure, 77 original epidemiological studies (70 on e-cigarette exposure, 5 on HTP exposure, 2 on both e-cigarettes and HTP exposure), 1 case-report on white snus, 1 human experimental study on e-cigarette exposure, 10 *in vivo* studies on e-cigarette exposure (all in mice models), 2 *in vitro* studies on e-cigarette exposure, and 1 *in silico* study on e-cigarette exposure.

In the summary below, epidemiological studies evaluating e-cigarette exposure and asthma or wheeze that were included in the systematic reviews or published during the period of inclusion are not described individually, but as part of the systematic reviews.

E-cigarettes/vapes

Epidemiological studies

In the past years, epidemiological studies on e-cigarette exposure and allergic airway disease, including asthma, have accumulated and a number of systematic reviews and meta-analyses have summarized the literature (Table 1). In 2021, several systematic reviews on primarily cross-sectional studies concluded that e-cigarette use was associated with asthma, for both current and ever use[21-23]. Pooled odds ratios (pORs) for ever e-cigarette use were between 1.24 (95% CI 1.13-1.36) and 1.39 (95% CI 1.28-1.51). When current and former use was analysed separately, a stronger association was found for current use than former use[21, 23]. The reviews included studies on adult or both adult and adolescent populations. In a systematic review and meta-analysis among adolescents only, pORs for asthma were similar for current (pOR 1.36, 95% CI 1.26-1.48) and ever e-cigarette use (pOR 1.31, 95% CI 1.22-1.42)[24]. In a later systematic review, a pOR of 1.24 (95% CI 1.19-1.30) was found for current e-cigarette use and asthma or wheeze, and the results were robust when restricting the analysis to never cigarette smokers and when assuming independency between e-

cigarettes and cigarette smoking[25]. However, there is a large overlap in included studies in the mentioned systematic reviews, which means that the pooled risk estimates are not independent of each other.

In a systematic review focused on respiratory symptoms among adults, a pooled prevalence of wheeze was found at 19% among exclusive e-cigarette users, which was comparable to dual users of e-cigarettes and cigarettes (21%), and to e-cigarette users transitioning from cigarette smoking (17%)[26]. All systematic reviews were based on self-reported information on e-cigarette use and asthma diagnosis or symptoms, primarily from cross-sectional studies, and all highlighted that there was large heterogeneity in the included studies. Further, the summarized literature could not distinguish whether e-cigarette use was related to onset of asthma or exacerbation of already established disease.

In the years following these systematic reviews, additional cross-sectional studies have been published that support previous findings. A large French population-based study in adults (n=121,186) found associations with higher asthma symptom score for ever, current and former e-cigarette users[27]. Current e-cigarette use was also associated with a higher score when restricting the analysis to never smokers. Further, two studies from the United States found that e-cigarette use was associated with higher odds ratio (OR) of asthma, both among adults (OR 1.47, 95% CI 1.21-1.78) and among high-school students aged 13-17 years (OR 1.18, 95% CI 1.02-1.37)[28-29]. On the other hand, a Swedish study found no association between e-cigarette use and any respiratory symptoms, including wheeze, in a sample of 17 325 participants[30]. However, wheeze was not evaluated as a separate outcome. In a sample of Swedish young adults, e-cigarette use was associated with experiencing breathing difficulties or wheeze, but only among those who smoked both e-cigarettes and conventional cigarettes[31]. However, in this study, the number of exclusive e-cigarette users was low (less than 1% of the population).

Currently, only a few longitudinal studies on e-cigarette exposure and subsequent allergic airway disease have been published. In the nationally representative Population Assessment of Tobacco and

Health (PATH) cohort from the United States, current exclusive e-cigarette use was borderline associated (OR 1.12, 95% CI: 0.99-1.27) with incident asthma during a 5-year follow-up in an adult sample[32]. However, another study in PATH with an additional follow-up wave found that past-30-day e-cigarette use was associated with an increased hazard ratio (HR 3.52, 95% CI 1.24-10.02) of asthma onset among young adults, compared to never smokers. On the other hand, e-cigarette use was not associated with earlier asthma onset among never smoking youths (ages 12-17 years)[33]. Similarly, in another study on adolescents in PATH, exclusive e-cigarette use was not associated with new onset of wheeze or asthma in the past year[34].

A smaller number of cross-sectional studies have examined the association between e-cigarette exposure and other allergic disease, including atopic dermatitis and allergic rhinitis. In a Japanese cohort (aged 40-69 years), the adjusted prevalence of ever e-cigarette users among men with atopic dermatitis was 13% compared 3.5% in the whole male sample, although the difference was not statistically significant. However, the number of e-cigarette users were few in the study, particularly among women[35]. In the Korea Youth Risk Behaviour, participants between the aged 12-18 years who had ever used e-cigarettes had a higher risk of a diagnosis of asthma, atopic dermatitis and allergic rhinitis compared to never smokers. However, when the analysis was restricted to exclusive e-cigarette use, only the association with atopic dermatitis remained borderline statistically significant (OR 1.34, 95% CI 1.00-1.80[36]. On the other hand, in an adult Korean cohort (Korean National Health and Nutrition Examination Survey (KNHANES)), current e-cigarette use was associated with allergic rhinitis (OR 1.38, 95% CI1.15-1.66) [37]. However, only 1.5% of e-cigarette users were never smokers[37]. Furthermore, in the National Health Interview Survey (NHIS) from the United States, e-cigarette use was associated with an increased occurrence of atopic dermatitis among adults (OR 1.35, 95% CI 1.16-1.58) in the total study population and among never smokers (OR 1.28, 95% CI1.28-2.02)[38]. In stratified analysis by sex, the risk was only higher among women. In another study from the NHIS, parental ever e-cigarette use was also associated

with atopic dermatitis or other skin allergy in children (OR 1.24, 95% CI 1.08-1.42), also among non-smokers (OR 1.37, 95% CI 1.05-1.78)[39].

In a pilot study from the United Stated, plasma levels of immunoglobin E (IgE) and immunoglobin G (IgG) were compared between never smoking e-cigarette users and never-tobacco users. E-cigarette users had significantly increased levels of IgE, while no difference was observed for IgG, indicating an altered immune response among e-cigarette users specifically related to allergic mechanisms[40].

Mechanistic studies

Several studies have examined underlying mechanisms of the association between e-cigarette exposure allergic disease, primarily allergic airway disease (Table 1). An experimental study on human participants found differences in sputum collected from e-cigarette users and non-smokers, including elevated levels of innate immune defence proteins (including matrix metalloproteinase-9 (MMP-9), increased neutrophil activity and higher concentrations of the MUC5AC protein. These changes have been observed in asthmatics, but also in other airway diseases including chronic obstructive pulmonary disease (COPD), making the findings more general to airway disease and not specific to allergic disease[41]. The smoking status of the participants was validated using serum cotinine, but the authors highlighted that 12 of the 15 e-cigarette users were previous cigarette smokers. Another study found increased levels of MMP-9, MMP-2 and neutrophil elastase in bronchial lavage fluid (BALF) from e-cigarette users as compared to non-smokers, which can be found in asthmatics, but also in patients with other airway diseases[42]. In an experimental study where small airway epithelial cells from human donors without airway disease were exposed to e-cigarette vapor, cells exposed to nicotine-free e-vapor exhibited increased levels of IL6 and IL6 dependent MUC5AC[43]. However, nicotine containing e-vapor did not exhibit any effect. Increased MUC5AC and altered small airway function occurs in asthma, but also in COPD.

A number of *in vivo* studies have used mice models that are intended to mimic asthma in order to study effects of e-cigarette exposure. In several studies, e-cigarette exposure pronounced asthmatic features

in asthmatic mice[44-49]. For example, one study found that e-cigarette exposure increased airway hyperresponsiveness, infiltration of immune cells in the airway, production of t-helper 2 (Th2) type cytokines and MUC5AC levels among asthmatic mice[47]. Additionally, one study found that nicotine vapour modulated airway allergic response through nicotine receptors, by suppressing eosinophil response to allergen challange[45]. In several studies, the observed effects were dependent on nicotine content and e-vapor flavour. In one study, both nicotine containing and nicotine-free e-cigarettes suppressed airway inflammation, but only nicotine-free flavours increased airway remodelling and hyperresponsiveness[46]. Another study showed that glycerine-based, but not propylene glycol-based, e-cigarette aerosols induced airway hyperresponsiveness, both with and without nicotine[50]. One study found that short term (3 days, 2 hours per day) e-cigarette exposure increased inflammatory response and features often seen in asthmatics and allergic disease, including increased immune cell infiltration in lungs and upregulations of asthma-related proteins[51]. Importantly, the observed effects varied by nicotine exposure, flavour and commercial brand. Another short-term exposure study found similar results in an asthmatic mouse model, along with increased IgE levels and mitochondrial damage in lung tissue[48]. The response differed somewhat between male and female mice but generally indicated that e-cigarettes exacerbated asthma features among asthmatic mice. A study on long-term exposure (daily for 3 months) in asthmatic mice similarly showed induced airway remodelling processes and increased production of asthma related proteins, in a nicotine dependent manner[49].
Furthermore, mice models have studied second-hand e-cigarette exposure in offspring to dams exposed during the gestation period. One study found that in utero exposure resulted in impaired lung development at birth. Further, in older offspring, asthma features were exacerbated[52]. Similarly, another study found that in utero exposure increased airway inflammation in adult offspring, as well as dysregulation of genes involved in asthma and allergies[53].

The existing *in vivo* or *in vitro* studies have all focused on airway allergic disease. However, one *in silico* study identified two classes of e-

cigarette flavouring chemicals, alkenylbenzenes and aldehydes, that are known to form DNA adducts in human cells. The authors then used predictive computational toxicology methods and found strong concordance between these DNA adducts and prediction of skin sensitization[54].

White snus

We found one case-report on white snus, in which a female patient had developed a contact allergy to carvone, a mint-flavour chemical, after using mint-flavoured white snus[55] (Table 2). The patient presented with chronic inflammatory lesions in the oral mucosa, which disappeared when she switched to a non-mint flavoured snus.

Heated tobacco products

Epidemiological studies

The literature on HTP use and allergic disease is limited, but a few cross-sectional studies have been published in recent years, all from Japan and Korea (Table 3). In a sample of Japanese adults (aged 40-69 years), the authors aimed to compare prevalence of chronic diseases (including asthma and atopic dermatitis) between HTP users and non-users. However, HTP use was too low in the study population for any comparisons to be made, with only 0,8% of men and 0% of women having ever used HTPs[35]. In the Japan Society and New Tobacco Internet Survey (JASTIS) past-30-day HTP use was more common among asthmatics aged 15-73 years (OR 3.97, 95% CI 1.73-911)[56]. However, in adults over 40 years in JASTIS, asthma was only associated with HTP use among current smokers, while no association was observed with HTP use among never smokers[57]. In a later study from JASTIS, also including data from the Japan COVID-19 and Society Internet Survey (JASCIS), frequent exposure to second-hand HTP aerosols in the past year was associated with an increased risk of asthma attacks or asthma-like symptoms (Prevalence ratio 1.49, 95% CI 1.21-1.85)[58].

Furthermore, data from JASCIS showed that both former and current HTP use by mothers in the antenatal period increased the prevalence ratio of any allergic disease diagnosis in children up to 2 years, in a

dose-response manner depending on number of heat sticks used[59]. Among 12–18-year-olds in the Korea Youth Risk Behaviour, ever HTP use was associated with increased risk of diagnosis of asthma, atopic dermatitis and allergic rhinitis. However, only the risk of asthma remained significant when excluding other tobacco use (OR 3.59, 95% CI 1.47-8.78)[36]. In KNHANES, current exclusive HTP use was associated with allergic rhinitis (OR 1.60, 95% CI 1.06-2.24) compared to never smokers, but not with asthma or atopic dermatitis[60].

Mechanistic studies

No mechanistic studies were identified on HTP exposure and allergic diseases.

Conclusions

The current literature on new nicotine products and allergic disease is largely focused on e-cigarette use and asthma or wheeze. The existing evidence suggests that e-cigarette use increases the risk of asthma and wheeze, but published studies are heterogeneous with regards to exposure classification and control of cigarette smoking. Nonetheless, several studies have confirmed increased risks among e-cigarette users without current and ever cigarettes smoking. Recent longitudinal studies confirm previous cross-sectional findings on e-cigarette use and new onset of asthma in adults, but not among adolescents, although there are few such studies. These findings mirror what is seen for conventional cigarettes, which have been related to asthma onset among adults. The epidemiological studies are supported by mechanistic data, where e-cigarettes have been shown to induce important asthma features including mucus production, tissue remodelling, airway hyperresponsiveness and airway infiltration of immune cells. However, *in vivo* data indicates that the relation may depend on e-liquid content. These results further highlight the need for future studies to characterize exposure in more detail. For e-cigarettes and other allergic disease, cross-sectional data suggests association with atopic dermatitis and allergic rhinitis as well, although the evidence is limited. Further, the research on HTPs is very limited, but indicates similar disease risks as studies on e-cigarettes. For nicotine pouches/white snus, no studies on associations with allergic disease

were found, presenting a large knowledge gap for this prevailing new nicotine product. Moreover, second-hand exposure has not been adequately studied, which is important given the second-hand cigarette smoke exposure is an important risk factor for allergic disease. This includes exposure during foetal and early life, for example through maternal use during pregnancy. Lastly, the current literature heavily relies on self-reported exposure and outcome data, which further needs to be supplemented by clinical or validated data.

TABLE 1. SUMMARY OF LITERATURE REVIEW: STUDIES INVESTIGATING E-CIGARETTE USE AND ALLERGIC OUTCOMES.

Author and year	Study design	Setting	Exposure	Outcome	Covariates	Results
Epidemiological studies						
Chand & Hosseinzadeh, 2022[21]	Systematic review and meta-analysis. Cross-sectional (n=13)	Adolescents and adults. Canada (n=1), Kuwait (n=1), South Korea (n=4), USA (n=7). 8 studies from nationally representative surveys. Search conducted March 2021.	Current and ever e-cig use	Asthma diagnosis	NA. 10 studies considered of "good" and 4 studies of "fair" quality according to AXIS criteria	Pooled OR (95% CI) current use: 1.36 (1.21, 1.52), ever use: 1.24 (1.13, 1.36).
Glantz et al, 2024[25]	Systematic review and meta-analysis. Cross-sectional (n=41)	Adults and children. Canada (n=1), China (n=1), Kuwait (n=1), South Korea (n=2), Sweden (n=1), USA (n=35). Several studies from nationally representative samples. Search conducted October 2023.	Current exclusive e-cig	Asthma diagnosis or wheeze	NA. Low risk of bias according to ROBINS-E criteria.	Pooled OR (95% CI): 1.24 (1.19, 1.30).
Wills et al, 2021[22]	Systematic review and meta-analysis. Cross-sectional (n=15)	Adolescents and adults from large representative samples. Canada (n=1), China (n=1), South Korea (n=3), USA (n=10). Search conducted in March 2020	Current and ever e-cig use	Asthma diagnosis or symptoms	NA.	Pooled OR (95% CI): 1.39 (1.28, 1.51)
Alqahtani et al, 2023[26]	Systematic review and meta-analysis. Cross-sectional studies (n=6)	Adults. Canada (n=1), USA (n=5). Search conducted April 2023.	Exclusive e-cig use	Wheeze	NA. 4 studies considered to have low, and 2 studies unclear risk of bias according to NIH	Pooled prevalence of wheeze (95% CI) 19% (0.12, 0.30)

					Study Quality Assessment Tools.	
Xian & Chen, 2021[23]	Systematic review and meta-analysis. Cross-sectional (n=11)	Adolescents and adults. From South Korea (n=4) & USA (n=7). Studies published before August 2020.	Current and former e-cig use	Asthma diagnosis	NA. 6 studies considered high quality, and 5 studies considered moderate according to AHQR scores.	Pooled OR (95% CI) current use: 1.30 (1.17, 1.45), former use: 1.22 (1.08, 1.39).
Li et al, 2022[24]	Systematic review and meta-analysis. Cross-sectional (n=10)	Adolescents. Studies published before February 2021.	Ever, current and ever e-cig use	Asthma	NA. All studies considered at moderate risk of bias according to AHQR scores.	Pooled OR (95% CI) for ever use: 1.31 (1.22, 1.42), current use: 1.36 (1.26, 1.48), former use: 1.20 (1.12, 1.28).
Delmas et al, 2024[27]	Cross-sectional	Adults aged 18-69 years from Constance's cohort, France, 2015-2019. n=121,186	Ever, current and former e-cig use	Asthma symptom score	Cigarette smoking, cannabis use, sex, age, education, BMI and history of chronic respiratory disease.	Mean score ratio (95% CI) for current use: 1.34 (1.28, 1.41), former use: 1.39 (1.33, 1.45). Mean score ratio for ever use among never smokers: 1.40 (1.14, 1.72)
Dirisanala et al, 2023[28]	Cross-sectional	Adults over 18 years from NHANES, USA, 2015-2018. n=178,157	Ever e-cigarette use	Prevalent asthma	Age, sex, race, annual household income, comorbid conditions	OR: 1.47 (95% CI: 1.21, 1.78).
Hedman et al, 2024[30]	Cross-sectional	Participants aged 16-69 years. From OLIN and WSAS cohorts, Sweden, 2016. n=17,325	E-cig use among never, former and current smokers	Any respiratory symptoms, including wheeze	Sex, age, socioeconomic status, number of symptoms as study baseline	OR (95% CI) for e-cig use among never smokers: 0.48 (0.10, 2.29)

Jackson et al, 2020[40]	Cross-sectional	Healthy adults without current respiratory symptoms from USA, 2016-2019. n=46	Current e-cig use, validated by plasma cotinine.	Plasma levels of IgE and IgG	No adjustments made	E-cig users had higher IgE levels than non-smokers, but not higher IgG levels.
Kioi & Tabuchi, 2018[35]	Cross-sectional	Adults aged 40-69, Japan, 2015. n=4,432	Current and ever e-cig use	Ever diagnosis or regular hospital visits for asthma and/or atopic dermatitis	Inverse probability weighting for, based on demographic factors including education and housing tenure	Adjusted prevalence of asthma for ever e-cig use was 13% as compared to 3.2% (p<0.17), and 1.2% (p<0.14) for current use. Adjusted prevalence of atopic dermatitis for ever e-cig use was 13% as compared to 3.5% (p<0.12), and 1.2% (p<0.15) for current use.
Lee et al, 2019[36]	Cross-sectional	Students aged 12-18, from KYRBWS, South Korea, 2018. n=58,336	Ever e-cig use	Diagnosis of asthma, allergic rhinitis, atopic Dermatitis within the past year.	Age, sex, obesity, residential area, family economic status, and physical activity.	OR (95% CI) for asthma: 1.23 (1.00, 1.52), allergic rhinitis: 1.08 (1.00, 1.18) and atopic dermatitis: 1.32 (1.18, 1.49). Exclusive e-cig use only associated with atopic dermatitis (OR 1.34 (1.00, 1.80).
Pérez et al, 2024[33]	Prospective	12-17 years and adults over 18 without asthma or COPD at baseline from PATH	Current e-cig use at baseline (wave 1)	Age of asthma onset	Sex, race, education level, parental education level,	HR (95% CI) for asthma onset was 3.52 (1.24, 10.02) for

		cohort, USA, 2013-2021 (6 study waves). n=24,789			alcohol use, cannabis use, weight status	adults and 1.79 (0.67,4.77) for youths.
Qeadan et al, 2023[32]	Prospective	Adults from PATH cohort, USA, 2014-2018 (wave 1-5). n=18,893	Current, exclusive, e-cig use	Self-reported adverse respiratory condition, including asthma diagnosis in past 12-months	Age, sex, race/ethnicity, region of residence, marital status, education, income, employment status, health insurance, cigarette smoking, heavy alcohol consumption, weight status, diabetes, prior adverse respiratory conditions.	OR (95% CI) of asthma over follow-up period: 1.12 (0.99, 1.27)
Rha et al, 2022[37]	Cross-sectional	Adults over 19 from KNHANES, South Korea, 2013-2019). n=38,413	Current and former e-cig use	Allergic rhinitis diagnosis	Age, body mass index, sex, income, education level, smoking history, alcohol consumption, hypertension, diabetes mellitus, asthma, atopic dermatitis, and pulmonary tuberculosis	OR (95% CI) of allergic rhinitis for current e-cig use: 1.38 (1.15, 1.66), OR for former use: 1.06 (0.94, 1.20).
Roh et al, 2023[29]	Cross-sectional	High school students aged 13-17 from YRBSS cohort, USA, 2015-2019. n=3042 in	Ever e-cig use (among never smokers)	Ever asthma diagnosis	Age, sex, race/ethnicity, BMI status, combustible product use, other	OR (95% CI) for asthma in Texas cohort: 1.32 (1.06–

		Texas cohort, n=32,885 in US cohort			substance use, and depression.	1.66), US cohort: 1.18 (1.02–1.37).
Smith et al, 2023[38]	Cross-sectional	Adults over 18 from National Health Interview Survey, USA, 2021. n=28,563	Ever e-cig use	Atopic dermatitis	Age, education, race, income, sex, diabetes, cigarette-smoking status, asthma, and BMI.	Ever e-cig use OR (95% CI): 1.35 (1.16, 1.58). OR excluding ever smokers: 1.61 (1.28, 2.02).
Sompa et al, 2022[31]	Cross-sectional	Young adults aged 22-25 from BAMSE cohort, Sweden, 2016-2019. n=3,055	Current exclusive e-cig use	Breathing difficulties or wheeze.	Sex, educational level, occupational status, waterpipe use, second-hand tobacco exposure, former tobacco use.	OR (95% CI) for exclusive e-cig use: 1.2 (0.3, 3.8).
Yao et al, 2024[34]	Prospective	Adolescents aged 12-17 at baseline from PATH, USA, 2017-2019. n=11,748	Current (past-30-days) exclusive e-cig use	Wheezing or whistling in the chest in past year, new asthma diagnosis, and asthma-related interference with activities in the past 30 days.	Current cannabis use, age, sex, race/ethnicity, household income, parents' education, home tobacco rules, living with tobacco use, wheeze at baseline.	OR (95% CI) for wheezing or whistling in chest:1.03 (0.69, 1.51), OR new asthma diagnosis: 0.37 (0.07, 1.87), OR asthma-related interference with activities: 1.39 (0.63, 3.09).
Youn et al, 2024[39]	Cross-sectional	Households with children under 18 years from NHIS, USA, 2014-2018. n=35,479	Parental ever e-cig use	Atopic dermatitis in past year in child.	Asthma, allergic rhinitis, respiratory allergies, parental smoking history, age, sex, race/ethnicity, insurance status, parents age, sex,	OR (95% CI) of atopic dermatitis in children for parental e-cig use: 1.24 (1.08, 1.42), among non-smokers: 1.37 (1.05, 1.78).

					income, region of residence.	
Experimental studies						
Cahill et al, 2022[53]	*In vivo*, adult off-spring of exposed mice, BALB/c	Dams exposed to mint-flavoured JUUL aerosol, 1-hr/d, 20 consecutive days during gestation. Offspring were sacrificed at birth or at 11-week of age after HDM challenge. 9 dams exposed, 8 control dams. Litter groups of 4-5 pups.	In utero exposure to mint-flavoured JUUL, air as control	Gene expression in uterine/placental tissue of the dams. Lung response in offspring.	NA	JUUL exposed mice showed gene dysregulation in both dams and offspring, including genes associated with organ development and inflammation. At 11 weeks of age, JUUL exposed mice exhibited pulmonary inflammation and dysregulation of several genes associated with allergies and asthma.
Chapman et al, 2019[46]	*In vivo,* BALB/c murine asthma model	Balb/c mice were challenged with HDM (Days 0, 7, 14–18) and exposed to room air or e-cigarette aerosol for 30 min twice daily, 6 days/week from Days 0–18 (n = 8–12/group).	Room Air (control), vehicle control (50%VG/%50PG) , Black Liquorice, Kola, Banana Pudding or Cinnacide without or with 12 mg/mL nicotine.	Airway hyperresponsiveness after methacholine challenge, BALF cell content, collagen content in lung, histopathology. Assessment 72 hours after the final HDM challenge.	NA	E-cig vapours containing nicotine suppressed airway inflammation but did not alter airway hyperresponsiveness or airway remodelling. Flavoured e-cig vapours without nicotine had significant but heterogeneous

						effects on features of allergic airways disease.
Gahring et al, 2020[45]	*In vivo*, unspecified mouse strain	12-min exposure of aerosols, twice daily for 5 days/wk. for 8 wk. Separate experiment exposed mice to oral nicotine through water (ad libitum). Mice were challenged HDM for 5 consecutive days, with maintained nicotine exposure. Each experiment used groups of 3–5 sex- and strain matched mice that were 3–6 months of age.	Nicotine aerosols or nicotine in water, 40 mg/ml concentration. Water aerosol and drinking water as controls	BALF cell counts, nicotine receptor modulator effects, alveolar macrophages. Assessment 96 h post final HDM challenge.	NA	Nicotine aerosols reduced HMD-induced recruitment of eosinophils through the alpha7 nicotine receptor, suggesting that e-cig vapor may modify allergic airway response.
Gellatly et al, 2020[43]	*In vitro* study on exposure to airway epithelial cells from human donors without known lung disease.	Small airway epithelial cells directly exposed to aerosol, 15 puffs over 24 h.	Tobacco flavour e-liquid with and without nicotine (2.4%), with PG/VG. Air as control	MUC5AC levels, MUC5B expression, cytotoxicity, Trans-epithelial electrical resistance, IL-6 levels.	NA	Nicotine free, but not nicotine containing e-vapor increased inflammatory response and mucus production in airway epithelial cells.
Ghosh et al, 2019[42]	*In vitro* study on human cells from healthy donor	Bronchoscopies performed on healthy non-smokers, cigarette smokers and e-cigarette smokers (14 in each group). Sputum and BAL collected. Also, neutrophils exposed e-liquid components for 4 h.	Never smokers, current cigarette smokers, e-cigarette smokers (9 of 14 were former cigarette smokers who quit >6 months	Protease activity. MMP-2/9 activity specifically by zymography.	age matched participants	E-cig users had increased protease activity in BALF. Similar cells count of neutrophils between all exposure groups. Nicotine containing vapor induced

			ago). Validated by cotinine measurements. Neutrophils exposed e-liquid components with and without nicotine (18 mg/ml) or an equivalent amount of nicotine in media.			neutrophil elastase release.
Kang and Valerio, 2020[54]	*In silico*, predictive modelling	Identification of chemical classes in e-cig liquids that are documented to form DNA adducts, using literature searching and *in silico* software.	Alkenylbenzene and aldehyde flavour chemicals (found in e-liquids)	Prediction of toxicity of DNA adduct formations.	NA	High concordance with computational predictions for skin sensitivity for both alkenylbenzene and aldehyde flavouring chemicals.
Larcombe et al, 2017[50]	*In vivo* study in BALB/c mice	Female mice were exposed for 8 wk. to tobacco smoke, medical air (control), or one of four different types of e-cigarette aerosol. 6 mice per group	Air (control), cigarette smoke, 0% nicotine PG, 12% nicotine PG, 0% nicotine VG, 12% nicotine VG.	Pulmonary inflammation, lung volume, lung mechanics, responsiveness to methacholine.	NA	Mice exposed to e-cigarette aerosol displayed decreased lung function but not increased inflammation. Mice exposed to glycerine-based e-cigarette aerosols displayed airway hyperresponsivenes s regardless of the

						presence or absence of nicotine.
Lee et al, 2024[47]	*In vivo* study using an OVA-induced murine asthma model	Asthma was induced in mice through sensitization to OVA. E-cig vapor exposure once daily (2x 30 min with 45 puffs). controls were exposed to room air. 4 exposure groups, 4 in each group per experiment, 3 experiments.	E-cig vapor exposure with and without OVA sensitization. Air as control.	Airway hyperresponsiveness, immune cell counts in BALF, lung tissue histology, mRNA levels of MUC5AC and MUC5B from lung tissue, levels of cytokines, MUC5AC and MUC5B.	NA	E-cig vapor exposed mice showed airway hyperresponsiveness, increased immune cell infiltration in airways, higher levels of Th2 mediated inflammatory cytokines and increased pathologic mucus production.
Muthumalage and Rahman, 2023[51]	Experimental, in vivo study using C57/BL67 and BALB/cJ mice	Exposure of 2 puffs/min for 2h exposure time, 3 days. 8-10 mice per strain and group, males and females, 8–10-week-old at start.	Brand A flavour (menthol), nicotine free and 6 mg nicotine. Brand B (tobacco), nicotine free and with 6 mg nicotine. PG/VG and air control.	Genotoxicity assessment, flow cytometry analysis in BALF for cell count, immunoblot, inflammatory mediators, gene expression, proteomics, mitochondrial bioenergetics.	NA	Outcomes in mice varied by nicotine content, flavour and mice strain. Several exposed groups displayed immune cell influx in airways and suppression of inflammatory cytokines.
Noël et al, 2023[52]	*In vivo* study in adult off-spring of exposed mice, BALB/c	Pregnant mice were exposed e-cig aerosols (1 puff every 30 s and a 55 mL puff volume) for 2 h per day throughout gestation (20 days). One group of off-spring were sacrificed at	Vanilla-flavoured e-cig aerosols (18 mg/mL of nicotine (50/50 PG/G)), filtered air as control	Lung function by plethysmograph, BALF cell content, histopathology evaluation, gene expression in lung tissue.	NA	Lung transcriptome was significantly altered in exposed newborns, in a sex specific manner. In males, genes involved in T-cell

		birth. A subgroup of male off-spring was immune challenged with HDM for 3 weeks, starting from 4 weeks of age. 15 control dams, 9 exposed dams.				immune response and signalling was up-regulated. Female offspring has dysregulated signalling in pathways involved in oxidative stress responses. In utero exposures to vanilla-flavoured e-cig aerosol exacerbated HDM-induced asthma in 7-week-old male mouse offspring. These mice had higher counts of neutrophils and eosinophils in lung tissue.
Riedel et al, 2018[41]	Experimental, samples from human participants	Healthy adults aged 18-50, USA. 14 current cigarette smokers, 15 current e-cigarette users and 15 never smokers.	Exposure status validated by serum cotinine and urine 4-(methylnitrosamino)-1-(3-pyridyl)-1butanol levels.	Mucin concentrations, peripheral blood neutrophils and NET levels, quantitative proteomics from induced sputum samples.	NA. 12 of 15 e-cig users were former cigarette smokers	E-cig users had altered mucus profile of innate defence proteins including degranulation of neutrophils, a feature of severe asthma and COPD.
Song, Kim et al, 2023[48]	*In vivo* study using C57/BL67 mice	Asthma induced and naïve adult mice exposed daily for 2 weeks (4 h/day, 5	Filtered air (control), PG/VG with nicotine (20	Immune cell count in BALF, PAS staining in lung	NA	E-cig exposed mice displayed toxic responses, with

		days/wk.). Groups of 8-16 mice.	mg/ mL) aerosols, PG/VG aerosol without nicotine	tissue, cytokines in BALF, total IgE in serum, protein assay from lung epithelial cells, RNA expression in lung tissue, mtDNA count in lung tissue.		stronger effects in males. including mitochondrial damage, inflammation and airway remodelling.
Song, Wold et al, 2023[49]	*In vivo* study using C57/BL67 mice	Asthma induced and naïve adult mice exposed 4 hours daily for 3 months.	Filtered air (control), PG/VG with nicotine (20 mg/ mL) aerosols, PG/VG aerosol without nicotine	Cytokines in BALF, PAS staining, mitochondrial DNA copy number, lung transcriptome.	NA	Aerosols containing nicotine increased inflammatory response in asthmatic mice and altered gene expression.
Taha et al, 2020[44]	*In vivo* study using BALB/c murine OVA asthma model	Adult male Balb/c mice exposed 1h daily for 4 weeks. 7-10 mice per group.	E-cig vapor exposure with and without OVA sensitization. Air as control.	BALF and lung tissue were evaluated for inflammatory cells and inflammatory mediators, respectively.	NA	E-cig exposed mice displayed increased immune cell infiltration in airways and airway remodelling, in both asthma model and control mice. Slightly higher response in asthma mice.

E-cig, Electronic cigarettes; NA, Not applicable; OR, odds ratio; 95% CI, 95% confidence interval; ROBINS-E, Risk Of Bias In Non-randomized Studies – of Exposure; NIH, National Institutes of Health; AHQR, Agency of Healthcare Research and Quality; BMI, body mass index; NHANES, National Health and Nutrition Examination Survey; OLIN, Obstruktiv Lungsjukdom I Norrbotten; WSAS, West Sweden Asthma Study; IgE, Immunoglobin E; IgG, Immunoglobin G; KYRBWS, Korea Youth Risk Behavior Web-based Survey; COPD, chronic obstructive pulmonary disease; PATH, Population Assessment of Tobacco and Health; KNHANES, Korea National Health and Nutrition Examination Survey; YRBSS, Youth Risk Behavior Surveillance System; BAMSE, Barn Allergi Miljö Stockholm Epidemiologi; NHIS, National Health Interview Survey; NET, Neutrophil extracellular traps; HDM, house dust mite; VG, vegetable glycerine; PG, propylene glycol; BALF, bronchoalveolar lavage fluid; OVA, ovalbumin; Th2, T-helper 2 cells; IL-6, interleukin 6.

TABLE 2. SUMMARY OF LITERATURE REVIEW: STUDIES INVESTIGATING WHITE SNUS AND ALLERGIC OUTCOMES.

Author and year	Study design	Setting	Exposure	Outcome	Covariates	Results
Epidemiological studies						
Adwa et al, 2024[55]	Case-report	Swedish woman aged 52 years, seeking care for inflammation of the oral mucosa.	Use of mint-flavoured white snus, 10 pouches per day for the past 10 years.	Patch-testing for carvone and mint-flavoured snus pouch	NA	Patient tested positive for contact allergy for carvone and snus pouch.

NA: Not applicable

TABLE 3. SUMMARY OF LITERATURE REVIEW: STUDIES INVESTIGATING HEATED TOBACCO PRODUCTS AND ALLERGIC OUTCOMES.

Author and year	Study design	Setting	Exposure	Outcome	Covariates	Results
Epidemiological studies						
Lee et al, 2019[47]	Cross-sectional	Students aged 12-18, from KYRBWS, South Korea, 2018. n=58,336	Ever use of HTP	Diagnosis of asthma, allergic rhinitis, atopic dermatitis within the past year.	Age, sex, obesity, residential area, family economic status, and physical activity.	Ever HTP use OR (95% CI) for asthma: 1.78 (1.37, 2.32), allergic rhinitis: 1.21 (1.06, 1.38) and atopic dermatitis: 1.58 (1.34, 1.87). Exclusive HTP use only significantly associated with asthma (OR: 3.59, 95% CI 1.47, 8.78).
Kioi and Tabuchi 2018[35]	Cross-sectional	Adults aged 40-69, Japan, 2015. n=4,432	Current and ever HTP use	Ever diagnosis or regular hospital visits for asthma and/or atopic dermatitis.	Inverse probability weighting for, based on demographic factors including education and housing tenure.	Underpowered to draw any conclusions of prevalences of asthma and atopic dermatitis among both current and ever HTP users
Nakama & Tabuchi, 2021[56]	Cross-sectional	Participants aged 15-73 from JASTIS cohort, Japan, 2019. n=9,008	Curren HTP use	Asthma	Age, sex, and cigarettes, equivalent household income, education, and drinking status.	OR (95% CI) of asthma for current HTP use: 3.97 (1.73, 9.11).
Noguchi et al, 2023[57]	Cross-sectional	Data from JASTIS, Japan, 2022, adults aged over 40 years.	Current asthma (self-report) or asthma together with COPD (ACO).	Current HTP use (past-30-days) among never smokers, current HTP use among former smokers, current HTP use	Sex, age, educational attainment, equivalent household income, and alcohol intake.	Asthma not associated with current HTP use among never smokers, but associated with lower odds of HTP use among past smokers and higher OR of HTP among current

				among current smokers.		smokers. ACO also associated with increased OR of HTP use among current smokers.
Seo et al, 2023[60]	Cross-sectional	Adults over 19 from KNHANES, South Korea, 2018-2020. n=18,230	Current exclusive HTP use	Diagnosis of allergic rhinitis, asthma, atopic dermatitis	Age, sex, education level, household income, alcohol consumption, physical activity, obesity, comorbidities.	OR (95% CI) for allergic rhinitis: 1.60 (1.06, 2.42), for asthma: 0.59 (0.15, 2.33), for atopic dermatitis: 0.87 (0.48, 1.58).
Yoshioka et al, 2023[58]	Cross-sectional	Current non-tobacco users' participants ages 15-80 from JASTIS/JACSIS cohort, Japan, 2021. n=18,839	Second-hand aerosol exposure from HTP, frequently in the past year	Asthma attack, asthma-like symptoms	Age, sex, education, marital status, household size, household income, past cigarette smoking, past HTP use, past other tobacco use, second-hand smoke, asthma, bronchitis or pneumonia.	Prevalence ratio (95% CI) of asthma attacks or asthma like-symptoms: 1.49 (1.21-1.85).
Zaitsu et al, 2023[59]	Cross-sectional	Postpartum mother-infant pairs from JACSIS cohort, Japan, 2021. n=5,688	Any HTP use period in (a) 3 months before pregnancy; (b) during pregnancy (c) after birth	Diagnosis of asthma, rhinitis, conjunctivitis and atopic dermatitis in infants ages 0-2 years	Maternal age, educational attainment, occupation, household income, combustible cigarette smoking before pregnancy, maternal history of allergic diseases, COVID- 19 infection before pregnancy, and partner's smoking status.	Prevalence ratio (95% CI) of any allergic disease in child: 1.98 (1.28, 3.05).

KYRBWS, Korea Youth Risk Behavior Web-based Survey; HTP, heated tobacco product; OR, odds ratio; 95% CI, 95% confidence interval; JASTIS, Japan Society and New Tobacco Internet Survey; COPD, chronic obstructive pulmonary disease; ACO, Asthma and COPD; KNHANES, Korea National Health and Nutrition Examination Survey; JACSIS, Japan COVID-19 and Society Internet Survey.

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Cancer

Kristian Dreij

Introduction

Cancer is a complex group of diseases characterized by uncontrolled cell growth, and it remains a leading cause of death worldwide. According to the World Health Organization (WHO), cancer accounted for nearly 10 million deaths in 2022[1], with the most common types being lung, breast, colorectal, prostate, and stomach cancer. Breast cancer, the most frequently diagnosed cancer in women, sees about 2.3 million new cases annually, while lung cancer, the leading cause of cancer death, results in approximately 1.8 million deaths each year, primarily due to smoking. Colorectal cancer, affecting the colon and rectum, accounts for around 1.9 million new cases yearly, with early detection significantly improving outcomes. Prostate cancer, the second most common cancer in men, has about 1.4 million new cases annually, often detected early through screening[2]. Stomach cancer, or gastric cancer, is more prevalent in East Asia, with about 1 million new cases each year[1].

Recent trends show a decrease in lung cancer incidence in high-income countries due to reduced smoking rates, while cancers related to obesity, such as colorectal and breast cancer, are on the rise[3]. Advances in treatment, including immunotherapy and targeted therapies, are improving survival rates and quality of life for many cancer patients[2].

Smoking and brown snus

Tobacco smoking is known to cause cancer of several organs, including the lung, oesophagus, pancreas, and bladder. Tobacco smoking is the leading cause of lung cancer globally and the relative risk for smokers is >20 times that of nonsmokers[1, 4]. Passive smoking is also classified as a human carcinogen with similar target organs [4]. For smokers, duration of smoking and number of pack-years are the most important determinants of cancer development. Tobacco smoke both initiates and promotes cancer development by inducing DNA damage, mutations and tumorigenesis[5]. This is attributed to several established organic and inorganic carcinogens present in tobacco smoke, including polycyclic aromatic hydrocarbons (PAHs), nitrosamines, carbonyl aldehydes, and cadmium. The association between the use of smokeless tobacco products, such as Swedish snus, and cancer development is not as well established as for tobacco smoke, but several studies show a moderately increased risk of cancer of the oral cavity, oesophagus, pancreas and similar to tobacco smoke due to the presence of carcinogens such as nitrosamines[4, 6, 7]. Several of these chemicals or their metabolites have been detected in the urine and blood of users, as has the DNA damage they induce, *e.g.*, in the respiratory tract of smokers, supporting the link between exposure and genotoxicity, an important mode of action in cancer development[4, 8].

Because of its addictive properties and central role in tobacco products, the potential carcinogenic properties of nicotine are of concern. Although there is no conclusive evidence that nicotine is carcinogenic in humans and animals, evidence from experimental studies indicate that nicotine may form carcinogenic tobacco-specific nitrosamines (TSNAs) and enhance tumour growth and progression[8-10].

Literature review

The literature search identified 15 epidemiological studies on e-cigarettes or HTPs and cancer, tumour promotion and progression, genotoxicity, or mutagenicity published between 2016 and 2024 (Table 3). An additional 29 experimental studies published between 2016 and 2023 were identified that examined the same effects and their possible mechanisms in animals or cell models (Table 3). Most of the studies focused on effects on lung or oral mucosa, so the text presents evidence from these two target organs separately, with support from studies involving other tissues (e.g., urinary bladder or urinary biomarkers). Most studies reported on the effects of e-cigarettes (40/44) and fewer on HTPs (6/44). No studies were identified that investigated the association between white snus and cancer.

In addition, three reviews were identified that summarized the existing literature on biomarkers of exposure from e-cigarette and HTP use, many of which are human carcinogens[11-13]. For e-cigarettes, users had higher urinary concentrations of several carcinogenic compounds compared to non-users, including volatile organic compounds (VOCs, *e.g.*, acrylamide), metals (e.g., cadmium), and PAHs (*e.g.*, 1-hydroxypyrene). For many other biomarkers such as TSNAs, the data were conflicting due to heterogeneous reporting. There is consistent evidence that most biomarkers of concern are lower in e-cigarette users compared to cigarette smokers and dual users, and that switching to e-cigarettes also reduces these biomarkers[11, 13]. For HTPs, biomarkers of exposure have only been compared with cigarette smokers, and show reduced levels of several biomarkers including nicotine, TSNAs and PAHs[12]. All three reviews concluded that the small number of available studies and heterogeneous reporting calls for additional rigorous studies that include both biomarkers and health outcomes to confirm their association.

E-cigarettes

Lung cancer

Epidemiological studies

The development of cancer can take several years or decades, depending on the specific type of cancer and individual factors. Since e-cigarettes and other new tobacco products were introduced to the global market in the early 21st century, it has not yet been feasible to investigate the association between their use and the development of cancer in humans.

Bittoni et al. addressed this challenge by instead examining the association of dual use of e-cigarettes and cigarette smoking with lung cancer in a case control study including 4975 newly diagnosed lung cancer cases and 27,294 controls[14]. The study found that individuals who both vaped and smoked chronically had a fourfold higher odds of lung cancer compared to those who only smoked cigarettes. Specifically, the odds ratio (OR) for lung cancer was 58.9 (95% CI 47.3–70.5) for those who vaped and smoked versus 13.9 (95% CI 12.7–15.3, p <0.001) for those who smoked only. Even after adjusting for other health conditions, the odds remained significantly higher for the combined use of vaping and smoking (OR 38.7, 95% CI 31.5–47.6) compared to smoking alone (OR 9.6, 95% CI 8.7–10.6, P<0.001). This association was consistent across sex, smoking history, and different types of lung cancer[14]. The results suggest that adding vaping to smoking further increases the risk of developing lung cancer.

Mechanistic studies

Carcinogenesis: Tang et al. were among the first to investigate the carcinogenic effects of long-term exposure to e-cigarette smoke (ECS) in an animal model[15]. FVB/N mice exposed to ECS for 54 weeks developed lung adenocarcinoma (9 of 40 mice, 22.5%, p 0.0154) and bladder urothelial hyperplasia (23 of 40 mice, 57.5%, p <0.001). The urothelial hyperplasia was characterized by a strong expression of several cell proliferation markers. These lesions were extremely rare in mice exposed to vehicle control or filtered air. In a previous study by the same group, FVB/N mice exposed to ECS for 12 weeks displayed increased levels of O^6-methyl-dG and γ-OH-1,N^2-propano-dG DNA adducts in the lung, bladder and heart, in parallel with decreased DNA repair activity in the lung compared to mice exposed to filtered air[16]. The exposure levels in both studies were equivalent to light e-cigarette smoking for ≥10 years. Importantly, nicotine and a nicotine-derived nitrosamine were shown to induce the same DNA adducts and inhibitory effects on DNA repair in human bronchial epithelial and urothelial cells *in vitro*[16].

Tumorigenesis: Animal studies indicate that ECS promotes tumour progression and colonization of cancer cells in the lung and mammary of exposed mice[17, 18]. A common mechanism underlying this process is reduced activation of apoptosis in metastatic cancer cells in these tissues. Experimental cell studies support these results, showing that human bronchial epithelial cells undergo premalignant transformation *in vitro* with increased anchorage-independent growth when exposed to ECS after weekly exposure over a 12-week period at doses calibrated to match the topography of e-cigarette users[19]. Cell transformation was found to be positively associated with increased levels of ROS and extensive transcriptional and epigenetic reprogramming, but not with increased levels of DNA damage[19]. Shorter exposure regimens (3-8 days) have also shown similar effects with induced epithelial-to-mesenchymal transition (EMT) of lung adenocarcinoma cells, accompanied by increased expression of EMT markers (*e.g.*, β-catenin) and cellular motility[20]. The role of nicotine was not investigated in these studies but has been shown to promote tumorigenic transformation in human bronchial epithelial and urothelial cells *in vitro*[16].

Genotoxicity and mutagenicity: The genotoxic effects of ECS observed by Lee et al, 2018[16] have been confirmed in a number of animal studies. Despite the use of different exposure regimens (i.e. nose-only vs. whole-body exposure, different nicotine levels, different puff volumes, durations and periods), acute, subchronic and chronic exposure to ECS has been shown to induce significantly increased levels of DNA damage in mice and rats[21-26]. Exposure to ECS for up to 6 months significantly induced oxidative DNA damage in the lungs and liver of exposed BALB/c mice, while no significant effects on chromosomal aberrations and gene mutations were observed[24]. In Sprague-Dawley rats, ECS increased lung free radicals and 8-oxo-dG levels and decreased the systemic antioxidant capacity after 4 weeks of exposure. The systemic effects of ECS were also demonstrated by increased levels of chromosomal aberrations in peripheral blood and a positive result in the Ames mutagenicity test for urine collected from exposed animals[21].

Moreover, ApoE-/- mice exposed to ECS for 12 weeks showed significantly induced levels of apurinic/apyrimidinic (AP) sites in liver DNA compared to control animals. This effect was associated with increased levels of hepatic oxidative stress and was only observed in animals exposed to nicotine-containing ECS[22]. Similarly, mice acutely exposed to tobacco-flavoured ECS containing nicotine showed higher levels of DNA damage in lung, measured as γH2AX levels, compared to the same product without nicotine[23]. Together with the study by Lee et al

(2018) described above, this suggests an important role for nicotine in the genotoxic effects of e-cigarettes. However, the opposite association has also been observed, with lower levels of DNA damage when nicotine has been added to a product[23, 25]. Further research is needed to determine which constituents are responsible for the genotoxic effects of ECS.

Data on the pulmonary genotoxic effects of e-cigarettes *in vitro* are conflicting, likely due to differences in the products tested and the way they were tested. Two main approaches have been used to assess lung genotoxicity *in vitro*: exposing lung cells at an air-liquid interface (ALI) to smoke, mimicking human respiratory conditions, and exposing submerged cells to ECS extracts. The identified ALI studies generally followed similar exposure regimens but varied puff numbers or power settings. Exposure to ECS via ALI neither induced genotoxicity or oxidative stress in human bronchial epithelial cells[27], caused chromosomal aberrations in hamster lung fibroblasts nor induced mutations in the Ames test[28]. DNA damage was observed only in lung adenocarcinoma cells exposed at high power levels (≥ 40 W)[29, 30] and in human primary lung fibroblasts following short time exposure[31]. For ECS extracts, results on oxidative stress and DNA damage in human bronchial epithelial cells have been mixed[32-34]. However, long-term exposure (up to 8 weeks) significantly increased DNA strand breaks and γH2AX in normal epithelial and head and neck squamous cell carcinoma lines, regardless of nicotine content[35]. ECS extracts did not induce mutations in transgenic mouse and human fibroblasts[36]. Using *in vitro* screening and *in silico* modelling, Hung et al, 2020[37] identified over 20 genotoxic flavouring compounds in e-cigarettes, suggesting that this approach could prioritize compounds for further testing.

Oral mucosa

Epidemiological studies

Carcinogenesis and tumorigenesis: As the first contact of the inhaled ECS occurs in the oral cavity, the impact on oral health, including cancer development, is of concern[38]. In the ongoing longitudinal US Population Assessment of Tobacco and Health study (PATH study, >10 000 respondents), no positive association has been found between e-cigarette use over 5 years (2014-2019) and the incidence of precancerous oral lesions (AHR 0.56, 95% CI 0.26-1.20, *p* 0.14)[39]. The PATH study has however reported significantly higher urinary levels of nicotine, TSNAs and some metals and VOCs among e-cigarette users compared to never users and that a transition from cigarette smoking to vaping significantly reduced levels of *e.g.*, TSNAs, PAHs and metals. Cigarette smokers who became dual users did not experience significant reductions in most markers[40, 41].

Cigarette users and e-cigarette users induce similar epigenetic effects in buccal epithelial cells that are predictive of lung cancer development including hypermethylation of genes involved in NOTCH1-mediated regulation of growth factor signalling and cell migration implicated in cancer. These findings were based on DNA methylation analyses in > 3500 tissue samples from long-term cigarette users and non-smokers and saliva samples from 116 e-cigarette users with limited tobacco smoking history (up to 6 months)[42]. In agreement, deregulation of growth factor signalling pathways with repressed expression of *e.g.*, *NOTCH1* was observed in a transcriptome study comparing oral epithelia of e-cigarette users and non-smokers[43].

Genotoxicity and mutagenesis: A significant increase in chromosomal aberrations and DNA damage has been observed in e-cigarette users compared to non-smokers in buccal mucosa cells[44-48], peripheral blood lymphocytes[49], and urine samples (*e.g.,* biomarkers 7-methyl-dG and

8-oxo-dG)[50, 51]. Notably, one study reported reduced levels of AP sites in oral mucosa DNA among e-cigarette users compared to non-smokers[52]. Only one study was identified that reported no increased levels of chromosomal aberrations among e-cigarette users compared to non-smokers[53]. These findings have been demonstrated in several smaller observational studies (N=12-160) with varying self-reported vaping histories among the participants (ranging from six months to eight years) including some former tobacco cigarette users, so the data should be interpreted with caution.

Mechanistic studies

Carcinogenesis and tumorigenesis: No experimental studies on cancer development in oral mucosa were identified. Consistent with the associations observed in lung, ECS extract can activate inflammatory signalling and invasion of human oral squamous cell carcinoma cells *in vitro*, in a cell type and flavour dependent manner[54].

Genotoxicity and mutagenicity: Three *in vitro* studies on the genotoxicity of e-cigarettes in human oral mucosa were identified[34, 55, 56]. Aerosols from eleven e-liquid products were evaluated for their ability to induce chromosomal aberrations in several oral epithelial cell lines. Six of the products caused dose-dependent aberrations in some of the cell lines that were not associated with nicotine content, chemical composition, or oxidative stress[56]. Similarly, ALI exposure to ECS caused increased DNA strand breaks and γH2AX in oral 2D and 3D epithelial models and was positively associated with increased oxidative stress[55]. In addition, ECS extracts induced oxidative DNA damage in oral epithelial cells in a dose-dependent manner independent of nicotine concentration[34]. Exposure for up to two weeks at concentrations mimicking human e-cigarette puff profiles resulted in a significant increase in cellular levels of 8-oxo-dG. This increase was associated with significantly increased oxidative stress, decreased cellular antioxidant capacity, and decreased levels of OGG1 protein, an enzyme essential for repairing oxidative DNA damage[34]. These latter findings are consistent with the animal study described above by Lee et al. in which a similar association between increased levels of mutagenic alkylated DNA adducts and decreased protein levels and repair activity was observed in ECS-exposed mice[16].

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Epidemiological studies

Similar to the ECS studies present above, a significant increase in chromosomal aberrations has been observed in smaller observational studies among users of HTPs compared to non-smokers in buccal mucosa cells[47] and for DNA damage biomarkers in urine[57]. In the study by Tadin et al, 2024[47], increased levels of genotoxicity were observed for both e-cigarette and HTP users compared to non-smokers, emphasizing the detrimental impact of these non-combustible alternatives on the oral mucosa.

Mechanistic studies

HTP smoke extracts have been shown to induce EMT in lung adenocarcinoma cells, accompanied by increased gene expression of EMT markers (*e.g.*, *Twist* and *Snail*)[58]. Comparing the genotoxicity in human bronchial epithelial cells ALI exposed to the same levels (number of puffs) of ECS and HTP smoke, showed that only the latter caused significantly increased levels of chromosomal aberrations and oxidative DNA strand breaks[27]. Similar increased levels of oxidative DNA damage have been observed in lungs of exposed rats and in human bronchial

and alveolar lung mucosal ALI models[59, 60]. All these studies observed concomitant increases of oxidative stress markers suggesting this as a central mechanism.

Conclusions

Taken together, these results indicate that ECS is a lung carcinogen and a potential bladder carcinogen in animals, with clear implications for human health. This concern is further supported by epidemiological studies showing an increased risk of lung cancer in dual users compared to tobacco smokers and pro-carcinogenic effects in the oral mucosa of vapers, including epigenetic reprogramming and induction of genotoxicity. There is also strong evidence that vaping is associated with human exposure to the same carcinogens found in tobacco smoke, although at lower levels. However, most of these studies were of cross-sectional design using self-reported data on e-cigarette use and including some former tobacco cigarette users.

A large number of experimental studies in mice and rats further support the associations found among vapers by showing increased levels of genotoxicity and promotion of tumorigenic cell transformation in ECS-exposed animals. Although data on the genotoxic effects of e-cigarettes *in vitro* are conflicting, likely due to differences in the products tested and the way they were tested, they suggest that the induction of oxidative stress and oxidative DNA damage may be important mechanisms for initiating carcinogenesis. In addition, a dose-dependent induction of chromosomal aberrations following ECS exposure has been observed in several *in vivo* and *in vitro* studies. The frequency of chromosomal aberrations in lymphocytes has been used to predict cancer risk in tobacco smokers.

Fewer studies concerning the influence of HTPs on cancer were identified, but similar to ECS, HTP exposure is associated with exposure to human carcinogens, increased genotoxicity among users and cell transformation in experimental settings.

Although the identified epidemiological and experimental studies show the same associations between exposure to ECS and HTP smoke and markers of carcinogenesis as those established for tobacco smoke, further studies are needed. Due to the long time-course of cancer development and the relatively recent introduction of new nicotine and tobacco products to the market, more large-scale prospective studies are needed to elucidate the influence of the products on cancer development. In addition, although some experimental *in vivo* and *in vitro* studies show clear evidence of a role for nicotine, there are some conflicting results that require additional research to elucidate how differences in habits, vape flavours and nicotine levels may affect cancer development.

TABLE 3. SUMMARY OF LITERATURE REVIEW: STUDIES INVESTIGATING THE NEW NICOTINE PRODUCTS IN RELATION TO CANCER DEVELOPMENT, TUMOUR PROMOTION AND PROGRESSION, AND GENOTOXICITY AND MUTAGENICITY.

Author and year	Study design	Country and setting	Exposure categorization	Outcome	Covariates	Results
			E-cigarettes			
Bitton et al., 2024[14]	Epi. Case-control	United States, 2013-2021, n=32,269	Dual e-cig and tobacco cigarette use, only tobacco cigarette use vs never use	Lung cancer	Age, sex, race and county of residence, comorbidity, cigarette smoking, and use of e-cigs (vaping)	OR (95% CI): Dual users: 58.9 (47.3-70.5), only tobacco users: 13.9 (12.7-15-3).
Camila et al., 2023[49]	Epi. Cross-sectional	Colombia, n=64	Current e-cig use vs non use	Genotoxicity (Chromosomal aberrations in peripheral blood lymphocytes)	None, groups had similar sex and age distribution	Significantly higher levels of genotoxicity in vapers compared to non-smokers ($p<0.05$)
Cheng et al., 2022[44]	Epi. Cross-sectional	United States, n=40	Current e-cig use vs non use	Genotoxicity (Acrolein-DNA adducts in oral mucosa)	None, groups had similar sex and age distribution	Significantly higher levels of genotoxicity in vapers compared to non-smokers ($p=0.001$)
Franco et al., 2016[53]	Epi. Cross-sectional	Italy, n=42	Current e-cig use vs non use	Genotoxicity (Chromosomal aberrations in oral mucosa)	Age and sex	No significant differences in genotoxicity in vapers compared to non-smokers
Guo et al., 2021[52]	Epi. Cross-sectional	United States, n=65	Current e-cig use vs non use	Genotoxicity (AP sites in oral mucosa)	None, groups had similar sex and age distribution	Significantly lower levels of genotoxicity in vapers compared to non-smokers ($p<0.05$)
Herzog et al., 2024[42]	Epi. Cross-sectional	5 European countries, n=233	Current e-cig use vs non use	Epigenetic effects (DNA methylation changes in oral mucosa)	None	E-cig use induces similar cancer-linked epigenetic changes as cigarette smoke in oral mucosa.

Podguski et al., 2022[50]	Epi. Cross-sectional	United States, n=12	Current e-cig use among EVALI subjects vs non-use in healthy subjects	Genotoxicity (8-oxo-dG in urine) and oxidative stress (myeloperoxidase and 8-isoprostane in urine)	None, groups had similar sex and age distribution	Significantly higher levels of genotoxicity biomarkers in EVALI vapers compared to non-smokers ($p<0.05$) but no significant differences for oxidative stress markers
Pop et al., 2021[45]	Epi. Cross-sectional	Romania, n=43	Current e-cig use vs non use	Genotoxicity (Chromosomal aberrations in oral mucosa)	None, groups had similar sex and age distribution	Significantly higher levels of genotoxicity in vapers compared to non-smokers ($p<0.01$)
Sakamaki-Ching et al., 2020[51]	Epi. Cross-sectional	United States, n=37	Current e-cig use vs non use	Genotoxicity (8-oxo-dG in urine) and oxidative stress (8-isoprostane in urine)	None, groups had similar sex and age distribution	Significantly higher levels of genotoxicity and oxidative stress biomarkers in vapers compared to non-smokers ($p<0.05$).
Schwarzmeier et al., 2021[42]	Epi. Cross-sectional	Brazil, n=47	Current e-cig use vs non use	Genotoxicity (Chromosomal aberrations in oral mucosa)	None, groups had similar sex and age distribution	Significantly higher levels of genotoxicity in vapers compared to non-smokers ($p<0.05$)
Silveira et al., 2022[39]	Epi. Cohort study	United States, PATH 2013-2019, n>10000	Current e-cig use vs non use	Self-reported oral health status including precancerous lesions (based on information from *e.g.*, dentist)	Age, sex, race, education, and household income, diabetes, heavy alcohol use, marijuana use, flossing	No positive associations were observed for e-cig users and oral precancerous lesions
Tadin et al., 2024[47]	Epi. Cross-sectional	Croatia, n=120	Current e-cig and HTP use vs non use	Genotoxicity (chromosomal aberrations in oral mucosa)	None, groups had similar sex and age distribution	Significantly higher levels of genotoxicity in vapers and HTP users compared to non-smokers ($p<0.05$)
Tommasi et al., 2023[48]	Epi. Cross-sectional	United states, n=48	Current e-cig use vs non use	Genotoxicity (LA-QPCR based analysis in oral mucosa)	Level of e-cig use	Significantly higher levels of genotoxicity in heavy vapers compared to light vapers and non-smokers ($p<0.05$)

Tommasi et al., 2019[43]	Epi. Cross-sectional	United states, n=69	Current e-cig use vs non use	Genome-Wide Gene-Expression Analysis in oral mucosa	None, groups had similar sex and age distribution	Deregulation of growth factor signalling pathways with repressed expression of *e.g.*, *NOTCH1*
Emma et al., 2023[28]	Experimental *in vitro*	Lung fibroblasts ALI and *S. typhimurium*	ECS vs filtered air	Genotoxicity (chromosomal aberrations) and mutagenicity (Ames test)	NA	No increased levels of genotoxicity or mutagenicity
Espinoza-Derout et al., 2019[22]	Experimental *in vivo*	ApoE-/- mice (male)	Whole body exposure to ECS (0% and 2.4% nicotine, 12 h/d) vs. saline aerosol for 12 wk.	Genotoxicity (AP sites in liver) and oxidative stress (MDA in liver)	NA	Significantly increased levels of genotoxicity and oxidative stress in the liver of mice exposed to ECS with nicotine ($p<0.05$). No effects in ECS without nicotine compared to control.
Canistro et al., 2017[21]	Experimental *in vitro*	Sprague Dawley rats (male)	Whole body exposure to ECS (1.8% nicotine, 3 h/d, 5 d/wk.) vs control for 4 wk.	Genotoxicity (chromosomal aberrations, DNA strand breaks, 8-oxo-dG), mutagenicity (Ames) test), and oxidative stress	NA	Significantly higher levels of oxidative DNA damage in lung, chromosomal aberrations and DNA strand breaks in blood and associated with increased oxidative stress in plasma and lung tissue ($p<0.05$). Urine samples induced positive Ames test.
Ganapathy et al., 2017[34]	Experimental *in vitro*	Oral and lung epithelial cells	5 ECS extracts (0-18 mg/ml nicotine) vs control	Genotoxicity (8-oxo-dG, q-PADDA assay, OGG1 protein levels) and oxidative stress (DCFDA assay)	NA	Dose dependent and significantly increased genotoxicity (both) independently of nicotine content (oral) associated with decreased OGG1 an increased oxidative stress (oral) ($p<0.05$)

Hung et al., 2020[37]	Experimental *in vitro* and *in silico*	TK6 lymphoblastoid cells	150 e-cig flavouring compounds	*In vitro* genotoxicity (γH2AX and p53 assay) and *in silico* mutagenicity, clastogenicity or carcinogenicity (QSAR)	NA	*In vitro* test identified 25 genotoxic compounds and silico 46 mutagenic, clastogenic or carcinogenic compounds with >80% concordance between the models.
Huynh et al., 2020[17]	Experimental *in vivo*	NSG mice (female)	Exposure to ECS (2.4% nicotine, 2 h/d, 5 d/wk.) vs air for 4 wk.	Tumorigenesis (Lung metastasis of tail vein injected cancer cells)	NA	ECS caused a higher lung localization of tumour cells with larger tumours and reduced apoptosis compared to control ($p<0.05$)
Khalil et al., 2021[30]	Experimental *in vitro*	A549 lung cancer cells ALI	ECS vs filtered air	Genotoxicity (Comet assay)	NA	Significantly increased genotoxicity by 3 of 4 ECS samples. ($p<0.05$)
Lee et al., 2018[16]	Experimental *in vivo* and *in vitro*	FVBN mice (male) and human lung and urothelial cells	Whole body exposure to ECS (1% nicotine, 3 h/d, 5 d/wk.) vs filtered air for 12 wk. Cells were exposed to nicotine and NNK.	Genotoxicity *in vivo* (DNA adducts in lung, bladder, heart and liver tissue & DNA repair activity in lung tissue). Genotoxicity, mutagenicity, tumorigenesis *in vitro* (DNA adducts, DNA repair activity, mutation frequency and colony formation)	NA	Increased levels of nitrosamine derived genotoxicity in the lung, bladder and heart tissue and reduced DNA repair activity in the lung of exposed mice. *In vitro* results showed that nicotine and NNK induced the same DNA damage and enhanced mutagenesis and tumorigenic transformation.
Lerner et al., 2016[31]	Experimental *in vitro*	human primary lung fibroblasts ALI	ECS vs filtered air	Genotoxicity (Comet assay)	NA	Significantly increased genotoxicity ($p<0.05$)

Ma et al., 2023[26]	Experimental *in vivo*	C57BL/6J mice (male)	Instilled aspiration of ECS extract from 2 different devices	Genotoxicity (8-oxo-dG in serum)	NA	Dose-dependent and significantly increased genotoxicity by both devices.
Muthumulage et al., 2023[23]	Experimental *in vivo*	C57BL/ 6J mice (male and female)	Whole body exposure to 2 different ECS +/- 0.6% nicotine (2 h/d) vs clean air for 3 d	Genotoxicity (γH2AX in lung tissue)	NA	One flavour induced nicotine independent genotoxicity and one flavour nicotine dependent ($p<0.05$)
Pearce et al., 2020[32]	Experimental *in vitro*	Normal Human Bronchial Epithelial Cells	ECS extracts from 3 devices vs. control	Genotoxicity (Comet assay) and oxidative stress (ROS and GSH)	NA	All devices caused increased levels of genotoxicity and oxidative stress ($p<0.05$)
Pham et al., 2020[18]	Experimental *in vivo*	BALB/C mice (female)	Whole body exposure to ECS (2.4% nicotine, 2 h/d, 5 d/wk.) vs air for 6 wk.	Tumorigenesis (Primary tumour behaviour and lung metastasis of mammary fat pad injected cancer cells)	NA	ECS enhanced breast cancer growth associated with reduced apoptosis, increased proliferation, and induced pulmonary metastasis.
Platel et al., 2023[24]	Experimental *in vivo*	BALB/c mice (male)	Nose-only exposure to ECS at 18 or 30 W (1.6% nicotine) for 4 d (30-90min/d), 3 mos and 6 mos (both 1 h/d, 5 d/wk.) vs fresh air	Genotoxicity (Comet assay, Micronucleus assay, *Pig-a* mutation assay, 8-oxo-dG)	NA	Only the high-power generated ECS induced oxidative DNA damage in the lung and liver of exposed mice ($p<0.05$). No significant increase of chromosomal aberrations or gene mutations.
Rankin et al., 2018[33]	Experimental *in vitro*	Human lung epithelial cell lines and distal lung tissue explants	ECS extracts (0 and 2.4% nicotine) vs control	Genotoxicity (Comet assay) and oxidative stress (ROS)	NA	No significant effects on genotoxicity and oxidative stress

Robin et al., 2022[54]	Experimental *in vitro*	Oral squamous cell carcinoma cell lines	ECS extracts (0 and 0.6% nicotine) vs control	Tumorigenesis (Cell invasion assay and inflammatory signalling)	NA	ECS extracts activated inflammatory signalling and cell invasion but in a cell specific and flavour dependent manner ($p<0.05$)
Ruth et al., 2023[29]	Experimental *in vitro*	Human A549 lung carcinoma cell line ALI	ECS from different power settings (0-75 W) vs control	Genotoxicity (Comet assay)	NA	Only high-power settings (>45 W) caused genotoxicity but was also associated with high cytotoxicity.
Sun et al., 2021[25]	Experimental *in vivo*	B6C3F1 mice (female)	Whole body exposure to ECS with 0, 1.2, 2.4% nicotine (2 h/d, 5 d/wk.) vs filtered air for 8 wk.	Genotoxicity (8-oxo-dG)	NA	Significantly increased levels of genotoxicity in plasma for ECS 0-2.4% compared to control ($p<0.05$) but with reduced levels of DNA damage for 2.4% compared to 0%.
Sundar et al., 2016[55]	Experimental *in vitro*	oral 2D and 3D epithelial ALI models	ECS vs air	Genotoxicity (Comet assay and γH2AX) and oxidative stress (protein oxidation)	NA	ECS caused increased DNA strand breaks and γH2AX and was positively associated with increased oxidative stress ($P<0.05$)
Tang et al, 2019[15]	Experimental *in vivo*	FVB/N mice (male)	Whole body exposure to ECS (3.6% nicotine, 4 h/d, 5 d/wk.) vs filter air for 56 wk.	Carcinogenesis (tumour formation)	NA	Exposed mice developed lung adenocarcinomas and bladder urothelial hyperplasia ($p<0.05$). No significant effects were observed in other organs.
Tellez et al., 2023[19]	Experimental *in vitro*	Human bronchial epithelial cell lines	ECS vs filtered air for 12 wk.	Genotoxicity (Comet assay), oxidative stress, and tumorigenesis (Soft agar assay)	NA	Significantly increased oxidative stress in 2/3 cell lines and cell transformation in 1/3 cell lines. No detected genotoxicity.

Tellez et al., 2021[56]	Experimental *in vitro*	Human Oral Epithelial Cell Lines	ECS from 11 e-liquids vs filtered air	Genotoxicity (Comet assay and micronucleus assay)	NA	Comet assay was negative. Six of the products induced chromosomal aberrations but consistently in all cell lines.
Tommasi et al., 2017[36]	Experimental *in vitro*	Human and mouse fibroblast cell lines	ECS extracts from 3 brands (1.6-1.8% nicotine) vs control	Mutagenicity (BigBlue *cll* mutations and *supF* mutations)	NA	No statistically significant increases in relative mutant frequency in either cell models.
Yu et al., 2016[35]	Experimental *in vitro*	Normal epithelial and head and neck squamous cell carcinoma cell lines.	ECS extracts from 2 brands +/- nicotine	Genotoxicity (Comet assay and γH2AX)	NA	Significantly induce genotoxicity independently of nicotine ($p<0.05$)
Zahedi et al., 2018[20]	Experimental *in vitro*	Human lung adenocarcinoma A549 cells	ECS extracts from 2 flavours vs control	Tumorigenesis (various EMT assays)	NA	ECS extracts induced EMT observed as increased motility, changed morphology, and upregulation of EMT markers ($p<0.05$)
Zarcone et al., 2023[27]	Experimental *in vitro*	Bronchial lung BEAS-28 cells ALI	ECS and HTP smoke vs. control	Genotoxicity (Comet assay w. hOGG1 and Micronucleus test) and oxidative stress (gene and protein expression)	NA	Only significantly increased levels of oxidative stress and DNA damage in response to HTP smoke. No effects on chromosomal aberrations.
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Kawasaki et al., 2021[57]	Epi. Cross-sectional	Japan, n=56	Current HTP use vs never use	Genotoxicity (levels of m^7Gua and 8-oxo-dG in urine)	Age	Significantly increased levels of urinary 8-oxo-dG in HTP users

						compared to non-smokers ($p<0.05$)
Tadin et al., 2024[47]	Epi. Cross-sectional	Croatia, n=120	Current e-cig and HTP use vs non use	Genotoxicity (chromosomal aberrations in oral mucosa)	No confounding adjustment, groups had similar sex and age distribution	Significantly higher levels of genotoxicity in vapers and HTP users compared to non-smokers ($p<0.05$)
Hirata et al., 2022[58]	Experimental *in vitro*	Lung cancer cell lines	HTP smoke extract vs. control	Tumorigenesis (sphere forming assay and expression of EMT markers)	NA	Significantly increased sphere formation and expression of EMT markers ($p<0.05$)
Rahman et al., 2022[59]	Experimental *in vitro*	Bronchial and alveolar lung mucosal ALI models	HTP smoke vs. control	Genotoxicity (8-oxo-dG and γH2AX) and oxidative stress (total cellular ROS)	NA	Significantly increased levels of oxidative stress and DNA damage in both models.
Vivarelli et al., 2021[60]	Experimental *in vivo*	Sprague-Dawley rats (male)	Whole body exposure to HTP smoke for 4 wk. (3 h/d, 5 d/wk.) vs. non-exposed	Genotoxicity (8-oxo-dG in lung) and oxidative stress	NA	Significantly increased levels of oxidative stress and DNA damage in lungs.
Zarcone et al., 2023[27]	Experimental *in vitro*	Bronchial lung BEAS-2B cells ALI	ECS and HTP smoke vs. control	Genotoxicity (Comet assay and Micronucleus test) and oxidative stress	NA	Only significantly increased levels of oxidative stress and DNA damage in response to HTP smoke. No effects on chromosomal aberrations.

WHO, World Health Organization; PAHs, polycyclic aromatic hydrocarbons; VOCs, volatile organic compounds; TSNA, tobacco specific nitrosamines; OR, odds ratio; ECS, e-cigarette smoke; EMT, epithelial-to-mesenchymal transition; AP site, apurinic/apyrimidinic site; ALI, air-liquid interface; PATH study, US Population Assessment of Tobacco and Health study.

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Cardiovascular diseases

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Introduction

Cardiovascular disease (CVD) is a collective term for several different diseases, including coronary heart disease (CHD), stroke, heart failure and arrhythmia diseases. CVD is the main cause of morbidity and mortality worldwide[1]. Even among youths and young adults, there is a substantial global burden of CVDs[2]. While long-term trends in age-standardized rates of CVD mortality and incidence show significant reductions in disease burden, the pace of decline appears to have slowed over the past decade[3]. A considerable proportion of cardiovascular morbidity is linked to atherosclerosis - a systemic inflammatory disorder of the vessel wall, which causes impaired blood circulation and ischemia. It often takes time before atherosclerosis manifests itself as CVD, and research therefore sometimes tries to identify subclinical CVD. This can be done, for example, by measuring plaque in the coronary arteries or in the carotid artery. However, subclinical cardiovascular disease does not necessarily lead to clinically manifested CVD but is a risk factor for this. Other cardiovascular risk factors include smoking, physical inactivity, unhealthful nutrition, dyslipidaemia, diabetes, high blood pressure, obesity, older age, male sex, kidney dysfunction and CVD heredity[4]. It has been estimated that 57% of incident CVD cases among women and 53% of cases among men, may be attributable to five modifiable risk factors: current smoking, diabetes, increased body-mass index, increased systolic blood pressure, and increased non-high-density lipoprotein cholesterol[5].

Smoking and brown snus

Tobacco smoking is one of the most important modifiable contributors to CVD and 2,370,000 [95% confidence interval (CI) 498,000-4,410,000] CVD deaths were attributed to smoking in 2021 according to the estimates produced by the global burden of CVD[6]. Among the several chemical compounds (approximately over 7,000) contained in tobacco smoke, some of them, including nicotine, have been consistently shown to trigger pathophysiological alterations including increased oxidative stress, inflammation, sympathetic activity, and platelet activity that, in turn, may lead to increase of heart rate, myocardial contractility, inflammation, endothelial dysfunction, and thrombus formation and ultimately contribute to atherosclerosis development and subsequent CVD[7-9]. The association between tobacco smoking and CVD has been confirmed in a recent mendelian randomization study involving 367,643 individuals. In that study, genetic variants linked to smoking initiation were strongly associated with higher odds of CHD, heart failure, abdominal aortic aneurysm, ischemic stroke, transient ischemic attack, peripheral arterial disease (PAD), and arterial hypertension[10]. Additionally, evidence suggests that there is no safe level of tobacco smoking in relation to CVD as an increased risk of CVD, especially CHD and stroke, has been noted even for a low number of cigarettes smoked (1-5 cigarettes)[11]. In addition, tobacco smoking has been associated with increased risk of hypertension[12, 13].

Studies on snus use and risk of CVD, primarily based on Swedish populations and with a clear predominance of male study participants, have mainly focused on CHD or myocardial infarction[14] and stroke[15]. Most studies show no association with CHD, MI or stroke[14, 15], although an association with increased cardiovascular mortality has been reported[16]. In addition, there are

findings of an association between snus use and increased lethality in the event of a first MI[14] or stroke event[15]. The observed increased lethality may relate to effects from nicotine on the cardiovascular system[9, 17]. Snus has been shown in repeated studies to be associated with an acute increase in blood pressure after its administration[18-21], probably explained by effects from nicotine on the blood vessels[9, 17-19, 22] . Only one study has addressed snus use in relation to long-term risk of hypertension; in a population of never smoking Swedish men, snus use was found to be associated with increased risk of hypertension[23].

Literature review

Based on the literature search, a total of 3,787 records were available for screening. We identified several updated systematic reviews and meta-analysis on e-cigarettes in relation to subclinical and clinically manifested CVD and therefore this chapter is based on the evaluation of the latest most comprehensive reviews (Table 4). We did not identify any studies investigating white snus or HTPs in relation to CVD.

E-cigarettes

Four systematic reviews / meta-analysis[24-27], two umbrella reviews[28, 29] and one review[30] were identified as relevant to our scope. All included review articles adhered to the PRISMA guidelines for reporting systematic reviews and meta-analyses, and/or were registered in PROSPERO, a database of systematic review protocols.

Epidemiological studies

Clinically manifested CVD: Two systematic reviews /meta-analysis[24, 25] and two umbrella reviews[28, 29] summarized the available evidence on the relationship between the use of e-cigarettes and CVD based on epidemiological studies. The two systematic reviews / meta-analyses represent the latest synthesis of epidemiological evidence linking e-cigarette use to CVD, primarily derived from cross-sectional studies. In the systematic review / meta-analysis by Chen et al.[24], the association between exclusive e-cigarette use and CVD was examined across seven cross-sectional epidemiological studies conducted in the United States of America (U.S., n = 864,888 participants). The definition of CVD in each of the included studies varied widely; some investigated myocardial infarction or CHD, and others stroke or overall CVD. The findings indicated that exclusive e-cigarette use, compared to never use, was associated with an increased risk of CVD, although this result was not statistically significant [pooled Odd Ratio (OR) 1.24, 95% CI: 0.93-1.67].

In an earlier systematic review / meta-analysis[25], stroke was investigated separately from CVD in relation to exposure to e-cigarette vs never use. The studies included (n = 11) were population-based epidemiological studies using national survey data in the U.S. (n = 1,112,152 participants), mostly cross-sectional except for four longitudinal studies (in total 4 studies of CVD outcomes and 2 of stroke). Pooled results showed significantly higher risk of CVD (pooled OR: 1.24, 95% CI: 1.05–1.46) in e-cigarette users compared to never users. Furthermore, an increased risk of stroke was suggested (pooled OR: 1.32, 95% CI: 0.99–1.76) among 818,855 participants. The 4 longitudinal studies, drawn from Population Assessment of Tobacco and Health (PATH, three studies) and Kaiser Permanente Research Bank (one study), had follow-ups ranging from 1.3 to 5 years. The results of the longitudinal studies and the cross-sectional studies were reported to be similar (no estimates were shown). We meta-analysed the individual results from the longitudinal studies and obtained a pooled OR of CVD: 1.02 (95% CI: 0.91-1.14) and of stroke: 1.70 (95% CI: 1.00-2.80).

The available evidence from systematic reviews and meta-analyses predominantly relies on cross-sectional studies that assess the prevalence of CVD outcomes, with only a few longitudinal studies included. Additional limitations of these studies include recall bias affecting both exposure and outcomes, which could have biased the pooled risk estimates. However, self-reported CVD diagnoses were often validated against medical records. Furthermore, the reported prevalence of CVD across the studies was in general found comparable. Differences in results between the systematic reviews / meta-analyses may arise for several reasons including how CVD outcomes were defined and varying study designs among included studies. As an example, as mentioned above, Glantz et al.[25] did not include stroke in their definition of CVD but Chen et al.[24] did. Further, Glantz et al.[25] included erectile function in the CVD outcome definition whereas Chen et al.[24] did not.

Regarding the umbrella reviews, both Banks et al.[28] and Travis et al.[29] concluded that there was insufficient quality of data to draw any conclusions on e-cigarette use in relation to CVD endpoints.

Our literature search did not identify any epidemiological studies investigating the relation between e-cigarette and any other CVD outcomes such as atrial fibrillation, abdominal aortic aneurysm or PAD.

Subclinical outcomes: The evidence on the association between e-cigarettes and subclinical outcomes has been summarized in three systematic reviews[25-27] and two umbrella reviews[28, 29].

The association between acute e-cigarette exposure and hemodynamic measures (i.e., measures reflecting the vessel function) as well as platelet function was investigated in a systematic review / meta-analysis[27]. Results of the meta-analysis, which include 27 randomized cross-over studies, primarily from the U.S. and Europe (n = 863 participants), showed that acute exposure to e-cigarette with nicotine was associated with increased heart rate, blood pressure (including systolic and diastolic blood pressure as well as mean arterial pressure), pulse wave velocity, augmentation index adjusted for heart rate, soluble CD40 ligand and soluble P-selectin and decreased flow mediated dilation (FMD). No significant changes were observed when e- cigarettes without nicotine were investigated in relation to the aforementioned endpoints, with exception of a significant decrease in mean artery pressure (weighted mean difference: -0.89, 95% CI: -1.26, -0.51)[27]. The latter result may suggest that the potential detrimental effect of e-cigarette on CVD health is mediated by nicotine. In turn, nicotine is known to modulate the cardiac sympathetic-vagal balance toward sympathetic predominance which may affect heart rate, blood pressure and vascular dilation. However, the detrimental effects on hemodynamic markers observed in relation to e-cigarette exposure were small and their clinical significance is uncertain.

An updated systematic review and meta-analysis by Lee et al.[26] assessed the relationship between e-cigarette exposure and FMD, a marker of endothelial function. Lee et al. included four studies, primarily cross-sectional studies from the U.S. (n = 769 participants). The pooled mean differences showed a non-significant reduction in FMD (MD: -1.47, 95% CI: -3.96,1.02) among exclusive e-cigarette users compared to never users.

The association between current e-cigarette use and cardiometabolic disorders (composite endpoint) was meta-analysed by Glantz et al.[25]. The pooled results were based on ten cross-sectional studies and one longitudinal study, primarily from the U.S. with the addition of one study from Sweden and one from Korea. An increased risk of cardiometabolic disorders —

including prediabetes (two studies), hypertension (six studies), metabolic syndrome (two studies), and waist circumference (one study) — was observed in relation to e-cigarette use vs never use (pooled OR: 1.25, 95% CI: 1.18–1.33 [25].

In an umbrella review by Travis et al.[29], the reviewed evidence suggests a potential cardiovascular harm of acute e-cigarette use in relation to heart rate, blood pressure (both systolic and diastolic), endothelial dysfunction, arterial stiffness, and biomarkers of oxidative stress. However, evidence regarding the long-term effects of e-cigarette use (vs never users) on chronic heart rate changes, blood pressure, cardiac geometry, and increased risk of CVD was deemed insufficient. In another umbrella review, by Banks et al.[28], the authors reported a lack of evidence on markers of subclinical atherosclerosis such as carotid intima-media thickness and coronary artery calcification and also deemed data insufficient, mainly in never smokers, for other cardiovascular subclinical outcomes (e.g., blood pressure, heart rate, autonomic control, arterial stiffness).

Mechanistic studies

The production of fine and ultrafine particulate matter from e-cigarettes may trigger cardio-pathophysiological processes leading to inflammation and platelet activation as shown in Siddiqui et al.[27]. Reactive oxidant species, produced by intermediate bioproducts such as acrolein, formaldehyde and acetaldehyde have been shown to induce cardiomyopathy. Flavouring products added to e-cigarettes may have cardiotoxic effects, likewise the presence of heavy metals in aerosols. Based on the current knowledge, it has been suggested[31] that the potential underlying biological mechanisms involving e-cigarettes in the development of a detrimental risk profile for CVD and, in turn, CVD may be due to: 1) activation of the sympathetic nerve system, 2) increase in oxidative stress, 3) damage to the endothelial dysfunction and 4) increased platelet activation. We here reviewed the available evidence from one umbrella review[29] and one systematic review[30] including experimental studies based on in vitro humans studies or in vivo animals studies. In the umbrella review, Travis et al.[29] did not draw strong conclusions from the included in vitro and in vivo systematic reviews. However, in vitro human studies consistently indicated increased production of reactive oxygen species, decreased antioxidant levels, and alterations in endothelial function and cellular interactions in relation to e-cigarette. In murine models, exposure to e-cigarette vapor displayed vascular inflammation, angiogenesis and increased atherosclerotic plaque as well as some effects on markers of oxidative stress and hemodynamic measures (e.g. heart rate and blood pressure). Kennedy et al.[30] reviewed the effects of e-cigarettes on oxidative stress, endothelial function including endothelial complement compounds, and platelet function using various cell types, including umbilical vein endothelial cells, pluripotent stem cell-derived endothelial cells, pulmonary microvascular cells, and coronary artery endothelial cells. The studies included (n=8) were both non-randomized and randomized controlled trials, with exposure durations ranging from 4 to 72 hours. Overall, the findings consistently demonstrated increased oxidative stress, reduced endothelial function and impaired platelet function. In the review by Kennedy et al.[30], five in vivo animal studies, primarily involving non-randomized and randomized controlled trials in mice were also included. These studies assessed the effects of e-cigarette exposure vs filtered air, room air, or saline aerosol on cardiac, vascular, platelet function and haemostasis. Results regarding cardiac remodelling[30] showed an altered cardiac function and structure, although the findings were inconsistent, particularly for concentric left ventricular hypertrophy, fractional shortening, and ejection fraction. Results related to vascular function[30] indicated generally increased dysfunction with enhanced mitochondrial damage, cytoplasmic

abnormalities, lipid accumulation, inflammatory and apoptotic gene expression and decreased cardioprotective gene expression following exposure. Regarding the long-term exposure to e-cigarette vapor, the studies included in the review by Kennedy et al. [30] found elevated surrogate markers of endothelial function (e.g., pulse wave velocity) and a shift in aortic mediators from vasodilation to vasoconstriction, potentially leading to hypertension, angiogenesis, and atherosclerotic plaque formation. Regarding platelet function and hemostasis[30], results showed increase in platelet aggregation, secretion, integrin activation, and resistance to prostacyclin, and reduction in bleeding time and occlusion time.

Conclusions

There are indications, from epidemiological studies as well as experimental and animal models, of adverse effects on the cardiovascular system from the use of e-cigarettes. However, studies that assess long-term exposure to e-cigarettes in relation to subclinical atherosclerotic markers or CVD endpoints such as CHD and stroke are scarce. Such long-term studies are essential to establish any potential association between novel nicotine products and CVD. Considering the total lack of studies addressing HTPs and white snus, no conclusions can be drawn. However, given the knowledge about the effects of nicotine on the cardiovascular system, and the high levels of nicotine that often occur in these new products, there is reason to suspect that these products may pose an increased risk of CVD.

TABLE 4. SUMMARY OF LITERATURE REVIEW: STUDIES INVESTIGATING THE NEW NICOTINE PRODUCTS IN RELATION TO CARDIOVASCULAR OUTCOMES.

Author and year	Type of study	Study design	Setting	Exposure	Outcome	Covariates	Results
Epidemiological studies							
Chen et al. 2024[24]	Systematic review / meta-analysis. Literature search: 2006 –April 2024	Cross-sectional (n = 7)	US, national representative surveys (n = 864,888)	Exclusive e-cig use vs never use	CVD composite (MI n = 3, stroke n = 4, CHD n = 2, overall CVD n = 2)	NA	Pooled OR of CVD: 1.24, 95% CI: 0.93,1.67
Glant et al. 2024[25]	Systematic review / meta-analysis. Literature search: January 2005 – October 2023	Cross-sectional (n =11). Longitudinal (n = 4)	US, national representative surveys (for CVD n = 1,112,152, stroke n = 818,855)	Exclusive e-cig use vs never use	CVD composite (CHD, MI, erectile function, HF and CVD) and stroke	Risk of bias: ROBINS-E considered low	Pooled OR for CVD: 1.24, 95% CI: 1.05,1.46. Pooled OR for stroke: 1.32, 95% CI: 0.99,1.76
Travis et al. 2022[29]	Umbrella review Literature search: May 2020 – January 2022	Systematic reviews /metanalyses and reviews of epidemiological studies	NA	Exclusive e-cig use vs never use	CVD clinically manifested endpoints	NA	No result reported, due to insufficient quality of data
Banks et al. 2023[28]	Umbrella review Literature search: 2017 – 2021	Systematic reviews /metanalyses and reviews of epidemiological studies	NA	Exclusive e-cig use vs never use	CVD clinically manifested endpoints	NA	No result reported, due to due to insufficient quality of data
Glantz et al. 2024[25]	Systematic review / meta-analysis	Cross-sectional (n = 10) and	US (n = 9), Sweden (n = 1), Korea (n = 1)	Exclusive e-cig use vs never use	Cardiometabolic composite (prediabetes n = 2,	Risk of bias: ROBINS-E	Pooled OR:

	Literature search: January 2005 – October 2023	longitudinal (n = 1)			hypertension n = 6, MS n = 2; WC n = 1)	considered low	1.25, 95% CI: 1.18,1.33
Lee et al. 2024[26]	Systematic review / meta-analysis. Literature search: January 2004 – March 2024	Cross-sectional (n = 4)	US (n = 769 participants)	Exclusive e-cig users vs never user	FMD	Adjusted, but not specified for what	Pooled MD: -1.47, 95 CI%: -3.96,1.02
Experimental studies in humans: randomized and non-randomized controlled studies							
Siddiqui et al. 2023[27]	Systematic review / meta-analysis Literature search: January 2006 – December 2022	Randomized cross-over studies (n = 27)	US, Belgium, Italy, Greece, Poland, Sweden and UK (n = 863 participants)	Acute exposure of e-cig	Changes (in general after mins/hours) in: HR (23 studies), BP (19 studies), MAP (5 studies), PWV (3 studies), Augmentation index adjusted for heart rate (4 studies), Soluble CD40 ligand (2 studies), Soluble P-selectin (2 studies), FMD (3 studies)	NA	HR pooled WMD: 1.22 bpm; 95% CI: 0.76, 1.68. SBP pooled WMD: 0.51 mmHg; 95% CI: 0.20, 0.82. DBP pooled WMD: 0.62 mmHg; 95% CI: 0.34, 0.91. MAP pooled WMD: 5.17; 95% CI: 3.33,7.02. PWV pooled WMD: 0.38; 95% CI: 0.13, 0.63. Augmentation index adjusted for heart rate pooled SMD: 0.58; 95% CI: .22, 0.94. Soluble CD40 ligand pooled WMD: 1.14; 95% CI: 0.41, 1.87. Soluble P-selectin pooled WMD: 4.73; 95% CI: 0.80, 8.66. FMD pooled

							WMD: -1.71; 95% CI: -2.97, 0.46
Travis et al. 2022[29]	Umbrella review. Literature search: May 2020 – January 2022	Systematic review / meta-analysis and reviews of experimental randomized and non-randomized studies	NA	Acute exposure to e-cig. Chronic exposure to e-cig	Changes in subclinical endpoints: HR, BP, endothelial dysfunction, arterial stiffness, biomarkers of oxidative stress	NA	Potential harm for acute (after mins/hours) exposure to e-cig. Insufficient evidence for effects of chronic (after months) exposure
Banks et al. 2023[28]	Umbrella review Literature search: 2017 – 2021	Systematic reviews / meta-analysis and reviews of experimental randomized and non-randomized studies	NA	E-cig (no other details)	Changes in subclinical atherosclerotic measures (e.g. C-IMT, CAC) and CVD subclinical endpoints (e.g. BP, HR)	NA	Lack of evidence for subclinical atherosclerotic endpoints Insufficient evidence for other subclinical outcomes
Animal and cell studies							
Travis et al. 2022[29]	Umbrella review Literature search: May 2020 – January 2022	Systematic review and reviews of randomized and non-randomized controlled trials In vitro human studies (n = 3) In vivo studies in mice (n = 2)	NA	E-cig (no other details)	Oxidative stress, endothelial function, inflammation, vascular function measures (e.g., HR and BP)	NA	In vitro human studies: increased production of ROS, decreased antioxidant levels, alterations in endothelial function and cellular interactions. In vivo studies in mice: Increased vascular inflammation, angiogenesis,

							increased atherosclerotic plaque, markers of oxidative stress and vascular function measures
Kennedy et al. 2019[30]	Systematic review Literature search: July 2017 – June 2019	Non-randomized and randomized controlled trials (n = 8). In vitro human studies	Human cell types: umbilical vein and endothelial, pluripotent stem cell-derived endothelial, pulmonary microvascular and coronary artery endothelial cells.	E-cig exposure 4 – 72 hours	Oxidative stress, endothelial function, endothelial complement compounds, platelet function	NA	Increased oxidative stress, endothelial dysfunction, platelet aggregation and activation
Kennedy et al. 2019[30]	Systematic review Literature search: July 2017 – June 2019	Non-randomized and randomized controlled trials (n = 5). In vivo animal studies	Mice	E-cig vs filtered or room air, or saline aerosol. Concentration of nicotine: 10mg / ml – 24 mg /ml. Dose: number of puffs/times per day. Duration of experiment: 1 week – 8 months	Cardiac functions and structure. Vascular function. Platelet function and haemostasis	NA	Altered cardiac function / structure. Endothelial dysfunction. Platelet activation and anticoagulation inhibition

NA, not available; OR, Odd Ratio; MD, mean difference; WMD, weighted mean difference; SMD, standard mean difference; bpm, beat per minute; mmHg, millimetre of mercury; CHD, coronary heart disease; HF, heart failure; MI, myocardial infarction; MS, metabolic syndrome; WC, waist circumference; FMD, flow-mediated dilation; HR, heart rate; BP, blood pressure; SBP, systolic BP; DBP, diastolic BP; MAP: mean arterial pressure; PWV, pulse wave velocity; C-IMT, carotid intima media thickness; CAC, coronary artery calcification; ROS, reactive oxygen species.

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Diabetes

Sofia Carlsson and Miranda Beck

Introduction

Diabetes is a common and growing[1] chronic disease that causes significant personal suffering and imposes substantial costs on society due to sick leave, healthcare expenses, and premature mortality, primarily due to its complications. Those include but are not limited to an increased risk of myocardial infarction, stroke, heart failure, kidney failure, painful diabetic neuropathy and ultimately, premature death[2].

Diabetes is a metabolic disorder characterized by high blood glucose levels. It is diagnosed based on fasting glucose or Haemoglobin A1c (HbA1c) levels or by oral glucose tolerance testing[3]. There are two main types of diabetes, type 1 and type 2 diabetes with distinct pathogeneses. Type 2 diabetes is most common accounting for 85-90% of all cases. This diabetes form primarily affects adults, and is characterised by insulin resistance in skeletal muscle, liver, and adipose tissue coupled with insulin deficiency[4]. In addition to insulin resistance and deficiency, several other factors contribute to hyperglycaemia. These include increased glucose production by the liver due to elevated glucagon, enhanced lipolysis, and the release of free fatty acids and pro-inflammatory cytokines from insulin-resistant fat cells. This worsens insulin resistance in the liver and muscles, reducing glucose uptake and contributing to glucose intolerance[4]. Oxidative stress, which promotes inflammation via reactive oxygen species (ROS), may also play a role in type 2 diabetes development. Type 2 diabetes is often preceded by prediabetes, a condition during which blood glucose levels are elevated but not yet high enough to meet the criteria for diabetes. Type 1 diabetes accounts for 99% of all diabetes in children but may occur at any age. It is an autoimmune form of diabetes, which occurs when autoreactive T-cells from the immune system destroy the pancreatic beta cells, resulting in reduced insulin production[5]. This process can lead to a complete lack of insulin, requiring external insulin administration to regulate blood glucose levels. Insulin resistance may also be implicated in the pathogenesis of type 1 diabetes, mainly by placing greater demand on beta cells, which may speed up the autoimmune process and progression to overt diabetes[6].

Smoking and brown snus

Smoking is associated with a 39% increased risk of type 2 diabetes (current vs. never smokers) and the risk increases in a dose-dependent manner by number of cigarettes smoked according to a meta-analysis based on 88 prospective cohort studies[7]. Mendelian randomization studies support that this is a causal association[8]. Nicotine, the primary addictive substance found in tobacco smoke, is probably instrumental in promoting type 2 diabetes in smokers. Smoking increases blood sugar levels in humans, and this elevation is associated with the amount of nicotine in the cigarettes[9]. Nicotine can raise blood sugar levels, disturb glucose regulation, and cause insulin resistance according to animal and human studies[9]. Nicotine can also stimulate release of cortisol[10], which promotes abdominal obesity that is key risk factor for type 2 diabetes. In support hereof, epidemiological and mendelian randomization studies link smoking to increased abdominal fat distribution[11-13].

Snus use is also associated with increased risk of type 2 diabetes. A study based on pooled data from five Swedish cohorts reported that people consuming one box of snus per day have

68% higher risk of type 2 diabetes compared to never users[14]. This effect size is on par with that observed for smoking: Pan et al. found a hazard ratio of 1.57 for individuals smoking one pack of cigarettes daily in their meta-analysis of 88 cohorts[7]. Snus provides similar or even higher nicotine exposure than cigarettes. One portion of snus contains more nicotine than a cigarette, but nicotine is absorbed faster from cigarettes, leading to a higher initial peak[15]. After 2 hours, nicotine levels tend to be higher in snus users and regular users of snus and cigarettes typically have similar blood nicotine levels[16]. Snus has lower levels of other potentially harmful compounds, such as nitrosamines. The similarly increased diabetes risk seen in smokers and snus users thus supports that the mechanism involves adverse effects of nicotine.

Fewer studies have investigated the influence of smoking and snus use on type 1 diabetes but recently, epidemiological studies from Scandinavia found an increased risk of adult-onset type 1 diabetes in smokers and snus users, and the strength of association was similar to that observed in relation to type 2 diabetes[17-18]. Further support for a link between smoking and autoimmune diabetes in adults was also provided by a Mendelian Randomization study[17].

Summary of literature review

652 articles were identified and after screening the titles and abstracts, 37 studies were considered potentially relevant. We screened the full-text of those articles and excluded 21 of them due to having the wrong outcomes, wrong study design, articles funded by the tobacco industry, wrong intervention, wrong comparator or the wrong setting. Among the 15 remaining studies[19-33], four studies from the US[19-22] were based on the same study populations and we only included the most recent publication from each setting[19-20]. This left us with 13 relevant articles of which one included both epidemiological and animal data which meant there were 14 studies overall, including eight epidemiological studies[19-20, 23-28] (seven on e-cigarettes, one on HTPs) and six *animal* studies conducted in mice or rats[26, 29-33] (five on e-cigarette exposure and one on oral nicotine) (Table 5).

E-cigarettes

Epidemiological studies

Our literature search identified seven unique studies on e-cigarettes and diabetes or prediabetes, glucose tolerance, insulin resistance or the metabolic syndrome published between 2019 and 2023[19-20, 23-27] (Table 5). All studies investigated exclusive e-cigarette use vs non-use while adjusting for use of other tobacco products[19-20, 24-25] or excluding smokers from the comparison[23, 26-27]. Five of the identified studies were from the US[19-20, 24, 26-27] and two from the Republic of Korea[23, 25]. They did not specify the type of diabetes they investigated, but since they focused on adults—where approximately 90% of cases are type 2 diabetes—it is reasonable to assume that they primarily studied type 2 diabetes or pre stages to type 2 diabetes, i.e. prediabetes. There were no studies on type 1 diabetes. The reason we included the studies on the metabolic syndrome which did not directly assess glucose tolerance or diabetes is that it is a cluster of conditions—including high blood pressure, high blood sugar, excess abdominal fat, and abnormal cholesterol or triglyceride levels—that increase the risk of type 2 diabetes[34]. The studies all adjusted for a range of potential confounders that may otherwise have contributed to an association including not only use of other tobacco products but also BMI, socioeconomic status and physical activity (Table 5).

Four studies investigated the prevalence of diabetes or prediabetes[19, 23-25] in e-cigarette users vs non-users. Two of the studies were from the US including one based on data from participants

of the National Health and Nutrition Examination Survey (NHANES) (n=5101) who had information on glucose levels from clinical screening[19] and one using data from the Behavioural risk factor surveillance system (BRSS) (n=600, 046) with self-reported information prediabetes[24]. The other two were from Korea; one consisting of 14,738 participants in the 6th Korea National Health and Nutrition Examination Survey (KNHANES) with screening detected diabetes and prediabetes together with information on use of glucose lowering drugs[25], and another based on Korean Community Health Surveys conducted 2021-2022 (N=460,603) with self-reported diabetes[23]. These studies all provided ORs that were compatible with a higher prevalence of diabetes/prediabetes in users of e-cigarettes (Table 5). We pooled these results together with the inverse variance method[35]. This yielded an OR of 1.18, 95% CI 1.06-1.31 in current users of e-cigarettes compared to non-users. For former users, the pooled OR of diabetes/prediabetes based on the same studies was 1.15 (95% CI 1.02-1.30). Ever users of e-cigarettes were also more insulin resistant than never users according to data collected 2015-2018 in the NHANES study[19]. A similar tendency, albeit not significant was observed in the 2013-2016 NHANES investigation while no association between e-cigarette use and glucose levels after an oral glucose tolerance test was noted in that study[26]. There was no data available on duration of e-cigarette use or dose.

Regarding the metabolic syndrome, analysis of data from the US NHANES study demonstrated that current e-cigarette users were 30% (95% CI 1.13-1.50) more likely to have the metabolic syndrome than never e-cigarette users[20]. Similarly, results of the Korean KNHANES study suggested that the metabolic syndrome was more prevalent in current e-cigarette users (OR 1.27, 95% CI 0.96-1.70) than never e-cigarette users[25]. Both studies defined metabolic syndrome in accordance with the U.S. National Cholesterol Education Programme Adult Treatment Panel III (NCEP ATP III) criteria[36].

There was also one study that investigated plasma metabolites in e-cigarette users, cigarette smokers, and non-smokers to explore potential metabolic effects[27]. E-cigarette use was associated with altered metabolites in the tricarboxylic acid (TCA) cycle, which could disrupt energy metabolism. This may impair glucose metabolism and increase oxidative stress, both of which are associated with insulin resistance and diabetes development.

Mechanistic studies

There were no human experimental studies, but we identified five animal studies[26, 29-32] that explored the effects of e-cigarette vapour exposure on glucose tolerance, insulin resistance or related traits, all conducted in mice. One of these studies showed increased insulin resistance following exposure to e-cigarette vapour with nicotine but not without nicotine[30]. In contrast, three other mice studies did not find effects on neither insulin sensitivity[26, 29] nor glucose tolerance[26, 32] in mice exposed to e-cigarette vapour with nicotine. Similar null results were seen in a study exploring effects of nicotine free e-cigarette exposure[31]. Another mice study showed that e-cigarettes induced oxidative stress, shown by elevated reactive oxygen species, oxidative damage and apoptosis. Additionally, metabolomics analysis revealed disruptions in amino acid metabolism and TCA cycle activity, suggesting impaired energy metabolism[32].

White Snus

Our literature search did not identify any epidemiological or human experimental studies investigating the association between white snus or nicotine pouches and diabetes, glucose tolerance or insulin resistance. Extending the search to the metabolic syndrome, overweight

and obesity did not yield any studies either. One animal study examined the effect of oral nicotine on insulin resistance in an obese rat model[33]. Since nicotine pouches and oral nicotine share similar absorption (via oral mucosa) and result in comparable blood nicotine levels, their health effects—including metabolic changes and addiction potential—are likely similar. It was found that chronic oral nicotine reduced plasma glucose after insulin loading, suggesting it may improve insulin resistance by lowering hepatic glucose release.

Heated Tobacco Products

There was only one epidemiological study that examined HTPs in relation to diabetes and related traits[28]. This was a Japanese cross-sectional study comparing the prevalence of diabetes and screening detected prediabetes in exclusive HTP users to that of never smokers. The findings indicate that exclusive HTP users had higher prevalence of prediabetes (OR 1.36, 95% CI 1.25–1.47) and diabetes (OR 1.68, 95% CI 1.45–1.94). Higher levels of HbA1c and fasting glucose were also noted in HTP users vs. never smokers. The literature search did not find any experimental animal or human studies that investigated the effects of HTP use on metabolic outcomes.

Conclusions

A wealth of studies from various countries show that smoking increases the risk of type 2 diabetes, and the risk increases with dose and duration of exposure. Snus use appears to carry a similar risk, according to a smaller number of primarily Swedish studies. Emerging evidence also suggests that smoking and snus may promote type 1 diabetes in adults, but further research is needed. Nicotine exposure has been shown to induce insulin resistance and disrupt glucose regulation, mechanisms that likely contribute to diabetes development[9].

New tobacco and nicotine products, such as e-cigarettes, white snus, and HTPs, contain similar or higher nicotine levels than cigarettes and traditional snus, suggesting they may also increase diabetes risk. A limited number of epidemiological studies on e-cigarettes support this hypothesis by showing higher prevalence of type 2 diabetes, prediabetes, insulin resistance and the metabolic syndrome in e-cigarette users than never users. However, all studies were of cross-sectional design and based on self-reports, which implies that reverse causation and recall bias is a concern. No study provided information on dose or duration of e-cigarette use and there was no study on type 1 diabetes. Animal studies suggest e-cigarettes may impair energy metabolism and increase insulin resistance, though findings are inconsistent. No studies have investigated the impact of white snus on diabetes risk, and only one has examined HTPs. However, given their high nicotine content, these products are unlikely to be less harmful to insulin sensitivity than conventional products.

Based on current knowledge on the influence of smoking and snus use on diabetes risk and the adverse effects of nicotine on insulin resistance and glucose tolerance it is reasonable to assume that the use of nicotine products —be it e-cigarettes, white snus, or HTPs—increases the risk of diabetes. Whether this is the case remains to be shown.

TABLE 5. SUMMARY OF LITERATURE REVIEW: STUDIES INVESTIGATING THE NEW NICOTINE PRODUCTS IN RELATION TO DIABETES, PREDIABETES, GLUCOSE TOLERANCE, INSULIN RESISTANCE AND THE METABOLIC SYNDROME.

Author and year	Study design	Country and setting	Exposure categorization	Outcome	Covariates	Results
			E-cigarettes			
Cai & Bidulescu, 2023[19]	Epi. cross-sectional	United States, NHANES 2015-2018, n=5101	Current and former e-cig use vs never use	Prediabetes (fasting glucose100 -<126 mg/dL or 5.6 to 6.9 mmol/L or HbA1c 39 - 47 mmol/mol), diabetes. HOMA-IR (Q3 vs Q1)	Age, sex, race/ethnicity, education, federal poverty level, family history of diabetes, BMI, other tobacco products, heavy alcohol use, PA, hypertension, high cholesterol.	OR (95% CI): Current users: Prediabetes 1.14 (0.68-1.92), Diabetes 0.73 (CI 0.30-1.93), HOMA-IR 1.33 (CI 0.77-2.30). Former users: Prediabetes 1.18 (0.82-1.72), Diabetes 1.54 (0.87-2.74), HOMA-IR 1.64 (1.04-2.59)
Jeong & Kim, 2024[23]	Epi. cross-sectional	Republic of Korea, KNHANES 2021-2022, n=460,603	Current e-cig use vs. non-use and non-smoking	Self-reported diabetes	Age, sex, education, region, occupation, alcohol consumption, PA, self-reported health, BMI	OR (95% CI): 1.15 (CI 1.01-1.31), dual smokers 1.39 (1.22-1.58) no results for former e-cig users
Zhang et al., 2022[24]	Epi. cross-sectional	US, BRSS, 2016-2018, n=600, 046	Current vs. never e-cig use (current and former smokers excluded)	Self-reported prediabetes	Age, sex, race, BMI, education; PA; heart disease, cancer, depressive disorder, COPD, asthma, other tobacco products.	OR (95% CI): Current users 1.54 (1.17-2.04) Former users: 1.13 (1.00-1.29) Dual users: 1.14 (0.97-1.34)
Cai & Bidulescu, 2023[20]	Epi. cross-sectional	US, NHANES, 2015-2018, n=5121	Current e-cig use vs. never use	Metabolic syndrome	Age, sex, race/ethnicity, education, income, and use of other tobacco products.	PR (95% CI): METS: 1.30 (1.13-1.50)
Kim et al., 2020[25]	Epi. cross-sectional	Republic of Korea, KNHANES 2013-2015, n=14,738	Current e-cig use vs. never use	Prediabetes or diabetes Metabolic syndrome.	Age, sex, education, income, cigarette use, alcohol consumption, PA.	Prediabetes or diabetes: PR (95% CI): Current user : 1.05 (0.78-1.40). Ever user: 0.89 (0.74-1.08) METS: 1.27 (0.96-1.69)

Orimoloye et al., 2019[26]	Epi. cross-sectional	US, NHANES 2013-2016, n=3,415	Current e-cig use vs. non-use and non-smoking	HOMA-IR 2h glucose levels after OGTT	Age, sex, race, PA, BMI, and heavy drinking.	β-coefficient (95% CI): HOMA-IR 0.20 (-0.09–0.49) Glucose (mg/dL) -0.05 (-0.21–0.11)
Wang et al., 2021[27]	Epi. cross-sectional	US, n=24	E-cig users, smokers of conventional cigarettes and never-users.	Plasma metabolites	No confounding adjustment, groups had similar sex and age distribution	Vaping led to changes in energy metabolism (TCA cycle).
Chen et al., 2021[29]	Animal experimental	Australia Balb/c mice (male)	Overfed and normal fed mice exposed for 6 weeks to e-cig vapour (with or without nicotine) vs. fresh air.	Glucose tolerance test. Marker of insulin sensitivity (PPARγ)	NA	No adverse effects on glucose tolerance or insulin sensitivity from e-cigs in neither overfed nor normal fed mice.
Lan et al., 2020[30]	Animal experimental	China ApoE gene knockout mice (male)	18 weeks exposure to e-cigs (with and without nicotine), conventional cigarettes, vs fresh air.	Blood glucose after insulin injection total cholesterol, triglyceride, low/high density lipoprotein. C-reactive protein and sTNF-α	NA	Insulin sensitivity decreases with e-cigs with nicotine (but not without nicotine) and conventional cigarettes. Blood lipids and chronic inflammatory indices increased.
Lechausseur et al., 2021[31]	Animal experimental	Canada Male and female C57bl/6N mice	Acute exposure (2h) and 9 weeks exposure to e-cig vapour (without nicotine) vs fresh air.	Fasting glucose and insulin. Glucose levels after glucose tolerance test.	NA	No effect on levels of fasting glucose or insulin and response to glucose tolerance test from exposure to e-cigs without nicotine
Orimoloye et al., 2019[26]	Animal experimental	United States, C57Bl6/J mice	E-cig vapour with and without nicotine vs. fresh air for 12 weeks.	Fasting glucose, insulin, HOMA-IR, glucose tolerance test.	NA	No effect on fasting glucose, insulin, HOMA-IR or glucose tolerance.

Ren et al., 2022[32]	Animal experimental	China C57BL/6J mice (male)	E-cig vapour for 0, 1, 2, 4 or 8h (acute exposure).	Glucose, total cholesterol and triglycerides. ROS levels and cell apoptosis rates in tissues. Metabolomics	NA	No effect on glucose or triglycerides, elevated cholesterol levels. Increased oxidative stress (ROS) and apoptosis in multiple organs. Metabolomics revealed disrupted amino acid TCA cycle activity.
White snus						
Liu et al., 2003[33]	Animal experimental	Japan, Zucker fatty rats	Oral nicotine administration vs. controls.	Blood samples	NA	Plasma glucose levels after insulin load were significantly lower in nicotine exposed group vs. controls.
Heated tobacco products						
Hu et al., 2022[28]	Epi. cross-sectional	Japan, Japan Epidemiology Collaboration Occupational Health Study, N=40,291	Exclusive HTP use vs. never smoking Dual use of HTPs and conventional cigarettes vs. never use.	Diabetes and prediabetes based on fasting blood glucose and HbA1c levels and self-reported diabetes treatment	Age, sex, BMI, alcohol, red and processed meat, dairy food, coffee, and sugar-sweetened soft drinks, PA, hypertension, and dyslipidaemia.	OR 95% CI: Exclusive HTP users: prediabetes 1.36 (1.25-1.47), diabetes 1.68 (1.45-1.94), higher fasting glucose and HbA1c levels Dual users: prediabetes: 1.26 (1.13–1.39). Diabetes 1.93 (1.63–2.29)

Metabolic syndrome: abdominal obesity, elevated triglycerides, elevated fasting glucose, reduced HDL-cholesterol, high blood pressure. BMI, Body mass index; BRSS, Behavioural Risk Factor Surveillance System; COPD, Chronic Obstructive Lung Disease; Epi, Epidemiological; HbA1c, Haemoglobin A1c; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance; KNHANES, Korean National Health and Nutrition Examination Survey; NHANES, National Health and Nutrition Examination Survey; OGTT, Oral Glucose Tolerance Test; OR, Odds Ratio; PA, Physical Activity; PR, Prevalence Ratio; ROS, Reactive Oxygen Species; sTNF-α, Serum Tumour Necrosis Factor-alpha; TCA, Tricarboxylic Acid; TyG index, Triglyceride-Glucose Index.

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Lung diseases

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Introduction

This chapter explores the respiratory health effects associated with tobacco and nicotine product use, focusing on conditions such as chronic bronchitis, chronic obstructive pulmonary disease (COPD), and acute lung injury. It examines how smoking, e-cigarette use, and heated tobacco products (HTPs) contribute to airway inflammation, impaired lung function, and increased respiratory symptoms (cough, mucous production, wheezing). Special attention is given to emerging concerns, including e-cigarette or vaping product use-associated lung injury (EVALI) and the toxicological impact of inhaled flavouring agents. Potential effects on asthma are covered in a separate chapter.

Smoking and brown snus

Traditional combustible cigarette smoking has been directly linked to cause and / or exacerbate all the-above mentioned outcomes[1]. The severity of the outcome/s is often associated with exposure levels (pack-years: the number of years of smoking multiplied by the number of packs of cigarettes smoked per day) and genetic susceptibility. Accelerated and early decline of lung function is common amongst traditional combustible cigarette smokers[1]. Since snus is an oral smokeless tobacco product that does not contribute directly to inhalation toxicity, we will not discuss traditional brown snus or the newer white snus/nicotine pouches in this chapter.

Literature review

Among the 4316 references identified in the "Lung" search, duplicate articles from the "Cancer" search and the "Asthma/Allergy" were identified and removed. This resulted in 2805 references for the lung chapter. Following that, 594 conference abstracts, 283 case reports, and 25 randomized control trials (RCT) that were all sponsored by the tobacco industry were identified through a search in "title" and "keywords" and thereafter excluded. This resulted in a reference list of 1903 articles (e-cigarettes: 1856; HTP: 47). Amongst the 1903 articles, 157 review articles (e-cigarettes: 152; HTPs: 05) were identified via. title search. Best possible attempts were made to exclude tobacco industry sponsored articles since automated search was not feasible. Recent systematic review articles and meta-analysis on respiratory health outcomes of e-cigarettes and HTPs were searched. No relevant meta-analysis or epidemiological studies on HTPs were identified. Two recent meta-analysis based on epidemiological studies on the relevant outcomes for lung were identified for e-cigarettes[2-3] and discussed in this chapter. Review articles on mechanistic insights of e-cigarette and HTP exposure on pulmonary toxicity and selected original research articles on experimental lung models using relevant exposure methods (*e.g.,* aerosol) form the basis of this chapter.

E-cigarettes

A particularly alarming acute condition linked to e-cigarette use so far reported was the outbreak of e-cigarettes, or vaping, product use-associated lung injury (EVALI). By February 2020, more than 2800 EVALI cases and 68 EVALI related deaths had been reported in the U.S[4-5] . The patients with EVALI (53 patients with median age 19 years) reported various respiratory symptoms such as respiratory distress, shortness of breath, chest pain, pleuritic chest pain,

and cough[6]. Bilateral infiltration in the lung, ground-glass opacification, lipid laden macrophages, mild and non-specific inflammation, acute diffuse alveolar damage with foamy macrophages, and interstitial and peri-bronchial granulomatous pneumonitis were reported[6].

E-cigarette use has been associated with chronic bronchitis since several of the aerosol constituents can damage the respiratory epithelium and impair mucociliary function[7-8]. A Swedish epidemiological study of young adults found that e-cigarette users experienced more chronic bronchitis-like symptoms, such as cough and mucus production, compared to non-users[8]. Those who used both e-cigarettes and regular cigarettes had an even higher risk of respiratory symptoms[8]. A systematic review involving eight studies (seven studying immediate effects and one studying long-term effects) and 273 participants indicated that e-cigarette use increases airway resistance but had no impact on lung function parameters such as forced expiratory volume in 1 second, forced vital capacity or their ratio[2].

A recent meta-analysis involving 94 cross-sectional and 30 longitudinal studies reported population-based disease odds for e-cigarettes and dual use (currently using e-cigarettes and traditional combustible cigarettes) versus traditional combustible cigarette use[3]. The authors reported the pooled adjusted odds ratio for current e-cigarette versus traditional combustible cigarette use to be lower for COPD (0.53) (Table 6). However, the pooled odds ratio for dual use was increased for COPD (1.41) when compared to only traditional combustible cigarette users (Table 6)[3]. The pooled odds ratio for COPD between traditional combustible cigarette users and non-users was 2.99 [3]. Importantly, the pooled odds ratio for COPD when compared between e-cigarette and dual users to non-users was increased (1.46 and 3.29 respectively). Therefore, it can be assumed that the risks of e-cigarette use are higher than previously estimated for COPD. It is also important to note that full manifestation of tobacco smoking associated COPD usually occurs at an age above 40 years. The lower COPD odds ratio for e-cigarette users may reflect that on average the e-cigarette users are younger than traditional combustible cigarette users[3].

Exposure of *in vitro* bronchial and alveolar lung mucosa models cultured at air-liquid interface to two common fruit flavoured e-cigarette aerosols using low intensity vaping regimen indicated pro-inflammatory response, oxidative stress, tissue injury, differential regulation of alarm anti-proteases and anti-microbial defence response, as well as alteration of epigenetic markers[9]. The findings of the study suggested that flavour, nicotine content, power settings of the e-cigarette instrument, and lung regions (bronchial and alveolar) impact the toxicological response[9]. Further, another *in vitro* study indicated that exposure of fruit flavoured e-cigarette aerosol may influence macrophage phenotype independent of nicotine content[10]. Altered lipid homeostasis, induction of the pro-inflammatory macrophages (M1-type), together with impaired phagocytosis were reported as one of the plausible mechanisms of e-cigarette mediated pulmonary toxicity[10].

TABLE 6. POOLED ADJUSTED ODDS RATIOS (95% CONFIDENCE INTERVAL) OF CHRONIC OBSTRUCTIVE PULMONARY DISEASE (COPD) AMONG E-CIGARETTE USERS, CONVENTIONAL CIGARETTE USERS, DUAL USERS (CURRENTLY USING E-CIGARETTES AND CONVENTIONAL CIGARETTES), AND NONUSERS (ADAPTED FROM GLANTZ ET AL)[3].

Comparison groups	COPD
E-cigarette use vs conventional cigarette use.	0.53 (0.38 - 0.74)
Dual use vs conventional cigarette use.	1.41 (1.12 - 1.64)
E-cigarette use vs non-use.	1.46 (1.31 - 1.61)
Dual use vs non-use.	3.29 (1.97 - 5.51)
Conventional cigarette use vs non-use.	2.99 (2.29 - 3-92)

Flavouring agents in e-cigarette liquids: One major concern is that many flavouring agents used in e-cigarette liquids, such as diacetyl and 2,3-pentanedione, are linked to severe respiratory conditions. Diacetyl, for example, has been associated with a condition known as bronchiolitis obliterans, or "popcorn lung," which causes permanent scarring of the lung's airways and leads to severe breathing difficulties. Though diacetyl has been banned in many e-cigarette liquids, it is still found in some products, particularly those with buttery or sweet flavours[11-12]. Assessment of pulmonary toxicity of diacetyl using the *in vitro* bronchial model at air-liquid interface indicated pro-inflammatory response, oxidative stress, and tissue injury[13]. Other chemicals, like cinnamaldehyde (found in cinnamon-flavoured e-liquids), can impair lung function by reducing the cilia's ability to remove mucous and other debris from the lungs[11-12]. Flavouring agents like vanillin and benzaldehyde, used in sweet and fruity flavours, have also been shown to increase inflammation and oxidative stress in lung tissues, contributing to the development of lung diseases such as chronic bronchitis[11-12]. It is important to note that flavourings added in e-cigarette liquid which are generally safe for ingestion (e.g. diacetyl) may be extremely toxic when inhaled. Moreover, thermal degradation of the flavourings at different power settings of the e-cigarette instruments may result in unknown by-products and correspondingly unknown risks[9]. Adulteration of e-cigarette liquid particularly when obtained from informal sources is another concern like the one observed in case of EVALI outbreak. Presence of vitamin E acetate in tetrahydrocannabinol containing e-cigarette liquid was strongly linked to EVALI outbreak[4].

Heated Tobacco Products

In contrast to traditional cigarettes HTPs do not involve combustion yet they produce toxic substances that can cause respiratory tract irritation and compromised airway function[14-15]. In fact, 2 - 137 times higher levels of twenty-two harmful and potentially harmful constituents have been reported in the HTP aerosol compared to mainstream smoke of traditional combustible cigarettes[16-17]. Since HTPs are a newer product compared to e-cigarettes, the scientific evidence from both human and experimental studies remains limited .

One study evaluated the toxicological response of HTP aerosol exposure to the *in vitro* bronchial and alveolar lung mucosal models cultured at air-liquid interface using the International Organization for Standardization puffing regimen[18]. To understand the plausible modes of pulmonary toxicity, the study assessed a broad spectrum of endpoints and identified

oxidative stress, DNA damage, lipid peroxidation, and ferroptosis as the key features of HTP aerosol exposure[18]. Importantly, the findings of this study demonstrated that HTP aerosol exposure is toxic by itself, and the adverse effects are consistent with those reported for traditional combustible cigarette smoke exposure[18]. The findings further indicated that similar as well as differential toxicological response may drive the effects in bronchial and alveolar lung regions.

Conclusions

Taken together, the increased respiratory symptoms seen among short-term e-cigarette users, increased airway resistance in e-cigarette users, higher odds of COPD compared to non-users, accumulating pulmonary toxicological evidence from mechanistic studies, and chemical profiling of e-cigarette and HTP aerosols indicate severe respiratory health risks associated with these products. Long- term assessments are warranted to further dissect the respiratory risks involved with e-cigarette and HTP use. Based on the current state of knowledge, use of e-cigarettes and/ or HTPs in turn pose significant risk towards developing both acute and chronic adverse respiratory health outcomes such as EVALI, chronic bronchitis, and COPD (Figure 3).

FIGURE 3: A SCHEMATIC REPRESENTATION OF THE CURRENT UNDERSTANDING OF INHALATION TOXICITY AND ASSOCIATED RISKS DUE TO E-CIGARETTE AND HEATED TOBACCO PRODUCT (HTP) USE.

Inhalation toxicity of e-cigarettes and/ or HTPs.

	Main outcomes	Plausible mechanisms	Anticipated risks
► **Acute :**	● EVALI.	● Inflammation. ● Oxidative stress.	● Nicotine addiction. ● Gateway to traditional combustible cigarette smoking particularly among adolescents and youth.
► **Chronic :**	● Respiratory symptoms. ● Chronic bronchitis. ● COPD.	● Impaired mucociliary clearance. ● Altered lipid homeostasis. ● Impaired phagocytosis.	● Thermal degradation of flavoring agents (unknown risks).

COPD: chronic obstructive pulmonary disease; **EVALI:** e-cigarettes, or vaping, product use-associated lung injury.

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Pregnancy and Women's Health

Maria Kippler and Donghao Lu

Introduction

Women's health and pregnancy represent critical aspects of public health, with far-reaching implications for maternal and child outcomes. Pregnancy is a particularly sensitive period, during which maternal exposures can influence foetal development and long-term health trajectories[1]. Moreover, broader aspects of women's health, including reproductive health and health issues that women are disproportionally affected by (*e.g.*, mental health outcomes), are central to ensuring equal well-being across the lifespan[2]. Understanding and mitigating risk factors that adversely impact these domains is essential for reducing health disparities and improving overall quality of life for all.

During the past decades, use of tobacco among women shows a decline in many regions, particularly among higher socio-economic groups[3]. Notably, there has been a recent shift towards the use of new nicotine products, such as e-cigarettes, especially among younger populations[4]. Moreover, the use of e-cigarettes among women, especially those of reproductive age and pregnant women, has significantly increased in recent years, partly due to the switch from conventional cigarette smoking. For instance, use of e-cigarette has increased from 15% in 2013 to 29% in 2016 among women aged 18-44 years in the US[5]. A similar trend has been found in Nordic countries. Use of snus before pregnancy has increased from 5.1% during 2012-2014 to 8.4% during 2015-2017 among pregnant women in Southern Norway[6]. Similar trend has been observed in Sweden (use of snus before pregnancy increased from 1.7% to 7.5% during 2000-2022; Figure 4), while smoking before and during pregnancy have declined over time. The introduction of new nicotine products, marketed as alternatives to conventional cigarettes, has raised concerns about their potential effects on women's health and pregnancy outcomes. This area warrants attention due to the rapid adoption of these products, particularly among younger women, and the existing evidence linking these emerging products to women's health outcomes.

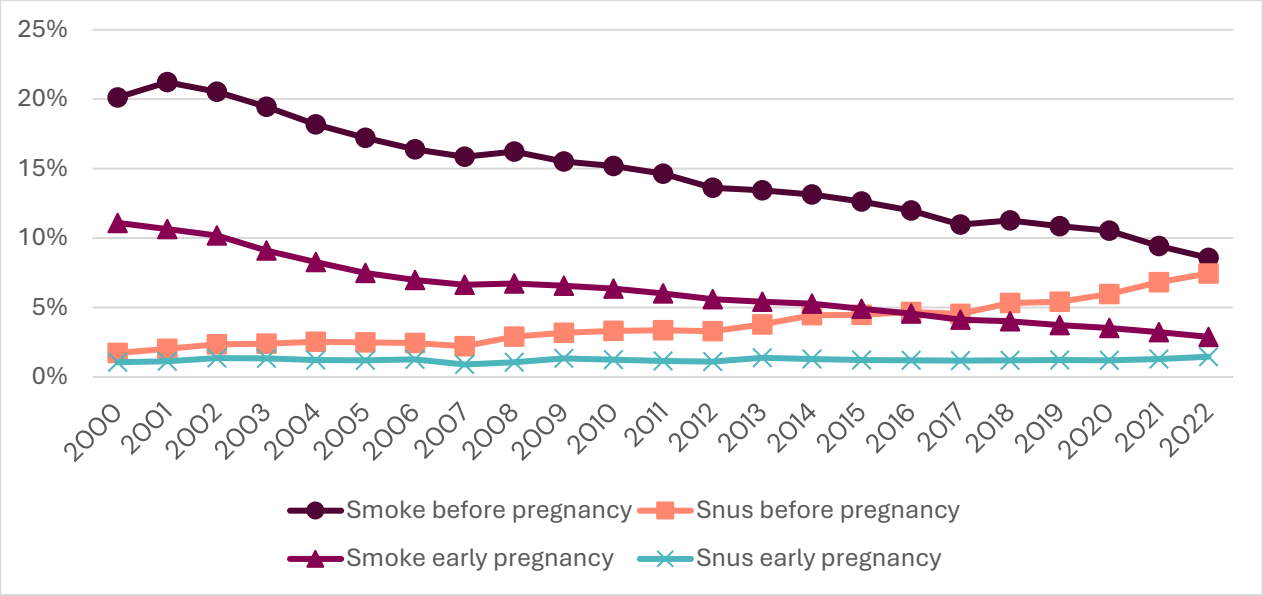
Smoking and brown snus

Pregnancy

Pregnant women who smoke during pregnancy have been shown to have an increased odds of miscarriage[7], placental abruption[8], placenta previa, and placental insufficiency[9]. Offspring exposure to conventional tobacco smoking *in utero* has consistently in a dose-response dependent manner been associated with preterm birth, small-for-gestational-age (SGA), and low birth weight[10,11] as well as with stillbirth, perinatal mortality[12], and sudden infant death syndrome (SIDS)[13]. Maternal tobacco use during pregnancy has also been associated with long-term adverse effects that extend into childhood and later life. There are a couple of epidemiological studies in which maternal tobacco smoking during pregnancy, especially >5-10 cigarettes/day, has been associated with reduced kidney size and volume both in foetal life and childhood[14]. In systematic reviews and meta-analyses, exposure to tobacco smoking *in utero* has been associated with risks of attention deficit hyperactivity disorder (ADHD)[15], anxiety, schizophrenia[16,17], and reduced academic achievement[18].

Several studies have revealed associations of *in utero* exposure to snus use with various pregnancy outcomes as well as early life health. In a systematic review, snus use during pregnancy was associated with increased odds of stillbirth, extremely and moderately premature birth, SGA, low birth weight, oral cleft malformations, and neonatal apnoea[19]. There were also indications that snus use during pregnancy may be associated with increased odds of very premature birth, preeclampsia, and antenatal bleeding. In a nationwide study of singleton births in Sweden from 1999 to 2010, comparing with non-users, women with snus use during early pregnancy had a higher risk of stillbirth but not for early neonatal mortality (1 week after birth)[20]. In a recent nationwide register-based study, including all infants with information on tobacco exposure in early pregnancy born in Sweden 1999-2019, snus use during pregnancy was associated with 65% increased risk of neonatal mortality (within first 28 days), 3.67-fold higher risk of SIDS, and 2.84 higher risk of sudden unexpected infant death compared to no use[21].

FIGURE 4. SELF-REPORTED SMOKING AND SNUS USE 3 MONTHS BEFORE PREGNANCY AND DURING THE FIRST TRIMESTER 2000-2022, BASED ON THE MEDICAL BIRTH REGISTER (2000-2021) AND PREGNANCY REGISTER (2022).



Women's health

Studies on the impact of tobacco smoking on women's health outcomes are not as abundant as those in pregnant women. Nevertheless, studies on tobacco smoking's impact on reproductive health and gynaecological conditions have been steadily increasing, although with inconsistent results. In more recent systematic reviews, it has been indicated that girls born to mothers who smoked during pregnancy enter menarche at an earlier age[22]. Moreover, both former and current smokers were more likely to develop dysmenorrhea than non-smokers[23]. On the contrary, a meta-analysis did not indicate any association between tobacco smoking and endometriosis[24]. However, concern was raised regarding the ascertainment of the presence or absence of endometriosis in many of these studies. There is emerging observational data suggesting that tobacco smoking women have a higher prevalence of infertility, lower fecundity, longer time to conception than non-smokers[25]. In a meta-analysis, including 11 studies,

tobacco smoking was suggested to be associated with early menopause[26], although it was noted that both exposure assessments and diagnosis were uncertain in some studies. In the Nordic countries, to our knowledge, no studies have been conducted on snus use and its potential impact on women's health.

Literature review

A comprehensive literature search was conducted to examine the impact of new nicotine products on women's health and pregnancy outcomes. Both searches utilized major databases, including Medline (Ovid), Embase (embase.com), Cochrane Library (Wiley), and Web of Science (Clarivate Analytics), with search strategies collaboratively developed with librarians from the Karolinska Institutet University Library. Searches were supplemented by snowballing techniques and conducted without language restrictions and included both MeSH terms and free-text keywords tailored for each database using the Polyglot Search Translator.

For the pregnancy outcome review, a total of 2,212 records were identified (Medline: 549, Embase: 979, Cochrane: 116, Web of Science: 568), with 1,206 unique records remaining after deduplication including both epidemiological and experimental studies. After the initial screening, full text was retrieved for 34 epidemiological studies for further assessment. After excluding 7 conference abstracts, 2 reports on long-term offspring outcomes (1 on asthma and 1 on facture), 2 non-original articles, 1 about perception of harm, and 6 on other types of smokeless tobacco products, we finally included 16 original studies. Among them, 13 studied e-cigarettes, 2 focused on HTPs, and one investigated both. After the initial screening of experimental studies, full text was retrieved from 49 studies and after excluding other exposure routes than inhalation, pre-pregnancy exposure to conventional tobacco, and outcomes covered by other chapters (lung function, asthma and allergy, metabolic and cardiovascular outcomes) 17 studies were included herein. Out of these 17 studies, 16 studied e-cigarettes and 1 studied HTPs. There were no studies on white snus and pregnancy outcomes.

For the women's health search, 593 records were identified (Medline: 81, Embase: 250, Cochrane: 140, Web of Science: 122), with 437 unique records after deduplication including both epidemiological and experimental studies. After initial screening, full text was obtained for 15 epidemiological studies for assessment. After excluding 4 conference abstracts, 2 studies on snus use, 1 about use behaviour, and 2 on pregnancy outcomes, we included 6 original studies. All of them investigated e-cigarette, while one study also assessed HTP use. Moreover, 4 experimental studies were identified from the initial screening and full text was retrieved for assessment. However, none of these studies applied e-cigarette exposure through inhalation or replicated exposure to white snuff, and therefore none was included in this report.

E-cigarettes

Pregnancy

Epidemiological studies

We identified 14 studies investigating e-cigarette use and pregnancy with sample sizes ranging from 248 to 190,707 (Table 7). Regarding study design, there were one randomized controlled trial (RCT), four cohort studies, eight cross-sectional studies, and one ecological study. One study was based in UK and one from Italy while the rest were from the US, of which eight reports were based on the same study population – the Pregnancy Risk Assessment Monitoring System (PRAMS), with varying study designs and study periods. To ensure the independence of

evidence, we referred to the latest or largest study based on PRAMS (Ammar 2023) whenever possible (i.e., when results were consistent); otherwise, we clarified in the summary if multiple studies were sourced from PRAMS.

The most studied pregnancy outcome is small-for-gestational age (SGA). Two studies[27,28] showed that exclusive e-cigarette use was associated with a higher risk of SGA compared to non-use (Risk Ratio (RR) ranges from 1.6 to 2.4), whereas null or non-significant associations were noted in four studies[29-32]. Of note, all these reports were based on PRAMS participants, and the conflicting results were likely due to different inclusion criteria and exposure categorizations.

More consistent findings were observed for preterm birth[27,28,30,33], while inconclusive results were found for low birth weight[27,30,31,33]. In an RCT of 1,140 pregnant women in UK, Hajek *et al.* illustrated that the e-cigarette arm had a lowered risk of low birth weight (RR=0.65, 95% CI 0.47-0.90) compared to the nicotine patch arm[34]. In a cohort study of 597 women, Lin *et al.* reported no significant difference on high-risk birth, a composite indicator of varying adverse birth outcomes, between e-cigarette use and no use[35]. In the same study, no difference was shown for foetal death[35], while in an ecological study by Cooper *et al.* a higher infant mortality rate was found in US counties adopted compared to those never adopted indoor vaping restrictions[36].

Beyond birth outcomes, Harlow *et al.* observed a non-significant reduction in fecundability among e-cigarette users compared to never users (RR=0.85, 95% CI 0.68-1.07)[37]. Among women who underwent intracytoplasmic sperm injection (ICSI) cycles, Galanti *et al.* found comparable oocyte quality endpoints, except for more germinal vesicles, in e-cigarette users compared to conventional cigarette users[38], although potential confounding was not addressed. Furthermore, in a cross-sectional study, Wen *et al.* showed that e-cigarette use was not associated with low weight gain during pregnancy[39].

Mechanistic studies

There are several experimental studies in rats or mice which have explored the link of e-cigarette aerosol exposure with both foetal as well as later life growth parameters (Table 8). Some studies report that gestational exposure or a combination of both gestational and lactational exposure to nicotine-containing e-cigarette aerosols have been associated with decreased foetal birthweight[39,40] or decreased weight from after birth up to adulthood [41], while other report that differences in early life weight disappear later on[42,43]. There are also studies where gestational exposure to nicotine-containing e-cigarette aerosols has been associated with decreased or increased adult weight in female, but not in male offspring [44,45]. Studies on maternal exposure to e-cigarette aerosols without nicotine before pregnancy and during pregnancy and lactation has been associated with significantly increased postnatal offspring weight[46,47], whereas exposure to e-cigarette aerosols with nicotine was associated with decreased postnatal weight compared with controls[47,48].

Several experimental studies have studied the impact of e-cigarette aerosol exposure on offspring neurodevelopment as well as underlying mechanisms of action (Table 8). Gestational exposure to e-cigarette aerosols with nicotine has been associated with altered behaviours[42,45], including increased risk of hyperactivity[45,49] and reduced anxiety [49] in adolescent or adult offspring. Studies of gestational or gestational and lactation e-cigarettes with nicotine have also been found to impact behaviour[48] and memory, both short-term and long-term memory deficit in adult offspring, although findings are still diverse[49,50]. Additionally, gestational and lactational

exposure to e-cigarette aerosols with nicotine has been associated with impaired locomotor, learning, and memory function in both adolescent and adult offspring[51]. Moreover, gestational exposure to e-cigarette with nicotine has been shown to worsen outcome in offspring hypoxic-ischemic brain injury[43]. At the cellular and molecular level, gestational e-cigarette aerosol exposure has been shown to disrupt offspring postnatal blood-brain-barrier integrity [51], induce epigenetic changes or alter gene expression in the brain[49,52], increased activity of superoxide dismutase in the hippocampus[43,50], alter neuronal lineage differentiation, calcium signalling and microglia[53], and induce neuroinflammation[41,45,54].

Women's Health

Epidemiological studies

We identified six studies investigating e-cigarette use and women's health outcomes (Table 9). All these studies are cross-sectional in design and based in US except one from Japan. Among these studies, five have a particular focus on mental health. Sung *et al.* reported a higher prevalence of depression among daily users (OR = 2.68, 95% CI: 2.08–3.46), with stronger associations in women compared to men[55]. However, in a cross-sectional study from Japan by Kioi *et al.*, comparable frequency of ever or current e-cigarette use was noted between women with and without mental disorders[56]. In addition, pregnancy-related depression, such as postpartum depression, was not associated with e-cigarette use according to one study[57]. Furthermore, disordered eating behaviours, including binge eating and weight preoccupation, were also more prevalent among users, as reported by Naveed[58] and Dunn[59], although potential confounders were not accounted for in both studies. Regarding overall health, Osibogun *et al.* showed a positive relationship between e-cigarette use and disability status (OR = 1.88, 95% CI: 1.15–3.07)[60]. Of note, all above studies employed cross-sectional design and reverse causation cannot be ruled out.

Mechanistic studies

In a fertility trial it was shown that following 4 months of exposure to e-cigarette vapour with nicotine the dams exhibited a significant delay in the onset of the first litter[44]. Moreover, exposure of new dams in early pregnancy impaired embryo implantation, despite presenting high levels of progesterone. Molecular studies at the transcriptional level revealed significant changes in the integrin, chemokine, and JAK signalling pathways.

Heated Tobacco Products

Pregnancy

Epidemiological studies

The evidence on HTPs and pregnancy is less developed, with only two reports identified from the literature search (Table 7). In a cohort study of 642 pregnant women in Italy, Incognito *et al.* reported a higher frequency of preterm births among HTP users compared to non-smokers (17% vs. 4%), but the lack of formal statistical analysis limits the strength of this finding[61]. In a cross-sectional study of 558 postpartum women in Japan, Zaitsu *et al.* found that ever use of HTPs appeared associated with higher prevalence of hypertensive disorders of pregnancy (OR=2.08; 95% CI: 0.80-9.15) and low birth weight (OR=2.78; 95% CI: 0.84-9.15), although the results did not reach statistical significance[62]. In addition, Galanti *et al.* found no significant differences in oocyte quality between HTPs and conventional cigarette users during ICSI cycles[37].

Mechanistic studies

Experimental data on the impact of exposure to heat-not-burn tobacco products on pregnancy or pregnancy outcomes is very limited (Table 8). Male offspring exposed to heat-not-burn tobacco prenatally had an altered testicular morphology and decreased spermatogenesis[63].

Women's Health

Research on HTPs and women's health is very limited. While Kioi *et al.* described information on ever or current HTP use, the number was too low to conduct any meaningful comparison[56] for mental disorder risk. To our knowledge, there are no experimental studies on the influence of HTPs on women's health.

Conclusion

In conclusion, while the current evidence from epidemiological studies is very limited in both quantity and quality, the findings suggest that the use of new nicotine products poses potential risks to pregnancy outcomes. So far, there is some evidence linking e-cigarettes to adverse birth outcomes, such as preterm birth, low birth weight, and small for gestational age, although results are not yet conclusive. Moreover, for the impact of e-cigarettes on other pregnancy outcomes (i.e., stillbirth and infant mortality), known to be associated with conventional tobacco smoking, either very few or no studies have been identified. Regarding experimental mechanistic studies, most of the studies have focused on e-cigarette exposure during pregnancy and lactation and its impact on offspring growth and neurodevelopment, although findings are often conflicting. Mechanistic studies for other new tobacco products and other offspring outcomes are either very limited or lacking. The epidemiological evidence on HTPs and pregnancy outcomes is limited, and to our knowledge there are no studies on white snus and pregnancy outcomes. However, it should be noted that the negative effect of snus on pregnancy outcomes, which is largely in line with conventional cigarette, has been well-documented in literature. Given the shared nature, for instance nicotine, of these tobacco products, similar effects would be anticipated for white snus, and therefore future studies are urgently needed in this emerging field.

Regarding women's health, there are very few studies available to date, primarily indicating a relationship between e-cigarettes and women's mental illness. As for experimental mechanistic studies, the data on new tobacco products and female health and reproduction is very limited. Future research on HTPs and white snus as well as other areas of women's health (*e.g.*, gynaecological conditions and reproductive health) are urgently needed.

Collectively, while data highlight concerning risks for both pregnancy and women's health, significant research gaps remain, particularly regarding use of HTPs and white snus. Continued investigations involving both preclinical and clinical/epidemiological approaches as well as experimental mechanistic studies is essential to inform public health policies and interventions to address these emerging health challenges for women and their families.

TABLE 7. SUMMARY OF THE LITERATURE REVIEW-EPIDEMIOLOGICAL STUDIES INVESTIGATING E-CIGARETTES AND HTPS IN RELATION TO PREGNANCY OUTCOMES.

Study	Type of study	Setting	Exposure categorization	Outcome	Covariates	Results
				E-cigarettes		
Cardenas 2019[65]	Cohort study	Pregnant women (n=248) seeking prenatal care at a low-risk pregnancy clinic of a university affiliated center in Little Rock, Arkansas.	Self-report at a prenatal visit (half before 20 gestational weeks): current use of e-cig only, cigarette only, dual use, or no use	SGA derived according to the 10th percentile of the sex-specific and gestational age-specific birth weight	Age, race/ethnicity	Compared to non-users, e-cig dual users (RR=1.9, 95% CI: 0.6–5.5) and e-cig-only users (RR=3.1, 95% CI: 0.8–11.7) have a higher risk of SGA birth. Excluding women who did not disclose their smoking status yet verified via biomarkers, the RR of SGA for e-cig-only use was 5.1 (95% CI: 1.1– 22.2), and 3.8, (95% CI: 1.3–11.2) any current e-cig users
Harlow 2021[37]	Cohort study	Women that were living in US or Canada and actively trying to get pregnant, nested from the Pregnancy Study Online (PRESTO)	Self-reported: never or ever (further classified into former or current) e-cig use; and < or ≥ 3 ml of liquid/day	Time to pregnancy	Age, income, education, baseline smoking status, pack-years of cigarette smoking, weekly alcohol intake, intercourse frequency, doing anything to improve conception chances, BMI, Major Depression Inventory score, multivitamin or supplement use, Healthy Eating Index, and parity.	Current and former e-cig use were associated small reductions in fecundability (current-use FR = 0.85, 95% CI: 0.68, 1.07; former-use FR = 0.89, 95% CI: 0.78, 1.00). The association did not get stronger with greater intensity of e-cig use (<3 ml per day, FR = 0.82, 95% CI: 0.58, 1.15; ≥3 ml per day, FR = 0.89, 95% CI: 0.65, 1.21). Time-varying estimates for current and former users were similar to baseline estimates (current-use FR = 0.84, 95% CI: 0.67, 1.06; former-use FR = 0.89, 95% CI: 0.79, 1.01).

Cooper 2022[36]	Ecological study	755 counties from 15 states and Washington DC in US.	Counties never adopting vs. adopting indoor vaping restrictions	Infant mortality rate during 2010-2015	Age, marital status, race, education, payment source, number of current births, minimal legal purchase age laws, and cigarette taxes in state at.	Counties adopting indoor vaping restrictions had a higher infant mortality (0.39 per 1000 live birth).
Hajek 2022[34]	RCT	Pregnant women (n=1140) were recruited from 23 hospital sites across England and one National Health Service Stop Smoking Service in Scotland.	E-cig arm vs. nicotine replacement therapy arm	A range of birth outcomes, including preterm birth, LBW, NICU admission, congenital abnormalities, C-section, perinatal death, and number of women with adverse birth outcomes	None	Compared to nicotine patch arm, LBW was less frequent in the e-cig arm (RR = 0.65, 95%CI: 0.47–0.90). No significant difference was found for other birth outcomes, there were few events.
Ammar 2023[30]*	Cross-sectional study	Women (n=190,707) who recently gave live birth and participated the Pregnancy Risk Assessment Monitoring System (PRAMS), in 2016-2020 in US	Self-reported in last 3 months of pregnancy: non-users, exclusive e-cig users, exclusive conventional cigarette users, and dual users	SGA (<10 percentile), LBW (<2500 g), and preterm birth (<37 weeks)	Age, race/ ethnicity, education, marital status, income, prenatal federal nutritional assistance, pregnancy intention, the Kotelchuck index, initiation of prenatal care in the first trimester, pre-pregnancy multivitamin use, pre-pregnancy alcoholic	Compared with non-use, e-cig only use was associated with a significantly increased risk of preterm birth (aRR: 1.29, 95%CI: 1.00, 1.65) and LBW (RR: 1.38, 95%CI: 1.09, 1.75), but not SGA (RR: 1.04, 95%CI: 0.76, 1.44).

					drinking frequency, parity, history of preterm birth, pre-pregnancy BMI, residency, and year of delivery.	
Kim 2020[27]*	Cross-sectional study	Women (n= 55,251) who recently gave live birth and participated the Phase 8 survey of the PRAMS collected between 2016 and 2018 in US.	Self-reported in third trimester: non-users, exclusive e-cig users, exclusive conventional cigarette users, and dual users	SGA (<10 percentile), LBW (<2500 g), and preterm birth (<37 weeks)	Maternal race, age, education, income, prenatal care adequacy, and conventional cigarette smoking status in the first and second trimester.	Compared to no use, e-cig use was associated with higher risks of SGA (OR 1.76; 95% CI 1.04, 2.96), LBW (OR 1.53; 95% CI 1.06, 2.22), and preterm birth (OR 1.86; 95% CI 1.11, 3.12). Between conventional and e-cig users, no significant difference was found for SGA (OR 0.67; 95% CI 0.30, 1.47), LBW (OR 0.71; 95% CI 0.37, 1.37), or preterm birth (OR 1.06; 95% CI 0.46, 2.48).
Wang 2020[28]*	Cross-sectional study	Women (n= 31,973) who recently gave live birth and participated the PRAMS in 2016 in US	Self-reported in third trimester: non-users, exclusive e-cig (and other electronic nicotine products) users, exclusive conventional cigarette users, and dual users	SGA (<10 percentile) and preterm birth (<37 weeks)	Age, education, race/ethnicity, marital status, previous preterm history, plurality, Kotelchuck index of prenatal care, pre-pregnancy BMI, drinking alcohol before pregnancy, gestational weight gain, and pre-pregnancy smoking/vaping.	Compared to no use, e-cig use was associated with a higher risk of SGA (sole use : OR 2.4, 95% CI 1.0–5.7; dual use OR 2.3, 95% CI 1.3–4.1), but not for preterm birth (sole use: 1.2, 0.5–2.7; dual use: 1.3, 0.8–2.3).
Regan 2021a[29]*	Cross-sectional study	Women (n=79,176) who recently gave live birth and participated the	Self-reported e-cig use 3 months last 3 months of pregnancy (further	SGA (<10 percentile), LBW (<2500 g), and	Age, race/ ethnicity, education, adequacy of prenatal care, use of WIC, combustible	Compared to no use, daily use of e-cig was associated with a higher risk of preterm birth (PR 1.94; 95% CI 1.28–2.93) and LBW (2.00; 95% CI

		PRAMS in 2016-2018 in US	grouped into daily or less than daily use), no use	preterm birth (<37 weeks)	cigarette use during pregnancy, and multivitamin use	1.34–3.00), but not for SGA (reported in figure, PR ~ 0.8). Less frequent use of e-cig was associated with a higher risk of LBW (1.76; 95% CI 1.04–2.65), while the association was not significant for preterm birth (1.26; 95% CI 0.71–2.22) and SGA (reported in figure; PR ~ 1.3).
Regan 2021b[33]*	Cross-sectional study	Women (n=16,022) who recently gave live birth and reported smoking combustible cigarette before pregnancy, nested from the PRAMS in 2016-2018 in US	Self-reported use of e-cig 3 months last 3 months of pregnancy	SGA (<10 percentile), LBW (<2500 g), and preterm birth (<37 weeks)	Age, race/ethnicity, adequacy of prenatal care, parity, multivitamin use, and presence of an obstetric risk factor.	Compared to current cigarette smokers, there was no significant difference in the prevalence of preterm birth (aPR 0.85; 95% CI 0.55, 1.31), SGA (aPR 0.56; 95% CI 0.29, 1.08), or LBW (aPR 0.81; 95% CI 0.54, 1.21) for e-cig users.
Wen 2023a[31]*	Cross-sectional study	Adolescents aged 10-19 (n=10,428) who recently gave live birth and participated the Phase 8 PRAMS in 2016-2021 in US	Self-reported in last 3 months of pregnancy: non-users, exclusive e-cig users, exclusive conventional cigarette users, and dual users	SGA (<10 percentile)	Age, race, ethnicity, marital status, health insurance, pre-pregnancy BMI, pre-pregnancy diabetes, pre-pregnancy hypertension, year of delivery.	Compared with no use, exclusive e-cig use appeared to have no significantly different odds of SGA birth (OR, 1.68 [95% CI, 0.89-3.18]).
Nian 2024[32]*	Cross-sectional study	Women (n= 29,505) who recently gave live birth and reported exclusive use of conventional cigarette 3 months before pregnancy,	Self-reported smoking behaviour in last 3 months of pregnancy: conventional	SGA (<10 percentile), LBW (<2500 g), and preterm birth (<37 weeks)	Age, race/ethnicity, education, household income, marital status, prenatal participation in the WIC program,	Compared to CC-exclusive users, E-cig initiators had an OR of 0.97 (95% CI 0.59-1.61) for preterm birth, 0.70 (95% CI 0.41-1.21) for LBW and 0.82 (95% CI 0.52-1.29) for SGA. Comparing E-cig initiators to quitters, the OR was 1.18 (95% CI 0.71-1.96)

		nested from the Phase 8 PRAMS in 2016-2020 in US	cigarette (CC)-exclusive users: reported exclusive CC use; e-cig initiators: Reported using e-cigs. Quitters: Reported neither e-cig nor CC use		pregnancy intention, flu vaccine receipt, the Kotelchuck index, initiation of first trimester prenatal care, parity, history of preterm birth, pre-pregnancy BMI, hypertension, preexisting and/or gestational diabetes, depression	for preterm birth, 1.52 (95% CI 0.88-2.61) for LBW and 1.42 (95% CI 0.90-2.25) for SGA
Lin 2023[35]	Cohort study	Women (n=597) who were in their pregnancy or encountered labour within one year of the interview date from the Population Assessment of Tobacco and Health (PATH) in US	No e-cig use, quit before pregnancy, and any use during pregnancy	High-risk birth (any of preterm birth, LBW, birth defects, placenta previa, placenta abruption, pre-eclampsia, and cleft lip or palate) and foetal death (any of miscarriage, abortion, ectopic or tubal pregnancy)	Age, education, race, physical and mental health before pregnancy, level of satisfaction with social activities /relationships, harmful perception of e-cig, smoking allowed at home, received advice to quit tobacco use in pregnancy, alcohol and marijuana use	Compared to no use, e-cig use before or during pregnancy was not significantly associated with high-risk birth (before pregnancy: OR 1.14, 95% CI 0.54-2.40; during pregnancy OR 1.19, 95% CI 0.38-3.73), nor foetal death (before: 0.39, 0.07-2.26; during 0.33, 0.04-2.83).
Heated tobacco products						
Galanti 2023[38]	Cohort study	Infertile women (n=410) referring to the Reproductive Physio- pathology and Andrology Unit, Sandro Pertini	Current use of conventional cigarette, e-cig, HTP, or no use	Quality of oocytes retrieved when performing intracytoplasmic sperm injection cycles	No	Compared to conventional cigarette users, e-cig users had a greater number of germinal vesicles (mean 0.48 vs. 0.33 per patient, *p*=0.04), but not for HTP users (mean 0.4). No

		Hospital, Italy, during 2019-2022				statistical difference was observed in terms of other quality results.
Zaitsu 2021[63]	Cross-sectional study	Women who were pregnant or within 1 year postpartum when participating in the Japan "COVID-19 and Society" Internet Survey study in Japan	Ever vs never use of HTP	Hypertensive disorders of pregnancy (HDP; systolic blood pressure ≥140mm Hg or diastolic blood pressure ≥90 mmHg after the 20th week of gestation), and LBW (LBW; <2500 g)	Age, combustible cigarette smoking, educational attainment, occupation, household income, and comorbidity of hypertension or diabetes	Compared to never HTP users, the ORs for HDP and LBW were 2.78 (95% CI 0.84 to 9.15) and 2.08 (95% CI 0.80 to 9.15) among ever HTP users. This analysis was restricted to postpartum women (n=558).
Incognito 2024[62]	Cohort study	Pregnant women (n=642) attending San Marco Hospital in Italy from 2021-2022	Non-smokers, ex-smokers, conventional cigarette smokers, and HTP smokers	Ultrasound evaluation and neonatal outcomes	None	No formal statistical comparison but descriptive presentation. Preterm birth was more frequent in HTP user than non-smokers (17% vs. 4%). The difference seems small for other outcomes.

* Studies were based on the PRAMS study (eight on pregnancy and one on women's health). Due to multiple studies on pregnancy outcomes, we referred to the latest and largest study based on PRAMS (Ammar 2023) whenever possible (i.e., when results were consistent). aPR, adjusted prevalence ratio; aRR, adjusted relative risk; CC, conventional cigarettes; CI, confidence interval; FR, fecundability ratio; HDP, Hypertensive disorders of pregnancy; LBW, low birth weight; PR, prevalence ratio; RR, relative risk; SGA, small-for-gestational age.

TABLE 8. SUMMARY OF THE LITERATURE REVIEW OF EXPERIMENTAL STUDIES INVESTIGATING THE NEW NICOTINE PRODUCTS IN RELATION TO PREGNANCY OUTCOMES

Study	Models	Exposures	Outcomes	Results
Smith 2015[49]	C57BL/6J mice	GD15 to GD19, and after birth both dams and pups were exposed from PD2 to PD16. -Ambient air - E-cig aerosol with nicotine E-cig aerosol without nicotine	-Weight (PD2 and PD7-16) -Gross locomotor activity and exploration (open field test) -Anxiety-like behaviours (elevated zero maze and light/dark transition text) -Spatial learning and cognitive flexibility (Morris water maze with reversed learning)	-PD7 pups exposed to e-cig were lighter than those exposed to e-cig with nicotine and pups exposed to e-cig with nicotine were lighter than controls, differences throughout the postnatal exposure -Adult male mice exposed to e-cig with nicotine during gestation and postnatal life had increased activity in the zero maze and open field tests -They also spent more than 25% of time in the new location in the water maze test after reversal training.
Lauterstein 2016[53]	C57BL/6 mice	Gestation and throughout lactation. -Ambient air - E-cig aerosol with nicotine E-cig aerosol without nicotine	1-month old male and female -Gene expression in frontal cortex	-E-cig constituents other than nicotine appear to cause changes in gene expression. -Transcriptome alterations in both sexes and exposure groups were associated with downstream adverse neurobiological outcomes.
Chen 2018[47]	BALB/C female mice	Before pregnancy, during pregnancy, and lactation: -Ambient air -Tobacco cigarette smoke -E-cig aerosol with nicotine -E-cig aerosol without nicotine	-Weight, fat mass, and fat % at PD20 -mRNA expression of brain metabolic regulators	-Offspring exposed to e-cigs (without nicotine) were the heaviest and with most body fat. -They also had increased mRNA expression of metabolic regulators.
Nguyen 2018[50]	BALB/C female mice	Before pregnancy, during pregnancy, and lactation: -ambient air -E-cig aerosol with nicotine -E-cig aerosol without nicotine	-Short-term memory (Novel object recognition), activity, and anxiety (Elevated plus maze) at week 12 -Global 5-methylcytosine DNA methylation at PD1, PD20, and week 13 (hippocampus only)	-E-cig exposed offspring showed deficits in short term memory, reduced anxiety and hyperactivity. -E-cig (without nicotine) exposed offspring had higher global DNA methylation in the brain than control at PD1 and PD20. -No difference in global DNA methylation of hippocampus at week 13.

			-Epigenetic mRNA gene expression of key chromatin-modifying enzymes at PD1, PD20, and week 13	-13 key genes in the brains of e-cig exposed offspring were significantly altered.
Chen 2018[48]	BALB/C female mice	Before pregnancy, during pregnancy, and lactation: -ambient air -E-cig aerosol with nicotine -E-cig aerosol without nicotine	-Blood cotinine level -Body weight and liver weight -Global DNA methylation -Lung developmental and inflammatory markers	-Postnatally E-cig (without nicotine) exposed offspring were heavier, while E-cig (with nicotine) exposed offspring were lighter. -Both groups had increased retroperitoneal fat mass. -E-cig (with nicotine) exposed offspring had increased liver weight. -In adult offspring, levels of TNF-a protein were increased, whereas IL-1b was suppressed in lung tissue in both exposure groups, in combination with changes in global DNA methylation.
Orzabal 2019[40]	Female Sprague–Dawley rats	GD5 to GD21: -Room air -Vaping e-cigs without nicotine -Vaping e-cigs with nicotine	-Foetal weight, foetal crown-rump length; birthweight -Foetal heart rate -Foetal umbilical artery blood flow	-E-cig (with nicotine) exposure resulted in decreased foetal weight and crown-rump length. - E-cig (with nicotine) exposure also led to reduced maternal uterine artery as well as foetal umbilical artery blood flow.
Wetendorf 2019[45]	C57BL/6J female mice	Preconception and/or during pregnancy: -Room air -Vaping e-cigs with nicotine	-Litter size; onset of the first litter -Embryo attachment -Weight in adulthood	-Gestational e-cig exposure led to significant weight reduction in female offspring at 8.5 months. -E-cig exposed dams exhibited a significant delay in the onset of the first litter. - E-cig exposed dams in early pregnancy significantly impaired embryo implantation.
Al-Sawalha 2020[51]	Female Wistar rats	During pregnancy and lactation: -Fresh air -E-cig aerosol with nicotine	-Brain oxidative stress biomarkers - Spatial learning and memory (the radial arm water maze)	-E-cig exposed offspring had impaired long-term memory in adulthood. -Increased activity of superoxide dismutase in the hippocampus. -BDNF and other tested oxidative stress biomarkers were not affected.

Sifat 2020[44]	CD1 female mice	GD5 to PD7: -Room air -Vaping e-cigs with nicotine	-Weight at PD8 and PD45 -Neuron viability, glucose utilization -Long-term motor and cognitive functions (open field, novel object recognition, Morris water maze, and foot fault tests).	-E-cig exposed pups had decreased weight at PD8, but not PD45 -E-cig exposed offspring had more severe brain injury and oedema following hypoxic-ischemic brain injury. -They displayed impaired memory, learning, and motor coordination in adolescence. -Also, expression of glucose transporters decreased.
Church 2020[46]	CD1 female mice	GD0.5 to GD17.5: -HEPA filtered air -E-cig aerosol -E-cig aerosol with nicotine	-Weight at PD21 and at 12 weeks of age -Stress-coping behavioural measures (elevated plus maze, open field exploration, forced swim test) -Memory performance -Brain region specific cytokine levels (novel object recognition).	-There was a treatment by sex interaction in relation to body weight. -E-cig (with nicotine) exposed offspring exhibited elevated locomotor activity and altered stress coping strategies. -Offspring from both treatment groups had lower object discrimination score. -Offspring from the e-cig nicotine group had a reduction in IL-4, IFN-gamma in the diencephalon, while offspring not exposed to nicotine had increased IL-6 in their cerebellum.
Chen 2022[54]	Female Sprague–Dawley rats	GD4-GD20 -Control air -E-cigs vapor	-Cell types in developing brain -Differential gene expression in neonatal neurons	Gestational e-cig exposure caused: -Disrupted calcium signalling and homeostasis. -Diminished number of microglia in the developing brain. -Elevated susceptibility to neonatal cerebral ischemic injury.
Cahill 2022[41]	BALB/C female mice	During pregnancy: -HEPA-filtered air -E-cig aerosol with nicotine	- Stillbirth, birthweight -Lung fibrillar collagen content, inflammatory lung markers, lung function (Newtonian resistance), chromatin modifying genes in the lungs	-Gestational E-cig exposure led to decreased body weight at birth which was sustained through PD5. -Altered lung structure and function and induced sex-specific molecular signatures during lung alveologenesis in neonatal mice.

Archie 2023[52]	CD1 female mice	During pregnancy and lactation (PD7): -filtered air -E-cig aerosol with nicotine	- Weight (PD7, 15, 30, 45, 60, 90) -Brain-to body weight ratio -Expression of structural elements in the blood brain barrier -Long-term motor and cognitive function (PD 40-45 and PD90-95 using open field test, novel object recognition test, and Morris water maze test)	- E-cig exposed offspring were lighter from PD0 to PD90. -E-cig exposed offspring had reduced expression of tight junction proteins and astrocyte markers until PD90. -They also had impaired locomotor, learning, and memory function in adolescence and adulthood.
Archie 2023[42]	CD1 female mice	GD5-PD7: -Room air -Vaping e-cigs with nicotine -E-cig aerosol -E-cig aerosol with nicotine	-Reactive oxygen species (ROS) -Antioxidative markers -Mitochondrial function -Proinflammatory markers	-E-cig exposed primary neurons had significantly higher levels of cellular ROS and mitochondrial superoxide. -Also, primary neurons displayed reduced antioxidative marker expression and increased proinflammatory markers.
AlHarthi 2023[43]	C57BL/6 female mice	From mating to GD11 -Room air -E-cig vapor -E-cig vapor with nicotine	-Body weight PD32-35 and PD45-46 -Anxiety-like behaviour -Typical behaviours (marble-burying test) -Recognition memory and locomotor activity (novel object recognition) -Brain gene expression	-E-cig exposure (with nicotine) increased weight in early adolescence. -They also had reduced number of buried marbles, lower discriminations index, increased locomotor activity, enhanced nicotine preference. -Gene expression of metabotropic glutamate receptors and transporters were not altered.
Awada 2024[55]	C57BL/6 female mice	During pregnancy and postnatally (PD 4-21) -HEPA filtered air -E-cig aerosol -E-cig aerosol with nicotine	-Gene expression and protein analyses in hypothalamus and hippocampus (offspring at 1-month of age)	-Offspring of both treatment groups displayed an increase in glucose metabolism protein levels. -They also had increased gene expression changes in several genes associated with neuroinflammation.
Yoshida 2022[64]	CD1 female mice	GD7 and GD14 -Control of clean air -Heat-not-burn tobacco aerosols	Male offspring (5 and 15-weeks-old)	-Exposure to heat-not-burn tobacco associated with abnormal seminiferous tubule morphology and decreased sperm production at 5 weeks. No

	-Conventional tobacco aerosols	-Spermatogenesis, sperm characteristics, serum hormone levels (testosterone, oestradiol and FSH). -Seminiferous tubule morphology	significant difference in sperm characteristics or hormone levels when comparing heat-not-burn with conventional tobacco.

BDNF, brain-derived neurotropic factor; GD, gestational day; IFN, Interferon; IL-4, Interleukin-4; IL-6, Interleukin-6; mRNA, messenger ribonucleic acid; PD, postnatal day; ROS, reactive oxygen species; TNF-a, Tumour Necrosis Factor-alpha.

TABLE 9. SUMMARY OF LITERATURE REVIEW-STUDIES INVESTIGATING THE NEW NICOTINE PRODUCTS IN RELATION TO WOMEN'S HEALTH

Study	Type of study	Setting	Exposure categorization	Outcome	Covariates	Results
Kioi 2018[57]	Cross-sectional study	Women (n=2239) aged 40–69 and participated in an internet survey in 2015 in Japan	Never, ever, or current use * This study also measured HTP use but had too few exposed persons	Self-reported chronic diseases, including mental disorders	Inverse probability weighting based on demographic and socio-economic factors, such as education and housing tenure	The prevalence of ever use or current use was statistically comparable between women without any chronic disease and those with a mental disorder (ever use: 3.5% vs 5.3%, *p*=0.34; current use: 0.0004% vs. 0.3%, *p*=1.00).
Naveed 2021[59]	Cross-sectional study	Adolescent girls (aged 13-17) recruited via Facebook advertisement to complete a survey in 2021 in US	Electronic Cigarette Dependence Index (ECDI), with a total score ranging from 0-20 and classified into 4 groups (no, low, medium and high dependence)	Disordered eating behaviours assessed with the Minnesota Eating Behaviour Survey, composed of 4 subscales including weight preoccupation, body dissatisfaction, binge eating, and compensatory behaviour.	None	ECDI scores was correlated with weight preoccupation (rho=0.13, P=0.02), binge eating (rho=0.15, P<0.002) and compensatory behaviour (rho=0.021, *p*<0.001), but not with body dissatisfaction (rho=0.06, *p*=0.28) using Spearman correlation test.
Sung 2021[56]	Cross-sectional study	Women (n=95,248) participating in the 2017 Behavioral Risk Factor Surveillance System (BRFSS) and Selected Metropolitan/Microp olitan Area Risk Trends (SMART) in US. * This study also	Never, former, current non-daily, and current daily e-cig use	Self-reported depression	Age, race, education, income, marital status, employment status, smoking status, and physical activity	Compared to never users, women with current daily use of e-cig had a higher prevalence of depression (OR=2.68, 95% CI 2.08-3.46). Weaker yet significant associations were found for current non-daily use and former use. The associations

		included men for comparison				were stronger in women than in men (daily use OR=1.37).
Dunn 2022[60]	Cross-sectional study	Female adolescents (n=915) attending a university-based adolescent clinic from 2016 to 2018 in US	Self-reported use in the past 30 days, use but not in the past 30 days, or no use	Disordered eating, assessed with self-reported intentional weight loss	No	Disordered eating was more common among e-cig users (in the past 30 days 25%; not in the past 30 days 14.8%) than non-users (5.5%, $p<0.001$).
Osibogun 2023[61]	Cross-sectional study	Reproductive-aged women (18-44 years; n=24,904) from the 2020 Behavioral Risk Factor Surveillance System (BRFSS) in US	Self-reported current use of e-cig, conventional cigarette, dual use, or no use	Disability status defined from questions about difficulty in hearing, seeing, concentrating, remembering, making decisions, walking or climbing stairs, bathing or dressing, or doing errands alone due to physical, mental, or emotional conditions.	Age, education, race/ethnicity, income, marital status, pregnancy status, depression, self-rated health, smokeless tobacco use, past-month marijuana use, and heavy alcohol consumption	Compared to no use, e-cig use (OR=1.88, 95% CI 1.15-3.07) and dual use (OR=2.37, 95% CI 1.55-3.62) were associated with a higher prevalence of disability status.
Choi 2024[58]*	Cross-sectional study	Women (n= 58,950) who recently gave live birth and participated the Phase 8 PRAMS in 2016-2019 in US	Self-reported e-cig use in the past 2-years, 3 months before pregnancy, and last 3 months of pregnancy.	Postpartum depression, assessed with two questions	Age, race, ethnicity, combustible cigarette, and/or hookah use, prenatal care during the last trimester, health insurance coverage during pregnancy, physical abuse during pregnancy, income, and survey type	Compared to no use, e-cig use during past 2 years (OR 1.09, 95% CI 0.98-1.29), pre-pregnancy (OR 1.00, 0.76-1.32), or during pregnancy (1.03, 0.73-1.46) was not associated with a higher risk of postpartum depression.

ECDI, electronic cigarette dependence score; OR, Odds Ratio; RHO, Spearman rank correlation coefficient.

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Conclusions

- The use of products contributing to nicotine addiction is changing, with declining cigarette smoking rates globally, and increased use of new products, such as e-cigarettes, heated tobacco products (HTPs) and tobacco free snus (white snus). In Sweden, the use of white snus has recently become popular among adolescents and young adults, particularly in women.
- This report evaluates health risks associated with the use of e-cigarettes, HTPs and white snus based on literature published until July 2024. It focuses on allergic, cardiovascular and respiratory diseases, cancer, diabetes, and adverse pregnancy outcomes, considering both epidemiological and experimental data.
- Use of e-cigarettes appears to increase the risk of asthma and chronic obstructive pulmonary disease (COPD), as well as other lung diseases, which is supported by mechanistic data. A link to cardiovascular disease, type 2 diabetes, cancer and adverse pregnancy outcomes is supported by limited epidemiological and experimental data. The epidemiological studies often had a cross-sectional design, which complicates assessment of causal associations.
- Less evidence is available on health risks associated with use of HTPs, but a few epidemiological studies indicate a link to asthma and COPD, with supporting data from experimental studies. An increased risk of respiratory diseases from HTP use seems plausible in view of the toxic properties of the emitted compounds. The evidence on other health effects is limited and often suffers from methodological inadequacies, complicating interpretation of the findings.
- Very few, if any, studies have been performed on different types of health risks related to use of white snus, particularly for long-term exposure. Given the high doses of nicotine in white snus users and the known effects of nicotine, e. g. in relation to type 2 diabetes and adverse pregnancy outcomes, it is reasonable to assume that such effects may occur among users of white snus. It is particularly disturbing that the use of white snus is rapidly increasing among women of childbearing ages.
- Despite the limited conclusive evidence on different types of health risks associated with long-term use of the new tobacco and nicotine products the knowledge is sufficient for decisive action to prevent exposure. Marketing is often focused new users, not earlier addicted to nicotine, and use of these products may serve as a gateway to smoking and other types of tobacco use. There is no natural law indicating that a certain percentage of the population will become nicotine addicts, and no nicotine addiction always is a better alternative than addiction from a public health perspective.

Research needs

- The use of different tobacco and nicotine products should be continuously monitored in the population, particularly in younger age groups. The information should be sufficiently detailed to permit disentanglement of the use of specific products. Furthermore, determinants of use should be included, such as age, sex and socioeconomic status, to guide preventive action.
- There is a need for high quality longitudinal epidemiological studies to assess different types of health risks associated with long-term use of the new tobacco and nicotine products. Some health effects have induction/latency periods of years to decades, such as cancer, which means that conclusive results from such studies will not be available for years to come regarding recently introduced products, such as white snus.
- Equally important are experimental studies in different systems to better understand toxic effects of specific exposures resulting from the use of the new tobacco and nicotine products. This may relate to additives used for flavouring and other purposes. Experimental studies may also shed light on etiologic mechanisms and early effect markers. A great advantage is that such studies can generate results earlier than prospective longitudinal epidemiological studies.
- In view of the increasing use of the new tobacco and nicotine products, such as white snus, among women of childbearing ages, with obvious risks for the offspring, there is an urgent need for research on the most effective means to curb this development. New communication and influence technology should be assessed and applied for these purposes.

Abbreviations

ADHD – Attention deficit hyperactivity syndrome
ALI – Air–liquid interface
AP site – Apurinic/apyrimidinic site
aPR – Adjusted prevalence ratio
aRR – Adjusted relative risk
BDNF –Brain–derived neurotropic factor
BP-Blood pressure
CC – Conventional cigarettes
CC users – Conventional cigarette users
CI – Confidence interval
CHD-Coronary heart disease
COPD – Chronic obstructive pulmonary disease
C–section – Caesarean section
CVD – Cardiovascular disease
ECDI – Electronic cigarette dependence score
E–cigarettes – Electronic cigarettes
ECS – E–cigarette smoke
EMT – Epithelial–to–mesenchymal transition
EVALI – E–cigarettes, or vaping, product use–associated lung injury
FR – Fecundability ratio
GD – gestational day
HOMA- homeostasis model assessment
HDP – Hypertensive disorders of pregnancy
HR – Hazard ratio
HTP – Heated tobacco products
ICSI – Intracytoplasmic sperm injection
IFN – Interferon
IL-4 – Interleukin–4
IL-6 – Interleukin–6
LBW – Low birth weight
mRNA – Messenger ribonucleic acid
OR – Odds ratio
p – P value
PAHs – Polycyclic aromatic hydrocarbons
PD – postnatal day
pOR – Pooled odds ratio
PR – Prevalence ratio
RCT – Randomized clinical trial
RHO – Spearman rank correlation coefficient
ROS – Reactive oxygen species
RR – Relative risk
SGA – Small for gestational age
SHS – Second–hand smoke
SIDS – Sudden infant death syndrome
TNF-a –Tumour Necrosis Factor–alpha

TSNA – Tobacco–specific nitrosamines
VOC – Volatile organic compounds
WHO – World Health Organization

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