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Adverse health effects of electronic cigarette use: an umbrella review and toxicological evaluation

Adverse health effects of electronic cigarette use: an umbrella review and toxicological evaluation

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Responsible Camilla Stoltenberg, Director-General

Authors Rune Becher, Norwegian Institute of Public Health, Nordic Institute of Dental Materials.
Håkon Valen, Norwegian Institute of Public Health, Nordic Institute of Dental Materials.
Gunn E. Vist, Norwegian Institute of Public Health
Bendik C. Brinchmann, Norwegian Institute of Public Health, The National Institute of Occupational Health
Jørn A. Holme, Norwegian Institute of Public Health
Tom K. Grimsrud, Cancer Registry of Norway
Ole Andreassen, Institute of Clinical Medicine, University of Oslo. Mental disorders
Vigdis Underland, Norwegian Institute of Public Health
Espen Mariussen, Norwegian Institute of Public Health
Miriam Bakkeli, Norwegian Institute of Public Health
Jon E. Dahl, Nordic Institute of Dental Materials. Non-malignant oral diseases.
Karin C. Lødrup Carlsen, Division of Pediatric and Adolescent Medicine, Oslo University Hospital, Oslo, Norway; Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway. Adverse pregnancy outcomes and effects on early life health
Jan Alexander, Norwegian Institute of Public Health

Quality assurance:
Johan Øvrevik, Director of research, NIPH
Gunnar Sæbø, Senior Scientist. Alcohol, tobacco and drugs, NIPH

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CONTENT

Hovedbudskap

Folkehelseinstituttet har på oppdrag fra Helse- og omsorgsdepartementet oppsummert kunnskapsoversikter (paraplyoversikt) om mulige skadelige helseeffekter ved bruk av e-sigaretter. Vi gjorde et systematisk litteratursøk og identifiserte og inkluderte systematiske oversikter som beskriver kunnskapen om innholdet i e-sigarettvæsker og aerosoler, helseeffekter hos mennesker og dyr, samt relevante funn i *in vitro*-studier. For systematiske oversikter som ikke hadde evaluert kvaliteten på resultatene for longitudinelle studier på mennesker, brukte vi GRADE-systemet for å indikere vår tillit til effektestimatene.

Vi oppsummerte funnene fra de inkluderte systematiske oversiktene i paraplyoversikten. I diskusjonen gjorde vi en toksikologisk evaluering av sykdommer og uønskede effekter identifisert i paraplygjennomgangen. I denne evalueringen inngikk i tillegg til studiene identifisert i de systematiske oversiktene også kunnskap fra andre relevante vitenskapelige rapporter og studier for å belyse sammenhenger mellom bruk av e-sigaretter og de skadelige helseeffektene som beskrives. De viktigste helseskadelige effektene ved bruk av e-sigaretter skyldes eksponering for stoffer gjennom innånding av e-sigarettaerosol dannet fra e-sigarettvæsken. Sammensetningen av aerosolen kan variere med blant annet type e-sigarett, stoffer som utløses fra oppvarmingsenheten, temperatur under aerosoldannelsen samt forskjeller i e-væskeinnhold.

E-sigaretter bør ikke betraktes som en enhetlig produktgruppe. E-sigaretter ble introdusert på markedet uten at det forelå tilstrekkelige toksikologiske studier (bl.a. dyrestudier og *in vitro* studier) som kunne bidra til å avklare et helseskadelig potensial ved bruk av e-sigaretter. Det er få gode langtidsstudier av e-sigarettbruk og sykdom hos mennesker og det trengs bedre eksponeringskarakterisering og lengre oppfølgingstid. Basert på vår systematiske litteraturgjennomgang og den toksikologiske vurderingen konkluderer vi med at bruk av e-sigaretter medfører økt risiko for skadelige helseeffekter. Den relative risikoen for de skadelige helseeffektene som kan tilskrives bruk av e-sigaretter er usikker.

Norsk tittel: Helseskadelige effekter ved bruk av e-sigaretter: En paraplyoversikt og toksikologisk evaluering

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Kvalitetssikring:
Johan Øvrevik
Fagdirektør for forskning
Folkehelseinstituttet

Gunnar Sæbø
Seniorforsker
Rusmidler og tobakk
Folkehelseinstituttet

Sammendrag

Innledning

Elektroniske sigaretter (e-sigaretter) består av et varmeelement, et batteri og en beholder som inneholder en væske. Ved oppvarming danner e-sigarettvæsken en aerosol (damp) som inhaleres. E-sigarettvæsken kan inneholde nikotin eller være nikotinfri. Den består ellers av en blanding av vann, propylenglykol (PG), vegetabilsk glyserin (VG), og ulike smakstilsetninger. Det tilgjengelige antallet av disse smakene smakene er høyt.

Ved oppvarming og fordampning av e-sigarettvæsken vil det dannes nye kjemiske forbindelser (nedbrytningsprodukter og syntese av nye forbindelser), og metaller kan frigjøres fra varmeelementet. Noen av forbindelsene som er identifisert i dampen/aerosolen eller senere dannet i kroppen etter innånding er kjente toksiske forbindelser som kan utgjøre en potensiell helsefare.

Helseskadelige effekter knyttet til bruk av e-sigaretter vil avhenge av en rekke forhold som sammensetningen av den inhalerte dampen, som igjen påvirkes av e-sigarett type, e-væske samt bruksmønsteret. I tillegg kommer individuell disposisjon (genetiske og miljømessige faktorer) som kan gjøre en mer mottagelig for helseskadelige effekter. En enhetlig og presis evaluering av helserisiko knyttet til bruk av e-sigaretter, er komplisert på grunn av den store variasjonen av produkter på markedet og brukernes heterogenitet (f.eks. brukstid, brukers alder, komorbiditet). Testing av aerosoler fra ulike e-sigaretter/e-sigarettvæsker i relevante dyreeksperiment er svært mangelfult. Videre er potensielle langtidseffekter av e-sigarettbruk hos mennesker så langt lite undersøkt.

Hensikt

På oppdrag fra Helse- og omsorgsdepartementet har vi gjennomført en systematisk gjennomgang av systematiske oversiktsartikler knyttet til skadelige helseeffekter ved bruk av elektroniske sigaretter. Mandatet var å gjøre en paraplygjennomgang (en systematisk gjennomgang av publiserte systematiske oversikter) av systematiske oversikter om e-væsker og aerosolinnhold, biomarkører for eksponering og effekter fra studier på mennesker og dyr og fra *in vitro* studier (eksponering av celler og vev) og oppsummere resultatene. I diskusjonen har vi kombinert kunnskapen fra de forskjellige studiene samt benyttet kunnskap fra andre relevante vitenskapelige rapporter/studier. Dette for å gjøre en samlet toksikologisk vurdering, der sammenhenger mellom bruk av e-sigaretter og de skadelige helseeffektene beskrives.

Metode

For å identifisere relevante systematiske oversiktsartikler, gjennomførte vi i henhold til vår protokoll (https://www.fhi.no/en/cristin-projects/ongoing/health-consequences-of-electronic-cigarettes---protocol -for-en-paraply-rev/) et systematisk søk etter litteratur i ulike databaser over vitenskapelige studier.

Kvaliteten på inkluderte systematiske oversikter ble vurdert med AMSTAR-2. For hvert helseutfall brukte vi generelt den mest oppdaterte systematiske gjennomgangen av høyest kvalitet for å oppsummere helseskadelige effekter. For de systematiske oversiktene som ikke hadde gradert kvaliteten på resultatene og der det forelå humane effektstudier, brukte vi GRADE systemet til å angi vår tillit til effektestimatene.

Referanser ble screenet av to forskere uavhengig, først etter tittel og sammendrag og deretter i fulltekst, for inkludering eller ekskludering. Titler og sammendrag ble vurdert i henhold til inkluderings- og eksklusjonskriterier som følger:

Inklusjonskriterier:
Alle typer e-sigaretter kombinert med innholdsstoffer, menneskestudier, dyrestudier og *in vitro* studier
Ingen begrensninger på sammenligninger: røyking, snus eller ingen bruk av tobakksprodukter tillatt som sammenligning
Alle helseutfall som følge av bruk av e-sigaretter
Studiedesign begrenset til systematiske oversikter med litteratursøk, klare inklusjonskriterier og risiko for skjevhetsvurdering av inkluderte studier
Litteraturen begrenses til dansk, engelsk og norsk eller svensk språk

Eksklusjonskriterier:
Forskning finansiert av eller på annen måte knyttet til tobakksindustrien
Publikasjoner om skadereduksjon uten bevis for helseutfall
Studier som kun beskriver eller diskuterer bruksmønsteret av tobakksvarer
Primært avhengighetsfokusert forskning
Anmeldelser og diskusjonsartikler uten systematisk litteratursøk, klare inklusjonskriterier og risiko for skjevhetsvurdering av de inkluderte studiene

I diskusjonen og helhetsvurderingen av resultater har vi også brukt rapporter fra internasjonale ekspertgrupper og oppdatert med relevant informasjon fra vårt systematiske litteratursøk.

Resultater fra de systematiske oversiktene

Vi inkluderte totalt 33 systematiske oversikter. De systematiske oversiktene ble gruppert etter helseeffekter. En systematisk oversikt kan inkludere data om ulike helseeffekter. Vi inkluderte 12 systematiske oversikter om ikke-maligne luftveissykdommer, 6 systematiske oversikter om hjerte- og karsykdommer, 4 systematiske oversikter om mental helse, 3 systematiske oversikter om ikke-maligne orale sykdommer, 2 systematiske oversikter om graviditet og barnehelse, 1 systematiske oversikt om kreft, 2 systematiske oversikter om forgiftning, 3 systematiske oversikter om eksplosjoner og brannskader og

2 systematiske oversikter om andre uønskede effekter. I tillegg inkluderte vi 4 systematiske oversikter om sammensetningen av e-sigarettaerosolen og biomarkører for eksponering, 3 systematiske oversikter om eksponering av dyr, og 4 systematiske oversikter som rapporterte *in vitro* studier.

Innholdsstoffer og eksponering for e-sigarettaerosoler
Ulike komponenter som karbonyl forbindelser, flyktige organiske forbindelser, reaktive oksygenforbindelser, frie radikaler, partikler samt ulike metaller er identifisert i e-sigarettaerosoler. Flere av forbindelsene er assosiert med ulike helseskadelige effekter. Det er stor variasjon i e-sigarett typer og e-sigarettvæsker som brukes, samt variasjoner i bruksmønster. Det er derfor vanskelig å forutsi hvor mye en vil få i seg av de ulike skadelige stoffene ved bruk av e-sigaretter.

Helseskadelige effekter

Luftveissykdom

Fra de systematiske oversiktene ble det ikke funnet noen oppfølgingsstudier som belyser hvorvidt bruk av e-sigaretter kan øke risikoen for utvikling av astma og kronisk obstruktiv lungesykdom (KOLS). To systematiske oversikter med metaanalyse av tverrsnittsstudier rapporterte økt forekomst (prevalens) av astma blant brukere av e-sigaretter sammenlignet med ikke-brukere.

En studie viste redusert funksjon av luftveienes slim-heis (viktig del av forsvaret mot infeksjoner) hos e-sigarettbrukere. En systematisk oversikt rapporterte økt hoste (tegn på irritasjon av luftveier) blant unge brukere av e-sigaretter sammenlignet med ikke-brukere.

En systematisk oversikt rapporterte at bruk av e-sigaretter kunne forårsake såkalt e-sigarett/ «vaping»-assosiert lungeskade (EVALI). Dette er en alvorlig lungeskade som kan forårsake død. De fleste rapporterte tilfellene var knyttet til bruk av e-væske fra det ille-gale markedet og som inneholdt tetrahydrocannabinol (THC; den viktigste psykoaktive for-bindelsen i cannabis) eller en kombinasjon av cannabinoider/THC og nikotin. Det er gode holdepunkter for at tilsatt vitamin-E acetat i e-sigarettvæsken var årsaken til lunge-skadene.

Ved bruk av nikotinholdige e-sigaretter ved røykeslutt er det usikkert om antall bivirkninger eller alvorlig bivirkninger er forskjellig fra bivirkningene som opptrer ved bruk av e-sigaretter uten nikotin, kun nikotinerstatningsprodukter eller ved atferdsstøtte.

Hjerte- og karsykdommer

Risiko for hjerteinfarkt, koronar hjertesykdom og hjerneslag: Vi fant ingen systematiske oversikter som direkte rapporterte risikoen for sykdom forbundet med bruk av e-sigaretter, blant personer som ikke tidligere har benyttet tobakk/nikotin produkter.

Basert på tre tverrsnitts studier var sannsynligheten (odds ratio) for å ha hatt hjerteinfarkt, koronar hjertesykdom eller hjerneslag tilsvarende for røykere som byttet til e-sigaretter og nåværende røykere. Tverrsnitt designet gjør det ikke mulig å konkludere for dette utfallet.

Hjertefrekvens, blodtrykk og karstivhet: Bruk av e-sigaretter med nikotin medførte en umiddelbar økning av hjertefrekvens, systolisk og diastolisk blodtrykk. Svært lav tillit til effektestimatene (GRADE). Bruk av e-sigaretter fører til akutte/umiddelbare effekter på pulsbølgehastighet i arteriene (mål på karstivhet). Lav tillit til effektestimatet (GRADE) Kronisk bruk av e-sigaretter med nikotin blant tidligere røykere reduserte systolisk og diastolisk blodtrykk. Det ble ikke rapportert endring i hjertefrekvens. Svært lav tillit til effektestimatene (GRADE).

Bruk av e-sigaretter med nikotin er assosiert med endringer i hjerterate variabilitet (et mål på den autonome kontrollen av hjertefrekvensen). Studiens forfattere rapporterte at tilsvarende effekter er knyttet til økt risiko for hjerte-kar sykdom.

Biomarkører for utvikling av hjerte- og karsykdom: De systematiske oversiktene rapporterte økte nivåer av biomarkører som kan knyttes til forstyrrelser av immunsystemet, oksidativt stress, aggregering av blodplater og skade på endotelceller i blodårene. Slike prosesser er knyttet til utvikling av hjerte- og karsykdommer.

Psykiske lidelser
Flere studier har rapportert en sammenheng mellom internaliserende (som angst og depresjon) og eksternaliserende (som ADHD) symptomer og lidelser og e-sigarettbruk. Flere studier rapporterte en økt sårbarhet for initiering og bruk av e-sigaretter blant personer med både internaliserende og eksternaliserende lidelser.

En langtidsstudie med 12-måneders oppfølgingstid, rapporterte en økning i depressive symptomer blant ungdom med vedvarende bruk av e-sigaretter. I tillegg rapporterte forfatterne om en sammenheng mellom økt brukerfrekvens de siste 30 dagene og økning i depressive symptomer. Tilsvarende funn som det siste ble også rapportert for unge voksne. Vi hadde svært lav tillit til effektestimatene for disse to funnene (GRADE).

En annen longitudinell studie rapporterte at ADHD-symptomer (Attention Deficit/Hyperactivity Disorder) kunne gi økt bruk av e-sigaretter, men bruk var ikke assosiert med fremtidige ADHD-symptomer. Vi hadde svært lav tillit til effektestimatet (GRADE).

Uønskede graviditetsutfall og helse tidlig i livet
Basert på to studier omtalt i en systematisk oversikt, er vi usikre på effektene på fødselsvekt. Både en økt risiko, og ingen effekt for at barnet ble lite for svangerskapsalder ble rapportert. Vi har svært lav tillit til effektestimatene (GRADE).

Ikke-maligne orale sykdommer
Bruk av e-sigaretter var assosiert med forskjellige symptomer fra munnhulen og slimhinnelesjoner.

Fra de kliniske studiene med tverrsnitts design som undersøkte tannkjøttsykdom (periodontal sykdom), var redusert blødning fra tannkjøttet ved sondering hos brukere av e-sigarett, det eneste kliniske funnet rapportert forskjellig fra ikke-brukere av tobakk.

I en longitudinell studie, med selvrapportert oral helse, ble det funnet økt betennelse i tannkjøtt og bentap rundt tennene hos brukere av e-sigaretter sammenlignet med aldri brukere. Fra tverrsnitts studier basert på selv rapporterte utfall, ble det generelt rapportert økt risiko for tannkjøttsykdom (gingival/periodontal sykdom).

De kliniske studiene som undersøkte peri-implantat sykdom inkludert i den systematiske oversikten rapporterte assosiasjoner mellom forverring av sykdomsparametere i tilknytning til tannimplantater og bruk av e-sigaretter sammenlignet med det man så hos aldri brukere av tobakk.

Det ble ikke presentert noen oppfølgingsstudier som kunne si noe om årsakssammenheng mellom karies og bruk av e-sigaretter.

Kreft
Det ble identifisert en systematisk oversikt hvor alle de humane studiene var evaluert til å være av lav kvalitet på grunn av studiedesignet.

En studie rapporterte om to tilfeller av oralt karsinom hos langvarige brukere av e-sigaretter. Andre studier vurderte biomarkører for eksponering og effekter med relevans for kreft (som dannelse av det kreftfremkallende stoffet N'-nitrosonornikotin (NNN) i spytt fra e-sigarettbrukere og lavere forekomst av mikrokjerner i celler fra munnslimhinnen til brukere av e-sigaretter sammenlignet med røykere. Nivåene hos brukere av e-sigaretter var tilsvarende de man så hos friske kontroller.

Forgiftning og skader
E-sigaretter kan overopphetes eller eksplodere og forårsake skader. Disse kan variere fra små rifter og brannskader til mer alvorlig vevsskade og i noen tilfeller er dødsfall rapportert. Videre kan utilsiktet eller tilsiktet inntak av e-væsker forårsake forgiftninger med alvorlighetsgrad som spenner fra milde symptomer som oppkast, økt hjertefrekvens og økt spyttutskillelse til tilfeller av død (utilsiktet eller selvmord). Hvor ofte disse hendelsene forekom var ikke rapportert.

Epilepsi lignende anfall er rapportert hovedsakelig hos ungdom og unge voksne etter bruk av e-sigaretter. Mange tilfeller var assosiert med samtidig bruk av andre stoffer. Det er usikkert i hvilken grad dette kan skje utelukkende som følge av e-sigarettbruk.

Andre helseutfall
Det var få systematiske oversikter om andre helseeffekter, og de to vi fant ga ingen klare svar. Vi vet ikke om bruk av e-sigaretter påvirker postoperativ helse etter fedmekirurgi eller påvirker søvn.

Vi fant ingen systematiske oversikter som omhandlet annenhånds- og tredjehånds eksponering for e-sigaretter.

Dyr og *in vitro*-studier

Respiratoriske funn: Avhengig av e-sigarett type, e-sigarettvæske, dose, varighet samt dyremodell, har bruk av e-sigarett blitt rapportert å påvirke ulike immunresponser knyttet til luftveissykdommer, inkludert: i) økt nivå av betennelsesfremmende cytokiner og immunceller i lungeskyllevæsken ii) økte allergi-induserte astmatiske symptomer og iii) redusert motstand mot både bakterielle og virale infeksjoner.

Kardiovaskulære funn: Dyrestudiene som er presentert indikerer at eksponering for e-sigarettaerosol med nikotin fremmer en protrombotisk tilstand.

Eksponering for e-sigarettaerosol var også assosiert med økning i arteriell stivhet etter kronisk eksponering (8 måneder), men ikke etter 4,5 måneder. I tråd med dette rapporterte forfatterne en redusert effekt av en vasodilator og økt effekt av en vasopressor.

I en musemodell som utvikler åreforkalkning, ble det rapportert at eksponering for e-sigarettaerosol som inneholder nikotin økte oksidativt stress, påvirket morfologien til hjerteceller så vel som hjertefunksjon og økte antallet aterosklerotiske lesjoner. Akutt eksponering for e-sigarettaerosol *in vitro* underbygger økt oksidativt stress og redusert antioksidativt forsvar etter eksponering for e-sigarettaerosol med nikotin. E-sigarettaerosol med nikotin har også blitt rapportert å øke området av hjerneinfarkt, muligens på grunn av økt permeabilitet av blod-hjernebarrieren. Eksponering av endotelceller *in vitro* for e-sigarettvæsker og aerosol ble rapportert å øke ROS-dannelsen og økte celledød. I tillegg påvirket eksponering av e-sigarettaerosol komplement faktorer og blodplateaggregering. Disse funnene underbygger at e-sigaretter kan ha effekter på kardiovaskulær sykdom.

Uønskede graviditetsutfall og helse tidlig i livet: Hos gravide mus eksponert for e-sigarettaerosol med og uten nikotin i nesten hele svangerskapsperioden og under amming ble det observert kjønnsavhengige endringer i genekspresjon som kan knyttes til uønskede nevrobiologiske og adferdsmessige utfall. Neonatale mus eksponert for e-sigarettaerosol med nikotin hadde redusert lungeutvikling. E-sigarettaerosol ekstrakt forårsaket også doseavhengige effekter på hjerteutvikling i en sebrafisk modell. I en froskemodell påvirket eksponering for e-sigarettaerosol ekstrakt, den kraniofaciale utvikling.

Oral: Det ble ikke funnet oversikter som oppsummerte dyreforsøk på oral helse. En systematisk oversikt inkluderte imidlertid *in vitro*-studier.

En studie rapporterte at eksponering av tannemalje fra storfe for e-sigarettaerosol med forskjellige konsentrasjoner av nikotin og forskjellige smaker resulterte i endringer i emaljefarge. En annen studie rapporterte at e-sigarettaerosol økte adhesjon (bindingstyrken) og at enkelte smaksstoffer økte biofilmdannelsen til *S. mutans.* Økt adhesjon og biofilmdannelse er faktorer forbundet med økt kariesrisiko

En studie rapporterte økt adhesjon til epitelceller og økt cytotoksisitet av *Candida albicans* (sopp) eksponert for e-sigarettaerosol med nikotin sammenlignet med kontroll og nikotinfri aerosol. Økt adhesjon til epitelceller kan mulig være knyttet til økt risiko for oral candida-infeksjon.

Ti *in vitro*-studier inkludert i den systematiske oversikten av Yang og medarbeidere rapporterte cytotoksiske effekter av e-sigarettvæsker og/eller aerosol. Seks av *in vitro*-studiene inkludert av Yang og medarbeidere rapporterte gentoksiske effekter av e-sigarettaerosol. Samlet tyder resultater fra *in vitro*-studier på at eksponering for e-sigarettaerosol kan øke oksidativ DNA-skade. Sannsynligvis skyldes dette forhold som økt dannelse av ROS, redusert antioksidantkapasitet samt redusert evne til reparasjon av DNA skader.

Kreft: Fra de systematiske oversiktene fant vi ingen relevante dyrestudier på kreft. Noen studier med gnagere indikerte effekter på redoksbalansen og effekter på immunsystemet, inkludert økte pro-inflammatoriske responser som kan ha implikasjoner for kreftutvikling. Videre fant vi noen *in vitro*-studier med humane celler som rapporterte at eksponering for e-sigarettaerosoler kunne føre til DNA-skader og ulike cellulære responser knyttet til DNA-skade.

Diskusjon/Overordnet toksikologisk evaluering

Sammensetning og eksponering av e-sigarettaerosoler
Flere skadelige kjemikalier samt ulike metaller/sporstoffer er identifisert i e-sigarettaerosoler. Den store variasjonen i e-sigaretter, både når det gjelder typer og væsker som brukes, samt hvordan selve inhaleringen foregår, gjør den individuelle eksponeringen svært variabel og det er vanskelig å forutsi hvor mye man får i seg av mulig helseskadelige stoffer.

Luftveissykdommer (utenom kreft)
Systematiske oversikter indikerer at bruk av e-sigaretter er assosiert med lokal irritasjon i luftveiene, økt hoste samt astma. Studier på mennesker, dyr og *in vitro* studier indikerer at e-sigaretter med nikotin kan påvirke biomarkører som: i) bronkokonstriksjon, ii) nedsatt hosterefleks, iii) redusert slimhinnetransport, iv) betennelse og v) redusert motstand mot bakterie- og virusinfeksjoner. En vedvarende effekt av slike parametere på luftveiene er ikke bare knyttet til astma, men også kronisk obstruktiv lungesykdom (KOLS). Bruk av e-sigaretter representere derfor en risiko for utvikling av luftveissykdommer og kan føre til forverring av allerede oppstått luftveissykdom.

Det nylige utbruddet av alvorlige lungeskader (EVALI) hovedsakelig i USA, var hovedsakelig knyttet til bruk av e-sigarettvæske med tetrahydrocannabinol (THC) fra det illegale markedet. Disse helseskadene forekom hovedsakelig i en begrenset tidsperiode på et par år. Vitamin E acetat i e-sigarettvæsken har vært sterkt knyttet til EVALI-utbruddet. En kan imidlertid ikke utelukke bidrag fra andre kjemiske komponenter i e-sigarettaerosolen. EVALI-utbruddet viser hvordan bruk av nye produkter kan gi uforutsette helseskader; og at enheten kan gi helseskader ved at den brukes til å inhalere andre stoffer enn det de opprinnelig er ment for.

Hjerte- og karsykdommer
De systematiske oversiktene viste at bruk av e-sigaretter hos mennesker og eksponering av dyr for e-sigarettaerosol kan føre til effekter knyttet til aktivering av det sympatiske nervesystemet, oksidativt stress og betennelses reaksjoner, endotelial dysfunksjon og blodplate-aktivering. Dette er sentrale mekanismer knyttet til økt risiko for hjerte- og

karsykdommer (CVD). For nye nikotin-brukere kan bruk av e-sigaretter øke risikoen for utvikling av CVD, og det kan bidra til økt risiko for mer alvorlige utfall etter kardiovaskulære hendelser. Vår samlede evaluering er at bruk av e-sigaretter kan utgjøre en økt risiko for CVD. Dette underbygges av funn knyttet til bruk av snus, nyere litteratur og en mekanistisk forståelse av hvordan ulike forbindelser i sigarettrøyk kan påvirke CVD.

Psykiske lidelser

Flere studier har vist en sammenheng mellom mental helse og økt bruk av nikotinprodukter. Årsaksfaktorene som ligger til grunn for sammenhengen er ikke kjente. Det er mulig at en felles genetisk og miljømessig sårbarhet er involvert. Det er rapportert at ungdom med psykiske problemer har større sannsynlighet for å begynne med e-sigaretter, noe som støtter hypotesen om "selvmedisinering" i stedet for en årsakssammenheng. På den annen side indikerer studier som fant at e-sigarettbruk var assosiert med depressive symptomer at mental helse kan påvirkes av e-sigarettbruk. Både studier på mennesker og dyreforsøk indikerer økt risiko for utvikling av avhengighet og varige endringer i kognitiv funksjon ved nikotineksponering i ungdomsårene. Effekter av nikotin under hjerneutvikling støtter at nikotin kan påvirke utvikling av psykiske lidelser som depresjon og angst samt ADHD. Det er imidlertid for tidlig å konkludere at det er en direkte årsakssammenheng.

Uønskede utfall ved graviditet, på mor, foster og nyfødt

Resultatene fra den systematiske oversikten om bruk av e-sigaretter under svangerskap, effekt på foster og nyfødt var spesifikt knyttet til fødselsvekt og liten for svangerskapsalderen (SGA), og effekten var usikker. Den kombinerte kunnskapen basert på økt risiko for uønskede graviditetsutfall assosiert med sigaretter samt røykfri tobakksbruk, *in vivo*-studier som viser skadelige effekter av nikotin og nikotinholdige produkter på fosterutviklingen og mekanistisk forståelse som underbygger toksiske effekter av nikotin på morkaken og barnet i fosterlivet, indikerer at bruk av nikotinholdige e-sigaretter under graviditet utgjør en helserisiko for mor og barn.

Ikke-maligne orale sykdommer

Bruk av e-sigaretter kan gi ulike symptomer fra munnhulen, i tillegg kan bruk forårsake skader på munnslimhinnen. Selv om det er lite resultater fra oppfølgingsstudier på bruk av e-sigaretter angående tannkjøttsykdom (periodontal sykdom) og peri-implantat sykdom (betennelse i relasjon til tannimplantater), tyder de resultatene man har på at det kan være en assosiasjon mellom e-sigarettbruk og slike sykdommer.

Kreft

Resultatene fra paraplygjennomgangen alene var utilstrekkelige til å konkludere om bruk av e-sigaretter utgjør en kreftfare. Det ble nylig publisert to studier med viktige funn relevant for evaluering av mulige kreftfare forbundet med bruk av e-sigaretter. E-sigarettaerosol ble rapportert å indusere lunge adenokarsinomer og urinblære hyperplasi hos mus. Forfatterne foreslo at nikotin kunne bidra til kreftutvikling ved å redusere DNA-reparasjonsaktivitet og økt dannelse av DNA-addukter ved at nikotin ble omdannet i kroppen til det kreftfremkallende nitrosaminet NNK.

Basert på våre opprinnelige funn, NASEM-rapporten og relevant informasjon kort oppsummert over, konkluderer vi: i) Det foreligger ikke studier på mennesker som har vist eller ikke vist at bruk av e-sigaretter er assosiert med markører som indikerer kreftutvikling; ii) Det foreligger derimot dyrestudier med lang oppfølgingstid som viser økt kreftrisiko knyttet til eksponering for e-sigarettaerosol. Det foreligger andre dyrestudier som har vist at bruk av e-sigaretter er assosiert med biologiske markører som indikerer kreftutvikling. Dette underbygger funnene i dyr som indikerer at langvarig bruk av e-sigaretter kan øke risikoen for kreft. iii) Det er vist at e-sigarettaerosol kan være mutagent eller forårsake DNA-skade hos mennesker, i dyremodeller og celler i kultur, iv) Det er vist at noen kjemikalier påvist i e-sigarettaerosol (f.eks. formaldehyd, akrolein) kan forårsake DNA-skade og fremme mutagenese.

Basert på en toksikologisk evaluering av samlet evidens konkluderer vi med at regelmessig, langvarig bruk av e-sigaretter mest sannsynlig representerer en økt risiko for å utvikle kreft. Hvorvidt eksponeringsnivåene er tilstrekkelig høye til å bidra til økt risiko for kreftutvikling av betydning for folkehelsen er imidlertid usikkert.

Forgiftninger og skader
E-sigaretter er forbundet med utilsiktede forgiftninger, tilsiktede forgiftninger og skader forårsaket av eksplosjoner, termiske og kjemiske skader på grunn av overoppheting av litiumbatterier. Vi er usikre på hvor ofte slike tilfeller skjer.

Eksponeringsnivåer og betydningen for helseskadelige effekter ved bruk av e-sigaretter
Forekomst av helse skadelige stoffer i e-sigarettaerosolen gir ikke nødvendigvis økt risiko for sykdomsutvikling og/eller forverring av sykdom. Hvorvidt helseskadelige effekter vil forekomme vil i stor grad også avhenge av faktorer som mengden av helseskadelige stoffer i aerosolen, alder når man begynner å bruke e-sigaretter og hvordan bruksmønsteret er (hvor ofte, hvor lenge hver gang og i hvor mange år), samt individuelle variasjoner i følsomhet/mottakelighet. Resultatene fra vår paraplygjennomgang samt informasjon fra internasjonale rapporter og nyere litteratur om e-sigaretter og andre nikotinprodukter, viser imidlertid at det er sannsynlig at nivåene av inhalert nikotin og andre komponenter fra bruk av e-sigaretter kan øke risikoen for skadelige helseeffekter.

Konklusjon

De viktigste helseskadelige effektene ved bruk av e-sigaretter skyldes eksponering for stoffer gjennom innånding av e-sigarettaerosol dannet fra e-sigarettvæsken.

Sammensetningen av aerosolen kan variere med blant annet type e-sigarett, stoffer som utløses fra oppvarmingsenheten, temperatur under aerosoldannelsen samt forskjeller i e-væskeinnhold. E-sigaretter bør ikke betraktes som en enhetlig produktgruppe.

E-sigaretter ble introdusert på markedet uten at det forelå tilstrekkelige toksikologiske studier (bl.a. dyrestudier og *in vitro* studier) som kunne bidra til å avklare et helseskadelig potensial ved bruk av e-sigaretter.

Det er få gode langtidsstudier av e-sigarettbruk og sykdom hos mennesker og det trengs bedre eksponeringskarakterisering og lengre oppfølgingstid.

Basert på vår systematiske litteraturgjennomgang og den toksikologiske vurderingen konkluderer vi med at bruk av e-sigaretter medfører økt risiko for skadelige helseeffekter. Den relative risikoen for de skadelige helseeffektene som kan tilskrives bruk av e-sigaretter er usikker.

Key message

On behalf of the Norwegian Ministry of Health and Care Services, the Norwegian Institute of Public Health has summarized systematic reviews (umbrella overview) on possible adverse health effects of using electronic (e)-cigarettes. We conducted a systematic literature search and identified and included systematic reviews that describe the knowledge about the content of e-cigarette liquids and e-cigarette aerosols, health effects in humans and animals, as well as relevant findings from *in vitro* studies. For systematic reviews that had not evaluated the quality of the results for longitudinal human studies, we used the GRADE system to indicate our confidence in the effect estimates. We summarized the findings from the included systematic reviews in the umbrella overview. In the discussion, we made a toxicological evaluation in relation to diseases and adverse outcomes identified in the umbrella review. In addition to the studies identified in the systematic reviews, this evaluation also included knowledge from other relevant scientific reports and studies to shed light on the connections between the use of e-cigarettes and the various adverse health effects described.

The main health concern linked to use of e-cigarettes arises from inhalation of harmful constituents in e-cigarette aerosol produced from the e-liquid. The composition of the aerosol varies due to device characteristics, e.g., temperature during aerosolization of e-liquid, substances released from the device/heating element as well as variation in e-liquid contents. E-cigarettes should not be considered a homogeneous product group.

E-cigarettes were introduced to the market without adequate animal and *in vitro* studies to clarify the harmful effects that use of e-cigarettes could cause. There are few high-quality human studies of e-cigarettes and disease, with longitudinal design, long-term exposure, and sufficient exposure characterization and follow-up time.

Tittel:
Adverse health effects of electronic cigarette use: an umbrella review and toxicological evaluation

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Quality assurance:
Johan Øvrevik
Director of research
NIPH

Gunnar Sæbø
Senior scientist
Alcohol, tobacco and drugs
NIPH

Based on our umbrella review and toxicological evaluation, we conclude that use of e-cigarettes leads to an increased risk for adverse health effects. The relative risks for these adverse health effects are still uncertain.

Executive summary

Introduction

Electronic cigarettes (e-cigarettes) consist of a heating element, a battery and a cartridge containing a liquid. When heated, the e-cigarette liquid forms an aerosol (vapour) that is inhaled. The e-cigarette liquid can contain nicotine or be nicotine free. In addition, the e-liquid contains a mixture of propylene glycol (PG), vegetable glycerine (VG), and various flavourings. The available number of these flavours and combination of flavours is exceedingly high.

Upon heating and aerosolization of the e-cigarette liquid, new chemical compounds will be formed (decomposition products and synthesis of new compounds), and metals can be released from the heating element. Some of the compounds identified in the aerosol or later formed in the body after inhalation are known toxic compounds that may pose a potential health hazard.

Health effects associated with the use of e-cigarettes will therefore depend on the composition of the inhaled vapour, which again is affected by the e-cigarette device, e-liquid as well as the vaping pattern. Any adverse outcomes will also depend on user/exposed specific (genetic) and environmental factors that may predispose for health effects. Thus, a precise evaluation of the health risks linked to e-cigarettes use is complicated due to the large variation of products on the marked and the heterogeneity of users (e.g., time of use, age of user, comorbidity). Testing of aerosols from various e-cigarettes/e-cigarette liquids in relevant animal experiments is mostly lacking. Furthermore, potential long-term effects of e-cigarette use in humans have so far only been scarcely investigated.

Objective

Conduct an umbrella review (systematic review of systematic reviews) of the adverse health effects from use of or exposure to electronic cigarettes. This includes summarizing findings from systematic reviews on e-liquids and aerosol content, biomarkers of exposure and effects in human, animal- and *in vitro* studies.

Method

We have previously conducted a literature search on the health effects from use of or exposure to electronic cigarettes which was updated for the present report. Since the amount of literature was large, the current report summarized the findings from systematic reviews in an umbrella review. In order to identify all relevant systematic reviews, we conducted a systematic search for literature according to our protocol

(https://www.fhi.no/en/cristin-projects/ongoing/health-consequences-of-electronic-cigarettes---protocol-for-an-umbrella-rev/).

References were screened by two researchers independently, first by title and abstract and subsequently in full text, for inclusion and exclusion according to predefined inclusion and exclusion criteria. The quality of included systematic reviews was assessed with AMSTAR-2. For each outcome, we used in general the most up-to-date systematic review of the highest quality to summarize data on constituents and adverse health effects. For the systematic reviews that had not graded the quality of the results and where there were human longitudinal effect studies, we used the GRADE approach to indicate our confidence in the effect estimates.

We separately summarized the findings from the systematic reviews included on exposure characterization, various human health outcomes/disease, and animal studies and *in vitro* studies. In the discussion we combined the human, animal- and *in vitro* results with other relevant data for the different health outcomes addressed and performed a toxicological evaluation of adverse health effects from use of or exposure to electronic cigarettes.

Results from the systematic reviews (umbrella review)

We included in total 33 systematic reviews. The systematic reviews were grouped according to health effects. One systematic review could include data on different health effects. We included 12 systematic reviews in relation to non-malignant respiratory diseases, 6 systematic reviews in relation to cardiovascular diseases, 4 systematic reviews in relation to mental health, 3 systematic reviews in relation to non-malignant oral diseases, 2 systematic reviews in relation to pregnancy and child health, 1 systematic reviews in relation to cancer, 2 systematic reviews in relation to poisoning, 3 systematic reviews in relation to explosions and burns and 2 systematic reviews in relation to other adverse effects. In addition, we included 4 systematic reviews in relation to composition of the e-cigarette aerosol and biomarkers of exposure, 3 systematic reviews in relation to exposure of animals and 4 systematic reviews that reported on *in vitro* studies.

Constituents and exposure of e-cigarette aerosols

Several harmful chemicals such as carbonyl compounds, volatile organic compounds (VOC), reactive oxygen species and free radicals as well as various trace elements/metals have been identified in e-cigarette aerosols.

The large variation in e-cigarette devises and liquids used as well as in vaping patterns make human exposure highly variable and complex. Thus, it is difficult to precisely know or predict the exposure levels of potentially harmful substances. However, several of the substances identified in e-cigarette aerosols have been linked to various adverse health outcomes including cancer.

Non-malignant respiratory diseases

Two systematic reviews with meta-analysis of cross-sectional studies with partly overlapping included studies reported increased prevalence of asthma among users of e-cigarettes compared with non-users. However, no prospective human studies elucidat-ing how exposure to e-cigarette aerosol may contribute to development of asthma and chronic obstructive pulmonary disease (COPD) were identified.

One study showed decreased mucociliary clearance associated with use of e-cigarettes. One systematic review reported that adolescent use of e-cigarettes is associated with increased coughing compared to non-users.

In one systematic review, use of e-cigarettes was reported to cause e-cigarette use or vaping associated lung injury (EVALI). Most cases described involved use of e-liquid from informal sources containing tetrahydrocannabinol (THC; the main psycho-active compound in cannabis) or a combination of cannabinoids and nicotine.

Regarding use of e-cigarettes for smoking cessation, we are uncertain if there is a difference in the number of side effects and serious side effects from the use of nicotine containing e-cigarettes compared with using non-nicotine e-cigarettes, nicotine replacement therapy or behavioural support only or no support for smoking cessation.

Cardiovascular diseases

Risk for myocardial infarction, coronary heart disease and stroke

We found no systematic reviews directly reporting the risk for disease associated with naive use of e-cigarettes in humans.

Based on three cross sectional studies included in one systematic review, the odds ratio of having had myocardial infarction, coronary heart disease or stroke was similar for smokers switching to e-cigarettes as for current smokers. However, the cross-sectional design contributes to difficulties regarding conclusions on this outcome.

Heart rate and blood pressure and arterial stiffness

Use of e-cigarettes leads to acute increased heart rate, systolic and diastolic blood pressure. We had very low confidence in the effect estimates (GRADE).

Use of e-cigarettes leads to acute effects on pulse-wave velocity (measure of arterial stiffness). We had low confidence in the effect estimates (GRADE).

Chronic use of e-cigarettes among former smokers reduced systolic and diastolic blood pressure. No change in heart rate was reported. We had very low confidence in the effect estimates (GRADE).

Use of e-cigarettes with nicotine is associated with changes in heart rate variability, with similarities reported to that associated with increased cardiac risk.

Biomarkers of effects with relevance for development of cardiovascular disease

The systematic reviews reported an increase in vascular biomarkers which are associated with imbalance in the immune system, oxidative responses, aggregation of platelets and damage to the endothelial vascular cells important processes linked to developmental of cardiovascular disease.

Mental disorders
Several studies have reported an association between internalizing (such as anxiety and depression) and externalizing (such as ADHD) symptoms and disorders and e-cigarette use. Many studies reported an increased vulnerability for e-cigarette initiation and use among individuals with both internalizing and externalizing disorders.

One longitudinal study among adolescents reported a greater rate of increase in depressive symptoms for sustained use of e-cigarettes during the 12 months study period. In addition, the authors reported an association between an increased user frequency for the past 30 days and increase in depressive symptoms. Similar finding to the latter was also reported for young adults. We had very low confidence in the effect estimates (GRADE).

Another longitudinal study reported that Attention-Deficit/Hyperactivity Disorder (ADHD) symptoms predicted e-cigarette use, however e-cigarette use did not significantly predict future ADHD symptoms.

Adverse pregnancy outcomes and effects on early life health
Based on two studies included in one systematic review, a similar and a non-significant reduction on birthweight was reported. Both an increased risk for and no effect on small for gestational age was reported. We had very low confidence in the effect estimates (GRADE).

Non-malignant oral diseases
Use of e-cigarettes was associated with different oral symptoms and mucosal lesions. From clinical studies with a cross-sectional design regarding periodontal disease, the only clinical periodontal measure reported to be different for e-cigarette users compared to never users of tobacco was reduced bleeding on probing (BOP). A longitudinal study based on self-reported oral health found increased gum disease and bone loss around teeth among e-cigarette users compared to never users.

From the cross-sectional population-based studies, mostly an increased risk for gingival/periodontal disease was reported.

The clinical studies included in the systematic review of Yang and co-workers regarding peri-implant disease reported associations between worse parameters of peri-implant disease among e-cigarette users compared to never tobacco users.

For dental caries, no longitudinal study with a design that could give a causal relation was presented.

Cancer
One systematic review was identified that reported on 5 studies (two cohort studies, two case control and one case series). Due to their study design, all were evaluated to be of low quality.

One case series reported on two cases of oral carcinoma in long time users of e-cigarettes. The cohort studies and the case control studies assessed biomarkers of expo-sure and effects with relevance for cancer (such as endogenous formation of the carcinogen

N'-nitrosonornicotine (NNN) in saliva from e-cigarette smokers and decreased prevalence of micronuclei in oral mucosal cells from e-cigarette users compared to smokers and similar to healthy controls).

Poisoning, injuries, and other adverse effects
E-cigarettes may overheat or explode and cause subsequent injuries. The injuries can range from small lacerations and burns to more serious tissue damage, even with fatal outcome. Furthermore, accidental, or intentional intake of e-liquids can cause poisonings with the severity ranging from mild symptoms such as vomiting, rapid heart rate, unsteadiness, and increased salivation to death as the most serious outcome (unintentional or suicide). How frequently poisoning occurs is not known. Seizures after use of e-cigarettes have been reported mainly in youth and young adults. Many cases were associated with concurrent use of other substances.

Other adverse health effects
There were few systematic reviews on other adverse health effects, and the two we found gave no clear answers. We do not know if use of e-cigarettes affects post-operative health following bariatric surgery or affect sleep.

We did not identify any systematic reviews regarding second or third hand exposure to e-cigarettes.

Animal and *in-vitro* studies
Adverse respiratory outcomes: Depending on e-cigarette device/liquid type, dose, duration as well as animal model, e-cigarette use has been reported to affect various immune responses linked to respiratory disease including: i) increased level of cytokines in the bronchoalveolar lavage and immune cell infiltration, ii) increased allergy-induced asthmatic symptoms and iii) decreased resistance to both bacterial and viral infections.

Cardiovascular disease: Exposure to e-cigarette aerosol with nicotine promotes prothrombotic state, shortened bleeding and occlusion time, hyperreactive platelets and reduced thrombomodulin expression. Chronic long-term exposure to e-cigarette aerosol has been associated with increase in arterial stiffness, reduced effect of a vasodilator and increased effect of a vasopressor.

In a mouse model that develops atherosclerosis, the authors reported that exposure to e-cigarette aerosol containing nicotine increased oxidative stress, affect the morphology of cardiac cells as well as cardiac function, indicative of cardiomyopathy and increased atherosclerotic lesions. Acute exposure to e-cigarette aerosol *in vitro* substantiates increased oxidative stress and reduced antioxidative defence after exposure to e-cigarette aerosol with nicotine. E-cigarette aerosol with nicotine has also been reported to increase area of brain infarction, possibly due to increased permeability of blood brain barrier. Exposure of endothelial cells *in vitro* to e-liquids and aerosol were reported to increase ROS formation and decrease viability. In addition, e-cigarette aerosol exposure increased endothelial deposition of complement factors and platelet aggregation. These finding support effects of e-cigarettes on CVD.

Adverse embryonic and postnatal growth and development: Exposure of pregnant mice with e-cigarette aerosol with and without nicotine during gestational period and lactation, induced sex-dependent changes in gene expression associated with enhanced risk of adverse neurobiological and neurobehavioral outcomes like those associated with early life exposure to tobacco cigarette smoke. Furthermore, neonatal exposure to nicotine containing e-cigarette aerosol reduced lung development and decreased lung cell proliferation. E-cigarette aerosol extract also caused dose-dependent effects on cardiac development in a zebrafish model. In a frog model, exposure to e-cigarette aerosol extract affected craniofacial development.

Adverse non-malignant oral health effects: One systematic review on human studies additionally included *in vitro* studies. Exposure to e-cigarette aerosol resulted in changes in enamel colour, increased the adhesive force and some flavours increased biofilm formation of *S. mutans*, the two latter factors are associated with increased caries risk. Increased adhesion to epithelial cells and increased cytotoxicity of *C. albicans* exposed to e-cigarette aerosol with nicotine potentially linked to increased risk for oral candida infection has been reported.

Cancer: From the systematic reviews included in this report, we found few relevant animal studies on cancer. Some rodent studies indicated effects on redox balance, effects on immune system including increased pro-inflammatory responses which may have implication for cancer development. Furthermore, we found some in vitro studies using human cells and reporting effects of e-cigarette aerosols extracts on DNA damage and various cellular responses linked to DNA damage.

Strengths, limitations, and applicability of the current umbrella review

An advantage of an umbrella review (a systematic review of systematic reviews) is that it collects and evaluate the literature in a transparent way. One of the limitations is that not all relevant issues for evaluation of possible health risk due to e-cigarette are covered by systematic reviews.

In this report, the umbrella review is presented according to the pre-published protocol. However, to compensate for the limitations of an umbrella review, we deviated somewhat from the pre-published protocol by summarizing the literature and performing an overall toxicological evaluation. This is presented in the section **Dicussion and overall evaluation**. To obtain an evaluation of acceptable quality, we included information on e-cigarettes from reports conducted by international expert groups and updated with literature from our systematic search. We also used information from our previous report on snus/snuff in the overall evaluations.

Discussion/Overall evaluations

Constituents and exposure of e-cigarette aerosols

Several harmful chemicals as well as various metals/trace elements have been identified in e-cigarette aerosols. The large variation in e-cigarette devices and liquids used as well as in vaping patterns make human exposure highly variable and complex. Thus, it is difficult to precisely know or predict the exposure levels of potentially harmful substances.

Non-malignant respiratory diseases

Systematic reviews indicate that use of e-cigarettes is associated with local irritation of the respiratory tract, increased coughing as well as asthma. Human, animal, and *in vitro* studies, indicate that e-cigarettes with nicotine may affect biomarkers such as: i) bronchoconstriction, ii) impairing cough reflexes, iii) reducing mucociliary transport, iv) inflammation and v) decreased resistance to bacterial, viral infection. A sustained impact of such parameters on the respiratory system is linked not only to asthma but also chronic obstructive pulmonary disease (COPD). Thus, use of e-cigarettes may represent a risk for development of respiratory disease and increase exacerbation of respiratory diseases.

The recent outbreak of serious lung injuries (EVALI), mainly in USA, was mostly associated with use of tetrahydrocannabinol (THC)-containing e-cigarette liquid from informal sources. Cases of EVALI were reported mainly during a period of two years. The presence of vitamin E acetate in the e-liquid has been strongly linked to the EVALI outbreak. Evidence is not sufficient to rule out the contribution of other chemicals of concern. The EVALI outbreak shows how use of new products may confer unpredicted health hazards, and that the device may result in adverse health outcomes as it may be used for inhaling other substances than those originally intended.

Cardiovascular diseases

The umbrella review shows that human use of e-cigarette and animal exposure to e-cigarette aerosol have reported effects linked to central nerve system (CNS, brain), more specifically activation of the sympathetic nerve axis, as well as effects on oxidative stress and inflammation, endothelial dysfunction, and platelet activation, all representing central pathways associated with increased cardiovascular disease risk. For the naïve tobacco users, use of e-cigarettes may represent an increased risk for development of CVD, and it may contribute to an enhanced risk for more severe adverse outcomes following acute cardiovascular events. Our overall evaluation that uses of e-cigarettes may a represent an enhanced risk for CVD is supported by findings related to the use of snus, recent literature, and the current mechanistic understanding of the effects of cigarette constituents on CVD.

Mental disorders

Several studies have shown an association between mental health and increased user prevalence of nicotine containing products. The causal factors underlying the association are unknown. It is possible that common vulnerability (genetic and environmental) is involved. Adolescents with mental problems have been reported to be more likely to start with e-cigarettes, supporting the "self-medication" hypothesis rather than a causal association. On the other hand, the currently reported studies that found e-cigarette use associated with depressive symptoms, indicate that use of e-cigarettes may also affect mental health. Both studies in humans and animal experiments indicate an increased risk of development of addiction and long-term cognitive impairments in adolescence upon nicotine exposure. Effects of nicotine on the developing brain supports that nicotine may affect development of mental problems, such as ADHD, depression, and anxiety. However, it is too early to conclude on causal inference of e-cigarettes and mental disorders.

Adverse pregnancy outcomes and effects on early life health
The information from the systematic review on use of e-cigarettes for pregnancy and early life health outcomes was restricted to uncertain effects on birthweight and being small for gestational age. However, the combined evidence of: i) increased risk of adverse pregnancy outcomes associated with cigarettes as well as smokeless tobacco use ii) *in vivo* studies showing deleterious effects of nicotine and nicotine containing products on fetal and early life development iii) mechanistic insights substantiating toxic effects of nicotine on the placenta, fetus and early life development, all indicate that use of nicotine containing e-cigarettes constitutes a potential threat to the mother and child.

Non-malignant oral diseases
The umbrella review show that use of e-cigarettes may cause symptoms of oral discomfort and oral mucosal lesions. Although there is scarce evidence from longitudinal studies on the use of e-cigarettes regarding periodontal and peri-implant disease, overall data indicate that there may be an association.

Cancer
The results obtained from the umbrella review alone were insufficient for a conclusion whether use of e-cigarettes constitute a cancer hazard. However, recently important new information relevant for evaluation of potential carcinogenic effects associated with e-cigarette use has been published. E-cigarette aerosol was reported to induce lung adenocarcinomas and bladder urothelial hyperplasia in mice. The authors suggested a role of nicotine in cancer formation by decreased DNA repair activity and increased DNA adduct formation by endogenously formed NNK from nicotine.

Based on results from our umbrella review, the NASEM report and the new information summarized above we conclude: i) There is no available evidence that e-cigarette use is associated with intermediate cancer endpoints in humans from human studies; ii) There are adequate long-term animal bioassays of e-cigarette aerosol exposures to inform cancer risk; there is evidence from *in vivo* animal studies using intermediate biomarkers of cancer to support the hypothesis that long-term e-cigarette use could increase the risk of cancer; iii) There is evidence that e-cigarette aerosol can be mutagenic or cause DNA damage in humans, animal models, and human cells in culture, iv) There is substantial evidence that some chemicals present in e-cigarette aerosols (e.g., formaldehyde, acrolein) are capable of causing DNA damage and mutagenesis.

Based on a toxicological evaluation of current literature, we conclude that regularly, long-term use of e-cigarette is likely to represent an enhanced risk for developing cancer. However, the impact on the prevalence of cancer in the general population is unknown.

Poisonings and injuries
E-cigarettes are associated with accidental poisonings, intentional poisonings and traumatic injuries caused by explosions, thermal and chemical injuries due to overheating of lithium batteries. We have no information regarding the frequency of such accidents.

Relevance of exposure levels following e-cigarette use and association to disease
The presence of hazardous constituents in e-cigarette aerosols does not necessarily confer an elevated risk for disease development and/or exacerbations. The outcome will depend on factors such as the level of hazardous constituents, age of initiation and quantity of exposure (frequency, duration, and years of exposure) as well as individual variations

in susceptibility. The results from the present umbrella-review as well as information from international reports and recent literature on e-cigarettes and other nicotine products, implies that it is likely that the levels of inhaled nicotine and other components from e-cigarette use may enhance the risk for adverse health effects.

Conclusion

The main health concern linked to use of e-cigarettes arises from inhalation of harmful constituents in e-cigarette aerosol produced from the e-liquid.

The composition of the aerosol varies due to device characteristics, e.g., temperature during aerosolization of e-liquid, substances released from the device/heating element as well as variation in e-liquid contents. E-cigarettes should not be considered a homogeneous product group.

E-cigarettes were introduced to the market without adequate animal and *in vitro* studies to clarify the harmful effects that use of e-cigarettes could cause.

There are few high-quality human studies of e-cigarettes and disease, with longitudinal design, long-term exposure, and sufficient exposure characterization and follow-up time.

Based on our umbrella review and toxicological evaluation, we conclude that use of e-cigarettes leads to an increased risk for adverse health effects. The relative risks for these adverse health effects are still uncertain.

Preface

The Ministry of Health and Care Services requested the Norwegian Institute of Public Health (NIPH) to elucidate the health effects of e-cigarettes, in a two-part assignment. The first part of the assignment was to perform a systematic literature search and prepare an interactive evidence and gap map of research on health effects of electronic cigarettes (e-cigarettes) use. The research map is completed and published (Becher, 2021).

The second part of the assignment was specified in September 2021 and is to perform an update of the literature search, and to evaluate possible adverse health effects linked to the use of e-cigarettes. This part will be based on a scientific evaluation of the systematic reviews found.

The systematic literature search should be restricted to studies addressing health effects and not include other e-cigarette related issues such as harm reduction and "gateway" (here the possibility that use of e-cigarettes leads to use of other tobacco or nicotine containing products) or the use of e-cigarettes in smoking cessation.

In agreement with the established policy of leading scientific journals, research funded by or otherwise linked to the tobacco industry should not be included. Otherwise, NIPH was free to organize the work as they find appropriate, including consultation with any external expertise.

Project leaders
Rune Becher, Norwegian Institute of Public health
Håkon Valen, Norwegian Institute of Public health

Working group
Gunn E. Vist, Norwegian Institute of Public health
Bendik C. Brinchmann, Norwegian Institute of Public health
Jørn A. Holme, Norwegian Institute of Public health
Tom K. Grimsrud, Cancer Registry of Norway
Vigdis Underland, Norwegian Institute of Public health
Espen Mariussen, Norwegian Institute of Public health
Miriam Bakkeli, Norwegian Institute of Public health
Jan Alexander, Norwegian Institute of Public Health

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Johan Øvrevik
Director of Research / Chief Scientist
Project responsible

Rune Becher and Håkon Valen
Senior scientists
Project leaders

Introduction

Electronic cigarettes (e-cigarettes) consist of a heating element, a battery and a cartridge containing a liquid. When heated, the e-cigarette liquid will form an aerosol (vapour) meant to be inhaled through a mouthpiece. E-cigarettes can be disposable, rechargeable with a cartridge, or manually refillable with e-cigarette liquid.

The e-cigarette liquid can contain nicotine or be nicotine free. In addition, the e-liquid usually contains a mixture of propylene glycol (PG), vegetable glycerine (VG), and various flavourings. The available number of these flavours/combination of flavours is high. Heating of the e-liquid can lead to thermal decomposition of these constituents and/or formation of new compounds, depending on the temperature, chemical composition, and duration of the heating. The composition of e-cigarette aerosol is thus unpredictably altered when compared to the original e-liquid. Additional constituents found in aerosols including metals and silicate particles from device may add to the toxicity of the aerosol.

The health effects will depend on the composition of the inhaled vapour, which again is affected by the e-cigarette device, e-liquid as well as the vaping pattern. Any adverse outcomes will also depend on user/exposed specific (genetic) and environmental factors that may predispose for health effects. Thus, a precise evaluation of the health risks linked to e-cigarettes use is complicated due to the large variation of products on the marked and the heterogeneity of users (e.g., time of use, age of user, comorbidity). Furthermore, potential long-term effects of e-cigarette use in humans have so far only been scarcely investigated.

It should also be noted that e-cigarettes may be used for vaping other liquids or additives that may be illegal or produced for other purposes, and thus not provided commercially from an e-cigarette producer.

Why it is important to conduct the present umbrella review

Since the introduction of e-cigarettes on the market, a development and diversification of both the e-cigarette and e-liquids has followed. E-cigarettes are currently introduced to new markets. Currently, in Norway, e-cigarettes with nicotine are not allowed sold. However, private import of e-cigarettes with nicotine for personal medicinal use is only

permitted from EU/EEA countries. Norwegian (The Norwegian Medicines Agency - Legemiddelverket). The popularity of e-cigarettes among adolescents is a matter of particular concern. In Norway, the life-time prevalence of e-cigarette use (used e-cigarettes at least once) among 15–19-year-olds is 31 % (ESPAD Group., 2020). The proportion of regular use of e-cigarettes is highest among the youngest age group (16-24 years), just under four percent (Tokle, 2022).

The amount of scientific literature on adverse health effects of e-cigarettes among users is increasing rapidly. Thus, there is an urgent need for an up-to-date overview of the available scientific information on adverse health effects of e-cigarettes to secure evidence-based information and regulations of e-cigarettes.

Aims

The aim of this project was to conduct an umbrella review (systematic review of systematic reviews) of the adverse health effects from use of or exposure to electronic cigarettes. This includes summarizing findings from systematic reviews on e-liquids and aerosol content, biomarkers of exposure and effects in human and animal studies. We also added results from *in vitro* studies when presented in the systematic reviews. A comprehensive risk assessment comparing the risk differences between use of e-cigarettes and cigarettes were not within the scope of this report or the mandate for the report.

Methods

We updated a previous systematic literature search performed in connection with the interactive evidence and gap map on health effects of e-cigarettes (Becher, 2021). The search was performed in 5 databases.

Project plan

Electronic cigarettes (e-cigarettes), both with and without nicotine, have increased in popularity in many countries. There is wide variation in product types and contents. The products are sold as less harmful alternatives to highly unhealthy conventional tobacco cigarettes. The need to systematically review existing evidence on consequences to human health from e-cigarettes is high and increasing.

On commission from the Ministry of Health and Care Services in Norway we systematically evaluated adverse health effects of use of e-cigarettes. The evaluation was based on scientific methods for collection and assessments of systematic reviews of health effects associated with use of e-cigarettes.

In order to identify relevant reviews, we conducted a systematic search for literature according to our protocol (https://www.fhi.no/en/cristin-projects/ongoing/health-consequences-of-electronic-cigarettes---protocol-for-an-umbrella-rev/). Titles and abstracts were considered according to the inclusion and exclusion criteria. References were screened by two researchers independently, first by title and abstract and subsequently in full text, for inclusion and exclusion.

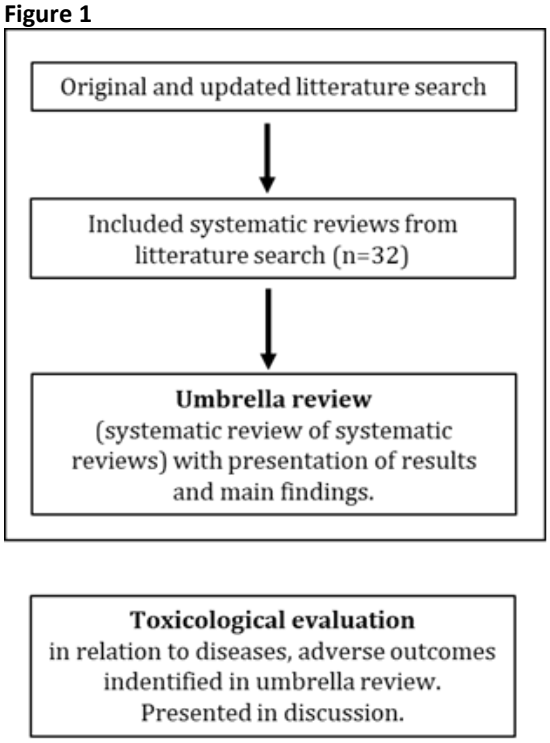
The quality of included systematic reviews were assessed using AMSTAR-2. For each (health) outcome, we used the most up-to-date systematic review of the highest quality to summarize health effects. For the systematic reviews that had not graded the quality of the results and where effect studies were available, we used the GRADE approach to indicate our confidence in the effect estimates.

The aim of this project was to conduct an umbrella review (systematic review of systematic reviews) of the adverse health effects from use of or exposure to electronic cigarettes. This includes summarizing findings from systematic reviews on e-liquids and aerosol content, biomarkers of exposure and effects in humans and animal. We also added results from *in vitro* studies when presented in the systematic reviews.

When conducting an umbrella review it is expected that the important and required information from primary studies are already summarised by the authors of the included systematic reviews. However, for several of the included systematic reviews in this umbrella, we consulted the primary studies to retrieve additional information. This was particularly useful for animal and *in vitro* studies.

In the toxicological evaluation presented in the discussion of the present report, we included reports from international expert groups and updated with relevant information from our systematic literature search. See figure 1 for an overview of the current work.

Figure 1



Inclusion criteria

We used the following inclusion criteria:

Population	Human and animal (*in vitro,* physical, and chemical characterization)
Intervention or exposure	All types of electronic cigarettes and additives
Comparison	No restrictions: smoking, snus/snuff or no use of tobacco product allowed as comparison
Outcomes	All health outcomes as a result of the use of e-cigarettes
Study design	Systematic review with a literature search, clear inclusion criteria and risk of bias assessment of included studies
Publication time	No restrictions
Country/ context	No restrictions
Language	Danish, English, Norwegian, Swedish

Exclusion criteria:
Research funded by or otherwise linked to the tobacco industry
Harm reduction publications without evidence of health outcomes
Studies that only describe or discuss the pattern of use of tobacco products
Primarily addiction focused research
In general, reviews and discussion papers without systematic literature search, clear inclusion criteria and risk of bias assessment of the included studies

Literature search

Research librarian Miriam Bakkeli updated the systematic literature search that she developed in collaboration with the project group and conducted in connection with the interactive evidence and gap map on the health effects of e-cigarettes (Becher et al., 2021). The strategy was peer reviewed by another research librarian before she conducted the searches first time. The following databases were searched; Ovid MEDLINE, Embase, PsycInfo, Web of Science and Cochrane Database of Systematic Reviews. The complete and updated search strategy is presented in appendix 1.

For the toxicological evaluation we used information from our systematic search as well as reports from National Academies of Sciences, Engineering, and Medicine (NASEM; https://www.nap.edu/catalog/24952/public-health-consequences-of-e-cigarettes), The Health Consequences of Smoking—50 Years of Progress A Report of the Surgeon General (US Surgeon General 2014; The Health Consequences of Smoking—50 Years of Progress - NCBI Bookshelf (nih.gov)), E-Cigarette Use Among Youth and Young Adults, a Report of the Surgeon General (US Surgeon General 2016; E-Cigarette Use Among Youth and Young Adults - NCBI Bookshelf (nih.gov)) and Scientific Committee on Health, Environmental and Emerging Risks (SCHEER; https://ec.europa.eu/health/scientific_committees/scheer_en) and Health risks from snus use (FHI, 2019; https://www.fhi.no/publ/2019/helserisiko-ved-snusbruk2/).

Selection of studies

Two authors from the working group (RB, HV, BCB, JAH, GEV and TKG) read and assessed each reference identified in the literature searches. Relevant references were selected according to our inclusion criteria. The first selection was based on the title and abstract, and the second selection on full-text evaluation of the publications. Any disagreements were resolved through discussion or contact with another researcher in the team. EPPI Reviewer 4 was used for study selection.

Assessing the quality of systematic reviews

We used AMSTAR-2 to assess the quality of included systematic reviews (Shea et al., 2017). Three authors from the author working group (HV, VU and GEV) assessed the quality of each included systematic review. Any disagreements were resolved through discussion or contact with another researcher in the team (BCB or RB).

Data collection and grading

For each health outcome, we used the most up to date systematic review of highest available quality to summarise the findings. Where deviations to this occur, it is noted in the text.

Two authors from the author working group (RB, HV, BCB, JAH, GEV and TKG) independently collected data from the systematic review. Disagreements were solved by consensus. We collected information on the full reference, the date of the literature search, number and type of studies included, when and where it was conducted, number and characteristics of participants in the studies, type and content of e-cigarettes used, type and content of comparison, outcomes measured, results including grading results if conducted. Where the systematic reviews have graded their confidence in the evidence, we looked to their assessments. When the evidence was not graded, we will use the Grading of Recommendations Assessment, Development and Evaluation (GRADE) approach to assess our confidence in the quality of the documentation (Balshem et al., 2011).

Peer review of project plan and report

The project plan had undergone internal peer review before the work on systematic review started. The present report underwent quality assurance by internal reviewers.

Evaluation of confidence in results

We summarized health risks in accordance with international standards for systematic reviews using the GRADE approach, when not performed in the systematic reviews. According to this approach, observational studies rarely achieve higher ratings than "low confidence" in the effect estimate. Therefore, most of the results on health risks in this report - which are from observational studies - are referred to by statements indicating that we have low to very low confidence in the effect estimates (according to GRADE). This does NOT indicate that the cohort studies that have assessed the health risks of using e-cigarettes are less trustworthy than cohort studies in general. For example, central evidence we have about risks from smoking and risks from air pollution also comes from observational studies. Some of the risk estimates show large effects and other results have established a dose-response relationship (which means that a larger dose produces a larger response). In these cases, we have upgraded to "moderate confidence" in the effect estimate, with conclusions formulated as "it is likely that the use of e-cigarettes increases the risk of …."

When the risk estimates for the use of e-cigarettes have wide confidence intervals, including the possibilities of both a considerable reduction in risk and a considerable increase in risk, we are uncertain whether e-cigarette use will affect the outcome in question and if so in which direction, and we downgrade to very low confidence in the effect estimate. It is important to distinguish between cases when there are so wide confidence intervals that we do not know whether or in which direction the outcome is affected, and

when narrow confidence intervals indicate little or no effect. The absolute number affected by an increased relative risk depends on the specific outcome's incidence in the population. We have explained our assessments in the relevant chapters. An overview of the statements used is given below in table 1.

We used study design as a starting point and then assessed five criteria to arrive at a degree of confidence in the documentation: risk of systematic bias (risk of bias), degree of consistency / consistency between the results (consistency), sparse data / precision of data (precision), directness and publication bias.

Table 1: GRADE -expressions used in this report GRADE assessment. The GRADE (Grading of Recommendations Assessment, Development and Evaluation)(Balshem et al., 2011) approach and the digital tool GRADEpro (GDT 2) were used to assess confidence* in the documentation. Degree of trust is a continuous quantity but is for practical reasons divided into four categories: high, medium, low, very low.

High confidence	⊕⊕⊕⊕	We have high confidence that the effect estimate is close to the true effect.
Moderate confidence	⊕⊕⊕◯	We have moderate confidence in the effect estimate: the effect estimate is probably close to the true effect, but the effect estimate can also be significantly different from the true effect. We use the term probably to express our confidence in the result.
Low confidence	⊕⊕◯◯	We have low confidence in the effect estimate: the true effect can be significantly different from the effect estimate. We use the term possibly to express our confidence in the result.
Very low confidence	⊕◯◯◯	We have very low confidence that the effect estimate is close to the true effect. We use the term unclear / uncertain to express our confidence in the result.

*By assessing confidence in the results, we mean an assessment of the extent to which we can trust that the research results show the 'truth' or the 'real' effect of the measures we are investigating. Another way of expressing it is how well documented the research results are.

When including observational studies, it is also possible to consider upgrading the documentation. This is done by considering the following three criteria: strong or very strong associations/connections between measures and outcomes (i.e., the calculated effect is so large that it is unlikely that it is due to coincidences), large or very large dose-response effects, where all probable confounders would have helped to reduce the effect estimate.

Two researchers (HV) and (GEV) assessed the confidence in the main outcomes together, and we resolved disagreements about the assessments by discussion or by conferring with a third project employee (RB).

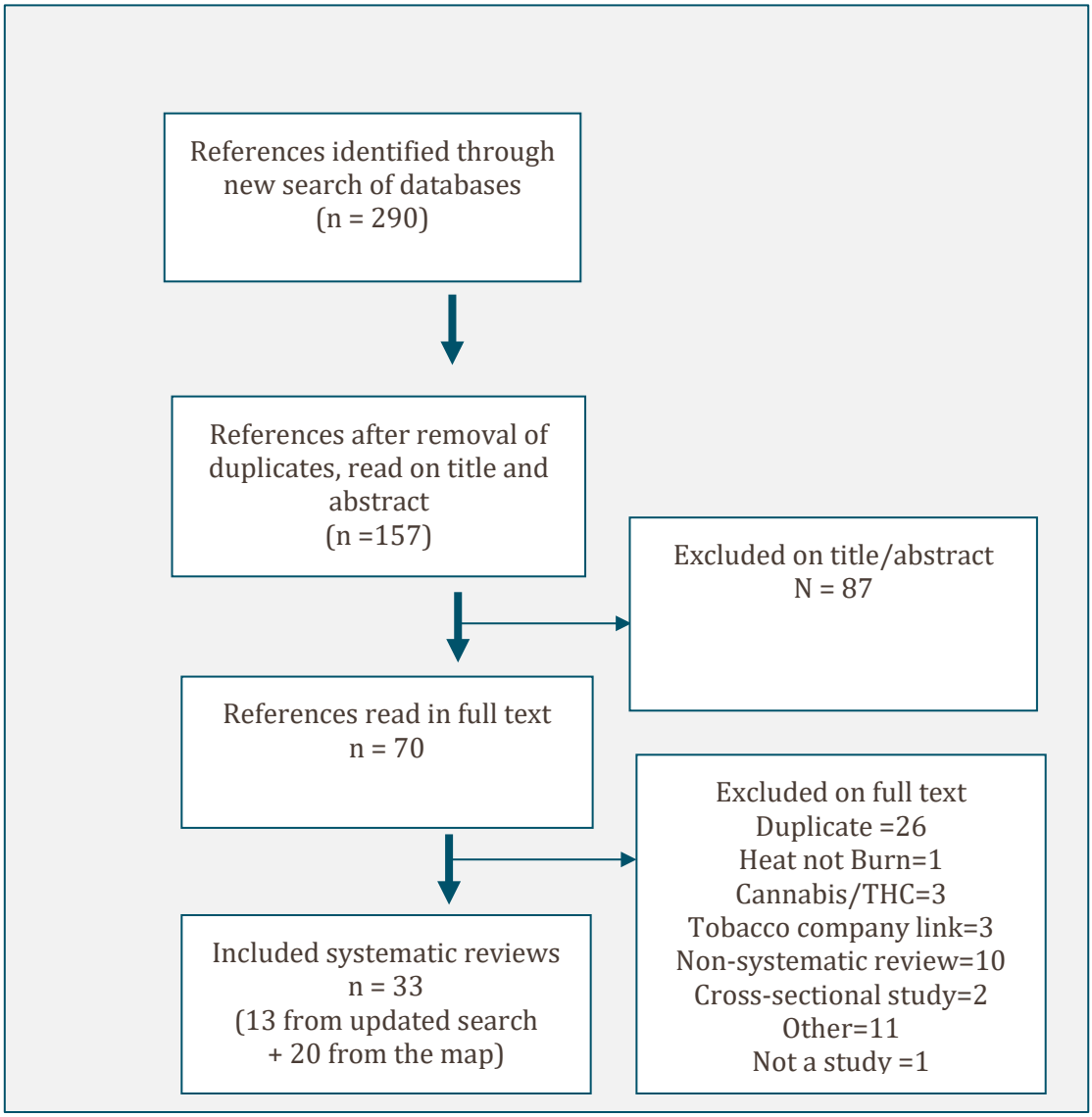
More details on use of GRADE to assess trust in the results can be found in Guyatt and colleagues (Guyatt et al., 2011) and www.gradeworkinggroup.org.

Results

Results from literature search and selection of studies

The systematic literature search retrieved 290 references before duplicate removal (Figure 2). After removing the duplicates, we were left with 157 references. Of these, we excluded 87 references that obviously did not meet our inclusion criteria. We obtained and evaluated 70 full-text publications. We excluded 57 publications of these and included 13 systematic reviews in addition to 20 systematic reviews from our previous search (Figure 2).

Figure 2: Flowchart over selection of studies



Description of included systematic reviews

We included in total 33 systematic reviews. The systematic reviews were grouped according to health effects. One systematic review could include data on different health effects. We included 12 systematic reviews in relation to non-malignant respiratory diseases, 6 systematic reviews in relation to cardiovascular diseases, 4 systematic reviews in relation to mental health, 3 systematic reviews in relation to non-malignant oral diseases, 2 systematic reviews in relation to pregnancy and child health, 1 systematic reviews in relation to cancer, 2 systematic reviews in relation to poisoning, 3 systematic reviews in relation to explosions and burns and 2 systematic reviews in relation to other adverse effects. In addition, we included 4 systematic reviews in relation to composition of the e-cigarette aerosol and biomarkers of exposure, 3 systematic reviews in relation to exposure of animals and 4 systematic reviews that reported on in vitro studies.

In Table 2 the included systematic reviews are presented according to which adverse health effects that were reviewed, marked as dark green in the box. Reviews are listed so that the one with the most recent literature search is presented first.

Table 2. Systematic reviews with the adverse health effects addressed in the review, the reviews with the newest searches are presented first.

Reference Search date	Oral	Airways and pulmonary	Cardiovascular and vascular	Digestive system	Central nerve system	Mental health	Cancer	Immune system	Metabolic disorders	Pregnancy	Mortality	Poisoning	Explosions and burns	Other adverse events	Sexual health	Kidney and urological tract
Prasetyo et al 2021 unclear date		■														
Chand & Hosseinzadeh 2021, Mar 2021		■														
Zakiyah et al 2021 Dec 2020		■				■										
Bourke et al 2021 Oct 2020		■														
Goniewicz et al 2020 Sep 2020		■	■													
Xian & Chen 2021 Aug 2020		■														
Srikanth et al 2021 Jun 2020											■			■		
Scarpino et al 2020 May 2020												■				
Becker et al 2020 Mar 2020						■										
Figueredo et al 2020 Mar 2020	■															
Calder et al 2021 Feb 2020										■						
Sreedharan et al 2021, Feb 2020		■														
Tzortzi et al 2020 Feb 2020		■										■	■			
Hartmann-Boyce et al 2020, Jan 2020		■	■													
Saz-Lara et al 2021 Jan 2020			■													
Yang et al 2020 Des 2019	■												■			
Rothrock et al 2020 Nov 2019						■										
Dekhou et al 2021 Oct 2019													■			
Flach et al 2019 Sep 2018							■									
Kennedy et al 2019 Jun 2019			■													
Claire et al 2019 May 2019										■						
Ralho et al 2019 Nov 2018	■															
Kwon et al 2019 Sep 2018														■		
Skotsimara et al 2019 Nov 2017			■													
Liu et al 2018 Jul 2017		■				■										
Riley et al 2016 Jun 2015			■													

Gualano et al 2015 Apr 2014																
Franck et al 2014 Sep 2013																
Biomarkers, animal studies, in vitro and or mechanistic																
Ward et al 2020 May 2020																
Bjurlin et al 2021 Jan 2019																
Wang et al 2019 Nov 2018																
Zhao et al 2020 Nov 2018																
Lee et al 2020 Jun 2019																

Critical appraisal of systematic reviews

We used the AMSTAR 2 critical appraisal tool (Shea et al 2017) to assess quality of the included systematic reviews. The 16 questions used for the AMSTAR 2 appraisal were:

1. Did the research questions and inclusion criteria for the review include the components of PICO?
2. Did the report of the review contain an explicit statement that the review methods were established prior to conduct of the review and did the report justify any significant deviations from protocol?
3. Did the review authors explain their selection of the study designs for inclusion in the review?
4. Did the review authors use a comprehensive literature search strategy?
5. Did the review authors perform study selection in duplicate?
6. Did the review authors perform data extraction in duplicate?
7. Did the review authors provide a list of excluded studies and justify the exclusions?
8. Did the review authors describe the included studies in adequate detail?
9. Did the review authors use a satisfactory technique for assessing the risk of bias (RoB) in individual studies that were included?
10. Did the review authors report on the sources of funding for the studies included in the review?
11. If meta-analysis was performed did the review authors use appropriate methods for statistical combination of results?
12. If meta-analysis was performed, did the review authors assess the potential impact of RoB in individual studies on the results of the meta-analysis or other evidence synthesis?
13. Did the review authors account for RoB in individual studies when interpreting/discussing the results?
14. Did the review authors provide a satisfactory explanation for, and discussion of, any heterogeneity observed in the results of the review?
15. If they performed quantitative synthesis did the review authors carry out an adequate investigation of publication bias (small study bias) and discuss its likely impact on the results of the review?
16. Did the review authors report any potential sources of conflict of interest, including any funding they received for conducting the review?

Each question was answered yes (Y), probably yes (PY), probably no (PN), no (N), no meta-analysis (NM) or not applicable (NA). Question #9 is answered twice, first for RCT and then for observational studies. Two authors critically appraised each systematic review independently before they compared and discussed.

Our AMSTAR 2 assessments of the included systematic reviews pertaining to health effects in humans of use of electronic cigarettes are presented in Table 3. Only 5 of these reviews obtained High quality score (H), 16 were of Moderate quality (M) and 7 of Low quality (L), 5 scored Critically Low (CL).

Table 3. AMSTAR 2 critical assessment of systematic reviews on the adverse health effects of use of electronic cigarettes

Reference	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15	Q16	Quality
Becker et al 2021	Y	PY	Y	PY	Y	Y	PY	PY	N PY	N	NM	NM	N	N	NM	Y	L
Bjurlin et al 2021	Y	PY	Y	PY	Y	Y	PY	PY	N N	Y	NM	NM	N	Y	NM	Y	CL
Bourke et al 2021	Y	N	Y	PY	Y	N	PY	Y	NA Y	N	NM	NM	Y	Y	NM	Y	M
Calder et al 2021	Y	PY	Y	PY	N	N	PY	Y	NA Y	N	NM	N	N	Y	NM	Y	M
Chand & H 2021	Y	PY	Y	PY	N	Y	PY	Y	NA PY	Y	Y	Y	Y	Y	Y	Y	H
Claire et al 2020	Y	Y	Y	Y	Y	Y	PY	Y	Y NA	Y	Y	Y	Y	Y	NM	Y	H
Dekhou et al 2021	Y	N	N	N	Y	Y	N	PY	NA PY	NA	NM	NM	N	N	NM	N	CL
Figueredo et al 2020	Y	PY	Y	PY	Y	Y	PY	Y	Y Y	N	Y	N	Y	N	NA	Y	M
Flach et al 2019	Y	N	Y	PY	Y	Y	N	PY	Y N	N	NM	NM	Y	Y	NM	Y	M
Franck et al 2014	Y	N	Y	PY	N	Y	PY	Y	Y N	N	NM	NM	Y	Y	NM	Y	M
Goniewicz et al 2020	Y	N	Y	PY	Y	Y	PY	PY	NA Y	Y	NM	NM	Y	Y	NM	Y	M
Gualanao et al 2015	Y	N	Y	PY	N	N	PY	PY	PY Y	Y	NM	NM	N	Y	NM	N	M
Hartmann-B 2020	Y	Y	Y	Y	Y	Y	Y	Y	Y NA	Y	Y	Y	Y	Y	Y	Y	H
Kennedy et al 2019	Y	N	Y	PY	N	N	PY	Y	Y Y	Y	NM	NM	Y	Y	NM	Y	M
Kwon et al 2019	Y	N	N	PY	N	Y	PY	Y	NA PY	N	NM	NM	N	Y	NM	Y	L
Lee et al 2020	Y	N	N	PY	Y	Y	PY	N	N N	Y	NM	NM	N	N	NM	Y	CL
Liu et al 2018	Y	N	Y	PY	N	Y	PY	PY	Y Y	N	Y	N	N	N	NM	Y	M
Prasetyo et al 2021	Y	N	Y	PY	Y	Y	PY	Y	Y PY	N	NM	NM	Y	Y	NM	Y	H
Ralho et al 2019	Y	PY	Y	PY	Y	N	PY	PY	NA Y	N	NM	NM	Y	N	NM	Y	M
Riley et al 2016	Y	N	Y	PY	Y	N	N	Y	PY PY	Y	NM	NM	N	N	NM	Y	L
Rothrock et al 2020	Y	Y	Y	PY	Y	Y	PY	Y	NA Y	N	Y	Y	Y	Y	Y	Y	H
Saz-Lara et al 2021	Y	PY	Y	PY	Y	Y	PY	PY	Y Y	N	N	N	N	Y	Y	Y	M

Reference	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Q14	Q15	Q16	Quality
Scarpino et al 2020	Y	N	Y	PY	N	Y	N	PY	N P	N	NM	NM	Y	NA	NM	Y	M
Skotsimara et al 2019	Y	N	N	N	Y	Y	N	N	NAY	N	N	N	N	N	N	Y	CL
Sreedharan et al 2021	N	N	Y	PY	Y	Y	PY	N	NA PY	NA	NM	NM	Y	N	NM	Y	CL
Srikanth et al 2021	Y	N	Y	Y	N	N	PY	PY	N PY	N	NM	NM	Y	N	NM	Y	L
Tzortzi et al 2020	Y	N	Y	PY	N	Y	N	PY	N N	NA	NM	NM	Y	NA	NM	Y	L
Wang et al 2019	Y	N	Y	PY	N	N	PY	PY	NA PY	N	NM	NM	Y	Y	NM	Y	L
Ward et al 2020	Y	N	Y	PY	N	N	PY	N	NA Y	N	NM	NM	NA	NA	NM	Y	M
Xian & Chen 2021	Y	N	Y	PY	N	Y	PY	PY	NA Y	N	Y	Y	Y	Y	Y	Y	M
Yang et al 2020	Y	N	Y	PY	Y	Y	PY	PY	N N	Y	NM	NM	Y	NA	NM	Y	L
Zakiyah et al 2021	Y	PY	Y	PY	Y	Y	PY	Y	Y Y	Y	NM	NM	N	N	NM	Y	M
Zhao et al 2020	Y	N	Y	PY	Y	Y	PY	Y	NA Y	N	NM	NM	Y	N	NM	Y	M

H = high quality; M = moderate quality; L = low quality; CL = critically low quality; N = no; NA = not applicable; NM = no meta-analysis; PN = probably no; PY = probably yes; Y = yes.

Constituents and exposure of e-cigarette aerosols

Before reviewing the health effects, we briefly summarize findings from systematic reviews on e-cigarettes e-liquids and aerosols.

Adverse health effects of e-cigarette use are highly depending on the quality and quantity of exposure. Important data for the final discussion of health effects includes aerosol composition, direct effects, uptake and distribution of hazardous elements and compounds, and evidence based on biomarkers of exposure.

E-cigarettes come in many shapes and sizes (cig-a-likes and tank). They have a battery, a heating element and a place to hold the liquid sample (bottle, cartridge, open wick tank). The e-liquid usually contains nicotine, solvents such as propylene glycol (PG) and, vegetable glycerine (VG), water as well as various amounts of other additives including flavourings, preservatives, thickeners, colouring ingredients and chemicals for pH adjustment. Upon heating and aerosolization, thermal decomposition of ingredients, synthesis of new compounds as well as release of metals from the e-cigarette device, alters the vapour composition compared to the original e-liquid. Many of these compounds and elements represent a health hazard.

We identified two systematic reviews that assessed the constituents of e-liquid aerosols (Ward et al., 2020; Zhao et al., 2020). More specifically, Ward and co-workers (2020) summarized data on potential toxicants in aerosols from different e-cigarette delivery systems and illustrated how device construction affects aerosol composition. The systematic review by Ward and co-workers (2020) was evaluated to be of moderate quality. Zhao and co-workers (2020) reviewed the content of various metals and metalloids (elements with properties intermediate between metals and non-metals such as silicon, arsenic, and antimony) in e-liquids and e-cigarette aerosols (Zhao et al., 2020). The systematic review by Zhao and co-workers (2020) was evaluated to be of moderate quality. The systematic review by Bjurlin and co-workers (2021) investigated biomarkers of exposure in the urine of e-cigarette users with potential implications for the development of bladder cancers (Bjurlin et al., 2021). The systematic review was evaluated to be of critically low quality. One systematic review included toxicant comparison for one specific e-cigarette brand compared with other forms of e-cigarettes and cigarettes, evaluated to have critically low quality.

Composition

Ward and co-workers (2020) identified substances and they were grouped into 6 categories: carbonyl compounds, volatile organic compounds (VOC), trace elements/metals, reactive oxygen species (ROS), free radicals, polycyclic aromatic hydrocarbons (PAHs) and tobacco-specific nitrosamines (TSNA).

Organic chemicals

The review by Ward and co-workers (2020) concluded that e-cigarette aerosols may contain harmful substances, and that the construction of the devices effects the level of harmful substances. Most of the studies measured carbonyl compounds/VOC in the aerosols, which includes substances such as formaldehyde, acetaldehyde, benzene, and

styrene. Location of the heating coil in the device was reported to affect formation of carbonyl compounds/VOCs. Several studies showed that increased power (watt) or voltage of the device increased the levels of carbonyls/VOCs in the aerosols. An increase is also observed when the device operates suboptimal, for example when there is not sufficient liquid to produce aerosols (dry puff). The systematic review of Lee and co-workers (2017) reported that JUUL e-cigarette aerosol had lower levels of certain harmful compounds compared to other e-cigarettes and cigarettes (Lee et al., 2020).

Thermal degradation of e-liquid and the presence of various components including flavouring agents, have impact on formation of carbonyls. The levels of ROS and other free radicals in e-cigarette aerosols are closely associated with numerous factors linked to the construction of the device as well as the composition of e-liquids. Thus, the final influence of flavouring agents and the ratio of PG and VG on radical formation are difficult to predict.

The concentrations of PAH in aerosols from e-cigarettes was reported by Ward and co-workers (2020) to be low. This is due to lower operation temperature of the e-cigarette device as well as lack of organic material (eg tobacco) when compared to tobacco smoking. However, a few PAHs are detected in low concentrations, such as naphthalene, acenaphtylene, and cadalene, of which the latter typically has an origin from plants.

Metals and metalloids

The levels of various metals and metalloids in e-liquids and e-cigarettes aerosols have been reviewed in two systematic reviews (Ward et al., 2020; Zhao et al., 2020). Zhao and co-workers (2020) reported the presence of numerous metals/metalloids in e-cigarette samples in the studies reviewed. For most metals/metalloids, levels were heterogeneous according to sample (aerosol, e-liquid), source of the sample (bottle, cartridge, open wick tank), and device type (cig-a-likes and tank). Overall, the metal/metalloid content was highest in e-liquid aerosol, followed by e-liquid in contact with metals in the device, especially coil or soldered joints of poor quality, followed by e-liquid in itself. Copper (Cu), zink (Zn), aluminium (Al) and iron (Fe) were in general the dominating metals in the aerosols, whereas metals such as chromium (Cr), Nickel (Ni) and lead (Pb) were found in lower concentration. In some aerosols, traces of arsenic (As), antimony (Sb) and Cadmium (Cd) were detected, and one study found high levels of tin (Sn). The review by Ward and co-workers (2020) reported that increasing power of the e-device can increase the metal content in the aerosols similar as for carbonyls and VOC.

Biomarkers of exposure

The exposure to constituents in e-cigarette aerosol depends not only on e-cigarette device and e-cigarette liquid, but are also closely linked to other variables, such as puff volume, the degree of air supply or smoke dilution with ambient air, the depth and speed of inhalation, as well as the rate of puffing.

Organic chemicals

One systematic review investigated biomarkers of exposure in the urine of e-cigarette users and potential implications for the development of bladder cancers (Bjurlin et al., 2021). Of the 22 included studies, only 6 were identified by Bjurlin and co-workers (2021) to have non-user control groups and 4 were sponsored by the tobacco or e-cigarette industry. Most studies compared biomarker levels in urine from e-cigarette users

with those in smokers. In general, the biomarker levels were higher in urine from tobacco smokers. Identified substances were metabolites of different VOCs such as benzene and acrylamide, PAHs and TSNA, which have been associated with various adverse health outcomes including cancer. In smokers switching to e-cigarettes, the urinary levels of VOCs and PAHs, decreased. In one study comparing e-cigarette users with non-users of tobacco, higher levels of acrylamide, O-toluidine and 2-naphtylamine were found in e-cigarette users.

Metals and metalloids

Zhao and co-workers (2020) reviewed metal/metalloid levels in blood and urine in e-cigarette users. The general conclusion was that most metal/metalloid levels found in biosamples of e-cigarette users were similar or higher than levels found in biosamples from tobacco users. Moreover, daily use of e-cigarettes was associated with significantly higher urinary Pb, and Sr levels compared to occasional use. In general levels of various metals in blood and urine of e-cigarette users as well as non-users are influenced by intake from other sources, making it difficult to use specific metals as biomarkers for e-cigarette exposure.

Summary: Constituents and exposure of e-cigarette aerosols

Several harmful chemicals such as carbonyl compounds, volatile organic compounds (VOC), reactive oxygen species and free radicals as well as various trace elements/metals have been identified in e-cigarette aerosols.

The large variation in e-cigarette devices and liquids used as well as in vaping patterns make human exposure highly variable and complex. Thus, it is difficult to precisely know or predict the exposure levels of potentially harmful substances. However, several of the substances identified in e-cigarette aerosols have been linked to various adverse health outcomes including cancer.

Non-malignant respiratory diseases

The airways involve all structures passing air from the tip of the nose to the bottom of the lungs, and effects on these structures may involve a broad spectrum of conditions, from trivial symptoms to life-threatening disorders. Minor symptoms may be acute or temporary but may also indicate an underlying process that over years may develop into a chronic airway disease.

Twelve systematic reviews pertaining to consequences for airways and pulmonary health were included. Six reviews assessed health effects from use of e-cigarettes and six reviews focused on smoking recording side effects from cessation with the use of e-cigarettes. Although the effect from e-cigarettes on smoking cessation is outside of our mandate, we included these six reviews as potentially informative on health effects.

The quality of the included systematic reviews was assessed using AMSTAR 2 (Table 2). Three of the reviews scored high quality (Chand & Hosseinzadeh, 2021; Hartmann-Boyce et al., 2020; Prasetyo et al., 2021), seven scored moderate (Bourke et al., 2021; Franck et al., 2014; Goniewicz et al., 2014; Gualano et al., 2015; Liu et al., 2018; Xian & Chen, 2021; Zakiyah et al., 2021), one scored low (Tzortzi et al., 2020) and one was assessed to have critically low quality (Sreedharan et al., 2021).

None of these twelve systematic reviews explicitly stated that they excluded studies sponsored by the tobacco industry or studies conducted by authors with link to the tobacco industry.

Respiratory outcomes addressed

Twelve systematic reviews reported on airways and pulmonary health effects in humans from use of electronic cigarettes. One of these reported on nasal mucociliary clearance (measured), two reported on asthma (self-reported diagnoses from doctors), one reported on coughing (probably self-reported), one reported on respiratory disease, case reports and case series, one reported on radiological findings on presentation with e-cigarette or vaping product use associated lung injury (EVALI) whereas six reported on respiratory symptoms reported by smokers switching to e-cigarettes.

Nasal mucociliary clearance

Research on nasal mucociliary clearance was summarised by Prasetyo and co-workers (2021). The review included 16 studies that had measured nasal mucociliary clearance, 15 studies investigating effects in traditional tobacco smokers, and one prospective randomised controlled trial including use of e-cigarettes (Kumral et al., 2016). Only the latter was relevant for this umbrella review, including 98 tobacco smokers, 58 of whom switched to e-cigarettes with nicotine, compared with the other 40, who quitted without using e-cigarettes. Impaired nasal mucociliary clearance was found in e-cigarette users as measured by saccharin transfer time (STT). STT (minutes ±SD) was higher in electronic cigarette users (11.9 ±1.8) than in former smokers who did not use e-cigarettes (10.4 ±1.6, p=0.0003).

Asthma

Use of e-cigarettes and the association with asthma was summarised in two systematic reviews (Chand & Hosseinzadeh, 2021; Xian & Chen, 2021). We note that these two systematic reviews appear to have very similar aim and similar inclusion criteria. Chand & Hosseinzadeh (2021) conducted their literature search seven months after Xian & Chen (2021) and included more studies. Still, only five of the 13 studies included by Chand & Hosseinzadeh 2021 overlapped with the 9 studies included by Xian & Chen (2021) (two studies listed results separately for males and females). All included studies were cross-sectional, and they relied on self-reporting of asthma diagnosed by a doctor. Both systematic reviews reported that the use of e-cigarettes is associated with asthma. Chand & Hosseinzadeh (2021) reported that current e-cigarette use (pOR = 1.36, 95% CI 1.21 to 1.52, from meta-analysis of 14 cross sectional studies) as well as ever e-cigarette use (pOR = 1.24 95% CI 1.13 to 1.36, from meta-analysis of 8 cross sectional studies) was associated with increased risk of asthma compared to unexposed participants. In accordance with this, Xian & Chen (2021) also found that the risk of asthma among e-cigarette users was significantly higher than that of non-e-cigarette users (OR = 1.27 95% CI 1.17 to 1.37). Furthermore, asthma was associated with dual use, both use of e-cigarettes and traditional cigarettes OR 1.47 (95% CI = 1.13 to 1.91), and with traditional cigarettes alone OR 1.33 (95% CI 1.19 to 1.49).

Coughing

One systematic review (Bourke et al., 2021) looked at association between coughing and e-cigarette use among children and adolescents. Six cross sectional studies (three from USA and one each from Canada, Hong Kong and Switzerland) with a total of 52 514 children and adolescents (range 135 to 44 662 participants per study) and one medical chart review (USA) with 13 children and adolescents were included.

Bourke and co-workers (2021) reported results by vote counting: 2/3 studies found association between coughing and e-cigarette use compared with non-users. Two studies found that adolescents reported coughing when they started to use e-cigarettes. Two studies reported coughing as a common symptom when adolescents (15 to 19 years) presented to a hospital or to a paediatrician following e-cigarette use, some of them later diagnosed with EVALI. The authors of the systematic review concluded that use of e-cigarettes is associated with increased coughing among children and adolescents compared to non-users.

Respiratory diseases including e-cigarette/vaping associated lung injury (EVALI)

One systematic review (Tzortzi et al., 2020) collected case reports on e-cigarette related illness and injuries. They found 41 publications describing 58 cases (28 published 2019–2020, and 13 published 2012–2018). The median age of a case was 23 years (IQR 19 to 33, range 14 to 64) and 40 of the 58 were male. Most cases were reported in the USA (36), one case in the UK and four cases elsewhere (Australia, Canada, Japan). The most common precise diagnosis was e-cigarette or vaping use-associated lung injury, EVALI (16 cases). The authors also found another eight publications presenting 104 respiratory aggregate cases of EVALI. Additionally, they identified 19 reports from the U.S. CDC pertaining to EVALI. The latest CDC-report dated 24th January 2020 reported a total of 2668 EVALI patients hospitalised in the USA. The first reported EVALI case was originally considered to be a patient with lipoid pneumonia published 2012 (McCauley et al., 2012).

EVALI was mainly associated with inhalation of vitamin E acetate in THC (tetrahydrocannabinoid) containing products from informal sources (Ghinai et al., 2019).

Radiological findings and disease characteristics on presentation of EVALI were summarised in one systematic review (Sreedharan et al., 2021). Thirty studies with a total of 184 patients were included, 76.6% male, mean age 24.5 years. Among the 172 who reported type of vaped substances, there were 65% THC, 62% nicotine, 9.5% cannabinoid oil and 2% marijuana. A variety of radiological findings and injury patterns were reported. Admission to the intensive care unit was necessary for 89 patients (48%), and 37 (20%) required intubation. Concurrent use of traditional cigarettes was not reported. The authors noted that results from the study should be interpreted with caution.

Other diagnoses included in the review by Tzortzi and co-workers (2020) were organizing pneumonia/bronchiolitis obliterans with organizing pneumonia (BOOP)/ respiratory bronchiolitis (n = 12), lipoid pneumonia (n = 9), vaping precipitated pneumothorax (4 cases) and exacerbation of pre-existing asthma (2 cases). One fatality was reported. For most of these cases, the user pattern of e-cigarettes and possibly other tobacco products was not specified. Some used cannabis (21 cases), combined cannabis and nicotine (6 cases), combined cannabis and another unknown liquid (6 cases), and some used solely nicotine (2 cases). It was not clear what user profile is connected to which diagnosis.

Respiratory symptoms when using e-cigarettes for smoking cessation

Smoking cessation is outside the aim of this umbrella review. However, respiratory adverse reactions in ex-smokers using e-cigarettes are relevant. Of the six systematic reviews, one (Hartmann-Boyce et al., 2020) had high quality score using AMSTAR 2, the other five scored moderate quality. Two of the reviews of moderate quality had search dates (nine and twelve months) later than the high-quality review respectively.

Hartmann-Boyce and co-workers (2020) reviewed studies on use of e-cigarettes for smoking cessation. All the included participants were smokers, and comparison was made according to the following characteristics: use of nicotine containing e-cigarettes versus use of non-nicotine containing e-cigarettes and compared with other nicotine replacement therapies, other medication, behavioral support, or no support. Follow-up ranged from 1 week to 1 year. The most reported adverse effects were throat/mouth irritation, headache, cough, and nausea, which tended to subside over time. In some studies, reductions in biomarkers were observed in people who smoked and then switched to vaping, consistent with reductions seen with smoking cessation. Hartmann-Boyce and coworkers (2020) used GRADE to assess their confidence in the results, the results relevant for this umbrella review are presented below.

The most recent systematic review on use of e-cigarettes for smoking cessation by Zakiyah and co-workers (2021) reported on several forms of alternative tobacco and nicotine products for smoking reduction and cessation with the objective to compare effectiveness and safety. Consistent with the results of the systematic review by Hartmann-Boyce and co-workers (2020) the most common adverse events reported by Zakiyah and co-workers (2021) were mouth and throat-related irritation, dry cough, headache, and changes in measured pulmonary function which were considerably milder than with conventional cigarettes. There was a lack of long-term studies.

Three older systematic reviews (Franck et al., 2014; Gualano et al., 2015; Liu et al., 2018) reported similar respiratory symptoms from the use of e-cigarettes for smoking cessation.

Goniewicz and co-workers (2020) reviewed studies recording respiratory symptoms and diagnoses of airway disease in regular e-cigarette users who were former smokers of conventional cigarettes and comparing with symptoms and disease in current smokers not using e-cigarettes. The systematic review by Goniewicz and co-workers (2020) included three studies and found a relative risk of reporting symptoms and having airway disease of about 0.6 comparing e-cigarette using ex-smokers with current smokers.

Grading our confidence in the evidence regarding use of e-cigarettes on respiratory health

The Summary of findings table 4a show our grading of available evidence on respiratory symptoms with the use of electronic cigarettes. There is a general lack of evidence on respiratory consequences of using e-cigarettes. What we do know is that e-cigarette or vaping use associated lung injury (EVALI) would not happen without the use of e-cigarettes, but we do not know how often it happens.

Table 4a. Summary of findings table for use of e-cigarettes and respiratory health

E-cigarette use and respiratory effects					
Patient or population: persons who use e-cigarettes **Exposure:** e-cigarettes **Comparison:** no-use of tobacco					
Outcome	Relative effect (95% CI)	Number of cases Number of studies	Certainty of the causal relationship (GRADE)	Certainty of the risk (incidence of cases) (GRADE)	Comments
E-cigarette or vaping use acute lung injury (EVALI)	-	16 cases case reports	⨁⨁⨁⨁ High[a]	-	CDC reported 2668 cases of EVALI in January 2020
Chronic obstructive pulmonary disease (COPD)	-	0	-	-	No prospective controlled studies reported on COPD
Asthma	-	0	-	-	No prospective controlled studies reported on asthma
GRADE Working Group grades of evidence **High certainty:** we are very confident that the true effect lies close to that of the estimate of the effect. **Moderate certainty:** we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different. **Low certainty:** our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect. **Very low certainty:** we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.					

Explanations: a. These events cannot happen without e-cigarettes. Most of these were in connection with use of cannabis, but EVALI would not have happened without e-cigarettes

The three tables below with Summary of findings were collected from the grading conducted by Hartmann-Boyce and co-workers (2020). Table 4b show their grading of evidence comparing use of nicotine containing e-cigarettes with use of non-nicotine containing e-cigarettes during smoking cessation. Table 4c shows their grading for use of nicotine containing e-cigarettes compared with use of other nicotine replacement therapy for smoking cessation. Table 4d includes users of nicotine containing e-cigarettes compared with behavioral support or no support for smoking cessation. The available evidence is collected from studies with few participants, and results are characterized by wide confidence intervals (imprecision).

Table 4b. Summary of findings table for use of nicotine containing e-cigarettes compared with use of non-nicotine containing e-cigarettes for smoking cessation, from Hartmann-Boyce and co-workers (2020)

Use of nicotine containing e-cigarettes compared with use of non-nicotine containing e-cigarettes for smoking cessation

Patient or population: smokers of cigarettes
Setting: Canada, Italy, New Zealand, UK, USA
Intervention: nicotine containing e-cigarettes
Comparison: non-nicotine containing e-cigarettes

Outcomes	Anticipated absolute effects		Relative risk (95% CI)	Number of participants (number studies)	Certainty of the evidence (GRADE)
	Risk with non-nicotine e-cigarettes	Risk with nicotine e-cigarettes			
Adverse events at 1 week to 6 months (self-reported)	35 per 100	35 per 100 (25 to 47)	RR 1.9 (0.73 to 1.36)	346 (2 RCTs)	⊕⊕◯◯ Low[a]
Serious adverse events at 1 week to 1 year (self-report and medical record)	2 per 100	0 per 100 (0 to 4)	RR 0.25 (0,03 to 2.19)	494 (4 RCTs)	⊕⊕◯◯ Low[a]

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Explanations: a. Downgraded two levels for imprecision, confidence intervals include both a clinically significant harm and clinically significant benefit.

Table 4c. Summary of findings table for use of nicotine containing e-cigarettes compared with use of other nicotine replacement therapy for smoking cessation, from Hartmann-Boyce and co-workers (2020)

Use of nicotine containing e-cigarettes compared with use of nicotine replacement therapy (NRT) for smoking cessation

Patient or population: smokers of cigarettes
Setting: New Zealand, UK, USA
Intervention: nicotine containing e-cigarettes
Comparison: nicotine replacement therapy (NRT)

Outcomes	Anticipated absolute effects		Relative risk (95% CI)	Number of participants (number studies)	Certainty of the evidence (GRADE)
	Risk with NRT	Risk with nicotine e-cigarettes			
Adverse events at 4 weeks to 6 months (self-reported)	45 per 100	44 per 100 (36 to 53)	RR 0.98 (0.80 to 1.19)	485 (2 RCTs)	⊕⊕◯◯ Low[a]
Serious adverse events at 4 weeks to 1 year (self-reported and medical record)	5 per 100	7 per 100 (4 to 13)	RR 1.37 (0.77 to 2.41)	727 (2 RCTs)	⊕⊕◯◯ Low[a]

Explanations: a. Downgraded two levels for imprecision, confidence intervals include both a clinically significant harm and clinically significant benefit.

Table 4d. Summary of findings table for use of nicotine containing e-cigarettes compared with behavioural support or no support for smoking cessation, from Hartmann-Boyce and co-workers (2020)

Use of nicotine containing e-cigarettes compared with behavioural support only or no support for smoking cessation

Patient or population: smokers of cigarettes
Setting: Canada, Italy, New Zealand, UK, USA
Intervention: nicotine containing e-cigarettes
Comparison: behavioural support only or no support

Outcomes	**Anticipated absolute effects**	Relative risk (95% CI)	Number of participants (number studies)	Certainty of the evidence (GRADE)
	Risk with behavioural support only or no support	**Risk with nicotine e-cigarettes**		
Adverse events at 12 weeks to 6 months (self-reported)	60 per 100	70 per 100 (62 to 78)	RR 1.17 (1.04 to 1.31)	516 (3 RCTs)	⊕⊕◯◯ Low[a,b]
Serious adverse events at 4 weeks to 6 months (self-reported and medical record)	1 per 100	1 per 100 (0 to 5)	RR 1.33 (0.25 to 6.96)	842 (5 RCTs)	⊕⊕◯◯ Low[a,b]

Explanations: a. Downgraded two levels due to high risk of bias. b. Downgraded due to imprecision.

Summary: Non-malignant respiratory diseases

Two systematic reviews with meta-analysis of cross-sectional studies with partly overlapping included studies reported increased prevalence of asthma among users of e-cigarettes compared with non-users. However, no prospective human studies elucidating how exposure to e-cigarette aerosol may contribute to development of asthma and chronic obstructive pulmonary disease (COPD) were identified.

One study showed decreased mucociliary clearance associated with use of e-cigarettes. One systematic review reported that adolescent use of e-cigarettes is associated with increased coughing compared to non-users.

In one systematic review, use of e-cigarettes was reported to cause e-cigarette use or vaping associated lung injury (EVALI). Most cases described involved use of e-liquid from informal sources containing tetrahydrocannabinol (THC; the main psychoactive compound in cannabis) or a combination of cannabinoids and nicotine.

Regarding use of e-cigarettes for smoking cessation, we are uncertain if there is a difference in the number of side effects and serious side effects from the use of nicotine containing e-cigarettes compared with using non-nicotine e-cigarettes, nicotine replacement therapy or behavioural support only or no support for smoking cessation.

Cardiovascular disease

Effects of e-cigarettes on development of cardiovascular disease (CVD) are expected to develop after extended exposure. However, studies examining effects of short-term exposure on parameters including blood pressure, heart rate and arterial stiffens as well as biomarkers linked to mechanisms involved in development of cardiovascular disease are presented in systematic reviews.

We identified 6 systematic reviews that assessed the effect of e-cigarette use on cardiovascular health effects (Goniewicz et al., 2020; Hartmann-Boyce et al., 2020; Kennedy et al., 2019; Riley et al., 2016; Saz-Lara et al., 2021; Skotsimara et al., 2019). We assessed the quality of the systematic reviews using AMSTAR 2 and found one to be of high quality; Hartmann-Boyce and co-workers (2020), three of moderate quality; Goniewicz and co-workers (2020), Kennedy and co-workers (2019) and Saz-Lara and coworkers (2021), one of low quality; Riley and co-workers (2015) and one of critically low quality; Skotsimara and co-workers (2019).

All studies included in the systematic review of Riley and co-workers (2015) were also included in one or more of the other systematic reviews. Thus, the review by Riley and co-workers (2015) is not considered further. Considering health outcomes assessed in more than one systematic review where one of them performed a meta-analysis, we have presented the result from the meta-analysis.

The systematic review by Kennedy and co-workers (2019) were used to assess risk of adverse health outcomes for which no meta-analysis was presented.

Myocardial infarction, coronary heart disease and stroke

The systematic review by Goniewicz and co-workers (2020) assessed how switching from tobacco cigarettes to e-cigarettes may affect the cardiovascular risk of users. The authors included three cross-sectional studies in their review, evaluated to be of acceptable quality after giving score according to the appraisal tool for cross-sectional studies. Goniewicz and co-workers (2020) estimated the odds ratio for smokers switching to e-cigarettes compared to current tobacco cigarette smokers for myocardial infarction (two studies, (Alzahrani et al., 2018; Farsalinos et al., 2019)) and coronary heart disease (one study,(Farsalinos et al., 2019)). The risk for stroke was presented in the original publication referred to in the systematic review (Parekh et al., 2020). The authors concluded that for former smokers switching to e-cigarettes, the odds ratio of having had myocardial infarction, coronary heart disease or stroke was not significantly different for smokers switching to e-cigarettes and current smokers.

Blood pressure and heart rate

Two of the systematic reviews identified, reviewed the literature on effects of e-cigarettes on blood pressure and heart rate (Kennedy et al., 2019; Skotsimara et al., 2019). The systematic review of Kennedy and coworkers had a more recent literature search date. The study by Kennedy and co-workers (2019) did not perform a meta-analysis because of concern for heterogeneity. Five studies included by Skotsimara and co-workers (2019), were not included in the review by Kennedy and co-workers (2019). Eighteen studies on heart rate were included by Kennedy and co-workers (2019), 8 of them included in the meta-analysis of Skotsimara and co-workers (2019). The number of studies for resting systolic and diastolic blood pressure reported included by Kennedy and

co-workers (2019) were 17 whereof 7 were included in the meta-analysis of Skotsimara and co-workers (2019).

Acute effects on blood pressure and heart rate
Skotsimara and co-workers (2019) included 11 studies that measured acute effects of using e-cigarettes with nicotine on heart rate. All exposures were performed among individuals that were either smokers of tobacco cigarettes or e-cigarette users (two studies) and smoked less than 5 cigarettes per day. The authors reported a pooled mean difference (increase) in heart rate of 2.27 (95% CI: 1.64 – 2.89) beats per min. However, large heterogeneity was observed, this was reduced in a sensitivity analysis without the study with a largest weight on the pooled estimate in the meta-analysis, however the effect of e-cigarette uses on heart rate remained significant.

Skotsimara and co-workers (2019) included seven studies in their meta-analysis on the effect of e-cigarette use with nicotine on systolic blood pressure (SBP) and diastolic blood pressure (DBP). The pooled mean difference for use of e-cigarettes on SBP and DBP was an increase of (mmHg) 2.02 (95% CI: 0.07 - 3.97) and 2.01 (95% CI: 0.62 - 3.39) respectively.

Chronic effects on blood pressure and heart rate
Three studies were included in the meta-analysis by Skotsimara and co-workers (2019) regarding chronic effects of switching from cigarettes to e-cigarettes (range: 5 – 365 days) on HR, SBP and DBP. One of the studies was performed among smokers with arterial hypertension switching to e-cigarettes without reporting the nicotine concentration of the e-cigarette liquids. All three studies may have conflict of interest regarding e-cigarette/tobacco industry. Skotsimara and co-workers (2019) reported no mean difference in heart rate for smokers switching to e-cigarettes –0.03 (95% CI: –2.57 to 2.52) but reported a lower SBP and DBP for former smokers using e-cigarettes, pooled mean difference –7.00 (95% CI: –9.63 to –4.37) and –3.65 (95% CI: –5.71 to – 1.59).

Kennedy and co-workers (2019) reported that most included studies in the systematic review on heart rate and blood pressure reported an increase. The authors did not differentiate between acute and chronic effects.

Two smoking cessation RCT-studies with smokers switching to e-cigarettes, were included in the analysis on effects of e-cigarette use on heart rate and blood pressure by Hartmann Boyce and co-workers (2020), but not included by Skotsimara and co-workers (2019). One of the RCT studies observed decreased heart rate in the nicotine containing e-cigarette arm compared to a study arm where the participants used e-cigarettes without nicotine after 12 weeks. This study was excluded by Skotsimara due to missing information and no response from authors when asked. The other study (Hatsukami et al., 2020), observed decreased heart rate among e-cigarette users using e-cigarettes with nicotine compared to tobacco cigarette users after 8 weeks. Both studies reported no difference in SBP between the groups.

Heart rate variability
Kennedy and co-workers (2019) included two intervention studies on heart rate variability (HRV). One study had participants that did not currently use e-cigarettes or tobacco

cigarettes. Differences in HRV variables were measured in the participants before and after use of e-cigarettes with or without nicotine or sham control (Moheimani, Bhetraratana, Peters, et al., 2017).

Compared to e-cigarettes without nicotine and sham control, e-cigarettes with nicotine increased the sympathetic tone of the autonomic nervous system, resulting in cardiac sympathovagal balance towards a sympathetic predominance,

When restricting the analysis to those participants who used e-cigarettes with nicotine and also had measurable differences in nicotine/cotinine levels in blood, the differences were increased. The authors concluded that the changes observed in heart rate variability was similar to the pattern of heart rate variability associated with increased cardiac risk in multiple populations. The other study included by Kennedy and co-workers (2019), reported differences in some HRV variables measured during exercise for the users of e-cigarettes with nicotine compared to when using e-cigarettes without nicotine (Sumartiningsih et al., 2019). A third study measuring effects of e-cigarettes on cardiac function, reported no differences after e-cigarettes use (Farsalinos, Tsiapras, et al., 2014).

Arterial stiffness

The systematic review of Saz-Lara and co-workers (2021) included four studies that assessed effects of e-cigarette use on arterial stiffness. Acute and chronic effects on arterial stiffness was investigated in the included studies by measuring pulse wave velocity (PWv). Since different methods were used to measure pulse wave velocity, the authors used the standardized mean difference for their meta-analysis of acute effects. Both e-cigarettes with nicotine and without nicotine were included in the analysis. The authors reported that e-cigarettes increased the pulse wave velocity (indicating increased arterial stiffness) by a pooled percentage of 4,7% (m/sec), with a pooled standardized mean difference of 0,37 (m/sec) (95% CI: 0,14 –0,61). The chronic effects of e-cigarettes were investigated in one study and thus, no meta-analysis was performed. The authors reported that for smokers switching to e-cigarettes with nicotine for one month, no difference in pulse wave velocity was reported compared to baseline.

The systematic review by Kennedy and co-workers (2019), included four studies assessing arterial stiffness by the augmentation index normalized to 75 heartbeats per min. The use of e-cigarette with nicotine increased the augmentation index in all studies. One additional study not discussed by Kennedy and co-workers (2019), in relation to arterial stiffness, also measured the augmentation index normalized to 75 beats per min and reported no difference after use of e-cigarettes with nicotine or after use of tobacco cigarettes (Kerr et al., 2019). Kennedy and co-workers (2019) also included one other study measuring arterial stiffness by other methods on participants using e-cigarette with nicotine. This study reported no difference in arterial stiffness index and reflective index (Szoltysek-Boldys et al., 2014).

Different markers have been used to elucidate how the use of e-cigarettes may enhance the levels of reactive oxygen species (ROS), as increased "oxidative stress" has been implicated in cardiovascular pathology. Seven studies that assessed oxidative stress in humans were included in the systematic review of Kennedy and co-workers (2019).

Two studies reported increased soluble NADPH oxidase 2 derived protein (sNOX2, marker of NADPH oxidase activation) and 8-iso-prostaglandin-F2α (8-iso-PGF2α, marker of lipid peroxidation), both indicative of increased ROS (Biondi-Zoccai et al., 2019; Carnevale et al., 2016). The NOX family of NADPH oxidases is considered as the key producers of ROS in many cells, whereas 8-iso-PGF2α is produced by non-enzymatic peroxidation of arachidonic acid located in membrane phospholipids. The authors also reported decreased levels of vitamin E and bioavailable nitric oxide, further supporting enhanced oxidative stress. One of the studies also measured increased hydrogen peroxide and decreased breakdown of hydrogen peroxide by human hemoglobin A (Biondi-Zoccai et al., 2019), a redox reaction closely linked to oxidative stress. The latter study also reported increased levels of soluble CD40 ligand (sCD40L). Both studies used e-cigarettes with nicotine. CD40L expressed and released from platelets are elevated in patients with acute coronary syndrome have been implicated in production induction of reactive oxygen and nitrogen species (RONS) by endothelial cells. Increased level of sCD40L was also for both smokers and non-smokers using an e-cigarette with nicotine (Nocella et al., 2018).

Others have reported increased plasma levels of the enzyme myeloperoxidase (MPO) after using e-cigarettes with nicotine compared to e-cigarettes without nicotine or sham vaping (Chaumont, de Becker, et al., 2018). Myeloperoxidase may utilize hydrogen peroxide to produce other oxidants and has been associated with increased cardiovascular risk.

In addition, vaping both with and without nicotine resulted in increased levels of malondialdehyde (Ikonomidis et al., 2018), a biomarker of lipid peroxidation. E-cigarettes without nicotine have been reported to increase levels of ROS and C-reactive protein and decrease nitric oxide metabolites (Chatterjee et al., 2019). Notably, in a study reporting increased oxidative stress, no differences in the serum protein-bound 3-chlorotyrosine/tyrosine ratio or homocitrulline/lysine ratio (two other parameters associated with oxidative stress) were found (Chaumont, de Becker, et al., 2018). Another study reported that e-cigarettes with or without nicotine caused changes in levels of paraoxonase-1 (Moheimani, Bhetraratana, Peters, et al., 2017), which is another biomarker of oxidative stress.

Overall, e-cigarette use with or without nicotine appears to have a partly unpredictable impact on several measures of oxidative stress.

Endothelial dysfunction

Ten studies in relation to endothelial dysfunction in humans, were included in the systematic review of Kennedy and co-workers (2019). The studies reporting on augmentation index are reported under arterial stiffness above.

One study reported reduction in flow mediated dilatation for both smokers and non-smokers after using e-cigarettes with a nicotine containing liquid (Carnevale et al., 2016). However, another study included by Kennedy and co-workers (2019) reported that use

of e-cigarettes with nicotine increased reactive hyperemia index (RHI)(Kerr et al., 2019). In addition, vaping with nicotine reduced the endothelium-dependent vasodilator effect of acetylcholine on microcirculatory blood flow measured in the skin compared to vaping without nicotine and sham vaping (Chaumont, de Becker, et al., 2018).

Vaping e-cigarettes without nicotine have reported to increase the levels of soluble intercellular adhesion molecule (ICAM) in the blood (Chatterjee et al., 2019), a marker of inflammation expressed by endothelial cells, while another study found no differences (Kerr et al., 2019).

Increased circulation of endothelial progenitor cells as well as e-selectin positive microvesicles commonly used as markers of vascular endothelial injury has been seen after using e-cigarettes with nicotine (Antoniewicz et al., 2016).

Platelet activation

Kennedy and co-workers (2019) included three studies that assessed effects of e-cigarette use on platelet activation. Activation of blood plates are associated with adverse cardiac outcomes. One study reported increased circulation of platelet micro-particles 5 min after vaping e-cigarettes with nicotine, while the authors reported no differences for endothelial micro-particles or total micro-particles (Kerr et al., 2019). In another study, where the participants also used e-cigarettes with nicotine, no difference was observed in platelet micro vesicles after vaping. Increased platelet aggregation among smokers and non-smokers is reported after vaping e-cigarettes with nicotine (Nocella et al., 2018). It has also been observed that use of e-cigarettes affects the levels circulating soluble adhesion molecules associated with platelet activity. Inconsistent findings effects of vaping have been found regarding the levels of soluble platelet selectin (Biondi-Zoccai et al., 2019; Kerr et al., 2019; Nocella et al., 2018); an adhesion molecule closely linked to platelet/endothelial cell activation. As reported under oxidative stress, increased levels of sCD40L were observed among participants using e-cigarettes with nicotine.

Grading our confidence in the evidence regarding use of e-cigarettes and acute and chronic effects on cardiovascular health

We used the GRADE approach when applicable, cross-sectional studies do not inform on effect, and thus only the outcomes reporting from longitudinal studies were graded.

The Summary of findings table 5a show our grading of available evidence on acute effects on cardio-vascular health. The Summary of findings table 5b show our grading of available evidence on mental health.

Table 5a. Summary of findings table for use of e-cigarettes and acute effects on cardiovascular health

Acute effect of e-cigarette use on cardiovascular health

Patient or population: Healthy smokers or healthy e-cigarette users
Exposure: e-cigarettes
Comparison: Compared to baseline for e-cigarettes users, smokers or non-e-cigarette users or smokers)

Cardiovascular Outcome Number of participants (Number of studies)	Relative effect (95% CI)	**Anticipated absolute effects (95% CI)** **Difference**	Certainty of evidence (GRADE)	Comment
Acute effects on heart rate Participants: 273 (1 meta-analysis with 11 studies, Skotsimara and co-workers, 2019)	-	MD **2.27 BPM higher** (1.64 higher to 2.89 higher)	⊕◯◯◯ Very low[a,b]	4 of 11 studies in the meta-analysis included one or more authors that may have conflict of interest regarding e-cigarette and/or tobacco industry.
Acute effects on systolic blood pressure Participants: 175 (1 meta-analysis with 7 studies, Skotsimara and co-workers, 2019)	-	MD **2.02 mmHg higher** (0.07 higher to 3.97 higher)	⊕◯◯◯ Very low[c]	3 of 7 studies in the meta-analysis included one or more authors that may have conflict of interest regarding e-cigarette and/or tobacco industry.
Acute effects on diastolic blood pressure Participants: 175 (1 meta-analysis with 7 studies, Skotsimara and co-workers, 2019)	-	MD **2.01 mmHg higher** (0.62 higher to 3.39 higher)	⊕◯◯◯ Very low[c]	3 of 7 studies in the meta-analysis included one or more authors that may have conflict of interest regarding e-cigarette and/or tobacco industry.
Acute effects on pulse wave velocity Participants: 143 (1 meta-analysis with 4 studies, Saz-Lara and co-workers, 2021)	-	SMD **0.37 SD higher** (0.14 higher to 0.61 higher)	⊕⊕◯◯ Low[d,e,f]	None of the included studies reported conflict of interest.

CI: confidence interval; **MD:** mean difference; **SMD:** standardized mean difference

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Explanations: a. Downgraded due to risk of bias as the studies addressing heart rate by Skotsimara and co-workers were evaluated according to ROBINS-I. For all included studies in the meta-analysis, one or more of the domains were evaluated to be of moderate or high risk of bias. Thus, no studies were evaluated to be of overall low risk of bias.
b. The meta-analysis that included all eleven studies addressing heart rate, had high heterogeneity (I2) 70%. The heterogeneity was reduced to 38%, when omitting the study with the largest weight in a sensitivity analysis.

Both meta-analyses concluded with significant increase, hence, we have not downgraded for heterogeneity for this outcome
c. Downgraded due to risk of bias as the studies addressing blood pressure by Skotsimara and co-workers were evaluated according to ROBINS-I. For all included studies in the meta-analysis, one or more of the domains were evaluated to be of moderate or high risk of bias. Thus, no studies were evaluated to be of overall low risk of bias.
d. Did not downgrade due to risk of bias. Risk of bias was not provided for each of the three RCTs or the one non-RCT included in the meta-analysis.
e. Downgraded due to inconsistency as both e-cigarettes with nicotine and without nicotine was included in the analysis.
f. Downgraded due imprecision because of only 143 participants in total.

Table 5b. Summary of findings table for chronic effects of e-cigarettes on cardiovascular health of smokers switching to e-cigarettes

Summary of findings:

Chronic effect of e-cigarettes on cardiovascular health of smokers switching to e-cigarettes

Patient or population: smokers switching to e-cigarettes
Setting: Use of e-cigarette
Intervention: Chronic effect of e-cigarettes on cardiovascular health
Comparison: Baseline measures

Outcome № of participants (studies)	Relative effect (95% CI)	**Anticipated absolute effects (95% CI)** **Difference**	Certainty of evidence	Comment
Heart rate Participants: 173 (1 meta-analysis with 3 studies, Skotsimara and co-workers, 2019)	-	MD **0.03 HR lower** (2.57 lower to 2.52 higher)	⊕◯◯◯ Very low[a,b,c]	All included studies in the meta-analysis by Skotsimara and co-workers included authors that may have conflict of interest regarding e-cigarette and/or tobacco industry.
Systolic blood pressure Participants: 173 (1 meta-analysis with 3 studies, Skotsimara and co-workers, 2019)	-	MD **7 mmHg lower** (9.63 lower to 4.74 lower)	⊕◯◯◯ Very low[a,c, d]	All included studies in the meta-analysis by Skotsimara and co-workers included authors that may have conflict of interest regarding e-cigarette and/or tobacco industry.
Diastolic blood pressure Participants: 173 (1 meta-analysis with 3 studies, Skotsimara and co-workers, 2019)	-	MD **3.65 mmHg lower** (5.71 lower to 1.59 lower)	⊕◯◯◯ Very low[a,c,d]	All included studies in the meta-analysis by Skotsimara and co-workers included authors that may have conflict of interest regarding e-cigarette and/or tobacco industry.

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Explanations:
a. Downgraded due to uncertainty in exposure information and measurement of effect.
b. Downgraded due to heterogeneity in meta-analysis (I^2=60,7%)
c. Downgraded due to imprecision as there were only 173 participants at baseline combined for all three studies.
d. Downgraded due to one study performed measures on smokers with arterial hypertension.

Summary: Cardiovascular diseases

Risk for myocardial infarction, coronary heart disease and stroke

We found no systematic reviews directly reporting the risk for disease associated with naive users of e-cigarettes in humans.

For three cross sectional studies, the odds ratio of having had myocardial infarction, coronary heart disease or stroke was similar for smokers switching to e-cigarettes as for current smokers. However, the cross-sectional design contributes to difficulties regarding conclusions on this outcome.

Heart rate and blood pressure and arterial stiffness

Acute use of e-cigarettes increased heart rate, systolic and diastolic blood pressure. We had very low confidence in the effect estimates (GRADE).

Use of e-cigarettes leads to acute effects on pulse-wave velocity (measure of arterial stiffness). We had low confidence in the effect estimates (GRADE).

Chronic use of e-cigarettes among former smokers reduced systolic and diastolic blood pressure. No change in heart rate was reported. We had very low confidence in the effect estimates (GRADE).

Use of e-cigarettes with nicotine is associated with changes in heart rate variability, with similarities reported to that associated with increased cardiac risk.

Biomarkers of effects with relevance for development of cardiovascular disease

The systematic reviews reported an increase in *vascular biomarkers* which are associated with imbalance in the immune system, oxidative responses, aggregation of platelets and damage to the endothelial vascular cells important processes linked to developmental of cardiovascular disease.

Mental disorders

Rates of smoking are markedly higher among people with psychiatric illness, including schizophrenia, mood disorders, anxiety disorders, attention-deficit/hyperactivity disorder (ADHD), eating disorders and substance use disorders, than in the general population. The causes underlying the association between mental disorders and smoking is unknown, but it is possible that nicotine´s effect on cognition, overlapping susceptibility, and psychosocial factors may be involved. The negative effects of tobacco and nicotine on comorbid somatic disease in mental disorders is well-known, however, concern has raised regarding negative effect on brain development and youth mental disease in adolescence and early adulthood (McGrath-Morrow et al., 2020).

Four systematic reviews reporting on outcomes related to mental health associated with the use of e-cigarettes were identified (Liu et al., 2018) (moderate quality), (Becker et al., 2020)(low quality), (Zakiyah et al., 2021) (moderate quality) and (Rothrock et al., 2020)(high quality).

Mental health comorbidity associated with e-cigarette use

We identified one systematic review that assessed mental health comorbidity associated with use of e-cigarettes among adolescents and young adults (12 to 26 years of age) (Becker et al., 2020). The authors grouped the mental health disorders into internalizing disorders (including depression, anxiety, suicidality, eating disorders, post-traumatic stress disorder), externalizing disorders (attention-deficit/hyperactivity disorder and conduct disorder), and transdiagnostic constructs (impulsivity and perceived stress) problems. In this umbrella review we grouped suicidality under transdiagnostic constructs.

Internalizing disorders

Six studies assessing internalizing symptoms (depressive symptoms, anxiety etc.) and use of e-cigarettes among adolescents, evaluated to be of weak to moderate quality, were included by Becker and co-workers (2020). Four of the five studies were based on the Population Assessment of Tobacco and Health study (PATH), which used the Global Appraisal of Individual Needs – Short Screener (GAIN-SS) to assess emotional (internalizing) and behavioral (externalizing) problems. Two of the included studies based on PATH found that high severity lifetime internalizing problems were associated with lifetime use of e-cigarettes by adolescents at baseline adjusted odds ratio (aOR) 1.6 (95% CI: 1.3 - 1.8). Initiation of use by naïve users was associated with past year high severity internalizing problems, adjusted relative risk ratio (aRRR) 1.61 (95% CI: 1.12 - 2.33).

Two studies were included by Becker and co-workers (2020) concerning young adults, they were evaluated to be of weak to moderate quality and had observations similar to that observed for adolescents based on PATH. An association between internalizing symptoms and use of e-cigarettes by youth at alternative high schools was also reported.

Depression

Becker and co-workers (2020) included seven studies evaluated to be of weak to moderate quality assessing the association between depressive symptoms and use of e-cigarettes among adolescents. A one-year longitudinal study reported an increased risk of escalating depressive symptoms associated with sustained e-cigarette use. The authors also reported that increased past 30 days user frequency among sustained users was associated with increase in depressive symptoms (Lechner et al., 2017). From five cross-sectional studies, Becker and co-workers (2020) reported that e-cigarette use was associated with depressive symptoms except for a Taiwanese based study that reported no association between e-cigarette use and depressive symptoms.

Becker and co-workers (2020) reported larger heterogeneity for the eight studies regarding depressive symptoms among young adults and e-cigarette use, most studies were of weak quality. One study with 2.5 years follow-up reported an association between past month use of e-cigarettes and depressive symptoms (Marsden et al., 2019). While another study, based on the same cohort with shorter follow up, did not observe an association between e-cigarette use and subsequent depressive symptoms, however, depressive symptoms predicted use of e-cigarettes (Bandiera et al., 2017). Two cross sectional studies reported an association between depressive symptoms and current e-cigarette use, while two longitudinal studies and one cross-sectional study did not.

Becker and coworkers reported on symptoms, not on depressive episodes associated with use of e-cigarettes.

Anxiety

Becker and co-workers (2020) included one cross sectional study addressing use of e-cigarettes and anxiety. The study reported that lifetime e-cigarette users had more panic disorders than those never using nicotine. The study was evaluated to be of weak quality.

Four studies were included by Becker and coworkers, concerning associations between anxiety symptoms and e-cigarette use for young adults. None of the studies reported an association between anxiety and e-cigarette usage. The quality of the evidence was evaluated to be weak to moderate.

Eating disorders

One cross sectional study from South Korea was included by Becker and co-workers (2020) that address associations of e-cigarette use and unhealthy weight control behaviors. The study reported an association between lifetime and current use of e-cigarettes and unhealthy weight control behaviors for females, whereas for male participants the association was statistically significant only for current e-cigarette users, with aORs stretching from 1.87 to 3.76 (Lee & Lee, 2019b). A US study on young adults reported no association between binge-eating disorder and use of e-cigarettes, assessed using part of the Minnesota Impulsive Disorders Interview tool (Grant et al., 2019). Both studies were evaluated to be of weak quality.

Post-traumatic stress

Two cross sectional studies considering associations of e-cigarette use and aspects of post-traumatic stress disorders among young adults were included by Becker and co-workers (2020) both of weak quality. One study did not observe a significant effect after adjustment for confounding factors between e-cigarette use and post-traumatic stress (Grant et al., 2019). Another study reported increased lifetime e-cigarette use associated with mistreatment during childhood (Shin et al., 2019).

Externalizing disorders

Five studies included by Becker and co-workers (2020) reported externalizing symptoms associated with use of e-cigarettes based on data from PATH. High severity lifetime externalizing problems were associated with lifetime use of e-cigarettes by adolescents at baseline aOR 1.5 (95% CI: 1.3 - 1.7) (Conway et al., 2018). Initiation of e-cigarette use by naïve users was associated with past high severity externalizing problems for both adolescent and young adults(Green et al., 2018; Riehm et al., 2019).

Attention-Deficit/Hyperactivity Disorder (ADHD)

Becker and co-workers (2020) included two studies that assessed associations between e-cigarette use and Attention-Deficit/Hyperactivity Disorder (ADHD) symptoms among adolescents, both evaluated to have moderate quality. Both studies reported an association between ADHD symptoms and later e-cigarette use. Two studies that included young adults did not report significant associations between ADHD symptoms and e-cigarette use. These two latter studies were evaluated to have weak to moderate quality of evidence.

Conduct disorder and delinquent behavior

Three studies were included that assessed associations between symptoms of conduct disorder and subsequent e-cigarette use among adolescents, all evaluated to have moderate quality of evidence. The risk for subsequent e-cigarette use was increased for adolescents with rule-breaking tendency aOR 1.93 (95% CI: 1.58 - 2.34) (Seo et al., 2020). Delinquent behavior was associated with both later e-cigarette use aOR 1.32 (CI not available) (Goldenson et al., 2018), and lifetime e-cigarette only users was associated with delinquent behaviors (Staff et al., 2020).

Transdiagnostic constructs

Suicidality

Concerning suicidality, one cross sectional study from USA and three from South Korea were included by Becker and co-workers (2020), all were evaluated to be of weak quality. The US based study reported an increased risk for past year suicidal ideation among current e-cigarette users aOR 1.23 (95% CI: 1.03 - 1.47) (Chadi et al., 2019). This was also observed in the Korean studies all based on the Korean Youth Risk Behavior Survey. The survey from 2016 reported increased risk of suicidal ideation aOR 1.58 (95% CI: 1.31 - 1.89), plans aOR 2.44 (95% CI: 1.94 - 3.08), suicidal attempt aOR 2.44 (95% CI: 1.85 - 3.22) and serious attempt aOR 3.09 (95% CI: 1.51 - 6.32) (Kim & Kim, 2021). The survey from 2017 reported that the associations for suicidality was consistently stronger among women (Lee & Lee, 2019a). No association with suicides has been reported.

Impulsivity and executive function

Becker and co-workers (2020) included three studies assessing impulsivity and e-cigarette use among adolescents. Two cross sectional studies reported increased e-cigarette use among adolescents with elevated impulsivity, and a longitudinal study showed increased e-cigarette use at follow up among adolescents with elevated baseline impulsivity. Two studies were included regarding executive function and e-cigarette use.
One of these, reported increased lifetime e-cigarette use among children with executive function deficit, aOR 4.99 (95% CI: 1.80 - 13.96) (Pentz et al., 2015).

Four studies were included for young adults, which in general supported the observation of increased e-cigarette use among young adults with elevated impulsivity, measured by different subcomponents of impulsivity. For all the studies on both adolescents and young adults, the quality of evidence was evaluated to be weak.

Perceived stress

One study regarding perceived stress among adolescents, with a four-year longitudinal design, evaluated to have moderate quality, was included by Becker and coworkers. Perceived stress was associated with lifetime and past month use of e-cigarettes (Leventhal et al., 2017). One included cross-sectional study reported perceived stress to be associated with past month e-cigarette use in young adults (weak quality).

Alcohol use and abuse

The systematic review by Rothrock and co-workers (2020) assessed whether use of e-cigarettes was associated with any alcohol use or binge drinking/drunkenness among adolescents (10 to 19 years of age). Meta-analysis based on results from 24 studies reporting on the association between e-cigarette use and any alcohol use revealed an Odds Ratio (OR) of 6.6 (95% CI: 5.66 - 7.7). For the association between e-cigarette use and binge drinking, the authors performed a meta-analysis based on 16 studies and reported an OR 6.73 (95% CI: 4.5 - 10.07). Moderate confidence in the effect estimates based on the GRADE approach were reported for both results.

Adverse events reported from smoking cessation/reduction studies related to mental health

Two of the systematic reviews identified were assessing e-cigarettes as smoking cessation and/or reduction aid and reported on adverse events related to the transition from combustion cigarettes to e-cigarette use. Among the most common adverse events associated with this transition Zakiyah and co-workers (2021) reported difficult to sleep and abnormal dreams. While Liu and co-workers (2018) reported anxiety and depressed mood as common adverse events. The two systematic reviews were not entirely overlapping in included studies and the results from both systematic reviews are therefore reported.

Grading our confidence in the evidence regarding use of e-cigarettes and effects on mental health, ADHD

We used the GRADE approach when applicable. GRADE is not designed to evaluate cross-sectional studies, and thus, only the systematic reviews reporting on longitudinal studies were graded. The Summary of findings table 6 show our grading of available evidence on mental health.

Table 6. Summary of findings table for available evidence on mental health and use of e-cigarettes

E-cigarette use and mental health

Patient or population: adolescents and young adults
Exposure: e-cigarettes
Comparison: same persons followed up over time

Mental harm disorder № of participants (studies)	Relative effect (95% CI)	**Anticipated absolute effects (95% CI)** **Difference**	Certainty of evidence
Depression in adolescents 104 sustained e-cigarette users (1 observational study (Lechner et al., 2017) from Becker and co-workers, 2020)	-	One study among adolescents reported an increase in depressive symptoms associated with sustained use of e-cigarettes and increased user frequency.	⊕◯◯◯ Very low[a,b] .
Depression in young adults Biannual follow up. (1 cohort with 2 publications (Marsden et al., 2019) from Becker and co-workers, 2020)	-	The first publication reported that e-cigarette use did not predict depressive symptoms (one year follow-up). The second publication based on the same cohort with a longer follow-up (2,5-year follow-up), estimated (hierarchical model) that increased user frequency of e-cigarette (past 30 days) increased depressive symptoms.	⊕◯◯◯ Very low[a,c]
ADHD, adolescents 150 participants (1 observational study (Dvorsky & Langberg, 2019) from Becker and co-workers, 2020)	-	Use of e-cigarettes did not predict ADHD symptoms.	⊕◯◯◯ Very low[a,d] .

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Explanation: a. Downgraded due to exposure information and symptoms (depression or ADHD) are self-reported.
b. Downgraded due to imprecision, only 104 sustained users of e-cigarettes.
c. Downgraded due to variation in outcome reporting at different time points from the same cohort.
d. Downgraded due to imprecision, 150 participants in study with a prevalence of approximately 10% taking ADHD medications.

Summary: Mental disorders

Several studies have reported an association between internalizing (such as anxiety and depression) and externalizing (such as ADHD) symptoms and disorders and e-cigarette use. Many studies reported an increased vulnerability for e-cigarette initiation and use among individuals with both internalizing and externalizing disorders.

One longitudinal study among adolescents reported a greater rate of increase in depressive symptoms for sustained use of e-cigarettes during the 12 months study period. In addition, the authors reported an association between an increased user frequency for the past 30 days and increase in depressive symptoms. Similar finding to the latter was also reported for young adults.

Another longitudinal study reported that Attention-Deficit/Hyperactivity Disorder (ADHD) symptoms predicted e-cigarette use, however e-cigarette use did not significantly predict future ADHD symptoms.

Adverse pregnancy outcomes and effects on early life health

Epidemiological studies have revealed that maternal use of tobacco products such as snus and cigarettes during pregnancy are associated with adverse pregnancy outcomes such as low birthweight, increased risks of preterm birth and low birth weight and stillbirth. Smoking during pregnancy is also associated with adverse effects later in the child's life.

Two systematic reviews, (Calder et al., 2021; Claire et al., 2020), that assessed health risks associated with maternal use of e-cigarettes during pregnancy were included. Both of the systematic reviews were assessed to be of moderate quality.

The systematic review of Claire and co-workers (2020) assessed side effects from pharmacological interventions for assistance in smoking cessation during pregnancy and the safety of such interventions. In addition to standard nicotine replacement therapy and Bupropione the search included studies on e-cigarettes. However, no parallel- or cluster-randomized controlled trials were included for e-cigarettes.

Birthweight, small for gestational age (SGA) and the newborn's health score

The systematic review of Calder and co-workers (2021) included three studies regarding health risks associated with use of e-cigarettes during pregnancy.

One study from Ireland, included a total of 620 singleton births: 218 births of exclusively e-cigarette-using pregnant women, 99 smokers, 195 dual users and 108 never smokers (McDonnell et al., 2020).

Compared to never smokers, newborns of exclusive e-cigarette using pregnant women, had no reduction in birthweight or difference in the proportions of newborns small for gestational age. However, the rate of breastfeeding at discharge was significantly higher for never smokers compared to e-cigarette users. For smokers, reduced birthweight, increased proportion of SGA and decreased breastfeeding at discharge was reported compared both to exclusive e-cigarette users and never smokers. The results for smokers were similar to those observed for dual users.

The health of the newborn as measured by mean Apgar (appearance (skin color), pulse (heart rate), grimace response (reflexes), activity (muscle tone), respiration (breathing rate and effort) score and admission to neonatal intensive care was not different between the groups. A limitation of the study was that the exposure information was based on one interview performed by midwives generally between week number 10 – 14 of pregnancy, no information on nicotine content or type of e-liquids used was collected and no validation of exposure was performed.

The two other studies included by Calder and co-workers (2021), were based on a single US cohort (Cardenas et al., 2019; Clemens et al., 2019). Exposure information was retrieved from the pregnant women when they visited a prenatal clinic during pregnancy. Saliva and carbon monoxide levels, as well as hair samples from a subset of the population, were taken and used for verification of exposure. The cohort included 232 singleton births, whereof only 6 women were exclusive e-cigarettes users. A non-significant reduced birthweight compared to non-smokers was reported for e-cigarette only users

and dual users. In a multivariate logistic regression model, the risk for SGA among e-cigarette only users were aRR 3.1 (0.8–11.7) and for dual users, aRR 1.9 (0.6–5.5).

In an analysis excluding self-reported non-smokers with cotinine or CO levels compatible with smoking, the risk estimate for SGA was aRR 5.1 (1.2–22.2) and aRR 2.5 (0.7–8.8) for e-cigarette and dual users respectively.

The second study based on the same cohort investigated the level of nicotine, cotinine, NNK and NNAL from hair samples from 76 women that also had registered birth outcome data. There was no significant difference in the levels of exposure biomarkers for dual users and smokers, however, both groups had higher levels of nicotine, cotinine and NNK than non-smokers. A limitation of the study is the small sample size, especially the low number of participants using e-cigarettes.

Grading our confidence in the evidence regarding use of e-cigarettes and effects on adverse pregnancy outcomes

We used the GRADE approach when applicable, cross-sectional studies do not inform on effect, and thus only the outcomes reporting from longitudinal studies were graded. The summary of findings table 7 show our grading of available evidence on adverse pregnancy outcomes.

Table 7. Summary of findings table for pregnancy outcomes with use of e-cigarettes

E-cigarette use and mental health

Patient or population: pregnant mothers
Exposure: e-cigarettes
Comparison: no use of tobacco

Mental harm disorder № of participants (studies)	Relative effect (95% CI)	**Anticipated absolute effects (95% CI)** **Difference**	Certainty of evidence
Birthweight (2 observational studies (Cardenas et al., 2019; McDonnell et al., 2020) from Calder and co-workers, 2021)	-	One study with 6 users reported a non-significant reduction compared to non-smokers. One study with 218 users reported no difference compared to non-smokers.	⊕◯◯◯ Very low[a,b] .
Small for gestational age (2 observational studies (McDonnell et al., 2020) from Calder and co-workers, 2021)	-	One study with 6 users reported an increased risk for SGA (2/6, 33%) compared to non-tobacco users (8%) One study with 218 users reported no difference (24/218, 11%) compared to non-tobacco users (13%)	⊕◯◯◯ Very low[a,b, c]

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

Explanation:
a. Downgraded due to risk of bias, as exposure information is based on self-reported e-cigarette use in the largest of two studies.

b. Downgraded due to imprecision, as one study has 218 pregnant mothers using e-cigarettes, the other study has only 6 pregnant mothers using e-cigarettes.
c. Downgraded due to heterogeneity, as the two studies included by Calder and co-workers are inconsistent. One of the studies reported no difference in SGA for mothers using e-cigarettes compared to non-smokers, whereas the other study reported an increased risk for SGA for mothers using e-cigarettes compared to non-smokers.

Summary: Adverse pregnancy outcomes and effects on early life health

Based on two studies included in one systematic review, a similar and a non-significant reduction on birthweight was reported. Both an increased risk for and no effect on small for gestational age was reported. We had very low confidence in the effect estimates (GRADE).

Non-malignant oral diseases

Various tobacco products including snuff and cigarettes, have adverse effects on oral health. Smoking is a known risk factor for periodontal and peri-implant disease. Although, associations between tobacco smoking and dental caries are reported, there is limited evidence for a causal effect.

Three systematic reviews assessing oral health outcomes of e-cigarettes were identified (Figueredo et al., 2020; Ralho et al., 2019) (moderate quality) and (Yang et al., 2020)(low quality). (Figueredo and co-workers (2020) was not included as it was unclear if they combined periodontal and peri-implant measure in the same analysis).

The systematic review of Yang and co-workers (2020) was used to summarize effects on oral health in this umbrella review, as it had a more recent literature search, than Ralho and co-workers (2019), and cowered more topics in relation to oral health. The authors summarized the effects on mouth and throat irritation, periodontal and dental disease in addition to effects on the oral microbiome. The systematic review included all the studies included in the other systematic reviews, except for one study included by Figueredo and co-workers (2020) assessing self-reported oral health symptoms and periodontal status among cigarette users and users of e-cigarettes.

Mouth symptoms and mucosal lesions

Thirty-five studies included in the systematic review by Yang and co-workers (2020) reported on oral symptoms and oral mucosal lesions associated with use of e-cigarettes. Most studies included were evaluated, to have weak quality, due to self-reported and mostly descriptive information. The symptoms reported included mouth irritation, dryness, a burning feeling, halitosis (bad breath) and bad taste. Yang and co-workers (2020) reported reduced oral symptoms, and improvements in taste and mouth odor for smokers switching to e-cigarettes compared to conventional smokers.

Regarding oral mucosal lesions, two case reports were included by Yang and co-workers (2020). There was one case with a lichenoid reaction associated with the use of e-cigarettes reported to have high levels of propylene glycol (Bartram et al., 2016). The other case had oral ulceration in the hard palate associated with the use of an e-cigarette (Cant et al., 2017).

In a randomized controlled trial 2 participants allocated to the e-cigarette intervention group reported oral ulcerations (Holliday et al., 2019). One clinical study included, investigated the prevalence of oral mucosal lesions among 45 former smokers and among 45 current e-cigarettes users (more detailed history of tobacco use not reported in the study), and reported increased frequency of nicotine stomatitis, hairy tongue, and hyperplastic candidiasis among e-cigarette users (Bardellini et al., 2018).

Throat

Similar symptoms to those reported for the oral cavity was also reported for the throat such as dryness, a burnt sensation and irritation. In addition, use of e-cigarettes was associated with cough. Users of nicotine containing e-cigarettes more often reported sore throat and cough. Some symptoms were associated with the use of particular flavors.

Yang and co-workers (2020) reported that switching from cigarettes to e-cigarettes may mitigate throat symptoms.

One case study (conference abstract) included in Yang and co-workers (2020) reported acute uvulitis in a 30-years old man switching to e-cigarettes two days prior to symptoms onset.

Oral microbiology/microbiome

Ten studies were included by Yang and co-workers (2020) for assessment of effects of e-cigarette use on the oral microbiome. Mokeem and co-workers (2019) investigated the carriage rate of candida in saliva among users of e-cigarettes with an average nicotine concentration of 3.4 mg/ml and reported an overall candida carriage rate of approximately 83% for e-cigarette users compared to 50% for never smokers. Furthermore, the authors reported a significant increased carriage rate for *Candida albicans* compared to never smokers, but similar to conventional smokers (Mokeem et al., 2019). In agreement with this, increased frequency of hyperplastic candidiasis among e-cigarette users was reported by Bardellini and co-workers (2018).

Three studies included in the systematic review by Yang and co-workers (2020) investigated how e-cigarette use may impact the oral bacterial microbiome. Using next generation sequencing (16R rRNA), Kumar and co-workers (2019) reported that the salivary oral microbiome among users of e-cigarettes may be distinct from never smokers (Kumar et al., 2019). Among 10 e-cigarette users, with mild to severe periodontal disease Kumar and co-workers (2019), reported lower relative abundance of Bacteroidetes and higher abundance of Actinobacteria compared to never smokers. Protobacteria was increased in saliva from e-cigarette users compared to smokers and non-smokers. The authors reported an enrichment of the opportunistic pathogens Rothia and Haemophilus among e-cigarette users. The authors also performed a study (presented in same article as the study above) characterizing the functional properties of the microbiome and reporting a unique profile among e-cigarette users, using at least one nicotine-containing cartridge per day. However, Stewart and co-workers (2019), reported no difference in the oral microbiome (saliva and buccal swab samples) among e-cigarette users (with nicotine) and never users of tobacco (Stewart et al., 2018).

Periodontal and peri-implant consequences

The systematic review by Yang and co-workers (2020) identified 20 studies assessing periodontal health and use of e-cigarettes. The included studies were clinical studies (clinical examination with or without radiographic examination), non-clinical studies, mainly with a cross-sectional design based on self-reported oral health measures, and one pilot RCTs. The quality of evidence was evaluated to be weak to moderate. Of the eight clinical studies assessing periodontal health, three had no follow-up whereas five studies had a follow-up from two weeks to six months.

Two longitudinal studies investigated how switching to e-cigarettes with nicotine from tobacco cigarettes affected periodontal health. One study with 20 smokers switching to e-cigarettes, with a follow up of 2 weeks, reported increased bleeding on probing compared to baseline (Wadia et al., 2016). The other study followed recent e-cigarette users for 6 months, all were former smokers (Tatullo et al., 2016). The participants were divided in two groups based on the self-reported smoking intensity of tobacco cigarettes.

The authors reported a decreased plaque index and reduction in the papillary bleeding index at 6 months. None of the two studies included a comparison to a control group.

Three clinical studies reported on periodontal health measurements among self-reported e-cigarette users and compared them to never tobacco users or cigarette smokers (BinShabaib et al., 2019; Javed et al., 2017), and one of the studies also included a group of water pipe smokers (Mokeem et al., 2018). Two of the studies reported specifically use of e-cigarettes with nicotine. From the measurements reported on, (plaque index, bleeding on probing (BOP), percentage of periodontal pockets ≥ 4 mm, clinical attachment loss, marginal bone loss and number of missing teeth) all three studies reported decreased BOP among e-cigarette users compared to never users of tobacco. Two of the studies reported on levels of proinflammatory cytokines from the gingival crevicular fluid or whole saliva, however no difference between e-cigarette users and never tobacco users were reported. In general, worse clinical periodontal status, reported in all three studies, and increased levels of proinflammatory markers, reported in two of the studies, was observed for cigarette smokers compared to e-cigarette users. Of note, all data was descriptive.

Two of the included studies by Yang and co-workers (2020) investigated the response to periodontal treatment among e-cigarette users. One of the studies, included groups of smokers, e-cigarette users and never tobacco users and performed a full-mouth ultrasonic scaling (no manual toot surface debridement with curettes) at the initial visit and performed follow up measures at 3 and 6 months (ALHarthi et al., 2019). For the e-cigarette group, no difference in periodontal measures was reported at baseline, except reduced BOP compared to never tobacco users. At follow up, reduction in plaque index and periodontal pocket depths was reported compared to baseline, while no difference compared to never tobacco users at follow up. However, the authors reported that the results should be interpreted with extreme caution due to limitation in study design. The other study that included periodontal treatment in its design, was a pilot randomized controlled study, where smokers with periodontitis were allocated to e-cigarettes or standard smoking cessation and given periodontal treatment (Holliday et al., 2019). The authors reported that the changes in the periodontal measures were very similar for both groups. A reduction in periodontal pocket depth, percentage of sites with periodontal pocket depth ≥ 5mm and BOP was observed.

One cohort study with 6 months follow up was included by Yang and co-workers (2020) (Ismail et al., 2019). The study included 45 e-cigarette using participants that had a dental and periodontal clinical examination at baseline and follow up. The authors of the study reported an increase in the gingival index and calculus index after 6 months. One study based on the populations assessment of tobacco and health survey with a longitudinal design, reported increased risk for new gum disease and bone loss around teeth for participants with long- term use of e-cigarettes (Atuegwu et al., 2019). The findings were based on self-reported oral health measures.

Two cross-sectional studies that assessed adverse outcomes among smokers switching to e-cigarettes with nicotine were included by Yang and co-workers (2019) (Farsalinos et al., 2013; Farsalinos, Romagna, et al., 2014). One of the studies reported increased gum bleeding among former smokers switching to e-cigarettes.

A study among Korean adolescents reported no association between e-cigarette use and gingival pain/bleeding for both users of e-cigarettes with and without nicotine (Cho, 2017). Another Korea based health survey among adults reported an association between e-cigarette use and periodontal disease for male participants, aOR 2.34 (95% CI: 1.52–3.59) (Jeong et al., 2020).

Two US based cross sectional studies were included by Yang and co-workers (2020) (Vora & Chaffee, 2019; Yao et al., 2017). The authors of these studies reported an association between use of e-cigarettes and gum disease. One additional study reported an association between poor oral health and use of e-cigarettes (Huilgol et al., 2019).

Four clinical studies with no follow up were included by Yang and co-workers (2020) assessing peri-implant health status among e-cigarettes users (Al-Aali et al., 2018; Alqahtani et al., 2019; AlQahtani et al., 2018; ArRejaie et al., 2019). One study reported nicotine content of the e-liquid. Three of the studies reported increased plaque index compared to never tobacco users. All four included studies reported reduced bleeding on probing. Three of the studies reported increased percentage of peri-implant pockets with depth ≥ 4mm and increased peri-implant bone loss compared to never tobacco users. In addition, three of the four studies reported on proinflammatory cytokines in peri-implant sulcular fluid and observed higher concentrations of the cytokines investigated compared non-tobacco users. Three studies included comparisons to tobacco cigarette smokers. Two of these reported increased bone losses for smokers compared to e-cigarette users. Two studies reported on percentage of pockets ≥ 4mm; one study reported increased percentage of pockets, while another study did not find a significant increase. No difference in pocket depths (mm) was observed in the study measuring this compared to smokers.

Dental caries

There were a limited number of studies regarding caries risk among e-cigarette users. Increased risk of reporting poor oral health, defined as number of teeth lost due to caries or periodontal disease, was found for e-cigarette users. One study, following 45 e-cigarette users for 6 months, reported increased number of decayed, missed, and filled teeth (DMFT) (Ismail et al., 2019). However, this study was evaluated to be of low quality and no control group was included in the design. One cross-sectional study reported increased risk of cracked /broken tooth among Korean adolescents using e-cigarette users (Cho, 2017).

Summary: Non-malignant oral diseases

Use of e-cigarettes was associated with different oral symptoms and mucosal lesions. From clinical studies with a cross-sectional design regarding periodontal disease, the only clinical periodontal measure reported to be different for e-cigarette users compared to never users of tobacco was reduced bleeding on probing (BOP). A longitudinal study based on self-reported oral health found increased gum disease and bone loss around teeth among e-cigarette users compared to never users.

From the cross-sectional population-based studies with self-reported health outcomes, mostly an increased risk for gingival/periodontal disease was reported.

The clinical studies in relation to peri-implant disease included in the systematic review of Yang and co-workers reported associations between worse parameters of peri-implant disease among e-cigarette users compared to never tobacco users.

For dental caries, no longitudinal study with a design that could give a causal relation was presented.

Cancer

Effects from e-cigarettes on risk of cancer in humans will be expected first to be seen after long-term exposure. However, one systematic review of a specific cancer type was found. This review had also included a few studies examining effects from short-term e-cigarette exposure on biomarkers associated with exposure to carcinogens and/or effects linked to mechanisms involved in development of cancer using cells isolated from oral cavity (see *in vitro* section).

Human studies

A single systematic review on use of e-cigarette and cancer in humans was identified (Flach et al., 2019), we assessed the review to be of low quality. The review evaluated the literature on carcinogenic effects of e-cigarettes as a risk factor for head and neck cancers. The review included 5 studies: two cohort studies, two case-control studies and one case series. (Due to type of studies/ lack of or short follow up time) they were all were graded at level 4 according to Oxford Centre for Evidence-Based Medicine, Level of Evidence.

Only the case series reported directly on cancer in humans, describing two cases of oral carcinoma, diagnosed as basaloid squamous cell carcinoma (SCC), associated with chronic e-cigarette use in otherwise healthy individuals (Hoang, 2017). In both cases, e-cigarettes were reported to be consumed 20 or 30 times per day for 13 years.

Biomarkers of exposure and effects.

The four other studies reported in the review by Flach and co-workers (2019) were presenting data on biomarkers of exposure and effects.

One of the cohort studies included 65 participants divided into tobacco smokers, e-cigarette smokers and non-smokers (Franco et al., 2016). Cells from the oral mucosa of the participants were subject to cytological examination. The prevalence of micronuclei (MN) was reported to be significantly decreased in e-cigarette users compared to tobacco smokers and was similar to that of healthy controls. MN is an indicator of chromosomal damage/instability, which is believed to play a central role in cancer development.

One case-control study analyzed saliva from e-cigarette smokers, tobacco smokers and non-smokers and demonstrated the endogenous formation of the carcinogen N'-nitrosonornicotine (NNN) in e-cigarette smokers (Bustamante et al., 2018).

A case-control pilot study investigated the acute effects of e-cigarettes on blood flow in the buccal mucosa in 10 subjects (Reuther et al., 2016). An initial increase of capillary perfusion of the buccal mucosa was observed with the use of nicotine-containing e-cigarettes.

One case-control study, also reported on under non-malignant oral diseases, enrolled outpatients for dental consultations into two groups (former tobacco smokers and current e-cigarette smokers) and examined them for possible oral mucosal lesions (OMLs) (Bardellini et al., 2018). The total prevalence of OMLs was higher among e-cigarette

smokers; however, the difference was not statistically significant. In terms of pre-cancerous oral mucosal lesions, no difference was identified between the two groups. The review by Flack and coworkers also reported 13 relevant laboratory-based studies on cancer related issues reported under biomarkers of exposure, animals, and *in vitro* studies below.

Summary: Cancer

One systematic review was identified reporting on 5 studies (two cohort studies, two case control and one case series). Due to their study design, all were evaluated to be of low quality.

One case series reported on two cases of oral carcinoma in long time users of e-cigarettes. The cohort studies and the case control studies assessed biomarkers of exposure and effects with relevance for cancer (such as endogenous formation of the carcinogen N'-nitrosonornicotine (NNN) in saliva from e-cigarette smokers and decreased prevalence of micronuclei in oral mucosal cells from e-cigarette users compared to smokers and similar to healthy controls).

Poisoning, injuries, and other adverse effects

As the prevalence of e-cigarette use among youth and adults has increased so has the number of observed cases of poisonings and injuries associated with e-cigarettes and vaping substances. Adverse events reported include accidental ingestion among children, intentional ingestion and/or injection of e-liquid at older age as well as traumatic injuries due to device battery explosion in the pocket or the hand and/or face among adults. A direct link between use of e-cigarette and this type of adverse health outcome is obvious. The prevalence of this type of adverse events among users are still unknown.

We found two systematic reviews on poisonings associated with accidental ingestion or intentional ingestion and/or injection of e-liquid (Scarpino et al., 2020; Tzortzi et al., 2020). Scarpino and co-workers (2020) included case reports on intentional poisoning, while the systematic review of Tzortzi and co-workers (2020) included both accidental and intentional poisoning. In addition, the authors included 10 studies presenting aggregate data on poisoning. Trauma, accidental injuries were reported in three of the systematic reviews identified (Dekhou et al., 2021; Tzortzi et al., 2020; Yang et al., 2020). The systematic review of Tzortzi and co-workers (2020) included all the cases, case series included in the systematic review of Yang and co-workers (2020), and all cases, case series in the systematic review of Dekhou and co-workers (2021) except for one case described in an abstract. The systematic review of Tzortzi and co-workers (2020) also included injuries in addition to medical case reports further divided into respiratory (described under the chapter on airways) oral, cardiovascular, immunologic, hematologic, allergic reactions, infant complications, and altered medication levels. We assessed the review by Scarpino and co-workers (2020) to be of moderate quality and the review by Tzortzi and co-workers (2020) to be of low quality.

Poisoning

Accidental poisoning

The systematic review by Tzortzi and co-workers (2020) included nine studies on accidental poisoning. The median age at poisoning was reported to be 2 years of age. Two cases resulted in death, and one case had complications with hearing.

Intentional poisoning

Both systematic reviews identified include studies on intentional poisoning by either ingestion and/or injection of e-liquids. The study by Tzortzi and co-workers (2020) included 16 studies involving 18 cases and the study by Scarpino and co-workers (2020) included 33 studies involving 38 cases. Fourteen of the included studies in the review by Tzortzi and co-workers (2020) were included in the systematic review by Scarpino and co-workers (2020). The reported median age of attempted suicide was in the late twenties in both reviews. Scarpino and co-workers (2020) reported that most of the cases were caused by ingestion of nicotine containing e-liquid alone, however in some cases the subjects combined e-liquids with other substances such as alcohol, methadone, or benzodiazepines.

Of 38 cases reported on by Scarpino and co-workers (2020), nine were found dead by an emergency team and 12 were admitted to an Emergency Department. Of the latter 12 cases, five resulted in brain death, two were fatal through the withdrawal of life support treatment, three cases died of a non-neurological injury, one patient remained in a persistent vegetative state, whereas one patient recovered consciousness.

Traumatic injuries

Overheating and explosion of e-cigarettes often due to lithium-ion battery dysfunction, may cause traumatic, chemical, and thermal injuries. The systematic review by Tzortzi and co-workers (2020) included 42 studies reporting on a total of 126 cases of injuries in addition to 10 publications on aggregated cases of traumatic injuries. The severity of injuries caused by explosion and burns varied. Facial injuries with loss of body parts, burns of facial skin, hands and thighs requiring surgical treatment and skin grafts have been reported. In addition, fatal explosion injury to the head has been reported.

Seizures after intended use of e-cigarettes

The systematic review of Becker and co-workers (2020) included one study that reported on seizures after e-cigarettes use (Faulcon et al., 2020). The Food and Drug Administration in the USA had received 122 voluntary case reports of seizures associated with the use of e-cigarettes. Most of the cases involved youth and young adults (14 – 24 years of age). Of those reports with available information, 62% experienced seizures within 30 minutes after last use. Different psychiatric disorders were the most often named comorbidity, and some used medications that are reported to increase risk for seizure. In addition, cannabidiol oil and marijuana was reported in 8 of 11 reported cases, where such information was available.

Grading our confidence in the evidence regarding effects from use of e-cigarettes on poisoning, injuries, and seizures

We used the GRADE approach for case reports on trauma, poisonings, and seizures as it was no doubt that these outcomes occurred. However, we cannot estimate the risk for such events. The summary of findings table 8 show our grading of available evidence on poisoning, injuries, and seizures.

Table 8. Summary of findings table for use of e-cigarettes and poisoning, injuries, and seizures

E-cigarette use and serious adverse events

Patient or population: e-cigarette users
Exposure: e-cigarettes

Serious adverse events	Relative effect (95% CI)	Number of cases (number of studies)	Certainty of the causal relation-ship (GRADE)	Certainty of the evidence for the risk (incidence of cases) (GRADE)
Accidental poisoning	-	9 (9 observational studies)	⊕⊕⊕⊕ High[a]	-
Intentional poisoning	-	18 (16 observational stu-dies)	⊕⊕⊕⊕ High[a]	-
Seizures (1 study (Faulcon et al., 2020) from Becker and co-workers, 2020)	-	122 (1 observational study)	⊕◯◯◯ Very low[b]	-
Traumatic injuries caused by explosions, thermal and chemical burns due to overheating of lithium battery	-	128 (42 observational stu-dies)	⊕⊕⊕⊕ High[a]	-

GRADE Working Group grades of evidence
High certainty: we are very confident that the true effect lies close to that of the estimate of the effect.
Moderate certainty: we are moderately confident in the effect estimate: the true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different.
Low certainty: our confidence in the effect estimate is limited: the true effect may be substantially different from the estimate of the effect.
Very low certainty: we have very little confidence in the effect estimate: the true effect is likely to be substantially different from the estimate of effect.

a. Events cannot occure without use of e-cigarettes
b. Downgraded due to uncertainty regarding concurrent use of other substances and psychological comorbidities.

Summary: Poisoning, injuries, and other adverse effects

E-cigarettes may overheat or explode and cause subsequent injuries. The injuries can range from small lacerations and burns to more serious tissue damage, even with fatal outcome. Furthermore, accidental, or intentional intake of e-liquids can cause poisonings with the severity ranging from mild symptoms such as vomiting, rapid heart rate, unsteadiness, and increased salivation to death as the most serious outcome (unintentional or suicide). How frequently poisoning occurs is not known, most cases are likely not reported. Seizures after use of e-cigarettes have been reported mainly in youth and young adults. Many cases were associated with concurrent use of other substances.

Other adverse health effects

Post-operative health

One systematic review in relation to post-operative health after metabolic and bariatric surgery was identified (Srikanth et al., 2021) (low quality). Srikanth and co-worker (2021) conducted a systematic review on the effect of smoking and use of e-cigarettes on the post-operative health outcomes on metabolic and bariatric surgery patients.

Srikanth and co-workers (2021) included 48 articles and concluded that use of combustible tobacco by metabolic and bariatric surgery patients was related to higher risk of mortality and complications, but not weight loss. However, none of the included studies had a focus on e-cigarettes. Hence, we do not know if use of e-cigarettes affects post-operative health.

Sleep disturbances

One systematic review that assessed adolescent substance use and its association with sleep disturbances was identified (moderate quality). Kwon and co-workers (2019) included 13 articles where one cross sectional survey included the use of electronic cigarettes. This study involved 2488 youths (mean age 17 years) and reported shorter total sleep time in weekends but not weekdays among those adolescents who used e-cigarettes. Since this evidence is from one cross sectional study, we cannot conclude about potential cause-effect relationship. Hence, we do not know if use of e-cigarettes affect sleep.

Second and third hand exposure

We did not identify any systematic reviews regarding second or third hand exposure to e-cigarettes.

Summary: Other adverse health effects

There were few systematic reviews on other health effects, and we found no clear answers. We do not know if use of e-cigarettes affects post-operative health following bariatric surgery or affect sleep.

Animal- and *in vitro* studies

There are several different genetic, physiological, and environmental factors to take into consideration when extrapolating from animal studies to human. The human genetic and environmental predisposition to various diseases may vary as some individuals are more susceptible than others to develop disease or having an exacerbation of an existing illness. Humans are often simultaneously exposed to numerous factors with implications for disease development or triggering of exacerbations. Notably, several of the health outcomes in humans are not as common or have no direct counterpart in rodents. Regarding e-cigarette aerosol exposure, there are several complicating issues linked to differences in toxicokinetic as well as anatomical and physiological differences such as the breathing pattern, horizontally positioned respiratory system in rodents, inhalation frequency and the distance and time from creation of aerosol to lung lining exposure and exposure through licking of the fur.

Obviously, results from animal experiments are not directly comparable with the health effects observed in human studies. However, the relevance of animal studies may be improved with the combined use of human *in vitro* studies on specific processes in relevant cells. Important issues linked to animal testing includes: i) exposure situation can be accurately controlled with regard to dose and duration, ii) implications for disease development or triggering of exacerbations can be easily followed, iii) mechanisms involved can be elucidated by examining effects of specific aerosol components and by manipulating hypothesized key event, iv) it is possible to test role of genetic or environmental factors that may predispose for the disease in question and v) human exposure to hazardous substances may be reduced.

Included systematic reviews on animal studies

We identified three systematic reviews that included animal studies (Flach et al., 2019; Kennedy et al., 2019; Wang et al., 2019). We grouped them according to adverse health outcomes. The systematic review of Wang and co-workers (2019) (assessed to be of low quality) divided animal studies between pulmonary/respiratory health effects (6 studies) and extra-pulmonary effects (9 studies). Among the 6 studies, one was grouped as related to cancer and 4 others were reported under direct lung toxicity and host defense. The last of the 6 studies were reported under “Adverse embryonic development and postnatal growth and development”. Among the 9 studies related to extra-pulmonary effects, 6 were reported under “Adverse embryonic development and postnatal growth and development”. The three others were reported under “Other adverse health outcomes (effects on liver, kidney, and testes)”.

The systematic review by Flach and co-workers (2019) (assessed to be of moderate quality) included one animal study in relation to cancer (Salturk and co-workers, 2015) that was also included in the review by Wang and co-workers (2019). The systematic review of Kennedy and co-workers (2019) included 6 animal studies in relations to cardiovascular disease, none of these were included in the systematic review of Wang and coworkers. The study of Lee and co-workers (2018) included in the systematic review of Kennedy and coworkers was reported and discussed in relation to cancer.

Included systematic reviews on in vitro studies
We identified 4 systematic reviews reporting *in vitro* results (Flach et al., 2019; Kennedy et al., 2019; Wang et al., 2019; Yang et al., 2020). The one by Wang and co-workers (2019) reported 32 *in vitro* studies which were grouped between exposure methods. In the majority of these studies the endpoints were related to respiratory toxicity. The studies are grouped under "*In vitro* studies with relevance for cytotoxicity, oxidative stress, and pro-inflammatory markers".

The systematic review of Kennedy and co-workers (2019) included 8 *in vitro* studies related to cardiovascular effects, not included in the systematic review of Wang and co-workers (2019) or Yang and co-workers (2020). The systematic review of Yang and co-workers (2019) included 14 *in vitro* studies related to oral cytotoxic, genotoxic, and oncogenic effects. Six of these were also included in the systematic review by Flach and co-workers (2019). These studies are grouped under "General studies on DNA damage and other effects linked to cancer development ". Yang and co-workers (2020) also included two *in vitro* studies in relation to dental effects and 4 *in vitro* studies in relation to effect on oral bacteria/ microbiome, one of these studies was also addressed under dental effects (Kim and coworkers).

Below are the animal and *in vitro* studies identified in the systematic reviews described above in greater detail.

Animal studies
Non-malignant adverse respiratory health effects
Direct lung toxicity and host defence: Whole body aerosol exposure generated from an e-liquid with classical tobacco flavour and nicotine increased the levels of the inflammatory cytokines, IL-6, MCP-1, IL-1α and IL-13, 24 hours after last exposure in bronchoalveolar lavage fluid of mice. No significant difference was observed for total cell number. The authors also observed reduced total glutathione level (Lerner et al., 2015).

Another study also with mice, reported that whole body e-cigarette aerosol exposure with nicotine resulted in increased oxidative stress and infiltration of macrophages compared to control, but no increase in the measured cytokines was observed. Exposure to e-cigarette aerosol conferred however, reduced pulmonary clearance after infection with *Streptococcus pneumoniae.* Using an *ex vivo* model, the authors showed, reduced phagocytosis by pulmonary macrophages. In addition, e-cigarette exposure enhanced susceptibility to influenzae (H1N1) infection (Sussan et al., 2015).

A study also investigating bacterial defence, showed that e-cigarette aerosol inhalation resulted in changes in the bronchoalveolar proteome. The authors suggested that the observed increase in TREM-1, was caused by necrotic cell death and contributed to a proinflammatory state by macrophages and monocytes. Interestingly, the authors reported decreased IL-3 and GM-CSF in the bronchoalveolar lavage of e-cigarette exposed mice, both cytokines are important for an activation of the host defence during early stages of infection. Increased systemic inflammation indicated by increased levels of Pentraxin-3, an acute phase protein produced by various cell types in response to pri-

mary inflammatory signals, was reported in mice. The authors also reported that e-cigarette vapour exposed *Staphylococcus aureus*, led to increased bacterial lung burden and mortality in a *Staphylococcus aureus* infection model. (Hwang et al., 2016).

One study using an ovalbumin (OVA), to induce asthmatic airway inflammation, reported that inhalation of e-liquids exacerbated allergy-induced asthmatic symptoms (Lim & Kim, 2014).

Developmental effects on lungs: One study included by Wang and co-workers (2019), reported on early life exposure to e-cigarette aerosol and possible effects on growth and lung development after birth (see section on Adverse embryonic development and postnatal growth and development below) (McGrath-Morrow et al., 2015).

Adverse cardiovascular health effects

Effects linked to the cardiovascular system may not only have implications for cardiovascular disease (CVD) such as atherosclerosis, but also for other adverse outcomes. While reduced angiogenesis may reduce growth of embryo and effect brain development, enhanced angiogenesis may increase growth of cancer in adult.

One study investigated the effect of whole- body e-cigarette aerosol exposure on thrombogenesis and platelet activation and function (Qasim et al., 2018). The e-liquid used contained in addition to propylene glycol and glycerol, nicotine, and menthol. The authors reported that e-cigarette exposure led to shortened tail bleeding time and shortened occlusion time, using the ferric chloride carotid artery injury-induced thrombosis model. Both findings are indicative of a pro-thrombotic state. The authors also reported that platelets from exposed mice were hyperreactive, showing increased aggregation, increased dense granules secretion, with content potentiating platelet activation, and α granules secretion, with content mediating both primary and secondary hemostasis.

In addition, e-cigarette exposure enhanced integrin activation, phosphatidylserine expression with functions related to aggregation and assembly of coagulation factor complexes, respectively. The authors also reported an increased Akt and ERK activation in blood platelets, cellular signaling pathways contributing to the hyperreactive platelet phenotype observed. Finally, the authors reported that the platelets were more resistant to prostaglandin-I_2 inhibition, a factor that may be released from endothelial cells to counteract platelet aggregation.

Two studies included by Kennedy and co-workers (2019) investigated the effect of e-cigarette on cardiovascular function. Both studies used whole body e-cigarette aerosol exposure with e-liquids containing nicotine. The chronic study used a cappuccino flavored liquid.

The authors of the two-weeks exposure study (Shi et al., 2019), reported no difference for the parameters measured of cardiac function, compared to air only exposed animals. However, the authors reported findings indicative of increased angiogenesis, as they observed increased CD31 (PECAM-1) and CD34 positive areas (endothelial markers) in both heart and kidney tissues, and increased CD31 expression from heart homogenates. No significant differences were reported for the investigated parameters indicative of heart collagen content/fibrosis.

The chronic study (Olfert et al., 2018), 8 months of exposure, included in addition to a control- and e-cigarette group, a tobacco cigarette exposed group. The authors reported no difference for the cardiac functional parameters measured for the e-cigarette group compared to the control group, whereas an increased left ventricular mass was found compared to the tobacco cigarette group. The authors observed an almost three-fold greater increase in pulse wave velocity (indicating increased arterial stiffness) after 8 months exposure compared to control for both e-cigarette and cigarette exposed animals. In addition, the authors reported, using and ex-vivo model to measure changes in aortic tension, a reduced thoracic aortic vascular relaxation to a vasodilator (methacholine) and increased vascular response to a vasoconstrictor (phenylephrine).

One study performed with apolipoprotein-E knockout mice, a mouse strain that develops atherosclerosis, exposed the animals to e-cigarette aerosol generated from e-liquids with and without nicotine with a tobacco flavor or saline only for 12 weeks (Espinoza-Derout et al., 2019). The authors of the study reported that mice exposed to e-cigarette aerosol with nicotine developed impaired ventricular systolic function, but no impaired diastolic function. From an RNA sequence analysis of tissue from the ventricles, the authors reported 40 nicotine dependent differential expressed genes. The authors also reported ultrastructural changes in cardiomyocytes indicative of cardiomyopathy, in addition to increased malondialdehyde levels (marker of oxidative stress) and increased mitochondrial DNA lesions for nicotine exposed animals. The authors also observed increased atherosclerotic lesion formation in the aortic root of nicotine exposed animals.

One study included by Kennedy and co-workers (2019) investigated effects of e-cigarettes on the cerebrovascular system and stroke, using both *in vitro* and *in vivo* models (Kaisar et al., 2017). The e-cigarette liquid used for the experiments, was commercially available and contained nicotine. Exposure to e-cigarette extraction and soluble cigarette smoke, induced increased levels of oxidative stress and expression of Nrf-2, a transcription factor regulating the antioxidative defense, and a downstream protein (NQO-1) in mouse primary brain microvascular endothelial cells. In addition, both exposures led to reduced expression of the junctional protein ZO-1, and increased intercellular permeability was reported. Interestingly, the *in vitro* experiments showed increased levels of platelet endothelial cell adhesion molecule-1 (PECAM-1), reported both for e-cigarette and tobacco exposure, a finding that was also observed in the *in vivo* brain homogenates for PECAM-1, in addition to ICAM-1 (intercellular adhesion molecule) and VCAM-1 (vascular cell adhesion molecule-1). Notably, increased expression of adhesion molecules by the activated endothelium is a critical feature of CVD including atherosclerosis. Furthermore, from brain tissue homogenates both e-cigarettes and tobacco cigarettes reduced expression for thrombomodulin, a membrane bound protein which functions as a cofactor for thrombin converting thrombin to an anticoagulant, and Nrf-2. In mice chronically exposed to both e-cigarette and cigarette aerosols increased brain infarction area was reported compared to control mice, using a brain infarction model. These findings were supported by *in vitro* experiments.

Adverse embryonic development and postnatal growth and development

Three studies treated pregnant mice with e-cigarette aerosol with and without nicotine for almost the entire gestational period and during lactation. In one of these studies, frontal cortex recovered from approximately one-month-old male and female offspring

were excised and analysed for gene expression by RNA Sequencing. The authors reported that e-cigarette aerosols, both with and without nicotine, induce sex-dependent gene expression changes associated with predicted adverse neurobiological and neurobehavioral outcomes like those associated with early life exposure to the smoke from conventional cigarettes (Lauterstein et al., 2016).

The other study assessed neurotoxicity resulting from early-life exposure to e-cigarette aerosols with or without nicotine (Zelikoff et al., 2018). Specifically, the study focused on biomarkers related to neuroinflammation and neurotrophins. The authors reported that both exposure conditions caused reductions in hippocampal gene expression of Ngfr (nerve growth factor receptor) and Bdnf (Brain-derived neurotrophic factor which plays an important role in neuronal survival and growth). The exposure also affected the serum level of several cytokines. Furthermore, exposure to e-cigarette aerosols without nicotine enhanced expression of Iba-1 (marker of microglia, a resident macrophage in the central nervous system (CNS)), suggesting that other constituents than nicotine such as PG, VG and flavourings may affect CNS development. Overall, the authors suggested that exposure to e-cigarette aerosols, with and without nicotine, may pose a considerable risk to the developing CNS.

A study where neonatal mice kept with their mothers received aerosol exposure via whole-body inhalation as well as through breast-feeding, investigated early life effects of exposure to e-cigarette aerosols with and without nicotine and no flavourings (McGrath-Morrow et al., 2015). After exposure the first 10 days of life, the pups weighed less after exposure to e-cigarette aerosol both with and without nicotine. The results indicated further that exposure to nicotine containing e-cigarette aerosol reduced lung development and decreased lung cell proliferation.

Investigations of developmental effects frequently use Zebrafish embryos as an alternative to use of higher animals. One study exposed zebrafish embryos in water containing varying doses of CSE (cigarette smoke extract) or ECE (e-cigarette aerosol extract) in the water, with comparable nicotine concentrations (Palpant et al., 2015). Both CSE and ECE caused dose-dependent effects on cardiac development, with ECE appearing less toxic than CSE.

One study exposed larval worms of *C. elegans* to e-cigarette liquid or e-cigarette extract. Both propylene glycol and nicotine independently influenced physiological measures of health and viability (Panitz et al., 2015). Flavourings in the e-liquid were not found to significantly affect the outcomes and there was no evidence that vaporization altered toxicity.

In a frog model, ECE exposure affected craniofacial development manifested as median facial clefts and midface hypoplasia (Kennedy et al., 2017).

Cancer

One study reported on histological changes, which can be indicative of a carcinogenic potential, in the laryngeal mucosa of adult female rats exposed to e-cigarette vapour (Salturk et al., 2015). The authors detected two cases of hyperplasia and four cases of metaplasia in the exposure group and one case of metaplasia in the control group but no significant differences between the study and control groups. It should be noted that

short exposure time and small sample sizes make firm conclusions regarding the different outcomes difficult. E-cigarette aerosol reduced lung glutathione levels (important for redox balance) and increased levels of pro-inflammatory cytokines in mice.

Other adverse health outcomes (effects on liver, kidney, and testes)
Three papers addressed e-liquid toxicity in adult rat kidneys, liver, and testes, respectively after intra-peritoneal injections of e-liquid with tobacco flavour, with or without nicotine but same solvent composition. E-liquid altered antioxidant defence, caused oxidative stress and minor changes in renal function parameters as well as induced adverse effects in rat testes (disturbed formation of steroid hormones). Nicotine did not enhance the severity of the outcomes, E-liquid also appeared to be associated with oxidative liver tissue injuries and addition of nicotine to e-liquid worsened the hepatotoxic outcome in this model.

In vitro studies
In vitro studies with relevance for cytotoxicity, oxidative stress, and pro-inflammatory markers
These effects are general effects of e-cigarette aerosol that in principle can be linked mechanisms involved in various adverse health outcomes not only respiratory disease, but also cardiovascular disease (CVD) and cancer discussed below.

Exposure to e-liquids: Wang and co-workers (2019) identified 10 publications investigating *in vitro* effects associated with exposure to e-liquids. Overall, the studies tested various e-cigarette liquids with different flavourings and with or without nicotine in primary cells as well as different cell lines. Cells were mainly of human origin. Nine of the 10 studies suggested that e-liquids caused cytotoxicity predominantly reflected in increased release of pro-inflammatory cytokines and cell death. Three of these studies indicated that cinnamon aldehyde was among the flavourings that gave the strongest cytotoxic effect of those tested.

Exposure to e-cigarette aerosol extracts (ECE): Wang and co-workers (2019) identified 10 publications investigating *in vitro* effects associated with exposure to e-cigarette aerosol extracts (ECE). Overall, the studies used various cell types of both animal and human origin as well as of both pulmonary and extra-pulmonary origin. Some of the studies reported the cytotoxicity and pro-inflammatory effects appeared to be associated with the flavourings, not with the nicotine levels of the e-liquid. The cytotoxicity also appeared to vary between different e-liquids. Cytotoxicity was also reported to increase when cells were exposed to e-liquid aerosol generated at higher voltage. Most of the studies comparing effects of CSE and ECE exposures on various cell types *in vitro* indicate that e-cigarettes were less toxic (i.e., cytotoxicity, oxidative stress, lung surfactant) than regular cigarettes. One study reported that ECE delayed maturation of human embryonic stem cells during cardiac differentiation.

Exposure to e-cigarette aerosol through air-liquid interface (ALI): Twelve studies reported on in vitro studies exposing cells directly to e-cigarette aerosol by using the air-liquid interface (ALI) system, an exposure model regarded as closer to *in vivo* situations than exposure through culture medium. Overall, 11 of 12 studies reported adverse effects after exposure to e-cigarette aerosol and 5 of the 12 studies showed that outcomes

such as cytotoxicity, oxidative stress, pro-inflammatory indicator levels, and transcriptomic modifications induced by e-cigarette aerosol occurred to varying extent, but the responses were lower than those induced by tobacco cigarettes. Both ECE exposure and direct e-cigarette aerosol exposure were reported to reduce antimicrobial activity in macrophages and neutrophil cells.

In vitro studies in relation to cardiovascular disease (CVD)
The *in vitro* studies summarized by Kennedy and co-workers (2019) were grouped under the headings of oxidative stress, endothelial cellular function, endothelial complement interaction and platelet function.

Oxidative stress: Three studies included by Kennedy and co-workers (2019) were reported to increase oxidative stress after exposure to e-cigarette aerosol extracts or e-liquids (one study). The results showed that the composition of the e-liquids, nicotine content and flavors, when applied directly or after aerosolization, affected the level of intracellular ROS formation. Lee and co-workers also showed increased hydrogen peroxide formation by endothelial cells subsequent to serum exposure from e-cigarette smokers.

Endothelial function and cytotoxicity: From the studies included, e-liquids and aerosol extracts may decrease cell viability, of note is that some flavors contributed significantly to the cytotoxic effects reported. Especially herbal- and cinnamon flavors were described as highly cytotoxic (Lee et al., 2019; Putzhammer et al., 2016). Regarding cell migration, one study reported decreased cell migration, suggesting reduced angiogenesis. This finding was, however, not verified in a study having conflict of interest regarding tobacco/e-cigarette industry. Another study reported that nicotine and e-cigarette liquids and condensed vapor extracts affected lung endothelial integrity (Schweitzer et al., 2015). Both e-liquids as well as the condensed extracts exerted barrier disruptive effects, which may have implications for infections/exposure to allergens.

Endothelial complement interactions: The complement system, a part of the immune system, is made up of a large number of distinct plasma proteins that react with one another to opsonize pathogens and induce a series of inflammatory responses that help to fight infection. Various steps involved can also be studied *in vitro.*

One study investigated complement factor deposition on endothelial cells after exposure to nicotine as well as various e-cigarette liquid and aerosol extracts with different flavors and nicotine concentrations including no nicotine, compared to cigarette smoke-extract and control without additions (Barber et al., 2017). Some differences in deposition of the individual complement factors investigated were reported for the various extracts. Increased deposition of C1q (part of the initial complement complex triggered in the classical complement pathway) was observed for all e-cigarette vapor extracts and mainstream smoke extracts. In relation to this finding, increased expression of complement receptors for C1q was observed. E-cigarette aerosol extracts were also suggested to cause an interaction with complement via particle exposure, thereby increasing deposition of some complement factors (C1q and C5b-9, and to some extent C3b).

Platelet function: Another study included by Kennedy and co-workers (2019), with three of the same authors as the previous, summarized that platelet aggregation rate and percent aggregation was significantly enhanced after exposure to e-vapor extracts (Hom et al., 2016). This was independent of nicotine, as it was also reported for e-cigarette vapor without nicotine and suggested to be mediated by fine particulate matter. In addition, exposure to e-cigarette vapor extract increased expression of platelet adhesion markers CD41, CD42b, and CD62P. As for platelet aggregation, the authors reported that platelet activation was increased and that this was independent of nicotine after exposure to e-cigarette aerosol.

In contrast to that reported for endothelial cells, no difference in deposition was reported for the complement factors C1q and C5b-C9 on platelets after exposure to e-cigarette aerosol although the complement receptors gC1qR and CC1qR were increased.

General studies on DNA damage and other effects linked to cancer developments
The systematic review on head and neck cancers Flach and co-workers (2019) also reported on *in vitro* studies using human cultivated cells originating from tissue in principle linked to oral, oropharyngeal, or middle ear tissue. This information is of value for evaluation of cancer risk.

In short, regarding *in vitro* studies, cytotoxicity of e-cigarettes in cellular experiments was demonstrated in eight studies, with varying extent of DNA damage and oxidative stress induced by toxic components. More specifically, they reported: i) A nicotine-independent but dose-dependent increase in DNA damage induced by e-cigarette aerosols. ii) Chronic exposure to e-cigarette aerosols increased ROS, caused mutagenic oxidative DNA damage, and a reduced expression of proteins essential for DNA damage repair. iii) An increase in DNA damage, cell arrest in G1 and G2, increased apoptosis, necrosis and cell death following exposure to e-cigarette aerosols. iv) Induction of DNA double-strand breaks in cells incubated with e-cigarette aerosols as well as an increase in migration of cancer cells and upregulation of epithelial-mesenchymal transition (EMT)–promoting genes following e-cigarette exposure. v) Increased cell migration in dysplastic oral keratinocytes following nicotine exposure was also reported by another group and vi) Reduction in cell viability as well as increased DNA damage following incubation with *fruit-flavoured e-liquids*.

In relation to oral cells, ten of the *in vitro* studies included in the systematic review of Yang and co-workers (2020) were reported to describe the cytotoxic effects of e-cigarettes liquids and/or vapor. Six of the *in vitro* studies included by Yang and co-workers (2020) were reported to show genotoxic effects of e-cigarette aerosol, and some of them are reported in greater detail below.

DNA damage is considered an important event related to cancer development. One study reported that e-cigarette aerosol from two different brands with and without nicotine caused DNA breaks in three different cell lines investigated, measure by increased tail length in comet assays and increased ϒ-H2AX immunostaining, both parameters representing DNA damage/DNA damage response (Yu et al., 2016).

The authors also reported increased cell death and reduced clonogenic survival compared to control. Sundar and co-workers (Sundar et al., 2016) showed that e-cigarette aerosol from e-liquid with tobacco flavor and nicotine caused increased protein

carbonylation, indicative of increased oxidative stress, but no significant increase from menthol flavored liquid without nicotine. However, both exposure increased IL-8 levels. An increase in COX-2, RAGE was seen for both exposures, but only significant for the no nicotine menthol flavored vapor. However, both exposure increased γ-H2AX, indicative of double stranded DNA breaks in periodontal ligament fibroblasts. Similar findings were replicated for human gingival epithelial progenitor cells.

Ganapathy and co-workers (Ganapathy et al., 2017) investigated DNA lesions in both oral and lung epithelial cells after exposure to different e-cigarette aerosol and smoke extracts. After one hour exposure, they observed a dose-dependent increase in DNA damage, which was reported to be independent of the nicotine concentration of the e-liquid, by using a quantitative primer anchored DNA damage assay. In addition, significant increase in oxidative DNA damage, 8-oxo-2'-deoxyguanosine, was reported. In a chronic *in vitro* exposure model (two weeks exposure) both e-cigarette aerosol extracts and smoke extracts caused increased levels of DNA damage compared to control. Smoke extracts were reported to confer increased DNA damage compared to e-cigarette aerosol. However, e-cigarette aerosol significantly increased oxidative DNA damage, as measured as 8-oxo-2'-deoxyguanosine, compared to smoke and control. Both exposures caused significant increase in cellular ROS and reduced total antioxidant capacity, reduced protein levels of OCG1 (base excision repair enzyme involved in oxidative DNA damage) and ERCC1 (nucleotide excision repair enzyme involved in bulky DNA lesions) in one oral epithelial cell line. These effects were only partly verified in another cell line.

Taken together, the *in vitro* studies suggest that e-cigarette aerosol exposure may enhance oxidative DNA damage. Likely mechanisms involved includes increased ROS generation and reduced antioxidant capacity, decreased DNA repair mechanisms resulting in an increased probability of more permanent DNA damage.

In vitro studies in relation to adverse non-malignant oral health effects

Dental effects: One study reported that exposure of bovine enamel to e-cigarette aerosol with different concentrations of nicotine and different flavors resulted in changes in enamel color (Pintado-Palomino et al., 2019). Another study investigated the cariogenic potential of e-cigarette aerosols with sweet flavors (Kim et al., 2018). The authors reported that e-cigarette aerosol increased the adhesive force to enamel for *Streptococcus mutans*. Biofilm formation was increased for 4 of the 5 flavors investigated, and three of the flavors led to decreased enamel hardness after 6 h of contact with *S. mutans*. Increased adhesion and biofilm formation are factors associated with increased caries risk

Two studies were included by Yang and co-workers (2020) which investigated how e-cigarette aerosol without flavors, but with and without nicotine affected planktonic growth, colony growth on agar and biofilm formation of oral streptococci (Cuadra et al., 2019; Nelson et al., 2019). Compared to cigarette smoke, e-cigarette aerosol had only minor effect on planktonic growth, survival of colony forming units, colony morphology and biofilm formation. One study investigated how e-cigarette aerosol affected *Candida albicans* growth, chitin production, hyphal length, virulence gene expression and interaction with gingival epithelial cells (Alanazi et al., 2019). E-cigarette aerosol increased growth, hyphal length, and virulence gene expression. The authors also reported increased adhesion to epithelial cells and increased cytotoxicity of *C. albicans* exposed to

e-cigarette aerosol. In general e-cigarettes aerosol with nicotine conferred increased effects compared to nicotine free aerosol, and highest effects were observed for cigarette smoke. These findings may potentially be linked to increased risk of oral candida infection.

Summary: Animal and *in-vitro* studies

Adverse respiratory outcomes: Depending on e-cigarette device/liquid type, dose, duration as well as animal model, e-cigarette use has been reported to affect various immune responses linked to respiratory disease including: i) increased level of cytokines in the bronchoalveolar lavage and immune cell infiltration, ii) increased allergy-induced asthmatic symptoms and iii) decreased resistance to both bacterial and viral infections. We found no systematic review covering or elucidating mechanisms behind EVALI.

Cardiovascular disease: Exposure to e-cigarette aerosol with nicotine promotes prothrombotic state, shortened bleeding and occlusion time, hyperreactive platelets and reduced thrombomodulin expression. Chronic long-term exposure to e-cigarette aerosol has been associated with increase in arterial stiffness, reduced effect of a vasodilator and increased effect of a vasopressor. In a mouse model that develops atherosclerosis, the authors reported that exposure to e-cigarette aerosol containing nicotine increased oxidative stress, affect the morphology of cardiac cells as well as cardiac function, indicative of cardiomyopathy and increased atherosclerotic lesions. Acute exposure to e-cigarette aerosol *in vitro* substantiates increased oxidative stress and reduced antioxidative defence after exposure to e-cigarette aerosol with nicotine. E-cigarette aerosol with nicotine has also been reported to increase area of brain infarction, possibly due to increased permeability of blood brain barrier. Exposure of endothelial cells *in vitro* to e-liquids and aerosol were reported to increase ROS formation and decrease viability. In addition, e-cigarette aerosol exposure increased endothelial deposition of complement factors and platelet aggregation. These finding support effects of e-cigarettes on CVD.

Adverse embryonic and postnatal growth and development: Exposure of pregnant mice with e-cigarette aerosol with and without nicotine during gestational period and lactation, induced sex-dependent changes in gene expression associated with enhanced risk of adverse neurobiological and neurobehavioral outcomes like those associated with early life exposure to tobacco cigarette smoke. Furthermore, neonatal exposure to nicotine containing e-cigarette aerosol reduced lung development and decreased lung cell proliferation. E-cigarette aerosol extract also caused dose-dependent effects on cardiac development in a zebrafish model. In a frog model, exposure to e-cigarette aerosol extract affected craniofacial development.

Adverse non-malignant oral health effects: One systematic review on human studies additionally included *in vitro* studies. Exposure to e-cigarette aerosol resulted in changes in enamel colour, increased the adhesive force and some e-cigarette flavours increased biofilm formation of *S. mutans*, the two latter factors associated with increased caries risk. Increased adhesion to epithelial cells and increased cytotoxicity of *C. albicans* exposed to e-cigarette aerosol with nicotine potentially linked to increased risk for oral candida infection has been reported.

Cancer: From the systematic reviews included in this report, we found few relevant animal studies on cancer. Some rodent studies indicated effects on redox balance, effects on immune system including increased pro-inflammatory responses which may have implication for cancer development. Furthermore, we found some *in vitro* studies using human cells and reporting effects of e-cigarette aerosols extracts on DNA damage and various cellular responses linked to DNA damage.

Discussion

Main findings from the systematic reviews

Constituents and exposure to e-cigarette aerosols

Several harmful chemicals such as carbonyl compounds, volatile organic compounds (VOC), reactive oxygen species (ROS) and other free radicals as well as various trace elements/metals have been identified in e-cigarette aerosols.

Non-malignant respiratory diseases

Findings from human studies

From the systematic reviews, no prospective studies elucidating how exposure to e-cigarette aerosol may contribute to development of asthma and chronic obstructive pulmonary disease (COPD) were found. However, two systematic reviews with meta-analysis of partly the same included cross-sectional studies, reported increased prevalence of asthma among users of e-cigarettes compared with non-users. One systematic review reported that adolescent use of e-cigarettes was associated with increased coughing compared to non-users. One study showed decreased mucociliary clearance associated with use of e-cigarettes.

Use of e-cigarettes was reported to cause e-cigarette or vaping use-associated lung injury (EVALI) a potentially life-threatening disease. However, the majority of cases was linked to use of e-liquid, from informal sources, containing tetrahydrocannabinol (THC; the main psychoactive compound in cannabis) or a combination of cannabinoids/THC and nicotine.

Findings from animal and in vitro studies

Depending on e-cigarette device/liquid type, dose, duration as well as animal model, e-cigarette use has been reported to trigger immune responses linked to respiratory disease including increased level of cytokines in the bronchoalveolar lavage and immune cell infiltration, increased allergy-induced asthmatic symptoms as well as decreased re-sistance to both bacterial and viral infections. We found no systematic review covering or elucidating mechanisms behind EVALI.

Cardiovascular disease

Findings from human studies

From the systematic reviews we found no prospective human studies showing that the use of e-cigarettes leads to development of new cardiovascular disease (CVD). However, the use of e-cigarettes was shown to have effects on various parameters that are closely linked to cardiovascular disease. In this context, use of e-cigarettes was reported to have acute effects on heart rate and blood pressure although with very low confidence in the

effect estimates. Also, use of e-cigarettes led to acute increases in pulse wave velocity, a measure of arterial stiffness with low confidence in the effect estimates.

Moreover, it was found that e-cigarettes increased biomarkers in the blood associated with imbalance in the immune system, oxidative responses, aggregation of platelets and damage to the blood vessel wall. It is currently assumed that such effects in the longer term may predispose to development, as well as contribute to exacerbation of cardiovascular disease.

Noteworthy, although based on cross-sectional studies, one systematic review reported no difference in the risk for stroke, coronary heart disease and myocardial infarction between current smokers and former smokers switching to e-cigarettes.

Findings from animal and in vitro studies
From the animal studies presented, exposure to e-cigarette aerosol with nicotine was associated with a pro-thrombotic state. Chronic exposure to e-cigarette aerosol was associated with increase in arterial stiffness. Increased area of brain infarction after exposure to e-cigarette aerosol with nicotine was reported in one study. These findings support the view that use of e-cigarettes with nicotine may worsen the adverse outcome of cardiovascular events. In addition, exposure to e-cigarettes with nicotine affected the cardiac function and increased the atherosclerotic lesion in a mouse model prone to developing atherosclerosis. Furthermore, there were measures suggesting increased oxidative stress and reduced antioxidative defense after exposure to e-cigarette aerosol with nicotine.

Exposure of endothelial cells to e-liquids and aerosol may increase ROS formation and decrease viability. In addition, e-cigarette aerosol exposure increased endothelial deposition of complement factors and platelet aggregation

Adverse pregnancy outcomes and effects on early life health
Findings from human studies
From one systematic review with two studies, we are uncertain (very low confidence, GRADE) if the use of e-cigarettes has an effect on birthweight or increases the risk of having small for gestational age (SGA) infants.

Findings from animal and in vitro studies
Exposure of pregnant mice with e-cigarette aerosol with and without nicotine during the gestational period and lactation induced sex-dependent changes in gene expression associated with enhanced risk of adverse effects on central nervous system (CNS) similar to those associated with tobacco cigarettes. Furthermore, neonatal exposure to nicotine containing e-cigarette aerosol reduced lung development and decreased lung cell proliferation. E-cigarette aerosol extract also caused dose-dependent effects on cardiac development in a zebrafish model. In a frog model, exposure to e-cigarette aerosol extract affected craniofacial development.

Mental disorders

Findings from human studies

Several studies have reported an association between internalizing (such as anxiety and depression) and externalizing (such as ADHD) symptoms and disorders and e-cigarette use, there is still however little evidence to make claims about causation. One systematic review reported associations between use of e-cigarettes and depressive symptoms in adolescents and young adults. We had very low confidence in the estimates.

Findings from animal and in vitro studies

No systematic reviews on this issue were identified. As reported in *Adverse pregnancy outcomes and effects on early life health*; pregnant mice exposed to e-cigarette aerosol with and without nicotine during the gestational period and lactation induced sex-dependent changes of gene expression in the brain. These changes were associated with enhanced risk of adverse effects similar to those associated with tobacco cigarettes.

Non-malignant oral diseases

Findings from human studies

Use of e-cigarettes was associated with some oral symptoms and mucosal lesions. From clinical studies with a cross-sectional design regarding periodontal disease, the only clinical periodontal measure reported to be different for e-cigarette users compared to never users of tobacco was reduced bleeding on probing (BOP). A longitudinal study based on self-reported oral health found increased gum disease and bone loss around teeth among e-cigarette users compared to never users.

Cross-sectional population-based studies with self-reported health outcomes, pointed mostly at an increased risk for gingival/periodontal disease. Worse parameters of peri-implant disease among users of e-cigarettes compared to never tobacco users was reported. For dental caries, no longitudinal study with a design that could give a causal relation was presented.

Findings from animal and in vitro studies

No systematic reviews that summarized animal experiment on oral health were identified. One systematic review included *in vitro* studies.

Dental effects: Exposure of bovine enamel to e-cigarette aerosol with different concentrations of nicotine and different flavors resulted in changes in enamel color. E-cigarette aerosol was reported to increase the adhesive force and some e-cigarette flavors increased biofilm formation of *S. mutans*. Increased adhesion and biofilm formation are factors associated with increased caries risk

Effects on C. albicans: Increased adhesion to epithelial cells and increased cytotoxicity of *C. albicans* exposed to e-cigarette aerosol with nicotine compared to control and nicotine free aerosol, was reported. Increased adhesion to epithelial cells may potentially be linked to increased risk for oral candida infection.

Poisoning and injuries

Findings from human studies

E-cigarettes may overheat or explode and cause subsequent injuries ranging from small lacerations and burns to more serious tissue damage or death. Furthermore, accidental, or intentional intake of available e-liquids can cause poisoning with the severity of outcomes ranging from mild symptoms such as vomiting, rapid heart rate, unsteadiness, and increased salivation to fatal outcomes (unintentional or suicide). While the causal association is obvious, the frequency of these incidents is unknown.

Findings from animal and in vitro studies

No systematic reviews were identified

Cancer

Findings from human studies

The systematic reviews identified few studies with implications for cancer development. However, reported results revealed that several of the compounds found in e-cigarette aerosols have been associated with cancer. Highly variable levels of some of these compounds were found in the urine.

Importantly, single studies included in the systematic reviews reported increased local and systemic pro-inflammatory responses associated with exposure to e-cigarette aerosol. If ongoing for longer periods, these types of effects may have implications for cancer development. Importantly some studies reported endogenous formation of the carcinogen N'-nitrosonornicotine (NNN) in saliva from e-cigarette smokers, probably as a result of exposure to nicotine.

Findings from animal and in vitro studies

From the systematic reviews included in this report, we did not find relevant animal studies on cancer. Some rodent studies indicated effects on redox balance, and effects on immune system including increased pro-inflammatory responses which may have implication for cancer development. Furthermore, some *in vitro* studies using human cells reported effects of e-cigarette aerosols extracts on DNA damage and various cellular responses linked to DNA damage.

Regarding oral cells, ten of the *in vitro* studies included in one systematic review reported cytotoxic effects of e-cigarettes liquids and/or vapor. Six of the *in vitro* studies included by Yang and coworkers were reported to show genotoxic effects of e-cigarette aerosol. Taken together, results from *in vitro* studies suggest that e-cigarette aerosol exposure may enhance oxidative DNA damage. Likely mechanisms involved includes increased ROS generation, reduced antioxidant capacity as well as decreased DNA repair mechanisms.

Strengths, limitations, and applicability of the current umbrella review

An advantage of umbrella reviews is that one can quickly get an overview of existing systematic reviews in the field, and thereby reduce the amount of literature needed to be covered. A strength of this umbrella review covering systematic reviews over a very broad field, is the systematic and transparent way in which literature has been collected and evaluated. It is based on systematic literature searches developed and conducted by a librarian, and the methodological approach is conducted according to a pre-published protocol.

An inherent limitation of umbrella reviews is that they may be quickly out of date as new research is published. The challenge may be more pronounced in new scientific fields if there is a high rate of new publications. An umbrella review is also limited by the choice of theme that researchers have previously chosen to summarize in a systematic review.

Effects of e-cigarette use on the development of diseases in humans are expected to be a result of exposure over several years and ultimately decades. Thus, at this stage, human studies are not expected to give a clear answer to possible contributing effects on development of i) asthma and chronic obstructive pulmonary disease (COPD), ii) cardiovascular disease such as myocardial infarction, coronary heart disease and stroke, iii) mental disorders, iv) adverse pregnancy outcomes with predisposition for adverse health effects later in life. Having said that, it is important to point out that studies reporting effects on parameters considered to predispose to disease development, and/or exacerbation of disease may be found.

Traditionally, information from animal-, *in vitro*- and mechanistic studies are not systematically reviewed, and will often be lost by this approach. However, herein, some of the systematic reviews included human, animal, and *in vitro* data. In general, information from animal and *in vitro* studies is required before testing new drugs on humans or introducing new food additives and chemical products into the market. More specifically, animal, and *in vitro* studies may add important information regarding relative toxicity associated with specific e-cigarette aerosol components, device characteristics as well as mechanisms involved in disease development. Furthermore, information from human, animal and *in vitro* studies of adverse health effects linked to use of closely related tobacco products including snuff and cigarettes are highly relevant when evaluating any potential health risk from e-cigarettes. Published data on health effects from snus and smoking are indeed plentiful, but a systematic literature review of original research in this field was not possible within the time frame available for this work.

In toxicological evaluations of possible adverse health effects, it is important to integrate all relevant information from exposure and link to the disease in question. Such information includes effects on biomarkers/mechanisms from human studies as well as relevant information from animal and *in vitro* studies.

To compensate for some of the limitations indicated above, we have for the discussion and overall evaluation used information from reports from highly recognized and authoritative international expert groups and updated results from other relevant published literature.

The evaluation of association between cancer and use of e-cigarettes is a relevant example of our approach. Effects on e-cigarettes on cancer will be expected to be seen in humans after lengthy exposure. Accordingly, we found no broad systematic review of high scientific quality linked specifically to possible effects of e-cigarette aerosols on cancer, including human and animal studies on cancer linked biomarkers of effects and *in vitro* studies. As the possible human cancer risk from the use of e-cigarettes is important to evaluate, we instead used the consensus study report: "Public health consequences of E-cigarettes" (prepared by a committee of National Academies of Sciences, Engineering, and Medicine (NASEM, 2018) as the basis of the cancer discussion section of our report. In the discussion we also added results on cancer developments from highly relevant animal studies (Canistro et al., 2017; H. W. Lee et al., 2018; Pham et al., 2020; Tang et al., 2019) published after the publication of the NASEM report.

Overall evaluation of the experimental approach
An advantage of an umbrella review (a systematic review of systematic reviews) is that it collects and evaluate the literature in a transparent way. One of the limitations of our umbrella review is that it may not include all relevant aspects of health risks from e-cigarettes sufficiently as some adverse health outcomes may not have been systematically reviewed.

In this report, the umbrella review is presented according to the pre-published protocol. To add to the systematic review of systematic reviews of adverse health effects on humans from the use of electronic cigarettes, we have additionally added a discussion including a toxicologically evaluation This is presented in the section below, **Discussion and overall evaluation.**

Discussion and toxicological evaluation

We identified few longitudinal cohort studies that address adverse health outcomes in humans associated with the of use of e-cigarettes. Such studies are important to gain knowledge on any possible causal relationship between use and health outcomes.

The risks of adverse health outcomes and changes in biomarkers of effects evaluated by the GRADE approach in the present review had low to very low confidence. Thus, information on health effects and mechanisms/mode of action obtained from other nicotine containing products may add useful information in the final evaluation of possible health hazard linked to use of e-cigarettes.

E-cigarette aerosol may contain nicotine, solvents and flavors added to the e-cigarette liquid and/or their decomposition products as well as synthesis of new compounds during heating (El-Hage et al., 2020; El-Hellani et al., 2020; Jensen et al., 2017). Compared to cigarettes, low levels of various carcinogenic tobacco specific nitrosamines (TSNAs) and polycyclic aromatic hydrocarbons (PAHs) are present in the aerosol. Swedish snus is another nicotine product where the levels of these compounds are relatively low. The orally absorbed ingredients from snus as well as the pulmonary absorbed ingredients from e-liquid aerosol transfer directly to the systemic circulation. Thus, we consider health outcomes associated with oral use of snus to be particularly relevant in the discussion of

possible health outcomes from e-cigarettes. However, the inhalation of e-cigarettes aerosol suggests that direct effects on the respiratory organs may be expected from e-cigarette use.

We have ended the discussion with some simple considerations regarding the relevance of e-cigarette exposure/dose and expected effects for disease developments.

Constituents and exposure of e-cigarette aerosols

Nicotine is a central component of e-cigarette aerosol with some well-known biological effects. After oral uptake or uptake from the gastrointestinal tract, most of the flavors found in e-cigarette liquids are generally recognized as safe (GRAS) by the FDA and approved by EFSA as showing low toxicity (SCHEER, 2021). An important critical issue to this approach is that the toxicity of the constituents in e-liquids is not evaluated after inhalation and/or after heating and aerosolization.

Several harmful chemicals such as carbonyl compounds, volatile organic compounds (VOC), reactive oxygen species (ROS) and other free radicals as well as various trace elements/metals have been identified in e-cigarette aerosols (Ward et al., 2020; Zhao et al., 2020). Furthermore, the e-cigarette aerosol formed after heating the liquid consists of droplets of different sizes. The relative particle size distribution and characteristics differs from that of tobacco smoke (NASEM, 2018). Harmful chemicals are identified in e-cigarette aerosols, however, lower levels of some of the toxicants compared to cigarette smoke has been reported among tested brands of e-cigarettes (Goniewicz et al., 2014). The large variation in e-cigarette -devises, e-cigarette liquids used as well as vaping pattern make the human exposure highly variable and complex. Several of the compounds found in e-cigarette aerosols have been associated with various adverse health outcomes.

Overall evaluation of constituents and exposure of e-cigarette aerosols

Several harmful chemicals as well as various metals/trace elements have been identified in e-cigarette aerosols. The large variation in e-cigarette devices and liquids used as well as in vaping patterns make human exposure highly variable and complex. Thus, it is difficult to precisely know or predict the exposure levels of potentially harmful substances.

Non-malignant respiratory diseases

The respiratory system from the oral cavity to the alveoli of the lungs, is the primary organ of exposure to constituents in the e-cigarette aerosol. The US Surgeons General (2014) concluded that smoking tobacco was the dominant cause of chronic obstructive pulmonary disease (COPD) in men and women in the United States, and that smoking causes all elements of the COPD phenotype, including emphysema and damage to the airways of the lung. The biological effects related to emphysema, are mediated by chronic exposure to cigarette smoke which leads to inflammation and immune cell recruitment, with subsequent release of proteinases leading to damaged lung tissues.

For tobacco smoking and asthma, the evidence has been evaluated to be suggestive for a causal relationship for the development of disease, but sufficient for a causal relationship for exacerbations (US Surgeon General, 2014). Irritative damage leading to chronic airway inflammation, impaired mucociliary clearance, and increased bronchial hyperre-

sponsiveness are mechanisms suggested to be involved in smoking mediated asthma development. In addition, smoking and nicotine exposure both pre- and postnatally may affect fetal and child lung development and confer increased risk for later respiratory disease (Gibbs et al., 2016; US Surgeon General, 2014). Although, the smoke produced by tobacco cigarettes is different from the vapor from e-cigarettes, also the latter contain constituent such as nicotine, particulate matter, aldehydes, ROS and other free radicals, which may represent common risk factors for adverse lung health (NASEM, 2018).

Earlier reports have concluded that there are moderate risks of local irritative damage to the respiratory tract in users of electronic cigarettes due to the cumulative exposure to polyols, aldehydes and nicotine (SCHEER, 2021). This in line with the NASEM report which concluded there was moderate evidence for increased cough and wheeze in adolescents who use e-cigarettes and for an association between e-cigarette use and an increase in asthma exacerbations. Whereas, for respiratory diseases, the NASEM report concluded that there was no available evidence whether or not e-cigarettes cause respiratory diseases in human. A challenge for risk evaluation based on human population studies for new products, is that a cumulative result of life-long general environmental exposures leads to deterioration of tissue and may increase the risk of disease which subsequently may confer increased susceptibility to an exposure(Risher et al., 2010). As e-cigarettes are most popular among younger populations, they may not have attained the age where disease occurs. A recent review reported that most of the clinical trials on e-cigarettes apparently had insufficient time of follow-up to detect significant differences in lung function (Tarran et al., 2021).

From the included systematic reviews in the present umbrella review, association between use of e-cigarettes and asthma was reported in two meta-analyses based on cross-sectional studies, with a partly overlap of included studies. No systematic review regarding COPD was identified.

Since there is currently a lack of high-quality prospective studies with sufficient follow up for respiratory diseases, current risk assessment has to use knowledge from acute effects and intermediate endpoints from human studies in addition to knowledge from animal and *in vitro* studies. The NASEM report presented a conceptual framework for how constituents in the inhaled aerosol could affect respiratory disease development for asthma and COPD by affecting biomarkers such as: i) bronchoconstriction, ii) impairing cough reflexes, iii) reducing mucociliary transport, iv) inflammation and v) decreased resistance to bacterial, viral infection.

From our previous systematic search several human studies investigated acute effects of e-cigarettes on the lung. Use of e-cigarettes with nicotine was observed to lead to conducting airway obstruction (Antoniewicz et al., 2019). Negative effects on different test of pulmonary function have also been observed in several other studies (Coppeta et al., 2018; Kerr et al., 2019; Kizhakke Puliyakote et al., 2021; Lappas et al., 2018; Palamidas et al., 2017; Vardavas et al., 2012) In addition, use of e-cigarettes with and without nicotine at high wattage, in large amounts induced sustained decreased transcutaneous gas tension and lead to airway epithelial injury, measured by increased serum CC16 suggested to leak from the lung in young occasional tobacco smokers. The authors suggested that the lung injury was primarily mediated by propylene glycol/glycerol (Chaumont, Bernard, et al., 2018; Chaumont et al., 2019). The vaping regime, also elicit a decrease in arterial oxygen tension in heavy smokers (Chaumont et al., 2019). Interestingly, Chaumont and co-workers (2019), reported that short term cessation in regular users of e-

cigarettes lead to measures compatible with slightly improved airway status (Chaumont et al., 2020).

Short term use of e-cigarettes with nicotine has been shown to inhibit the cough reflex sensitivity (Dicpinigaitis et al., 2016a, 2016b). Inhalation of nicotine has been suggested to lead to acute increase cough through effects on peripheral located receptors in the lung, and a delayed inhibition of cough reflex mediated through effects on the central nervous system (Dicpinigaitis, 2017). In addition, one study included in the umbrella review, reported decreased mucociliary clearance after use of e-cigarettes (Kumral et al., 2016).

Significant increases in aldehyde-detoxification and oxidative stress–related proteins were reported in a study among e-cigarette users. In addition, the authors reported an increase in proteins associated with COPD. In relation to COPD, a study by Ghosh and coworkers (2019) concluded that e-cigarette use induced nicotine-dependent protease release from lung immune cells whereas the antiprotease levels remained unchanged. This implies that chronic vaping is able to increase lung proteolysis by disrupting the protease–antiprotease balance (Ghosh et al., 2019). Accordingly, this may increase the risk of developing chronic lung disease among users of e-cigarettes. Furthermore, some animal studies of emphysema development have reported significant emphysema development after exposure to e-cigarette aerosol, while others did not (Tarran et al., 2021) , but the exposures may have differed in quality and quantity.

Increased infection after smoking tobacco cigarettes, mediated via both effects on the immune system and structural changes in the lung has been reported for tobacco smoking in humans (Arcavi & Benowitz, 2004). A study in never smokers, showed altered gene expression in small airway epithelial cells and alveolar macrophages, and increased total endothelial microparticles in serum after using e-cigarettes with nicotine (Staudt et al., 2018). Furthermore, in a study among non-smokers, smokers and e-cigarette users, the authors reported that use of e-cigarettes led to unique changes in alveolar macrophages and bronchial epithelia compared to non-smokers and smokers which may impact pulmonary host defense (Davis et al., 2022).

The effects of e-cigarettes on lung inflammation have been investigated in several animal studies included in the present umbrella review. Depending on e-cigarette device/liquid type, dose, duration as well as animal model, e-cigarette use was reported to affect various immune responses linked to respiratory disease including: i) increased level of cytokines in the bronchoalveolar lavage and immune cell infiltration, ii) increased allergy-induced asthmatic symptoms and iii) decreased resistance to both bacterial and viral infections. Furthermore, exposure to e-cigarettes has been shown to cause a transient effect on the often-used human biomarkers bronchoconstriction and decreased mucociliary transport (Chung et al., 2019; Khosravi et al., 2018; L. Y. Lee et al., 2018).

The *in vitro* studies in the current umbrella review show that e-cigarette aerosol increased the permeability of the lung endothelial integrity. A more recent study also showed that e-cigarette aerosol affected the lung epithelial barrier (Ghosh et al., 2020). In addition, the *in vitro* studies reported illustrated that the added flavor as well as the operation of the device may differently affect cytotoxicity and inflammatory responses. A general finding was, however, that the effects induced by e-cigarette aerosol was lower than that induced by smoke from tobacco cigarettes.

The NASEM report concluded that there was moderate evidence for an association with e-cigarette use and an increase in asthma exacerbations. Supported by acute effects of e-cigarettes on pulmonary function and inflammation reported to be more prominent in smokers with asthma (Kotoulas et al., 2020; Lappas et al., 2018).

From one systematic review included, the use of e-cigarettes was reported to cause e-cigarette or vaping use-associated lung injury (EVALI), which is a serious disease that may have fatal outcome. In the US, the number of cases reported showed a sharp increase in August 2019, a peak in September 2019, and then gradually decreased (CDC, 2021).

As reported by CDC, most of the EVALI patients were young and previously healthy. Patients had impaired general health where the most common symptom was fever and almost all had respiratory symptoms (shortness of breath, chest pain and cough). Most also had gastrointestinal symptoms (nausea, vomiting, diarrhea and abdominal pain), low blood oxygen levels, elevated blood levels of neutrophil cells (inflammatory cells). (Layden et al., 2020). Bronchioalveolar lavage revealed macrophages with lipid accumulations. Some patients were diagnosed with acute lung failure. Upon scanning and computed tomography (CT) some were found to have abnormal lung tissue typically characterized by opaque areas in the lungs (Layden et al., 2020). The EVALI cases represented a small percentage of those who used e-cigarettes, but the severity of the disease was high. Most cases had a history involving use of e-liquid from informal sources containing tetrahydrocannabinol (THC; the main psychoactive compound in cannabis) or a combination of cannabinoids/THC and nicotine. However, a few cases reported using e–liquid with nicotine but without THC.

Assessment of health hazards associated with individual components in e-cigarette liquid has linked vitamin E acetate to the EVALI outbreak. Vitamin E acetate was found in the lung fluid of 48 out of 51 patients with EVALI and in none of controls (Blount et al., 2020). These results are in accordance with the analyzes from the Food and Drug Administration where vitamin E acetate were detected in products used by EVALI patients (FDA, 2020). Recently it was suggested that inhalation of vitamin E acetate could increase the fluidity of lung surfactant, causing the surfactant layer to collapse, contributing to symptoms such as shortness of breath and lung inflammation, part of the characteristics for EVALI (DiPasquale et al., 2020). However, the complete explanation to all symptoms/characteristics of EVALI as well as its prevalence and the role of individual health status is still not clarified. Furthermore, although vitamin E acetate seemed to be associated with EVALI, it is still possible that more than one compound or ingredient in e-liquid are involved in the development of disease.

As illustrated above, it is important to recognize that some new devices can be used for inhaling other substances than originally intended. Most importantly, the EVALI outbreak shows how new products may confer unpredicted and serious hazards.

Overall evaluation of non-malignant respiratory diseases

Systematic reviews indicate that use of e-cigarettes is associated with local irritation of the respiratory tract, increased coughing as well as asthma. Human, animal, and *in vitro* studies, indicate that e-cigarettes with nicotine may affect biomarkers such as: i) bronchoconstriction, ii) impairing cough reflexes, iii) reducing mucociliary transport, iv) inflammation and v) decreased resistance to bacterial, viral infection. A sustained impact

of such parameters on the respiratory system is linked not only to asthma but also chronic obstructive pulmonary disease (COPD). Thus, use of e-cigarettes may represent a risk for development of respiratory disease and increase exacerbation of respiratory diseases.

The recent outbreak of serious lung injuries (EVALI), mainly in USA, was mostly associ-ated with use of tetrahydrocannabinol (THC)-containing e-cigarette liquid from informal sources. Cases of EVALI were reported mainly during a period of two years. The presence of vitamin E acetate in the e-liquid has been strongly linked to the EVALI outbreak. Evi-dence is not sufficient to rule out the contribution of other chemicals of concern. The EVALI outbreak shows how use of new products may confer unpredicted health hazards, and that the device may result in adverse health outcomes as it may be used for inhaling other substances than those originally intended.

Cardiovascular diseases

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels, including coronary heart disease, cerebrovascular disease, peripheral arterial disease, various heart diseases, deep vein thrombosis and pulmonary embolism. Tobacco use is one of the most important risk factors for myocardial infarction, coronary heart disease and stroke (cerebrovascular events). Central acute mechanisms involved, are activation of the sympathetic nerve system, oxidative stress/inflammation, endothelial dam-age/dysfunction as well as platelet activation with formation of blood clots or throm-bosis. Atherosclerosis is well-known as a chronic, low-grade inflammatory process in the arterial wall that predisposes to acute CVDs like myocardial infarction and stroke. In principle, tobacco smoke may induce atherosclerosis either by i) activation of a lung au-tonomic reflex, ii) triggering inflammation in the lungs resulting in systemic "spill over" of pro-inflammatory mediators, and/or iii) the translocation of particulate matter or ad-hered constituents into circulation, thereby reacting with endothelial cells as well as components in the blood. Long term exposure to toxic agents in cigarette smoke, includ-ing carbon monoxide, nicotine, reactive oxygen species (ROS), carbonyl compounds, vol-atile organic compounds (VOC), polycyclic aromatic hydrocarbons (PAHs) as well as par-ticulate matter may via these mechanisms enhance the risk of developing CVD, but may also directly trigger myocardial ischemia and contribute to smoking's immediate cardi-ovascular risk.

Compared with smoke from tobacco cigarettes, e-cigarette aerosol contains less amounts of many compounds central for effects of tobacco smoke on CVD (Goniewicz et al., 2014; Middlekauff, 2020). Thus, the risk of CVD from the use of e-cigarettes may be lower than that from tobacco cigarettes. However, e-cigarette aerosols contain nicotine, which is considered to be an important contributor to CVD, as well as other compounds with a known/partly unknown potential to affect CVD.

In the following, we summarize possible direct effects of e-cigarette use on CVD, as well as on some clinical parameters and biomarkers linked to CVD reported in the systematic review of human studies; and combine and discuss these findings with other relevant information including results from animal and *in vitro* studies.

Cardiovascular diseases in smokers switching to e-cigarettes
The review by Goniewicz and co-workers (2020) reported no reduction in risk for coronary heart disease, myocardial infarction or stroke when switching from regular cigarettes to e-cigarette compared to cigarette smokers. The data were based on cross-sectional studies; thus, it is not possible to draw conclusions on causal effects from these results. In addition, since the participants were former smokers, they may have accumulated substantial damage due to cigarette smoking. In relation to the latter, Benowitz and Burbank reported an enhanced risk for cardiovascular toxicity of nicotine (myocardial infarction or stroke) only for users with CVD. The enhanced risk for users without CVD was reported to be low. They concluded that long-term nicotine use, such as in smokeless tobacco users, may contribute to acute cardiovascular events in the presence of CVD, but appears to be without effect on the atherogenic process (Benowitz & Burbank, 2016).

The hypothesis that nicotine may enhance the risk for a more sever outcome following a cardiovascular event, is supported by findings from studies on snus, an oral smokeless tobacco product that contains low amounts of PAHs which in itself may confer increased risk of CVDs. The findings on human CVD risk for snus, showed that use of snus increased the risk of high blood pressure and lethality after myocardial infarction and stroke. There was no clear evidence whether use of Swedish snus affected the risk of myocardial infarction, stroke, atrial fibrillation, and chronic heart failure (NIPH, 2019). A more recent study, based in eight prospective cohorts, reported that use of snus was associated with increased cardiovascular mortality (Byhamre et al., 2021). There are also animal studies giving support to the notion that nicotine may exacerbate cardiovascular events. Increased brain infarction area was observed after an ischemic injury in mice exposed to e-cigarette aerosol with nicotine. Accordingly, exposure to e-cigarette aerosol was found to increase intercellular permeability, additionally implying the possibility of increased the blood brain barrier permeability (Kaisar et al., 2017).

Effects of e-cigarette use on biomarkers of effects and mechanism associated with CVD
Sympathetic nerve activation: Autonomic dysregulation of the vascular system has been suggested to affect both development and progression of CVD (Hadaya & Ardell, 2020). Accordingly, pronounced alteration in sympathetic control of the circulation has been associated with several type of CVDs.

The results from our umbrella review indicate that the use of e-cigarettes have acute effects on the cardiovascular system by increasing heart rate, blood pressure as well as pulse wave velocity (arterial stiffness). The acute hemodynamic effects are argued to be largely mediated by nicotine (Moheimani, Bhetraratana, Peters, et al., 2017). Furthermore, the use of e-cigarettes with nicotine has been associated with acute changes in heart rate variability, sharing similarities to changes associated with increased cardiac risk (Moheimani, Bhetraratana, Peters, et al., 2017). In addition, an increased low-frequency/ high-frequency (LF/HF) ratio among chronic e-cigarette users, indicating a shift in the cardiac autonomic balance toward sympathetic predominance compared to non-tobacco users has been observed (Moheimani, Bhetraratana, Yin, et al., 2017).

Some studies of chronic hemodynamic effects from use of e-cigarettes were found. Specifically, one study with a meta-analysis reported that smokers switching to e-cigarettes had a decrease in blood pressure without changes in heart rate. These results reporting

on chronic effects of e-cigarette use should be interpreted with caution since they were or may be associated with e-cigarette/tobacco industry.

Overall, the acute increase in heart rate, blood pressure and changes in heart rate variability indicate that use of e-cigarettes with nicotine increase sympathetic nerve activity. In addition, changes in HRV among habitual e-cigarette users compared to non-tobacco users was reported in one study. The findings from studies with humans are supported by the increased blood pressure reported from chronic exposed mice and the known toxicodynamic effects (mechanism/mode of action) of nicotine.

Oxidative stress and inflammation: Oxidative stress occurs when there is an imbalance in the generation and detoxification of ROS. ROS is involved in both vascular and cardiac signaling and function. Imbalance in ROS levels and oxidative stress may lead to dysfunction and development of CVD through several mechanisms such as macromolecular and cellular damage (Munzel et al., 2017). Several of the human studies included in this umbrella review have reported on different biomarkers indicating increased oxidative stress after use of e-cigarettes. That e-cigarette aerosol exposure may increase cellular oxidative stress, was also reported from *in vitro* studies in the present umbrella review. Furthermore, findings suggested increased oxidative stress and reduced antioxidative defense after chronic exposure of mice to e-cigarette aerosol with nicotine (Kaisar et al., 2017).

Inflammation is closely connected with oxidative stress and considered central in the pathogenesis of CVD including atherosclerosis. One of several suggested mechanisms linking cigarette smoke to atherosclerosis, involves damage to lung epithelial cells and/or endothelium in blood vessels. This type of damage may cause a low grade chronic inflammatory process, that may amplify the effect. Indices of acutely and transiently increased oxidative stress and inflammation have also been observed after inhalation of e-cigarette aerosol without nicotine (Chatterjee et al., 2019) implying that other constituents in the e-cigarette aerosol than nicotine may also pose a hazard. Furthermore, a cross-sectional study with separate groups of chronic e-cigarette users, tobacco cigarette users and non-users, showed a dose-response increase of proinflammatory monocytes, of lymphocytes in blood, and total cellular and cytoplasmic ROS content, lowest for non-users and highest for users of tobacco cigarettes (Kelesidis et al., 2020). Overall, use of e-cigarettes seems to increase markers of oxidative stress and inflammation.

Endothelial dysfunction: Endothelial cells are critical for hemostasis and vascular health. Damage to the endothelium which may occur in relation to smoking or diabetes, may result in increased endothelial permeability, inflammation, de-differentiation and alternations in vascular tone (Alexander et al., 2021). Endothelial dysfunction is indicated by registration of flow mediated dilatation, pulse wave velocity and augmentation index (Shahandeh et al., 2021).

One study included in the review of Kennedy and co-workers (2019) reported acute reduced flow mediated dilatation (FMD) after use of e-cigarettes for both smokers and non-smokers. The same was reported for healthy smokers using e-cigarettes (Kuntic et al., 2020). This was supported by studies showing an acute increase in pulse wave velocity after using e-cigarettes (Saz-Lara et al., 2021). In addition, several studies included in

the systematic review of Kennedy and co-workers (2019), reported an increased augmentation index when using e-cigarettes with nicotine. However, a study among healthy young e-cigarette users and smokers at equivalent nicotine doses reported no difference in baseline FMD, whereas only smoking decreased FMD after an acute exposure (Haptonstall et al., 2020).

Reduced flow mediated dilatation among users of Swedish snus, indicative of endothelial dysfunction has also been reported, implying a that nicotine may mediate such effects (Skaug et al., 2016). Increase in pulse wave velocity after chronic exposure to e-cigarettes aerosol with nicotine of mice has also been observed (Olfert et al., 2018).

Endothelial progenitor cells and endothelial derived micro vesicles are involved in vascular repair (Zhang et al., 2014). Acute increased in levels of endothelial progenitor cells and endothelial derived micro vesicles, indicative of vascular damage was observed after using e-cigarettes (Antoniewicz et al., 2016). The increase in micro vesicles was suggested to be mediated by nicotine (Mobarrez et al., 2020).

Overall, the findings indicate that using e-cigarettes may cause damage to endothelial cells and impair endothelial function.

Platelet activation: Platelet mediated vascular inflammation and platelet aggregation and subsequent thrombus formation, have been described to play a role in CVD progression, atherosclerosis, myocardial infarction and stroke (Lebas et al., 2019). From the human studies included in the work of Kennedy and co-workers (2019), use of e-cigarettes may affect platelet functions, such as platelet activity and aggregation. Furthermore, in a mouse model exposure to e-cigarette aerosol with nicotine promoted a pro-thrombotic state, supported by the shortened bleeding and occlusion time reported. In addition, hyperreactive platelets and reduced thrombomodulin expression was observed (Qasim et al., 2018). Overall, although scarce literature is available, e-cigarette use may affect platelet activity.

A potential bias in many of the human studies, is that the participants are often current or former smokers. A recent review reported that for several parameters associated with CVD risk, tobacco cigarette smoking showed increased responses compared to e-cigarettes. However, most studies did not report on nicotine levels, leaving uncertainty as to whether exposures were comparable (Shahandeh et al., 2021). Exposure levels may vary considerable for e-cigarette users.

In addition to the studies above, a study performed with male mice reported that chronic e-cigarette exposure led to cardiovascular dysfunction, including elevated blood pressure, increased adrenergic vasoconstriction, impaired vascular endothelial relaxation, cardiac hypertrophy, and vessel wall thickening. The authors summarized that long-term exposure to e-cigarette aerosol induced cardiovascular disease with similarities to that of tobacco smoke. The severity of the toxicity was reported to increase with both exposure duration and the nicotine content of the e-cigarette liquid used. Of note, is that also non-nicotine containing e-cigarette liquid resulted in elevated blood pressure after sustained (16 weeks) exposure along with increased oxidative stress in aorta and heart tissues (El-Mahdy et al., 2021). In addition, in a mouse model that develops atherosclerosis, the authors reported that exposure to e-cigarette aerosol containing nicotine affected

the cardiac function as well as the morphology of cardiac cells (Espinoza-Derout et al., 2019).

Overall evaluation of CVD

The umbrella review shows that human use of e-cigarette and animal exposure to e-cigarette aerosol have reported effects linked to central nerve system (CNS, brain), more specifically activation of the sympathetic nerve axis, as well as effects on oxidative stress and inflammation, endothelial dysfunction, and platelet activation, all representing central pathways associated with increased cardiovascular disease risk. For never users of tobacco, use of e-cigarettes may represent an increased risk for development of CVD, and it may contribute to an enhanced risk for more severe adverse outcomes following acute cardiovascular events. Our overall evaluation that use of e-cigarettes may a represent an enhanced risk for CVD is supported by findings related to the use of snus, recent literature, and the current mechanistic understanding of the effects of cigarette constituents on CVD.

Mental disorders

The main findings support an association between e-cigarette use and a range of mental symptoms and disorders in adolescence and early adulthood, but little evidence to claim any causal inference. Still, the results are of concern, due to knowledge about brain development. The childhood and adolescence period, as well as young adulthood, is regarded as a sensitive developmental period. The growth of the brain is largely finished before adolescence, but during adolescence, extensive remodeling of the brain occurs. Brain maturation occurs up to the late twenties (Arain et al., 2013). This is necessary for core human features, including cognitive function, reward processing, emotional regulation, and motivated behavior (Yurgelun-Todd, 2007).

Nicotine is a common constituent in tobacco products including e-cigarettes. Nicotine exerts many biological effects by binding to nicotinic acetyl cholinergic receptors (nAChRs), including those regulating critical parts of brain maturation. Thus, disturbance of the cholinergic systems during this period with nicotine containing products may have consequences on brain development during adolescence (Munafo et al., 2016; Yuan et al., 2015). Both studies in humans and animal experiments indicate that nicotine exposure during adolescence can cause addiction and harm the developing brain including an increased risk of development of long-term cognitive impairments (Holliday & Gould, 2016)US Surgeon General, 2016).

Many studies have shown an association between mental health and increased user prevalence of nicotine containing products (Becker et al., 2020; Kutlu et al., 2015). The causal factors underlying the association are unknown. It is possible that common vulnerability (genetic and environmental) is involved (Tsuang et al., 2004). Adolescents with mental problems have been reported to be more likely to start with e-cigarettes (Becker et al., 2020), supporting the "self-medication" hypothesis rather than a causal association. Some studies have tried to shed light on whether use of nicotine containing products per se may increase the risk for developing and/or exacerbate mental illnesses, such as the currently reported study in the systematic review of Becker and co-workers (2020), that found that e-cigarette use may predict future depressive systems. For Swedish smokeless tobacco, there are data indicating that its use is associated with an in-

creased risk for non-affective psychosis (Munafo et al., 2016). This is in line with longitudinal studies associating use of smoking tobacco with mental disorders (Chaiton et al., 2009; Gurillo et al., 2015). However, even longitudinal studies that might indicate a causal relationship, are possibly disturbed by reverse causality.

Becker and coworkers argued that there could be several potential causal relationships including (1) attempts of study participants to self-medicate symptoms, such as cognitive deficits in ADHD or low mood, (2) study participants efforts to counteract sedating side effects of psychotropic medications, (3) common underlying genetic or environmental risk factors for smoking and mental illness, or (4) neurotoxic impacts of nicotine on mental health (Becker et al., 2020). A combination of individual-specific factors likely contributes.

Effects of nicotine on the developing brain give some support to effects of nicotine on the development of mental problems, such as ADHD, depression, and anxiety (US Surgeon General, 2014; 2016; Dwyer et al., 2009). However, it is too early to conclude on causal inference of e-cigarettes and mental disorders.

Overall evaluation mental disorders
Several studies have shown an association between mental health and increased user prevalence of nicotine containing products. The causal factors underlying the association are unknown. It is possible that common vulnerability (genetic and environmental) is involved. Adolescents with mental problems have been reported to be more likely to start with e-cigarettes, supporting the "self-medication" hypothesis rather than a causal association. On the other hand, the currently reported studies that found e-cigarette use associated with depressive symptoms, indicate that use of e-cigarettes may also affect mental health. Both studies in humans and animal experiments indicate an increased risk of development of addiction and long-term cognitive impairments in adolescence upon nicotine exposure. Effects of nicotine on the developing brain supports that nicotine may affect development of mental problems, such as ADHD, depression, and anxiety. However, it is too early to conclude on causal inference of e-cigarettes and mental disorders.

Adverse pregnancy outcomes and effects on early life health
Potential harm due to e-cigarette exposure on the pregnant mother and her child has not been thoroughly investigated. Thus, we have used knowledge obtained from reports and new systematic reviews on other tobacco- and nicotine containing products to reveal possible health risk associated with its use.

Exposure to components from snus and cigarette smoke may in principle affect the fetal development via four main pathways by: i) effects on mother with implication for placenta development/function, ii) effects on mother with implication for development of fetus, iii) effects on placenta development/function, and iv) direct effects on the development of fetus. Still, the relative contributions of various pathways are mostly unknown.

Both smoking and use of moist smokeless tobacco (snus) are associated with adverse effects on the fetus and new-born child as well as the pregnant mother. Both use of snus and smoking has been associated with increased risk of prematurity (Dahlin et al., 2016), neonatal apnea (Gunnerbeck et al., 2011), oral cleft malformations (Gunnerbeck et al.,

2014), stillbirth (Baba et al., 2014), reduced birth weight (Juarez & Merlo, 2013a, 2013b) and increased risk of having SGA infants (Baba et al., 2013). However, available studies suggest a higher risk for SGA and a greater reduction in birthweight for smokers compared to snus using pregnant women. The differences observed between snus and smoking, suggest that additional constituents in the smoke such as PAHs contribute to the adverse pregnancy outcomes in addition to nicotine. Thus, the adverse effects associated with use of snus may largely by mediated by nicotine. Use of snus during pregnancy is also associated with altered heart rate variability and increased blood pressure in the offspring (Nordenstam et al., 2017; Nordenstam et al., 2019).

From the systematic review included in this review, two studies assessed the risk for SGA among e-cigarette using pregnant women. One of them found no elevated risk for SGA, whereas the other reported increased risk. A limitation of the studies were few participants and pregnant women using e-cigarettes. After publication of the systematic review, two studies assessing SGA, based on the U.S Pregnancy Risk Assessment Monitoring System (PRAMS), were published (Shittu et al., 2021; Wang et al., 2020). Based on exclusive e-cigarette using women, where approximately 19 % used e-cigarettes throughout pregnancy, the risk of having an SGA infant was aRR 1.52 (95% CI 1.45 - 1.60) (Shittu et al., 2021). Quitting e-cigarettes and for smokers switching completely to e-cigarettes, the risk for SGA was normalized.

Adverse effects of nicotine on the mother, placenta, fetus and the developing child, are supported by animal studies. Many of nicotine effects are mediated through binding and activation of nicotinic cholinergic receptors, which are widely distributed in the developing fetus. These receptors are important for development and physiological functions *in utero* (Lambers & Clark, 1996). Nicotine exposure during pregnancy in animal models have shown that nicotine affects brain and lung development, associated with adverse neurobehavioral outcomes and lung function (Lauterstein et al., 2016; McGrath-Morrow et al., 2015; US Surgeon General, 2016; Zelikoff et al., 2018). Nicotine exposure during pregnancy is further associated with postnatal obesity, type 2 diabetes and hypertension (Bruin et al., 2010). Furthermore, animal studies suggest that some of the effects of nicotine exposure on the developing fetus is mediated by its effects on the placenta development/function (Holloway et al., 2014; Suter & Aagaard, 2020).

When using information from animal studies, it is importance to consider the internal dose/concentration of harmful substance. As discussed under CVD, the level of systemic nicotine among adult e-cigarette users has been reported to be comparable to that seen in smokers of tobacco cigarettes and snus. Thus, it is likely that use of e-cigarettes will result in similar health effects as snus, and those due to nicotine in cigarettes. Furthermore, higher concentrations of nicotine in placenta, amniotic fluid and fetal serum compared to maternal serum levels are possible after snus and e-cigarette use as has been reported for smoking mothers (Luck et al., 1985).

Overall evaluation adverse pregnancy and effects on early life health

The information from the systematic review on use of e-cigarettes for pregnancy and early life health outcomes was restricted to uncertain effects on birthweight and being small for gestational age. However, the combined evidence of: i) increased risk of adverse pregnancy outcomes associated with cigarettes as well as smokeless tobacco use ii) *in vivo* studies showing deleterious effects of nicotine and nicotine containing products on fetal and early life development iii) mechanistic insights substantiating toxic effects of

nicotine on the placenta, fetus and early life development, all indicate that use of nicotine containing e-cigarettes constitutes a potential threat to the mother and child.

Non-malignant oral diseases

The oral health implications of the use of e-cigarettes are just beginning to emerge. Smoking is a well-known risk factor for malignant and non-malignant oral diseases. As to the latter, smoking is a recognized risk factor for initiation and progression of periodontal diseases (Leite et al., 2018), and smoking cessation has been reported to reduce the risk of and improve the outcome of non-surgical periodontal therapy (Leite et al., 2019).

By several potential mechanisms, smoking may affect different oral tissues, predisposing the individual to increased risk for periodontal disease. The mechanisms may be many, such as interference with the immune system, bone biology, tissue healing, vasculature, and changes in the oral microbiome (Johnson & Guthmiller, 2007; Kinane & Chestnutt, 2000). Some of these mechanisms may be linked to nicotine exposure as shown in clinical studies included in the systematic review by Yang and co-workers (2020), where reduced periodontal and peri-implant bleeding on probing was found in e-cigarette users compared to never tobacco users. Similar findings have been reported for smokers in several studies (Palmer et al., 2005). Of note, is the short-term longitudinal study included by Yang and co-workers (2020), that showed increased bleeding on probing after smokers switching to e-cigarettes, suggesting that other factors in the tobacco smoke or e-cigarette aerosol may also contribute. A recent clinical study with 6 months follow-up among patients with periodontitis, reported increased clinical attachment level among e-cigarette users, compared to smokers and non-smokers (Xu et al., 2021).

Smoking has been shown to affect the composition of the buccal (Karabudak et al., 2019) and the subgingival microbiota (Jiang et al., 2020). Interestingly, studies included in the systematic review of Yang and co-workers (2020) reported that e-cigarette use may also have distinct effects on the oral microbiome. This is supported by recent findings, showing a unique subgingival microbial profile among e-cigarette users, which shared similarities with both smokers and non-smokers (Thomas et al., 2022). One study reported in the systematic review of Yang and co-workers (2020), also reported increased prevalence of oral candida carriage among e-cigarette users, an observation also reported for smokers (Soysa & Ellepola, 2005). In this regard, nicotine has been shown *in vitro* to increase biofilm formation of two candida species (Gunasegar & Himratul-Aznita, 2019), and that e-cigarette vapor increases growth and epithelial adhesion of candida albicans (Alanazi et al., 2019).

Dental caries is a disease that develops after fermentation of carbohydrates to acids that may dissolve the dental hard tissues and is the result of an unfavorable balance between the hosts mechanical removal or disturbance of the oral biofilm, use or intake of fluoride and the dietary intake of fermentable carbohydrates. No larger prospective studies were included by Yang and co-workers (2020) covering caries risk among e-cigarette users, however the authors of one *in vitro* study reported that a combined effect of the viscosity and some chemicals in sweet-flavored e-liquids may increase the cariogenic potential of *S. mutans*.

There were no included studies assessing dental erosion. A concern has been expressed that the low pH of some e-liquids (Fairchild & Setarehnejad, 2021) may affect dental erosion.

Overall evaluation non-malignant oral diseases

The umbrella review show that use of e-cigarettes may cause symptoms of oral discomfort and oral mucosal lesions. Although there is scarce evidence from longitudinal studies on the use of e-cigarettes regarding periodontal and peri-implant disease overall data indicate that there may be an association.

Cancer

Cancer caused by environmental exposure is a complex, multistep process, often developing over several decades. Important steps may include formation of DNA damage due to reactive chemical intermediates or increased formation of reactive oxygen species (ROS). Other chemicals may interfere with DNA- repair mechanisms, or receptors modifying expression of genes involved in proliferation or cell death which also are processes relevant for cancer development. Induced chronic pro-inflammatory reactions may create a microenvironment involving immune cells promoting cancer development, while other part of the immune system may protect against cancer development. Also, these processes may be modified by chemical exposure.

Since e-cigarette aerosol contains lower amounts of known carcinogens, including TSNA (tobacco specific nitrosamines) and combustion products such as PAHs compared with tobacco smoke, it is assumed that the cancer risk from use of e-cigarettes may be lower than that from smoking conventional tobacco cigarettes. E-cigarette aerosols contain compounds that may affect cancer development, the overall effect is partly a question of the device and the pattern of use (frequency, intensity, strength, duration of habit). Considering the potentially long induction time for cancer, early signs of a carcinogenic effect must rely on toxicological information, such as biomarkers, animal experiments as well as *in vitro* studies.

Human studies

The NASEM report identified two additional studies relevant for cancer in humans not reported in the review by Flach and co-workers (2019). The latter review also reported a study detecting the carcinogen N'-nitrosonornicotine (NNN) in saliva from e-cigarette users, a marker of carcinogen exposure. Several studies reported by NASEM have investigated the impact of e-cigarette use on biomarkers of effect linked to cancer development. In their evaluation of the study on micronuclei (MN), NASEM reported that the average micronuclei burden was 21% higher in e-cigarette users, although confounder control was not satisfactory. Furthermore, NASEM described a case report suggesting that e-cigarettes were associated with substantially less inflammation than cigarette smoking. However, a combined case reports and clinical study provided some indication that e-cigarettes are a strong enough source of inflammation to elicit symptoms that could be potentially misdiagnosed as a form of cancer. The presence of multinucleated giant cells in the lung as well as apparent lung nodules and liver lesions in this study was considered linked to e-cigarette aerosol as the findings disappeared after stopping use of e-cigarettes.

Animal studies

The systematic review of animal studies (Wang et al., 2019) reported a study where nicotine containing e-cigarette aerosol may enhance hyperplasia and metaplasia in laryngeal mucosa, although, the changes were not statistical different from controls. Notably, the small sample size prevented firm conclusions. The NASEM report identified no animal studies on cancer.

The overall literature search identified one single study (H. W. Lee et al., 2018) reporting that mice inhaling e-cigarette aerosol with nicotine for 54 weeks developed lung adenocarcinomas (9 of 40 mice, 22.5%) and bladder urothelial hyperplasia (23 of 40 mice, 57.5%) (Tang et al., 2019). The authors pointed out that these lesions were extremely rare in mice exposed to vehicle control or filtered air. In a previous 12-week mice study on *biomarkers linked to cancer* (H. W. Lee et al., 2018), the same authors reported that e-cigarette smoke induced DNA damage (detection of mutagenic O^6-methyldeoxyguanosines and γ-hydroxy-1, N^2-propano-deoxyguanosines) in lung, bladder, and heart. DNA-repair activity and -repair proteins XPC and OGG1/2 in lung tissues was reduced. From the combined set of results from these two studies, the authors implicated e-cigarette aerosol as a lung and potential bladder carcinogen in mice (Tang et al., 2019).

In a study linked to *biomarkers of carcinogen exposure and effects* (Canistro et al., 2017) referred by NASEM, male rats exposed to aerosols of e-liquid with nicotine had urine that was directly mutagenic in *Salmonella* TA100. They reported increased levels of CYP enzymes, free radicals, and oxidative DNA damage (8-OHdG) in lung tissue compared to the control animals (exposed to ambient filtered air). Additionally, the authors reported reduced levels of antioxidant enzymes. E-cigarette aerosol also induced DNA damage in blood cells, including DNA strand breaks (peripheral blood leucocytes) and formation of micronuclei (measured in reticulocytes).

NASEM commented that although oxidative damage is widely regarded as a potentially significant contributor to carcinogenesis, most of the oxidative DNA damage occur via endogenous processes. On the other hand, in the systematic review by Wang et al (Wang et al., 2019), there were additional studies reporting that e-cigarette aerosol impaired redox balance and increased levels of pro-inflammatory cytokines when compared to untreated controls.

Pham and co-workers (Pham et al., 2020) reported that e-cigarette aerosol enhanced growth of breast cancer cells injected in the mammary fat pad and enhanced lung metastasis after injection in the tail vein. Detailed mechanistic studies revealed that e-cigarette exposure created a *tumor-promoting microenvironment* by modifying tumor-immune cell crosstalk and tumor metastasis. NASEM also pointed out that although *carcinogenic susceptibility* to e-cigarettes have not been specifically studied, there may be populations with particularly high-risk characteristics including race/ethnicity, sex, and socioeconomic status. Children and adolescents are likely vulnerable populations for lung cancer, as indicated by studies on tobacco cigarette smoke (US Surgeon General, 2014).

In vitro studies

NASEM reported two studies collecting aerosol from e-cigarettes with blended tobacco flavour and testing this in *Salmonella* TA98 and TA100 with and without metabolic activation *in vitro.* The authors reported no increased mutagenicity in either strain at any dose. Neither did the study on micronucleus in Chinese hamster ovary cells detecting

clastogenic (structural chromosomal aberrations) and aneugenic (numerical chromosomal aberrations) DNA damage. NASEM emphasised experimental design as an explanation for the difference in results between *in vivo* (mutagenic) and *in vitro* studies (no effect). NASEM further reported one cell-transformation assay, detecting some non-genotoxic chemical carcinogens with tumor promoter activity in e-cigarette aerosol. Breheny and colleagues (Breheny et al., 2017) found that collected aerosol particulate matter from an e-liquid cartridge containing blended tobacco flavour with nicotine was negative, while tobacco smoke total particulate matter was positive. In the review of Flach and co-workers (2019) it was reported that exposure to e-cigarette aerosols increased cell migration in dysplastic keratinocytes and cancer cells.

Studies of effects of major components of e-cigarettes on cancer related outcomes
NASEM committee examined existing evidence of major components of e-cigarettes (nicotine, PG, and glycerol) on cancer outcomes. The report summarised the potential carcinogenicity of nicotine, which has been studied extensively in *in vitro* and *in vivo* (Grando, 2014; IARC, 2000; US Surgeon General, 2014).
The report concluded that there is a lack of *epidemiological evidence* assessing the potential association between exposure to nicotine per se and the risk of cancer in humans. No epidemiological studies have addressed the long-term adverse health effects, including cancer, of propylene glycol and glycerol.

Animal studies: NASEM identified no properly conducted rodent bioassays for carcinogenesis associated with nicotine exposure. However, there were several relevant long-term studies on cancer where no clear conclusion about carcinogenic effects could be drawn (Haussmann & Fariss, 2016; Toth, 1982; Waldum et al., 1996). Most interestingly, nicotine was reported to enhance development of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung tumors (Murphy et al., 2011), and induce urothelial hyperplasia (increased cell proliferation) (Dodmane et al., 2014), indicating synergistic effects with other carcinogens. Animal bioassays also show that nicotine can be metabolized to form carcinogenic tobacco-specific N-nitrosamines (TSNA), including N-nitrosonornicotine (NNN) and a nicotine derived nitrosamine ketone (NNK) (Hecht, 2003).

In vitro studies: Studies have reported that chemical constituents of e-cigarette aerosols can react with DNA and in some instances induce mutations *in vitro* as well as following *in vivo* exposure (Canistro et al., 2017). Some chemicals in e-cigarette aerosols, including the reactive aldehydes formaldehyde and acrolein, are DNA-reactive human carcinogens. NASEM pointed out that compared to cigarette smoke, the levels of DNA reactive carcinogens in e-cigarettes are low. More recently Lee and co-workers (H. W. Lee et al., 2018) reported that nicotine and NNK induce the same type of DNA adducts as well as inhibited DNA repair in human bronchial epithelial and urothelial cells *in vitro*.

Overall evaluation cancer
The results obtained from the umbrella review alone were insufficient for a conclusion whether use of e-cigarettes constitute a cancer hazard. However, recently important new information relevant for evaluation of potential carcinogenic effects associated with e-cigarette use has been published. E-cigarette aerosol was reported to induce lung adenocarcinomas and bladder urothelial hyperplasia in mice. The authors suggested a role of nicotine in cancer formation by decreased DNA repair activity and increased DNA adduct formation by endogenously formed NNK from nicotine.

Based on results from our umbrella review, the NASEM report and the new information summarized above we conclude: i) There is no available evidence that e-cigarette use is associated with intermediate cancer endpoints in humans from human studies; ii) There are adequate long-term animal bioassays of e-cigarette aerosol exposures to inform cancer risk; there is evidence from *in vivo* animal studies using intermediate biomarkers of cancer to support the hypothesis that long-term e-cigarette use could increase the risk of cancer; iii) There is evidence that e-cigarette aerosol can be mutagenic or cause DNA damage in humans, animal models, and human cells in culture, iv) There is substantial evidence that some chemicals present in e-cigarette aerosols (e.g., formaldehyde, acrolein) are capable of causing DNA damage and mutagenesis.

Based on a toxicological evaluation of current literature, we conclude that regularly, long-term use of e-cigarette is likely to represent an enhanced risk for developing cancer. However, the impact on the prevalence of cancer in the general population is unknown.

Poisonings and injuries

With high evidence for a causal relationship, e-cigarettes are associated with accidental poisonings, intentional poisonings and traumatic injuries caused by explosions, thermal and chemical injuries due to overheating of lithium batteries. For all outcomes the incidence of cases is not estimable, in other words, we do not know how often this happens. For all such events, risk for underreporting may occur, since less severe cases go undetected/not reported.

Accidental poisonings occur mainly in infants and children of young age. To reduce the risk for such events the Tobacco Products Directive demands that all e-cigarettes and e-liquid containers (bottles) should be child- and tamper proof. Nevertheless, accidental poisonings occur. In 2016 the United Kingdom National Poisons Information Service received 278 enquiries regarding e-liquid poisoning in children under 16 years of age, an increase as compared to 2008 (Ang et al., 2018). Of note, is that the service only receives calls from clinicians seeking advice. The most prevalent clinical features were vomiting, tachycardia, dysaesthesia, irritation and increased creatine kinase. In accordance with this observation, an estimated 885 (95% CI: 397–1374) cases of poisoning by e-liquid ingestion among children under 5 years of age were treated in the emergency departments in the United States in 2018 (Chang & Rostron, 2019).

Regarding nicotine containing products, explosions associated with e-cigarettes are a new phenomenon. Based on the available literature, some of the injuries confer serious debilitating outcomes and even death has been reported. In the US, 676 visits to emergency departments due to explosion and/or burn injuries was estimated for 2019 (Rossheim et al., 2020).

Overall evaluation poisonings and injuries

E-cigarettes are associated with accidental poisonings, intentional poisonings and traumatic injuries caused by explosions, thermal and chemical injuries due to overheating of lithium batteries. We are uncertain how often such accidents occur.

Relevance of exposure levels following e-cigarette use and association to disease

The presence of hazardous constituents in e-cigarette aerosols does not necessarily confer an elevated risk for disease development and/or exacerbations. The final outcomes will be highly dependent on factors such as the quality and quantity of exposure as well as individual variations in susceptibility. Thus, it is difficult to know precisely or predict the human exposure levels of the substances identified in the aerosol. Unintentional/accidental intake of e-cigarette liquid as well as accidental explosions of device represent a clear hazard, although incident rate is uncertain. Changes in several biomarkers relevant for disease development have been measured in exposed humans, animals, and cells.

Several adverse health outcomes are associated with nicotine exposure. The absorption of nicotine into the systemic circulation in e-cigarette users have been found to be comparable to adult smokers (NASEM, 2018). Notably, for both product types, the initial concentrations of the aerosol constituents in the lungs are much higher than in the serum, thus increasing the possibility for local adverse effects (Herman & Tarran, 2020). The peak level of nicotine in serum following snus are somewhat lower than for adult tobacco smokers, but detectable levels are sustained for longer periods. Taken together, this implies that also e-cigarettes will be expected to have effects on adverse health outcomes similar to those caused by nicotine in snus and cigarette smoke.

Exposure limits for several of the constituents identified in e-cigarette aerosol are proposed by international bodies such as The Agency for Toxic Substances and Disease Registry (ATSDR), The National Institute for Occupational Safety and Health (NIOSH), Occupational Safety and Health Administration (OSHA) and World Health Organization (WHO). The exposure limits may differ depending on toxicological endpoints (cancer or non-cancer effects), exposure duration (acute, intermediate, or chronic exposure) and which uncertainty factors are used as well as who the guidelines are set to protect (vulnerable individuals, general population, occupational exposure).

The risk of cancer is dependent on environmental exposure levels, co-exposures, and susceptibility factors, but in the end also partly the result of chance (probability). Nevertheless, exposure to potential or known carcinogens are regulated to protect the general population. In a regulatory context, no safe (risk-free) dose threshold for genotoxic carcinogens is set as the risk of cancer associated with such carcinogens are regarded to be linear with dose (measured e.g., as exposure levels and duration). Thus, when exposed to a very low dose during a short period late in life, in general, it is assumed that the risk will be very low/negligible. In contrast, persons with life-long exposure to higher doses starting early in life will have a correspondingly much higher risk of disease. These general principles have been elucidated in animal experiments for other genotoxic carcinogens (Hattis et al., 2004).

One study reported average concentrations of IARC type 1 and 2 carcinogens measured in tobacco smoke and other forms of nicotine delivery products including e-cigarettes, as well as unit risks and cancer potencies for the various nicotine products (Stephens, 2017). Mean life-time cancer risk for e-cigarettes 30-liter vapor/day (average intake by

an e-cigarette user) was estimated to 9.5×10^{-5}. This was higher than nicotine inhaler (8.9×10^{-6}). These risk estimates are substantially lower than for cigarettes (2.4×10^{-2}) (Stephens, 2017). However, the estimated cancer risk for e-cigarette use is close to 1×10^{-4}. The US EPA suggests an increase in lifetime cancer risk in the range 1×10^{-4} to 1×10^{-5} as a risk level where measures (to reduce exposure) must be considered (US EPA, 1999).

In a study modelling intake of toxic compounds from e-cigarette aerosol, the authors reported that the daily dose of formaldehyde, acrolein and diacetyl intake were comparable to or exceeded those derived from occupational health guidelines when used with a relatively high number of puffs per day (250 puffs/day) (Logue et al., 2017). Notably, different vaping regimes had major effects on the predicted toxicant intakes. The authors further suggested that for formaldehyde and diacetyl, the predicted daily intakes from e-cigarettes could exceed NIOSH limits even with a lower, more typical vaping rate such as 100 puffs/day. For formaldehyde, NIOSH recommended 8 h time-weighted average exposure at 20 μg/m^3 (EPA, 2016). However, the minimal risk level (MRL) for chronic inhalation established by Agency for Toxic Substances and Disease Registry (ATSDR) is 0.003 ppm (4 μg/m^3). This is based on respiratory effects in humans. (MRL is an estimate of the daily human exposure to a hazardous substance likely to be without appreciable risk of adverse non-cancer effects only over a specified duration of exposure) (EPA, 2016).

None of the calculations above included the role of nicotine in relation to carcinogenesis as elucidated in two animal studies on cancer and exposure to e-cigarette aerosols (H. W. Lee et al., 2018; Tang et al., 2019). These studies suggest a role of inhaled nicotine in the development of lung and bladder cancer by decreasing DNA repair activity and increasing formation of DNA adducts due to endogenously formed NNK from nicotine. The levels of the inhaled nicotine from e-cigarette aerosol, and the genotoxic mechanism have been suggested to be relevant for extrapolations to human cancer. The importance of experimental conditions in the study by Tang and co-workers on cancer and their relevance to human consumption has been questioned (Li Volti et al., 2018; Queimado et al., 2018). However, Tang replied and believed that their e-cigarette aerosol exposure conditions in mouse were comparable to a human, light e-cigarette smoker's exposure situation (Tang, 2018a), and showed the relevance of concentrations used as well as the proposed mechanisms involving formation of NNK (Tang, 2018b). Most importantly, the highly reputed cancer expert CC Harris published a commentary supporting the potential importance of studies by Lee and co-workers (Harris, 2018). He points out that any relevant epidemiological study regarding cancer risk from e-cigarettes will require following cohorts using e-cigarettes in future decades. While more studies are needed, this type of *in vivo/in vitro* approach will have value in the decision making.

Another important question to answer is if the levels of e-cigarette exposure are high enough to contribute to the other human diseases discussed in the present umbrella review. As revealed by the human studies summarized above, e-cigarettes have an impact on biomarkers of effects, but we mostly still not know the exact risk for adverse health outcomes/diseases. The risk will depend on age of initiation/exposure, frequency, and duration of exposure. Also considering other knowledge, we conclude that it is likely that

exposure to e-cigarette aerosol will increase the risk for diseases linked to nicotine exposure. Exposure to other e-cigarette constituents may also enhance the risk for adverse health outcomes/disease.

Overall evaluation relevance of exposure levels following e-cigarette use and association to disease

The presence of hazardous constituents in e-cigarette aerosols does not necessarily confer an elevated risk for disease development and/or exacerbations. The outcome will depend on factors such as the level of hazardous constituents, age of initiation and quantity of exposure (frequency, duration, and years of exposure) as well as individual variations in susceptibility. The results from the present umbrella-review as well as information from international reports and recent literature on e-cigarettes and other nicotine products, implies that it is likely that the levels of inhaled nicotine and other components from e-cigarette use may enhance the risk for adverse health effects.

Conclusions

The main health concern linked to use of e-cigarettes arises from inhalation of harmful constituents in e-cigarette aerosol produced from the e-liquid.

The composition of the aerosol varies due to device characteristics, e.g., temperature during aerosolization of e-liquid, substances released from the device/heating element as well as variation in e-liquid contents. E-cigarettes should not be considered a homogeneous product group.

E-cigarettes were introduced to the market without adequate animal and *in vitro* studies to clarify the harmful effects that use of e-cigarettes could cause.

There are few high-quality human studies of e-cigarettes and disease, with longitudinal design, long-term exposure, and sufficient exposure characterization and follow-up time.

Based on our umbrella review and toxicological evaluation, we conclude that use of e-cigarettes leads to an increased risk for adverse health effects. The relative risks for these adverse health effects are still uncertain.

Appendix 1: Search strategy

Søk: Miriam Bakkeli
Fagfelle: Nataliya Byelyey
Kommentar: Oppdateringssøk; WoS 181220, Medline 171220, Embase 171220, samt Cochrane 080221.

Dublettsjekk i EndNote:

	1: oversiktsartikler	2: andre referanser
Før dublettkontroll:	290	6392
Etter dublettkontroll:	157	3755

Database: **Web of science,**
Dato: 23.11.2021
Antall treff: 78 (oversiktsartikler), 1977 (andre referanser)

	Søk	Avgrensning	Treff
#5	#2 NOT#4	Web of Science Core Collection	**1,977**
#4	#2 AND #3	Web of Science Core Collection	**78**
#3	TS=(("systematic*" NEAR/1 "review*") or ("review" and (("structured" or "database*" or "systematic*") NEAR/1 "search*")) or "integrative review*" or ("evidence" NEAR/1 "review*")) OR TI=("metaanal*" or "meta anal*") OR AB=("metaanal*" or "meta anal*")	Web of Science Core Collection	455,492
#2	TS=(("electronic cigarette$" or "e-cigarette$" or "ecigarette$" or "eCIG*" or "e-CIG*" or "electronic nicotine delivery system$" or "electronic nicotine delivery device$" or "nicotin* vapor*" or "nicotin* vapour*" or "vaporised nicotin*" or "vaporized nicotin*" or "vapourised nicotin*" or "vapourized nicotin*" or "e-hookah$" or "Electronic Hookah$" or "Hookah Pen$"))	**Timespan: 2020-12-18 to 2021-11-23 (Index Date)** Web of Science Core Collection	2,055
#1	TS=(("electronic cigarette$" or "e-cigarette$" or "ecigarette$" or "eCIG*" or "e-CIG*" or "electronic nicotine delivery system$" or "electronic nicotine delivery device$" or "nicotin* vapor*" or "nicotin* vapour*" or "vaporised nicotin*" or "vaporized nicotin*" or "vapourised nicotin*" or "vapourized	Web of Science Core Collection	9,348

	nicotin*" or "e-hookah$" or "Electronic Hookah$" or "Hookah Pen$"))		

Database: **Ovid MEDLINE(R) and Epub Ahead of Print, In-Process, In-Data-Review & Other Non-Indexed Citations, Daily and Versions(R)** <1946 to November 22, 2021>

Dato: 23.11.2021

Antall treff: 119 (oversiktsartikler), 2734 (andre referanser)

#	Searches	Results
1	Electronic Nicotine Delivery Systems/ or ("Electronic Cigarette?" or "E-Cigarette?" or "E Cigarette?" or "E-Cig?" or "E Cig?" or "ecigarette$" or "eCIG*" or "Electronic Nicotine Delivery System?" or "Electronic Nicotine Delivery Device?").tw,kf.	8542
2	("nicotin* vapor*" or "nicotin* vapour*" or "vapori#ed nicotin*" or "vapouri#ed nicotin*").tw,kf.	104
3	("e-hookah?" or "e hookah?" or "Electronic Hookah?" or "Hookah Pen?").tw,kf.	29
4	Vaping/ or (Vape? or vaping).tw,kf.	3735
5	or/1-4	9119
6	2021*.ed,ep,yr,dp,dt.	2201335
7	("20201216" or "20201217" or "20201218" or "20201219" or "20201220" or "20201221" or "20201222" or "20201223" or "20201224" or "20201225" or "20201226" or "20201227" or "20201228" or "20201229" or "20201230" or "20201231").ep,ed,dt.	119014
8	or/6-7	2258950
9	5 and 8	2855
10	limit 9 to "reviews (maximizes specificity)"	98
11	Meta-Analysis/ or Network Meta-Analysis/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kf,bt.	415423
12	9 and 11	113
13	**10 or 12 [IMPORT]**	**119**
14	**9 not 13 [IMPORT]**	**2736**

Database: **Embase** <1974 to 2021 November 22>

Dato: 23.11.2021

Antall treff: 90 (oversiktsartikler), 1562 (andre referanser)

#	Searches	Results
1	electronic cigarette/ or ("Electronic Cigarette?" or "E-Cigarette?" or "E Cigarette?" or "E-Cig?" or "E Cig?" or "ecigarette$" or "eCIG*" or "Electronic Nicotine Delivery System?" or "Electronic Nicotine Delivery Device?").tw,kw.	10640
2	("nicotin* vapor*" or "nicotin* vapour*" or "vapori#ed nicotin*" or "vapouri#ed nicotin*").tw,kw.	141
3	("e-hookah?" or "e hookah?" or "Electronic Hookah?" or "Hookah Pen?").tw,kw.	36
4	vaping/ or (Vape? or vaping).tw,kw.	4480

5	or/1-4	11511
6	conference abstract.pt.	4252265
7	5 not 6	9753
8	limit 7 to embase	6909
9	2021*.yr,dd,dp,dc.	2206592
10	("20201216" or "20201217" or "20201218" or "20201219" or "20201220" or "20201221" or "20201222" or "20201223" or "20201224" or "20201225" or "20201226" or "20201227" or "20201228" or "20201229" or "20201230" or "20201231").dd,dc.	112861
11	or/9-10	2312744
12	8 and 11	1652
13	limit 12 to "reviews (maximizes specificity)"	58
14	exp Meta-Analysis/ or "systematic review"/ or ((systematic* adj2 review*) or metaanal* or "meta anal*" or (review and ((structured or database* or systematic*) adj2 search*)) or "integrative review*" or (evidence adj2 review*)).tw,kw.	596679
15	12 and 14	90
16	**13 or 15 [IMPORT]**	**90**
17	**12 not 16 [IMPORT]**	**1562**

Database: **Cochrane Database of Systematic Reviews,** Issue 11 of 12, November 2021
Cochrane Central Register of Controlled Trials, Issue 10 of 12, October 2021

Dato: 23.11.2021

Antall treff: 3 (oversiktsartikler) Cochrane-reviews
117 (andre referanser) Cochrane Central Register of Controlled Trials

ID	Search	Hits
#1	[mh ^"Electronic Nicotine Delivery Systems"]	173
#2	((Electronic NEXT Cigarette?) or (E NEXT Cigarette?) or (E NEXT Cig?) or ecigarette? or eCIG* or (Electronic NEXT Nicotine NEXT Delivery NEXT System?) or (Electronic NEXT Nicotine NEXT Delivery NEXT Device?)):ti,ab	773
#3	((nicotin* NEXT vapor*) or (nicotin* NEXT vapour*) or (vapori?ed NEXT nicotin*) or (vapouri?ed NEXT nicotin*)):ti,ab	24
#4	((e NEXT hookah?) or (Electronic NEXT Hookah?) or (Hookah NEXT Pen?)):ti,ab	5
#5	[mh ^"Vaping"]	65
#6	(Vape? or vaping):ti,ab	198
#7	OR #1-#6	860
#8	**#7 with Cochrane Library publication date Between Feb 2021 and Dec 2021, in Cochrane Reviews []**	**3**
#9	#7 with Publication Year from 2021 to 2021, in Trials	98
#10	#7 with Cochrane Library publication date Between Feb 2021 and Dec 2021, in Trials	110
#11	**#9 or #10**	**117**

Appendix 2: Excluded studies table

[Tekst eller liste i tabell]

Studie	Eksklusjonsårsak
[Studie]	[Forklaring]

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