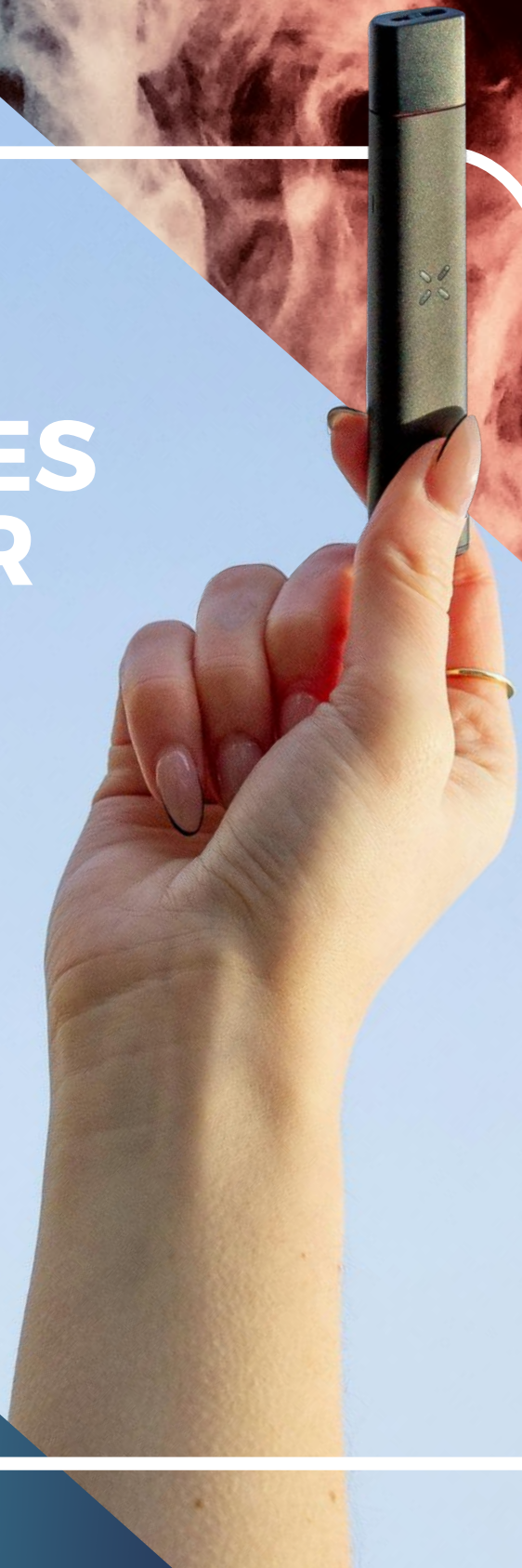
Clinical Oncology Society of Australia

RESEARCH REPORT

E-CIGARETTES AND CANCER

A QUALITATIVE RISK ASSESSMENT



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Contents

1. Executive summary

In Australia, an increasing number of young people are using vapes, and transitioning to tobacco smoking. There is no perception in the community of a cancer risk from e-cigarettes. The Clinical Oncology Society of Australia (COSA) has the breadth of expertise to assess such a risk. One in seven Australian adults have used e-cigarettes, as have a third of those aged 15-30 years. Since their introduction, the design of e-cigarettes has changed, but delivery of a nicotine-containing aerosol is fundamental. From 2024, e-cigarettes will only be available in Australia through pharmacies under restricted conditions.

Cancer patients who smoke survive longer if they quit. At present, only a small proportion of cancer survivors use vapes compared with those who smoke (data from the USA). Perceptions concerning e-cigarettes have altered from notions of safety to being as harmful as smoking, though perceptions don't align with behaviour. There are few policy statements about e-cigarette usage by cancer patients undergoing active treatment or long-term survivors, and scant guidance for oncology clinicians as to whether e-cigarettes have worth.

Almost from their introduction, the potential of e-cigarettes to cause cancer has been notified, initially because aerosol components include known carcinogens. To evaluate the particular spectrum of carcinogenicity data for e-cigarettes, qualitative risk assessment affords the best option. Certain restrictions are specified: peer reviewed publications as the only source; tobacco industry publications not addressed and risk attributable to using both cigarettes and vapes at the same time is beyond scope.

On the basis of levels of the respective compounds and/or their metabolites in tissue and biofluids, people who vape are exposed to carcinogens, such as formaldehyde, acetaldehyde, acrolein and acrylonitrile as well as to other carcinogens, such as the nitroso-derivatives of nicotine, propylene oxide derivatives and heavy metals. Many exposure studies have included data from smokers, but investigators are increasingly identifying exposure to e-cigarette aerosols an issue in its own right.

Inflammation and toxic injury to oral and respiratory tissue from vaping is evident histologically. A broad range of clinical studies have monitored biomarkers of harm in people using e-cigarettes. Molecular endpoints have included indicators of DNA damage, oxidative stress, epigenetic change, inflammation and immunosuppression, variously subject to focussed reviews. Focusing on assessments made between 2018 to 2024, there has been an unequivocal expression of concern specifically in relation to carcinogenesis.

E-cigarette aerosol is carcinogenic to experimental animals. In a single study of inhalation exposure for 54 weeks, about a quarter of exposed mice developed lung adenocarcinoma. Mechanistic data reviewed with reference to the 'key characteristics of carcinogens' revealed clear positive findings for electrophilicity, genotoxicity, DNA repair, inflammation and cellular immortalization, with some evidence for epigenetic change, oxidative stress and altered cellular behaviour.

Reviews of e-cigarette carcinogenicity have transitioned from assertions of credibility to indications of likelihood for lung cancer and oral cancer in particular. Taking into account all findings concerning clinical studies, animal bioassay and mechanistic data presented in this report, the following assessment is made:

Nicotine-based e-cigarettes are likely to be carcinogenic to humans who use them.
E-cigarettes are likely to cause lung cancer and oral cancer.

2. Introduction

In Australia and like countries, an increasing number of young people are using vapes, and transitioning to tobacco smoking. There is no perception in the community of cancer risk attributable to usage of e-cigarettes. COSA has the leadership authority and breadth of cancer-based expertise to assess all aspects of e-cigarettes. In this report the term e-cigarettes is identified with electronic nicotine delivery systems. This report is intended for a multidisciplinary healthcare professional readership.

Recognition of a public health crisis

The Cancer Council Australia (CCA) Position Paper *E-cigarette use in young people – urgent action needed to avert a public health crisis* published in October 2022 offers a perspective in line with government and health authorities across Australia. Namely, the uptake and continued usage of e-cigarettes by an increasing proportion of young people across Australia is alarming. Nicotine is addictive and once initiated, usage of e-cigarettes is likely to become habitual. Epidemiological studies indicate a variety of adverse health outcomes attributable to e-cigarettes.[1]

Beyond addiction, moderate to strong evidence of injury and disease caused by e-cigarettes includes poisoning, heat injuries, nicotine toxicity from inhalation or accidental ingestion of vape fluid – including seizures and death, e-cigarette or vaping associated lung injury, increased blood pressure, increased uptake of tobacco smoking, and indoor air pollution, environmental waste and fires. Injury to smokers attributable to their nicotine intake includes outcomes from maternal smoking and behavioural changes associated with smoking during adolescence and all such outcomes are expected following nicotine intake from e-cigarettes.[1]

Young people who use e-cigarettes are three times more likely to become tobacco smokers than non-smokers.[2-4] Such a transition has been documented in Australian youth.[5] Tobacco smoking has long been recognised as the major preventable cause of premature death worldwide, and specifically the major preventable cause of cancer worldwide. There is, therefore, the immediate and recognisable prospect of a burden of cardiovascular disease, cancer and other diseases caused by smoking independent of disease attributable to earlier vaping.

Cancer as a matter of community concern and confusion

The community is more concerned about cancer than any other disease. This concern extends from cancer being reportedly caused by food contaminants, multiple consumer products and local pollution to achieving a 'breakthrough' cure for cancer as the principal goal of medical research. Tobacco smoking is correctly recognised as causing lung cancer. Unfortunately, smoking is often presumed to account for all instances of lung cancer, resulting in a prejudicial approach to all people diagnosed with this malignancy.

More generally, evidence of community misunderstanding about what does, and what does not cause cancer is clear.[6, 7] Misunderstanding is exemplified by a failure to appreciate that drinking alcohol is a major known contributing cause to the cancer burden in Australia[8] through to an understanding that cancer causation by environmental factors may be self-evident as cancer clusters.[9]

Experience of the tobacco epidemic over decades revealed that fear of cancer motivates smoking cessation in people over age 50, but no such scenario is evident in teen-aged smokers.[10, 11] Accordingly, there is no prospect of reduced vaping by young people concerned about cancer or ill health later in life. However, evidence of harm caused by e-cigarettes is likely to generate community concern.[12] The likelihood of effective regulatory action to limit availability seems likely to be enhanced by medico-scientific evidence implicating cancer as a consequence of e-cigarette usage.[13, 14]

Leadership by COSA

COSA is the national oncology community bringing together multidisciplinary health professionals across all cancers to advance care and improve outcomes. COSA is recognised as an activist organisation whose views are valued in all aspects of cancer care, and provides high-level clinical advice to CCA. COSA, through its Council, formally endorsed the Cancer Council Position Paper *E-cigarette use in young people – urgent action needed to avert a public health crisis*. That statement delineated the alarming uptake of e-cigarettes by adolescents and specified the proven adverse health effects.

Members of COSA Council were aware of the complexity inherent in any assessment of the risk of cancer due to e-cigarettes. Other considerations, arising from the overarching mission of COSA to improve cancer care and control through collaboration, involve any role for e-cigarettes in smoking cessation by cancer patients and related matters. There was broad recognition that all aspects of e-cigarettes and cancer came within the scope of one or more of respective COSA Groups or affiliated clinical trials organisations.

The proposal for COSA to develop a Position Statement on e-cigarettes and cancer was developed by COSA Council with the support of CCA as represented. The current manuscript is a summation of the research evidence relating to e-cigarettes and cancer and will be used to directly inform the COSA Position Statement.

Terminology

In the medico-scientific literature, e-cigarettes are sometimes described as ENDS: electronic nicotine delivery systems. The term 'ENDS' is sometimes understood to include e-cigarettes, e-pens, e-pipes, e-hookah and e-cigars. Otherwise, in the medico-scientific literature, 'ENDS' is interchangeable with 'e-cigarettes'. ENDS that are not e-cigarettes are rarely, if ever, addressed in the studies cited in this literature. Accordingly, in this report and for practical purposes, specification of e-cigarettes is equated with ENDS, and the term 'e-cigarettes' is used throughout.

The terms 'vaping' and 'vaper' are used in the media and in the scientific literature to refer to the usage of e-cigarettes, and these terms are so used here. As will be subject to specific discussion, the medico-scientific literature concerning e-cigarettes is largely focused on making comparisons between vaping and tobacco smoking. Concerning the latter, a variety of terms are used including conventional cigarettes, traditional cigarettes, combustible cigarettes and tobacco cigarettes. These terms are not adopted when reporting relevant findings in this report. Rather the term 'cigarettes' always refers to tobacco-based products and the terms 'smoking' and 'smoker' always refer only to the usage of tobacco-based products.

Readership

This report has been prepared in response to a resolution by COSA Council and, in the first instance is for COSA members. COSA policy is to make research reports and policy statements available via the COSA website. Typically these COSA publications address specific matters warranting multidisciplinary professional engagement as the foundation for any assessment offered. The resultant publications are based on professional standards, in anticipation of a professional readership that is not limited to a particular craft, discipline or expertise.

Professional readership is implicit in the rigorous citing of journal publications and related peer-reviewed sources of information throughout this report. Multidisciplinary accessibility is an editorial policy and is achieved in part by providing explanations for specialised types of investigations. An effort has been made to provide explanatory information for terminology particular to fields such as clinical medicine, molecular biology, or behavioural psychology. We have sought to avoid narrowly-recognised jargon (including acronyms) or otherwise give sufficient detail as is required for lucidity.

The necessity for professionals outside medicine and health, as exemplified by regulators and lawyers, to be aware of findings made in the report is recognised, as has interest and concern of the wider community. This requirement for accessible information has been met most immediately by the provision of a plain language version of the executive summary. Beyond that, accessibility will also be taken into account by COSA through the later publication of a Policy Statement on e-cigarettes which will integrate the findings made in this report with wider considerations relating to the availability and impact of e-cigarettes in Australia.

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3. Faced with an epidemic, regulatory measures in Australia and worldwide

One in seven Australian adults have used e-cigarettes, as have a third of those aged 15-30 years. Since their introduction around 2008, the design of e-cigarettes has changed, but for all, delivery of a nicotine-containing aerosol is fundamental. This report does not address illicit drugs delivered via e-cigarette design. From 2024, e-cigarettes will only be available in Australia through pharmacies under restricted conditions.

This assessment of the carcinogenicity of e-cigarettes, despite being of inherent medico-scientific concern, comes in a specific context. There is worldwide recognition that e-cigarettes, though originally touted as an option for smoking cessation, are being aggressively marketed to young people in high income countries, including Australia. In Australia, the USA and other countries, young people are increasingly using e-cigarettes such that the level of prevalence has been characterised as epidemic.

Around 2023 in particular, Australian research contributed improved understanding regarding practice and ramifications of e-cigarettes by young people as summarised in this section.

Globally, a key response to this 'public health crisis' has been national regulatory measures. The scope of such regulations is remarkably broad, ranging from outright prohibition through to minimal limitations on availability. In Australia, such regulation addresses different designs through which e-cigarettes have evolved.

As is also explained in this section, vaping is now a means of not only inhaling nicotine, but also of inhaling illicit drugs, which, to the extent this practice is documented, is a major issue in the USA and UK. This report is restricted to the carcinogenicity of nicotine-based e-cigarettes.

Recent assessments of vaping by Australian youth and its consequences

The 2022 National Health Survey data from the Australian Bureau of Statistics (as released in December 2023)[1] records that one in seven (14.4%) Australian adults had used vaping devices at least once in their life, while 4.0% reported currently using a device:

- Males were more likely than females to have used vaping devices at least once (17.4% compared to 11.4%)
- Almost twice as many males reported current use of vaping devices than females (5.2% compared to 2.9%).

Vaping among 15-17 year olds more than doubled between 2020-21 and 2022, from 7.6% to almost 18%, while over the same period the proportion of young adults aged 18-24 years who had tried an e-cigarette at least once almost doubled from 21.7% to 38%.

As reported in 2023 by Pettigrew et al.,[2] a national sample of 1,006 Australians aged 15-30 years revealed that almost half had used/tried e-cigarettes, being either current users (14%) or had tried/used e-cigarettes in the past (33%). Factors positively associated with ever use were being a past or present user of tobacco cigarettes and number of friends who vape. Stronger perceptions of addictiveness were inversely associated with use.

Increasing usage of e-cigarettes by young people is evident in similar countries. In the USA, Nelson[3] reports in 2023 that about one in 10 middle school and high school students (more than 2.5 million) currently use e-cigarettes. More than a quarter (27.6%) of these used an e-cigarette product daily, and there was an overwhelming preference for the flavoured varieties. In the UK as reported in 2023, analysis

of cigarette and e-cigarette use among 9,731 adolescents indicated that 45.8% abstained from cigarettes or e-cigarettes; 21.3% used cigarettes and/or e-cigarettes once but not currently by age 17; 19.0% had low levels of use at age 14 but high levels of use at age 17; and 13.9% exhibited high levels of use at ages 14 and 17.[4]

In concert with data on vaping by young Australians, there are indications of increased tobacco smoking as a consequence. Scully et al.[5] determined that tobacco smoking susceptibility was independently associated with ever use of e-cigarettes (adjusted odds ratio = 3.26) with behaviour influenced by a range of perceptions including a desire to be more popular, having a close friend/s who smoked, not perceiving smoking one or two cigarettes occasionally as personally dangerous, and having symptoms of depression. These investigators concluded that the strongest smoking-initiation risk factor identified was ever use of e-cigarettes, with social norms, harm misperceptions around low-rate tobacco use, and mental health also linked to smoking susceptibility.

These findings are consistent with transitions by adolescent users of e-cigarettes to the smoking of regular cigarettes observed in other countries, and again, the most recent data confirm what had been observed earlier. Data from 10,229 study participants aged 10–25 years in the UK indicated that there was a 14% probability that e-cigarette users went on to smoke cigarettes after one year, rising to 25% after three years.[6]

Vaping rates in Australia have tripled in the past four years, with one in five Australians having tried e-cigarettes, according to recent (2024) data from the Australian Institute of Health and Welfare.[7] More than 10% of teenagers aged 14-19 years are regular vapers, a significant increase from six years ago. However, the majority of teenagers (66%) had never tried an e-cigarette. Nearly one in three Australians aged 20-29 years regularly vaped in the last financial year, 13 times more than in 2019.

Evolving designs of e-cigarettes

E-cigarettes have common main components: battery, atomizer based on a coil of varying electronic resistance, and a tank or cartridge containing e-liquid. Addressing patterns of e-cigarette usage, commentators stress the importance of being aware of different device types.

As described by Vivarelli,[8] two main classes of e-cigarettes are identified: ‘closed’ e-cigarettes, non-refillable with non-replaceable battery or atomizer; and ‘open’ systems, designed to be refilled by users, allowing them to customize the atomizer (single or multiple coil), or battery, with a range of electrical resistances in terms of Ohm, voltage applied, and composition of the e-liquid.

Early e-cigarette devices looked like conventional cigarettes and contained a fixed amount of nicotine. Subsequently, vape-pens or hookah-pens, modified devices or mods (like advanced personal vaporizers, box-style mods and mechanical mods) allowed users to change temperature/voltage, nicotine concentrations, and other constituents of e-liquids including flavours in addition to adding accessories to generate more vapour and enhance the vaping experience. More recent market entrants include ‘pod devices’, which are discreet, generate less vapour, and are enormously popular among young people; an example is the JUUL, which resembles a USB memory stick and has surpassed all other e-cigarette devices in sales in the past few years in the USA. Of concern, many young people do not use the term ‘e-cigarettes’ to refer to JUULs and also use terms like ‘JUULing’ to indicate use of this device.[9]

Thus JUUL products belong to the fourth generation of e-cigarettes and have been one of the largest selling nicotine vape products in the USA. In 2018, JUUL took most of their flavoured products off the market after public and government concern over their popularity with high school students and young adults.[10] This study examined chemical exposure (dose), retention, symptoms during vaping, and the

environmental accumulation of exhaled propylene glycol, glycerol, nicotine, and menthol in a cohort of human participants who vaped JUUL 'Menthol' e-cigarettes.

Early e-cigarettes were disposable products. As outlined above, new rechargeable e-cigarette types were developed over time to deliver nicotine more effectively via refillable tanks or replaceable pods. These devices came to dominate the global e-cigarette market: by 2018, just 2% of adults who vaped in the UK used disposables.[11] However, in 2021 a new form of disposable e-cigarette entered the market, and from 2021 to 2022, use of disposables rose sharply in Great Britain as these new products rapidly became popular among young people.[11]

Similar trends were observed among young people in the USA. A study in southern California found that disposable pod users reported lower prevalence of lifetime smoking and daily vaping and were younger in age. Given their findings, the authors suggest that regulations addressing non-tobacco flavours and nicotine concentration in disposable pod devices merit consideration in efforts to reduce vaping in younger adults that have never smoked.[12]

Beyond nicotine: inhalation of illicit drugs

Some types of e-cigarettes are used for the inhalation of illicit chemicals, specifically as identified in the USA. Thus Gordon and Fine[13] refer to the hazards of inhaling aerosols generated by the heating of e-liquids containing nicotine, illicit drugs, and flavours or fragrances in a mix of solvents and carriers.

As reviewed by Breitbarth et al.,[14] the drug primarily concerned in this context is cannabis (marijuana) which is usually administered orally or by inhalation. The theory behind vaping cannabis is a reduction in inhalation of smoke-related toxins and carcinogens including tar, carbon monoxide and ammonia. Other narcotic drugs delivered via e-cigarettes include synthetic cannabinoids, methamphetamine and related agents, synthetic cathinones, fentanyl and derivatives, cocaine and heroin.

The extent of such practices, specifically in relation to illicit drugs, currently in Australia is not clear. In any event, this report is confined to the impact of nicotine-based e-cigarettes, and studies involving electronically-based inhalation delivery of illicit drugs are not included in the identified publications and in the assessments made.

Regulation of e-cigarettes in Australia

Under the subheading 'Regulation' in the context of a 2021 critique of current policy options, Al-Delaimy and Sim[15] observe that inadequate regulation of e-cigarettes in jurisdictions all over the world has led to manufacturers using chemicals in e-liquids that are a risk for human health and are used by millions. They note that in the USA, there is scant regulation and widespread advertising for these products targeting young people by use of celebrity endorsement on social media, by introduction of candy, dessert and fruit flavours and even promotion by purporting to increase sex appeal. In contrast, some countries, including Japan, Brazil, Australia, India and Norway, have strict restrictions or bans on e-cigarettes.

National regulatory options for e-cigarettes have been recognised to include prohibition, component ban, and regulation as medicinal products, poisons, tobacco products, consumer products, and/or unique products. However, these various options are not mutually exclusive, and in a 2021 survey of initiatives taken by 97 countries, Australia was identified with component ban, poisonous or hazardous substances and consumer products; no specific national references are given in the relevant Table.[16]

In Australia, importation and marketing of nicotine-based e-cigarettes is primarily regulated through the Therapeutic Goods Administration (TGA, tga.gov.au). Otherwise, and in the absence of nicotine, the safety of the Australian community in relation to e-cigarettes is a responsibility of the Australian Industrial Chemical Introduction Scheme (AICIS, www.industrialchemicals.gov.au). AICIS has published an

assessment titled *Non-nicotine liquids for e-cigarette devices in Australia: chemistry and health concerns*.[17]

In 2024, the TGA notified regulation of e-cigarettes on the basis of whether these products are intended for therapeutic use in the context of smoking cessation and the management of nicotine dependence or not. Therapeutic use will be restricted to reusable vapes. Beyond that context, the importation, supply and manufacture of e-cigarettes is prohibited, the only exception recognised being supply for clinical trials and scientific research. The details concerning these regulatory changes were provided at tga.gov.au/products/unapproved-therapeutic-goods/vaping-hub/reforms-regulation-vapes (accessed 18 January 2024).

The regulations outlined below make no distinction between nicotine-based vapes and any other such products.

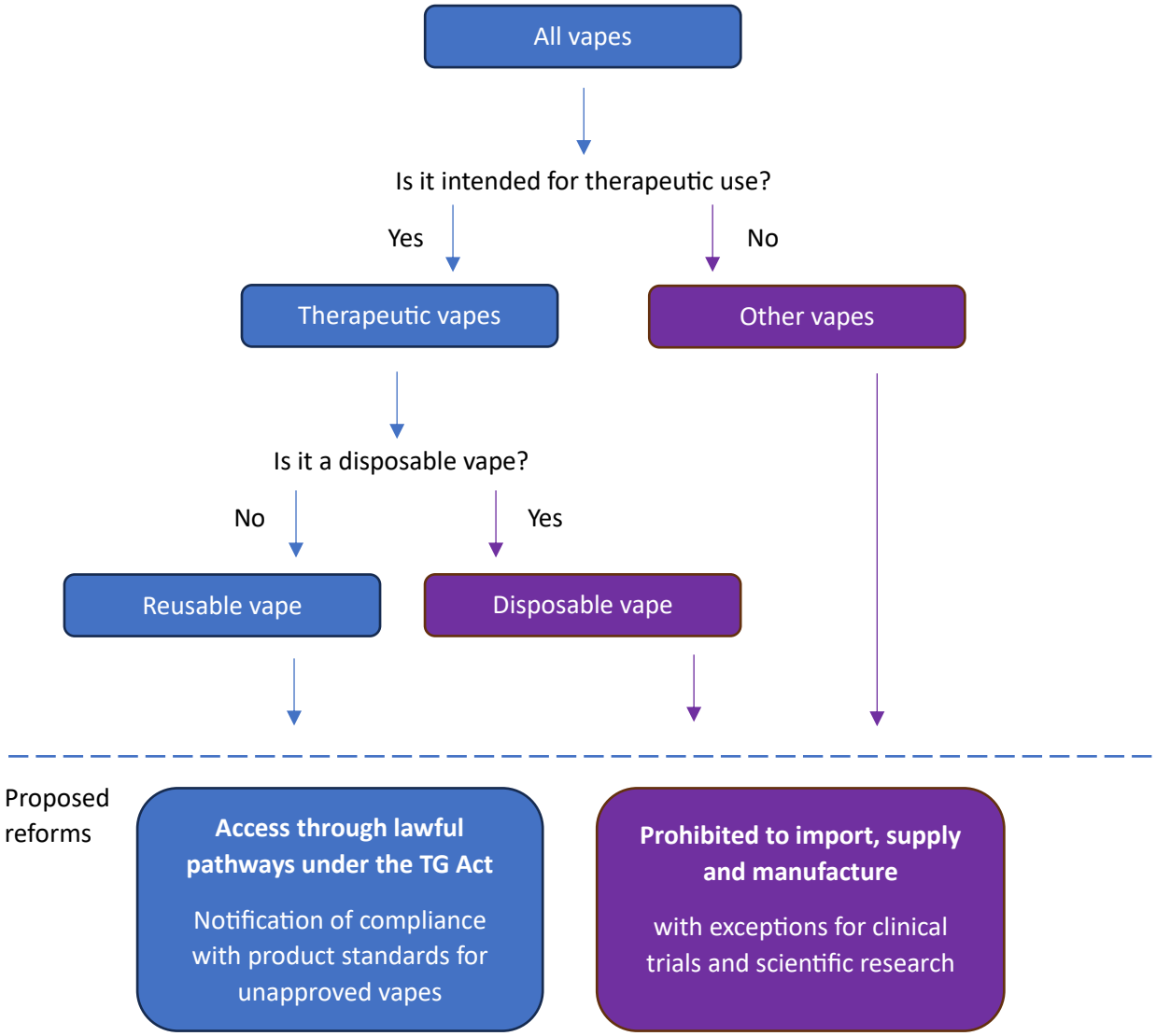
From 1 January 2024:

- The importation of all disposable vapes is banned, with very limited exceptions
- The Special Access Scheme C (SAS C) pathway is available to facilitate legitimate patient access to therapeutic vapes for smoking cessation and the management of nicotine dependence
- A form for importers and manufacturers of therapeutic vapes is available to notify the TGA about compliance with the relevant product standards prior to importation into Australia, or release for supply of vapes manufactured domestically (notices are required for goods imported or released for supply on or after 1 March 2024)
- An application form for therapeutic vape importers is available to apply for licences and permits for importing therapeutic vapes (licences and permits are required for goods imported on or after 1 March 2024).

From 1 March 2024 there is:

- A ban on the importation of all vapes without an import licence and permit from the Office of Drug Control
- A requirement for therapeutic vape importers and manufacturers to notify the TGA about compliance with the relevant product standards before importation to Australia or release for supply in Australia
- Closure of the Personal Importation Scheme for vapes
- An exemption for travellers bringing a small quantity of vapes into Australia
- Some changes to the quality requirements for therapeutic vapes for smoking cessation and the management of nicotine dependence, including restrictions on flavours to mint, menthol and tobacco.

The operation of these regulations was presented diagrammatically by the TGA as shown below:



Arguing that *Closing loopholes in Australian vaping laws: Why Australia's proposed vaping reforms are sound public health policy* Freeman, Dessaix and Buchanan[18] describe how comprehensively addressing access and supply of vaping products is crucial if Australia hopes to roll back the alarming rise in use by young people.

Passage of the Therapeutic Goods and Other Legislation Amendment (Vaping Reforms) Bill 2024 means that from 1 October 2024 pharmacists can sell vapes up to 20mg/ml strength to adults after deeming the use 'clinically appropriate'. Prescriptions are required for under 18s and for strengths >20mg/ml. It also standardises branding, include graphic warnings on vape packaging, and restricts additives to the product.

Despite pharmacies being permitted to sell vapes to individuals without a prescription, media reports as of 2 October 2024 suggest that many pharmacists are hesitant to stock the devices (edition.smh.com.au/shortcode/SYD408 2 October 2024, p12).

E-cigarettes as a prescribed therapeutic product

This assessment of e-cigarettes and cancer is not intended to address the worth of e-cigarettes, if any, as a therapeutic support for smoking cessation in the general population. That matter continues to be

subject to investigation. One particular aspect of this matter concerns COSA, and many COSA members, directly.

Smoking cessation subsequent to a cancer diagnosis is established as contributing to increased survival. Knowledge concerning use of e-cigarettes in that context, including perspectives held by both oncology clinicians and cancer survivors is a particular consideration. Short of a finding that e-cigarettes are innocuous in relation to any carcinogenic risk, any imputation regarding such a risk may be subject to qualification in respect of the usage of e-cigarettes by cancer survivors. This matter is addressed in the next section.

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4. E-cigarettes and cancer survivors

People who smoke and are diagnosed with cancer of any type or site survive longer if they quit. At present, data from the USA indicates that only a small proportion of cancer survivors use vapes compared with those who smoke. Perceptions concerning e-cigarettes have altered from notions of safety to being as harmful as smoking, though perceptions don't align with behaviour. There are few policy statements about e-cigarette usage by cancer patients undergoing active treatment or long-term survivors, and scant guidance for oncology clinicians as to whether e-cigarettes have worth.

Possible use of e-cigarettes by cancer patients as a means of facilitating smoking cessation has been subject to discussion in the peer-reviewed literature and the matter is of immediate relevance to COSA. Indeed, because of its multidisciplinary engagement, COSA is in a unique position to make an informed assessment of findings.

Consideration of whether there is any basis for endorsing e-cigarette usage as of benefit to cancer patients wishing to quit raises the issue of whether e-cigarettes are of benefit in smoking cessation generally. The question may be addressed in relation to multiple considerations, primarily with reference to multiple criteria for assessing cessation but also with reference to ongoing nicotine addiction. The results of relevant trials continue to be reported. Multiple reviews and meta-analyses are published each year.

No attempt will be made in this report to evaluate the worth of e-cigarettes for smoking cessation in the wider community. The matter is contentious. There are at least some assessments that are positive,[1] but many qualify a clear endorsement.[2-4]

Benefits to cancer patients from smoking cessation after diagnosis

In the context of a Position Statement on embedding smoking cessation care in Australian oncology health services, in 2020 COSA summarised evidence that smoking cessation benefits cancer patients.[5] The adverse impact on treatment of continued smoking after a cancer diagnosis continues to be documented. The following is restricted to some of the studies published since the COSA Position Statement was published.

Smoking cessation following cancer diagnosis is beneficial to lung cancer patients; the cancer patients who must be anticipated to include a marked proportion of lifelong smokers. Following review and meta-analysis, Caini et al.[6] reported in 2022 that quitting smoking at or around diagnosis was significantly associated with improved overall survival (summary relative risk: 0.71, 95% CI 0.64–0.80) consistently among patients with non-small cell lung cancer and small cell lung cancer.

Smoking by cancer patients has an adverse effect on clinical care, and this scenario is not restricted, or indeed primarily an issue, for lung cancer patients specifically. Smoking adversely affects the outcomes from treatment irrespective of tumour type. Smoking cessation after diagnosis increases patient survival.[7]

Smoking and vaping by cancer survivors

A proportionate number of adults diagnosed with cancer are smokers. While Australian data are not readily available, assessments of smoking and vaping among cancer survivors have been published regularly.

In the USA the prevalence of e-cigarette use among adult cancer survivors was lower than the general population when assessed in 2016.[8] 2.8% of cancer survivors reported currently using e-cigarettes and an additional 6.3% had previously used e-cigarettes but were not currently using them. Use of e-cigarettes was most common among cancer survivors who currently smoked cigarettes: 34.3% of current smokers

were ever e-cigarette users and 15.6% were current e-cigarette users, compared with former smokers (2.7% ever and 1.4% current e-cigarette users) and never smokers.

Sanford et al.[9] examined trends in e-cigarette use by patients compared with smoking and other nicotine-related options. Although the rates of smoking remained stable from 2014 to 2017 (50.7% to 51.9%), the prevalence of e-cigarette use increased from 8.5% to 10.7%.

In a USA patient survey involving 26,742 respondents aged 18 years or older published in 2020, current smoking prevalence was higher among smoking-related cancer survivors compared with non-smoking-related cancer survivors (19.78% vs 10.63%). After cancer diagnosis, the odds of continued cigarette smoking were twice as high among those with smoking-related cancers compared with those with non-smoking-related cancers.[10]

Based on a cross-sectional analysis involving 12,984 cancer survivors, those with tobacco-related cancers, when compared with survivors of other cancers, had a significantly higher prevalence of current smoking (18.2% compared to 9.7%) and e-cigarette use (2.7% compared to 1.6%).[11]

Streck et al.[12] observed in 2023 that among cancer patients generally, there is scant transition to e-cigarettes by smokers. Among patients with cancer (typically 59 years old, 70% male), and with a mean time since cancer diagnosis of 26 months, 21% smoked while markedly fewer used smokeless tobacco (5%), cigars (4%) or e-cigarettes (2%).

A 2024 review and meta-analysis by Lopez-Olivo et al.[13] reported that in the USA, the pooled rate of lifetime e-cigarette use among cancer survivors was 15% (95% CI 6-27%); current use was 3% (95% CI 0-8%). Among survivors who currently smoked, 63% also vaped (so-called 'dual use'). The rates of lifetime e-cigarette use differed between age groups (18-44 years, up to 47%, 45-64 up to 27% and >64 years up to 25%). E-cigarettes were used as a quit resource by 75% of survivors reporting dual use.

Most cancer patients are diagnosed after age 50 years. In contrast to e-cigarette usage by typical cancer patients – that is by middle-aged to elderly adults – one study has addressed e-cigarette usage by young adult cancer patients and a different situation is evident.[14] Patients aged 18-39 years with a cancer history were more likely to report having ever used e-cigarettes than their peers without a cancer history (total with cancer history, 1,444, any use, 658 (46.7%) compared with a total with no cancer history, 53,487, any use, 20,517 (39.1%). The investigators found disproportionally higher rates of vaping among young adult cancer survivors across nearly all demographic subgroups.

Perceptions regarding e-cigarettes

Typically, in the period a few years after their widespread availability, examinations of how e-cigarettes were perceived focused on the general population. In an online survey published in 2012,[15] 40.2% of respondents had heard of e-cigarettes, with awareness highest among current smokers. Utilisation was higher among current smokers (11.4%) than in the total population (3.4%), with 2.0% of former smokers and 0.8% of never-smokers. The authors note that consistent with and extending previous work, their results show that e-cigarettes are perceived as less harmful than combustible cigarettes.

However, since 2020, the overwhelming focus of studies on perceptions regarding e-cigarettes have involved attitudes and beliefs held by youthful populations. Diverse, to the point of contradictory, findings have been reported as typified by the following publications.

As published in 2023,[16] a sample of 4,617 Australians aged 12+ years completed an online survey that assessed (i) smoking and e-cigarette user status, (ii) e-cigarette risk perceptions, (iii) beliefs about e-cigarettes as a smoking cessation tool, and (iv) positive e-cigarette outcome expectancies. Among the findings, significantly more adolescents and young adults than adults held positive outcome expectancies,

and a substantial minority of non-users and never users in all age groups believed that using e-cigarettes confers social and mental health benefits. The authors conclude that these findings highlight the importance of (i) adequately communicating the risks associated with non-nicotine e-cigarette use and (ii) addressing misperceptions about use, especially among youth.

In 2022[17] an online USA national survey of adolescents and young adults (n=4,315, age 13-24 years, 50% never users) to address perceptions of risk, including respiratory problems, was reported. Among those surveyed who had ever used e-cigarettes, those who did not believe that such usage increases the risks of respiratory problems were more likely to have used e-cigarettes in the past month.

Burnley et al.[18] examined how individual e-cigarette use perceptions differ between adolescents based on e-cigarette use status and susceptibility to future use of e-cigarettes. E-cigarette use perceptions among youth differ by e-cigarette use and susceptibility status. In general, risk-related use perceptions around e-cigarette use were endorsed more often than benefit-related use perceptions.

Most recently in 2024, Toll et al.[19] delineated considerations regarding e-cigarettes for healthcare providers in the USA. Young people regarded tobacco products (taken to include e-cigarettes) as unsafe. The authors note that many people in the USA believe e-cigarettes are just as harmful, if not more harmful than cigarettes, with only 11.4% thinking otherwise. These perceptions were shared by physicians.

Policy statements regarding patient needs

Authoritative policy statements concerning e-cigarettes in a general context have been made in the USA. However, a 2014 policy statement of the American Heart Association[20] and the *Updated Policy Statement from the American Association for Cancer Research and the American Society of Clinical Oncology* published in 2022[21] do not address the specific issue of e-cigarettes for patients with either heart disease or cancer. Likewise, from the perspective of thoracic medicine, an assessment of 'what the healthcare provider needs to know' is concerned with community needs rather than patient needs.[22] Similarly, when the regulation and incentivisation of e-cigarettes across 97 countries were investigated, the term '(cancer) patient needs' was not among those specified as a basis for assessment.[23]

In Australia, the TGA has offered 'practical guidance' to medical and nurse practitioners on the use of vapes for smoking cessation or the management of nicotine dependence, available at tga.gov.au/resources. Comprehensive statements about e-cigarettes are exemplified by those from the Thoracic Society of Australia and New Zealand.[24] Clearly, policy statements of this type are not the source of recommendations to oncology healthcare professionals specifically concerning any possible role for e-cigarettes in patient care.

Guidance for oncologists

Ten years ago, a commentary titled *E-cigarettes and cancer patients*[25] was introduced with the following perspective.

In the absence of sufficient evidence that e-cigarettes are effective and safe for treating nicotine dependence in cancer patients, the International Association for the Study of Lung Cancer advises against recommending their use at this time. However, this recommendation may change if new data become available.

No update or change in recommendations is immediately evident.

One assessment in relation to lung cancer patients was provided in 2017. Dautzenberg and Garelik[26] advised that based on current knowledge, for patients with lung or other forms of cancer who would otherwise continue to smoke, e-cigarettes offer an alternative way to quit smoking while they undergo

medical treatment. However, there is no body of publications addressing an evidence-based position on the use of e-cigarettes by cancer patients.

Assessing e-cigarettes and cancer risk, Mravec et al.[27] include a discussion under 'E-cigarettes and cancer patients', noting that patients with cancer may assume that e-cigarettes are almost harmless from the point of view of their disease. However, if patients with cancer are informed that even if e-cigarettes are a less hazardous alternative to smoking, the risk of potentiating the progression of their disease by e-cigarettes still exists, this might motivate some patients with cancer to quit smoking.

Most participants in one survey of cancer patients who vape (n=121) identified smoking cessation as the reason for initiating (81%) and continuing (60%) e-cigarette use.[28] However, 51% of patients reported current dual use of combustible cigarettes and e-cigarettes, and most patients reported never having discussed their use of e-cigarettes with their oncology provider (72%).[28]

D'Angelo et al.[29] reported on e-cigarette use at 42 NCI-designated Cancer Centers and established that 25 Centers (60%) assessed e-cigarette use in the first half of 2019, increasing to 30 in the last half of 2019. Of these, 17 Centers assessed smoking status at every patient visit while 6 assessed e-cigarette use at every visit. The authors concluded that their study identifies a gap in the systematic assessment of e-cigarette use among patients seen at NCI-Designated Cancer Centers.

Clinician attitudes

Oncology clinicians are not wholly convinced enough to advocate e-cigarettes to patients, specifically in UK. Members of the British Thoracic Oncology Group (n=154) explored perceptions of patient e-cigarette use, practitioner knowledge regarding sources of guidance pertaining to e-cigarettes, and practitioner advice. Practitioners frequently observed e-cigarette use among patients with lung cancer. The majority of practitioners (81.4%) reported responding to patient queries pertaining to e-cigarettes within the past year; however, far fewer (21.0%) felt confident providing patients with e-cigarette advice.[30]

Clinician surveys in UK indicate diversity of opinion concerning e-cigarettes. Brett et al.[31] reported that 29% of 506 clinicians involved in adult cancer care responding to an online survey would not recommend e-cigarettes to patients with cancer who continue to smoke. Lung cancer specialists in Korea doubted the safety of e-cigarettes and use of e-cigarettes as smoking cessation treatment, and supported strict regulation.[32]

In UK general practice, a qualitative analysis found barriers obstructing clinicians and patients from easily accepting e-cigarettes for harm reduction, rather than as aids to support smoking cessation: clinicians had difficulty reconciling harm reduction with their existing ethical models of practice, even following targeted training, and patients saw e-cigarettes as quitting aids.[33]

As indicated by one study,[34] physicians do not routinely assess e-cigarette use among patients and reported that discussions were often initiated by patients. In this USA study a minority of participants discussed e-cigarettes in conjunction with other best-practice recommendations for smoking cessation.

Patient beliefs and experiences

As might be expected, the clear majority of studies addressing user (or potential user) attitudes and beliefs concerning e-cigarettes have addressed this matter in relation to youth in the general community. In relation to cancer patients, two studies were identified.

Adult patients being treated at a Florida cancer hospital in 2016-2017 characterised e-cigarettes as less addictive, less expensive, less stigmatizing, and less likely to impact cancer treatment than combustible

cigarettes (Ps < .05), and more satisfying, more useful for quitting smoking, and more effective at reducing cancer-related stress than nicotine replacement therapies.[28]

Antwi and Rhodes[35] examined the association between e-cigarette use and self-reported clinical depression in cancer survivors (7,498) among whom 22.1% reported a history of clinical diagnosis of depression. Analysis showed 52% of current users, 40% of former users and 19% of those who had never used e-cigarettes reported clinical depression.

Conclusions and implications

Smoking cessation by cancer patients is desirable to reduce the adverse impact of smoking-related toxins on the efficacy of virtually all treatments variously available to patients diagnosed with any form of cancer; reducing the likelihood of a second primary cancer attributable to smoking is not an immediate or assessed benefit to cancer patients who quit.

To the extent that e-cigarettes have been characterized as 'safer' than smoking on the basis of the nature and level of toxins variously inhaled by vapers compared to smokers, qualified endorsement by oncology clinicians for their patients transitioning from tobacco smoking to e-cigarette usage might have been anticipated. Rather than this being the situation, our literature search indicates that oncologists and cancer professionals have not engaged with this matter. Surveys of cancer patients provide little, if any evidence that those who smoke are turning to e-cigarettes.

With reference to all issues addressed in this section, there are insufficient publications to allow description of findings particular to Australia.

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5. Recognition and assessment of carcinogenicity data

Almost from their introduction, the potential of e-cigarettes to cause cancer has been notified, initially because aerosol components include known carcinogens. To evaluate the particular spectrum of carcinogenicity data for e-cigarettes, qualitative risk assessment affords the best option. Certain restrictions are specified: peer reviewed publications as the only source; tobacco industry publications not addressed and risk attributable to using both cigarettes and vapes at the same time is beyond scope.

E-cigarettes and cancer as currently addressed

The prospect of e-cigarettes presenting a carcinogenic hazard because users are exposed to a range of carcinogens is not a recent development. Fifteen years ago in 2009, the results of a US Food and Drug Administration (FDA) analysis of two widely marketed e-cigarette products were reported in the authoritative *Journal of the American Medical Association* because these devices may contain some of the same toxic or carcinogenic compounds as traditional cigarettes.[1] The commentary explained that e-cigarettes are battery-powered devices that vaporize nicotine, flavouring, and/or other chemicals into an inhalable vapour. Chemical analyses of several samples of products by FDA scientists detected tobacco-associated chemicals that may be harmful to humans, including known human carcinogens.

The prospect that e-cigarettes present a carcinogenic hazard to users has been a specifically recognised aspect of the public health assessment of e-cigarettes ever since. Thus commentaries published since 2020 on the adverse health impact of e-cigarettes consistently include a specific section concerning cancer.[2-4] By sharp comparison with the initial focus on exposure on e-cigarette users to known carcinogens, contemporary commentaries include cellular and molecular evidence of tissue injury consistent with, if not indicative of, malignant transformation.

This report addresses a wide spectrum of investigations calculated to provide insight regarding the possible carcinogenic risk posed by e-cigarettes.

Relevant publications have involved monitoring of carcinogen exposure consequent upon vaping (section 6) and tissue injury which has been evidenced by clinical examinations and determination of tissue and cellular harm using biomarkers with reference to the lung, oral cavity and bladder (section 7). Evidence of carcinogenicity also includes tests (bioassays) using experimental animals and carcinogen-related mechanistic indicators which are documented with reference to the 'key characteristics of carcinogens'[5] as used in IARC *Monographs* (section 8). These key characteristics include carcinogen metabolism, DNA damage and repair, oxidative stress, inflammation and changed cell growth characteristics among others.

The carcinogenicity of e-cigarettes remains an immediate concern, as the matter was reviewed in 2023.[6] Newer insights are exemplified by the cancer-related effects of increased activation of the sympathoadrenal system induced by the inhalation of nicotine, which, in the view of Mravec et al.[7] have been completely overlooked.

Evaluating the carcinogenicity of complex mixtures, exemplified by tobacco smoke or diesel emissions, requires reference to effects mediated by the mixture taken together with findings that have been made in relation to individual agents contained in the mixture. Knowledge of the chemical components, and specifically the recognised carcinogens, in e-liquids and inhaled aerosols is crucial to a reasonable interpretation of tissue injury and adverse health outcomes which are attributable to e-cigarettes.

Substances inhaled by e-cigarette users

Tobacco smoke, the major carcinogen in most countries by population-attributable risk,[8] is a mixture of several thousand chemicals a number of which are known to be carcinogenic to humans, including

polycyclic aromatic hydrocarbons, nitroso derivatives of nicotine and nornicotine, and certain aromatic amines. The potential carcinogenicity of emissions from e-cigarettes gives rise to the need for a comparable analysis.

There is comprehensive literature concerning analytical procedures and the usage of valid procedures to assess the levels of particular chemicals in aerosols generated by various types of e-cigarettes. Such levels vary, for example, in respect of the composition of e-liquids and the coil temperature used for volatilization.[9] With all such research and variable parameters acknowledged, the present summation is restricted to a qualitative specification of the chemicals which have been identified and their source in respect of chemicals not specified as a component of e-liquids.

The e-liquid is composed of polypropylene glycol and/or vegetable glycerin containing nicotine, and a multitude of different flavouring agents. These include aldehydes (vanillin, vanilla; benzaldehyde, berry/fruit; cinnamaldehyde, cinnamon; damascenone, tobacco), benzyl alcohol, terpenes (linalool, flowery; farnesol, apple), pyrazines (coffee, chocolate), menthol, menthone, and other minty compounds, and sweet flavours, including ethyl maltol.[10, 11]

Because of its biological activity and physiologically-significant concentration, nicotine is an immediate concern when considering the toxicology of e-cigarette aerosols. Nicotine itself also has potent potential carcinogenic activity by means of its conversion to nitrosamine compounds – especially nitrosamine ketone and nitrosonornicotine.[12] This does not infer that usage of nicotine-free devices are of no interest in the present context.

Analyses of e-liquids revealed traces of aldehydes such as acrolein, formaldehyde and acetaldehyde, and some metals.[12] These aldehydes, together with the volatile organic compounds toluene and xylene, have been subsequently detected in e-cigarette emissions.[13] Formaldehyde and acetaldehyde formation from the heating of glycerol and propylene glycol during vaporization, and these compounds may also be generated from flavouring agents.[14]

The presence of heavy metals in the aerosol of an e-cigarette is highly dependent on the e-liquid which may contain cadmium, lead, nickel, copper, arsenic and/or chromium. During vaporisation, there may be transfer of nickel from the device coil to the aerosol.[15]

Beyond the findings outlined above, this report will not provide details concerning the chemical analysis of e-liquids and e-cigarette aerosols. Rather, the discussion of exposure in the next section will involve clinical and physiological evidence of exposure, particularly in regard to carcinogen metabolites as measure.

Qualitative risk assessment

Evaluation of carcinogenicity data primarily involves two considerations: hazard identification and risk assessment.

Hazard identification addresses the matter of whether an agent is capable of causing cancer in humans.[16] Relevant findings may include evidence from epidemiological data indicating that humans known to have been exposed to the agent exhibit an increased incidence of cancer, or rodent bioassay data indicative of or establishing carcinogenicity. Other findings may include evident carcinogenicity by related agents, encompassing, for example, related chemical structure or biological type.

Critical to hazard identification is the notion that capacity to cause cancer is the only consideration; particular circumstances of human exposure are not addressed (irrespective of what may be evident from epidemiological findings which have been considered). Specification of one or more cancer types (or 'target organs') is not required and, in theory, is not involved.

Risk assessment, in contrast, must involve evidence of human exposure, which may involve environmental monitoring and/or physiological determinations. Quantitative risk assessment is the most definitive determination because it involves numerical expression of risk. Clearest evidence is from epidemiological studies which, in addition to having the potential of numerically determining an increased risk, almost invariably establishes risks related to a particular tumour type/s.[17]

Qualitative assessment of carcinogenic risk is predicated on consideration of relevant carcinogenicity and exposure data. It does not involve or require a numerical expression of risk. Once evidence of carcinogenicity and evidence of exposure have been appraised, a qualitative assessment of the carcinogenic risk may be made. A key element in quantitative risk assessment, dose–response, is not taken into account as a separate parameter in qualitative risk assessment.[18-20]

This determination of the carcinogenicity of e-cigarettes is undertaken as a qualitative risk assessment. Therefore, the determination will go beyond hazard identification, and make reference to a specific mode of exposure and will address specific organ sites. However, in respect of exposure, no quantitative risk assessment will be undertaken.

A qualitative approach to e-cigarette-related harm has been previously published.[21] Commitment to a qualitative risk assessment in the present context has ramifications in respect of exposure data in the present context. Specifically, unless qualitative differences are demonstrated, distinction between different types of vapes is not required and only relevant if a particular study is confined to, or deemed to only apply to a particular design of vape. Likewise, findings made by investigators which are predicated on, or deemed only applicable to a particular level of vaping (for example, in terms of number of occasions per day) do not require integration into any overall determination.

Search procedure

Concerning the potential carcinogenicity of e-cigarettes, the state of knowledge and scope of findings are not amenable to determination by meta-analysis. Indeed, while many relevant reviews with titles including ‘e-cigarettes’ or ‘ENDS’ have been published, none are designated as a meta-analysis with reference to, for example, particular biomarkers.

No single search term recovered the bulk of publications cited in this report. A multiplicity of terms were used. Primary search terms were generated by combining ‘e-cigarettes’, ‘electronic delivery systems’ or ‘ENDS’ with terms identifying an affected organ or tissue, nicotine and the chemical components or categories of components in e-cigarette aerosols, all relevant biomarkers of exposure, all relevant biomarkers of harm, and each of the 10 ‘key characteristics of carcinogens’. Subsequent searches involved terms indicated from the title of papers identified by primary searches such as ‘*in vivo*’, ‘*in vitro*’, ‘clinical’, ‘genetic’ and so on. Recourse was made to publications cited in recent reviews, together with relevant ‘Special issues’ of particular journals addressing e-cigarettes across different disciplines or specialised readerships.

Qualitative risk assessment is not predicated on due reference to all publications recovered using a particular search term/s. This report is predicated on a reasonable assessment of journal publications addressing each subsection heading and immediately related matters. No tabulation of all research papers addressing particular types of investigation was made. The availability and scope of such tables in reviews is recorded. Consistently in relevant reviews, however, such tables only served to achieve comprehensive citation, while failing to provide insight beyond what was evident from individual investigations.

Priorities concerning relevant journal publications

Research findings about e-cigarettes have been prominent since 2009, and reviews of the available data (including in some cases, cancer as a foreseeable risk) have been published regularly. No attempt has been made to trace the development of particular lines of investigation from the outset till now. To that extent, earlier reviews must be consulted to determine who first notified particular determinations or specified relevant concerns. This report is focused on results and reviews published since 2018.

Primary focus was on publications since 2018; no attempt was made to establish 'first reporting' except where such context provided specific insight.

Short of documenting all available publications addressing a particular issue, certain individual studies are cited, and findings summarised. The purpose of these summations is to provide insight concerning the determinations that underlie broader conclusions. Where possible, use is made of reviews to indicate the scope, often in terms of the number of publications, of particular lines of investigation. Where summations or conclusions from available data are required, one or more assessments from relevant review/s are provided.

As noted in section 4, the term 'dual use' refers to people who vape while continuing to smoke. Dual use is particularly identified with a failed attempt at smoking cessation and is subject to extensive investigation.[22, 23] There is evidence that such dual use may result in worse toxin exposure than smoking alone.[24] Dual use is evident in cancer survivors and has been addressed in that context.

In assessing the carcinogenicity of e-cigarettes however, no reference is made to findings involving dual use. Recognised exposure to tobacco smoke obviously compromises any inference that could be made concerning the impact of e-cigarettes in this context. Data on dual use may be included in particular cited publications, but relevant findings are not recorded here and have not been considered in respect of any determinations that have been made concerning carcinogenicity.

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6. Physiological evidence of exposure to carcinogens

On the basis of levels of the respective compounds and/or their metabolites in tissue and biofluids, people who vape are exposed to carcinogens, such as formaldehyde, acetaldehyde, acrolein and acrylonitrile, as well as to other carcinogens, such as the nitroso-derivatives of nicotine, propylene oxide derivatives and heavy metals. Many exposure studies have included data from smokers, but investigators are increasingly identifying exposure to e-cigarette aerosols as an issue in its own right.

This section is restricted to physiological evidence of exposure to e-cigarette aerosols as that matter is required for a qualitative risk assessment of the carcinogenicity of e-cigarettes. Accordingly, an examination of all circumstances of exposure has not been made. Specifically, investigations of foetal exposure and 'second hand' exposure to e-cigarette aerosols have not been addressed.

User-exposure to a range of chemicals can be anticipated following chemical analysis of e-liquids and the aerosols generated by and emitted from e-cigarettes. The previous section outlined the detection and monitoring of known or likely human carcinogens in that context. Regarding exposure to such agents, a separate and more immediate body of research involves measurements made in the tissue or body fluids of people who have used e-cigarettes. These parameters are often categorised as 'biomarkers of exposure'.

Many such biomarker studies of exposure involving e-cigarette users have compared findings for vapers and smokers. This includes studies provided by the tobacco industry exemplified by *Biomarkers of Tobacco Exposure Decrease after Smokers Switch to an E-Cigarette or Nicotine Gum.*[1] Other such publications[2] are found through relevant searches. Subject to being detected, industry studies are not addressed in this report.

Relatively few exposure studies are focused on, or even incorporate, a comparison between people who use e-cigarettes and those who don't: the *a priori* basis for any assessment of exposure to e-cigarette aerosol. However, this latter comparison will be the focus for data summation on exposure and other parameters in this report, with, at most, secondary attention being paid to data for smokers where such findings are provided in relevant studies.

Categories of carcinogens implicated

Nicotine and its metabolites

E-cigarettes may be perceived as a means of inhaling nicotine without the other toxins in tobacco or generated by combustion. The manufacturers intend that e-cigarette users will inhale nicotine at physiologically effective concentrations to achieve the neurophysiological outcome experienced by smokers. Accordingly, concerning potential carcinogen from e-cigarettes, the immediate issue is nicotine.

In brief, nicotine is an alkaloid that is secreted from plants of the nightshade family as an insecticide. Nicotine binds to nicotinic acetylcholine receptors. Certain neuronal excitatory effects are down-regulated by chronic exposure.[3] Relevant receptors rapidly desensitise upon nicotine binding, and upregulation or desensitisation leads to nicotine craving.[4]

In humans, nicotine is predominantly metabolised to cotinine. Although both nicotine and cotinine levels can be measured in biofluids and tissues, the longer half-life of cotinine (16 to 18h compared to 2h for nicotine) make this metabolite the more attractive biomarker of nicotine exposure.[5]

There is no simple relationship between the concentration of nicotine in e-liquids and the intake of nicotine from e-cigarettes as measured by blood levels. The amount of nicotine received by vapers is influenced by many factors including e-liquid composition, user behaviour and device characteristics.

Notably, open-tank (refillable) e-cigarettes can deliver high nicotine levels to consumers, sometimes at greater doses than received by smokers.[6]

Nicotine in e-liquids can exist in a free-base or protonated (salt) form, the latter being less aversive and favoured by manufacturers seeking to deliver high nicotine concentrations. In a clinical trial, acid additives in e-cigarettes that change nicotine from free base to salt appeared to enhance the appeal and sensory experience of vaping.[7] Christen et al.[8] reported that free-base 20 mg/ml formulations achieved lower blood nicotine concentrations than nicotine salt 20 mg/ml, while 40 mg/ml nicotine salt yielded concentrations similar to cigarette smoking. With patented nicotine salt technology, JUUL devices dominate the USA e-cigarette market.[9]

The complex relationship between nicotine levels in e-liquids and biofluids may involve compensatory behaviour. Among e-cigarette users who opted to reduce the concentration of nicotine in their e-liquid over time, blood level determinations indicated that over 12 months they maintained their nicotine intake possibly through self-titration via more intensive puffing.[10]

Singh et al.[11] recorded plasma cotinine levels of 165 $\pm$ 40 ng/ml in e-cigarette users which were significantly higher than that in non-smokers (4 $\pm$ 3 ng/ml). Other such studies focus on comparisons between vapers and smokers. For example, Rapp et al.[12] analysed 428 participants, 379 (87%) of whom smoked and 49 (13%) smoked e-cigarettes. Serum cotinine levels increased linearly with consumption in both e-cigarette and tobacco smokers.

The 2021 review by McDonough et al.[13] includes tabulation of six studies of cotinine levels based on levels in either serum or urine from e-cigarette users. They conclude that collectively these data show that it is possible to reliably differentiate nicotine-containing e-cigarette users from non-users based on cotinine levels, but question whether such data allow differentiation between vapers and smokers.

Since that review, another study determined serum cotinine values in samples from smokers (n=112), e-cigarette users and controls which, after adjustment for age and gender, showed no difference in the levels for smokers and vapers.[14]

Concerning urinary cotinine, as reviewed in 2023 by Goniewicz[15] in respect of difference between e-cigarette users and non-users, the respective geometric means were 124.3 as against 0.42 ng/mg creatinine; a difference in excess of 100-fold.

N-nitroso derivatives of nicotine

Studies of the tobacco-specific nitrosamines in biofluids predominantly involve *N'*-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridul)-1-butanone (NNK) as indicated by its metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL). Full information including their generation from nicotine and metabolism of NNN and NNK is published by the International Agency for Research on Cancer (IARC) in the IARC *Monographs*, available at monographs.iarc.who.int.

Studies monitoring these nitroso compounds in urine from e-cigarette users almost invariably involve comparison with smokers, and the perspective of harm reduction. Thus in 2015, levels of NNAL and other carcinogen metabolites were primarily described as significantly lower in e-cigarette users compared to smokers.[16]

With samples from 20 participants, Bustamante et al.[17] reported that the mean of NNN in saliva of e-cigarette users was 14.6 (±23.1) pg/mL. Total findings including data from smokers prompted them to conclude that NNN is formed endogenously in e-cigarette users.

Goniewicz et al.[18] involved 5,105 participants aged 35-54 years in a study of nicotine and toxicant exposure. Compared with exclusive e-cigarette users, never users had 19% to 81% significantly lower

urinary concentrations of biomarkers of exposure to nicotine and tobacco-specific nitrosamines. These investigators also concluded that NNN is formed endogenously in vapers. In a 2023 review, Goniewicz[15] determined that geometic mean urinary levels of NNAL for e-cigarette users and non-users were 4.887 and 0.921 pg/mg creatinine respectively.

A randomised clinical trial compared six weeks of e-cigarette use with no change in smoking habit among African American and Latinx adult smokers. Those randomised to e-cigarette usage maintained their cotinine levels and significantly reduced urinary NNAL levels compared with continuing smokers.[19]

Eight studies reporting urinary levels of NNN or NNAL in samples from e-cigarette users are tabulated in a 2021 review.[13] The findings of each of these studies are almost invariably summarised as recording lower levels in vapers compared to smokers. In respect of tobacco-specific nitrosamine measurements among other indicators, a national cohort study provides evidence of harm reduction associated with transitioning from exclusive cigarette use to exclusive e-cigarette use, indicating a lower ***relative risk*** (bold italics in the original).[20]

Volatile organic compounds

E-cigarette users are exposed to multiple volatile organic compounds which are identified in this context as toxins that can cause injury to oral and respiratory tissue.[21] In this report, distinction is made between toxins and carcinogens as is indicated in the title of some studies.[22]

Volatile organic carcinogens inhaled from e-cigarettes are not declared ingredients of e-liquids, but typically arise as degradation products. Benzene can be formed from e-cigarette constituents such as benzoic acid. Propylene oxide can be formed by thermal degradation of propylene glycol. The carbonyl compounds, namely acetaldehyde, acrolein and formaldehyde, are mainly formed through a thermal decomposition of glycerol and propylene glycol, the two common aerosol formers present in e-liquids.[23]

Multiple toxins and/or their metabolites have been detected in the urine of e-cigarette users, although this does not establish the source of agents which include acrolein, benzene, 1,3-butadiene, formaldehyde, ethylene oxide, *o*-toluidine, trichloroethylene, acrylamide, N.N-dimethylformamide, styrene, acetaldehyde, acrylonitrile, 1-bromopropane crotonaldehyde and propylene oxide. All of these chemicals have been evaluated as carcinogens by IARC *Monographs* (monographs.iarc.who.int). All of these chemicals occur in tobacco smoke and for many, exemplified by acrolein and crotonaldehyde, tobacco smoke is the major source of community exposure. Similar assessments are now made with reference to e-cigarette aerosols.[23]

Many determinations of exposure biomarkers for these agents in vapers involve comparison with smokers. Thus, Dai et al.[24] monitored such biomarkers in three mutually exclusive groups: smokers, e-cigarette users, and dual users. There was a significant reduction in urinary concentrations of volatile organic compounds (among other classes of agent) when users transitioned from exclusive cigarette to exclusive e-cigarette use. Shahab et al.[25] made similar findings.

In contrast, the biological impact of e-cigarettes in their own right was addressed by Samburova et al.[26] who measured levels of carbonyls in exhaled breath of e-cigarette users while they were vaping and estimated the uptake of specific aldehydes, including formaldehyde and acetaldehyde. Levels of aldehydes and methyl ethyl ketone were significantly higher (2–125 times) in exhaled e-cigarette breaths than before vaping started. The mean retention of formaldehyde in the respiratory tract was 99.7% for all participants, while acetaldehyde retention was 91%.

In a study involving adolescents, Rubinstein et al.[27] contrasted findings for groups which among others included e-cigarette only users and ever-user controls. Excretion of metabolites of acrylonitrile, acrolein, propylene oxide, acrylamide, and crotonaldehyde were significantly higher in e-cigarette–only users

compared with controls, indicating that young vapers should be warned of potential risk due to carcinogenic compounds from e-cigarettes.

On volatile organic compounds in a 2023 review of biomarkers of exposure in e-cigarette users,[28] thirteen studies addressed acrolein, 10 were on 1,3-butadiene, nine on acrylonitrile, eight on acrylamide and on crotonaldehyde, and seven were on benzene, with lesser number of studies for other compounds. No agent or small group of agents were identified as being of primary concern.

Polycyclic aromatic hydrocarbons (PAHs)

PAHs, which include pyrene, identify a class of potent carcinogens accounting in a major way for lung and other cancers in humans as a result of smoking together with a range of occupational exposures. In cigarettes, these carcinogens are generated by combustion and, to that extent are carcinogens which users of e-cigarettes might be expected to avoid *a priori*. In commentaries on chemical exposure from e-cigarettes, PAHs are often distinguished from volatile organic compounds.

By comparison with smokers, markedly less exposure to PAHs in vapers has been confirmed. Thus, in an early study, significantly lower levels of urinary 1-hydroxypyrene, a metabolite and hence a biomarker of pyrene exposure, were recorded for e-cigarette users than for smokers.[16] However, never users have been reported to have 20% significantly lower urinary concentrations of 1-hydroxypyrene than e-cigarette-only users.[18]

Few studies have addressed human biological indicators of exposure to multiple PAHs. Monitoring exposure to nicotine and selected toxicants in smokers who switched to vaping, urine samples of 20 smokers collected before and two weeks after switching were analysed, among other indicators for metabolites of four PAHs: naphthalene, fluorene, phenanthrene, and pyrene. Levels of some such metabolites did not change but others significantly decreased.[29]

Wang et al.[30] quantified seven PAH urinary biomarkers in 8,327 participants categorised as never tobacco user (n=1,700), current combustible products user (n=5,767) and exclusive non-combustible products user (n=860), the latter including smokeless product user (n=509) and e-cigarette user (n=280). Among many comparative assessments made, levels of 3-hydroxyfluorene and 1-hydroxypyrene were significantly higher in e-cigarette and in smokeless product users than in never users.

Metals

E-cigarettes are a potential source of exposure to toxic metals, specifically Cr, Ni, and Pb, and to metals that are toxic when inhaled, namely Mn and Zn.[31] More than six other metals are also implicated. Metal exposure from e-cigarettes is thought to come in part from the heating coils as well as from soldered joints and other metallic components of these devices. A 2023 investigation found wide variability in aerosol metal concentrations within and between the different e-cigarette device types, brands, and flavours.[32]

Among other matters, Sakamaki-Ching et al.[33] sought to determine if increased e-cigarette usage was associated with elevated metal exposure as assessed in urinary samples from non-users (n=20), e-cigarette users (n=20) and smokers (n=20). Zinc was significantly elevated in the electronic cigarette users (585 µg/g) compared with non-smokers (414 µg/g).

Prokopowicz et al.[34] examined blood Cd and Pb levels in non-smokers and e-cigarette users who had switched from cigarettes for $\geq$ 6 months and determined significantly higher blood Cd levels in e-cigarette users in comparison to non-smokers. Another study analysed urinary levels of several metals and reported significantly higher geometric mean concentrations of Cd and Pb, 23% and 19% respectively in e-cigarette users by comparison with never users.[18]

Reviewing metal/metalloid exposure from e-cigarettes in 2020, Zhao et al[35] noted studies of levels in biosamples (urine, saliva, serum, and blood) of e-cigarette users. Most levels were similar or higher than levels found in biosamples of smokers. Other authors concluded that due to the serious health effects associated with metal exposure, and the limited data available, metals as biomarkers of e-cigarette use is an area of research warranting additional attention.[13]

Flavouring agents

Flavours in e-cigarette liquids are recognised as having the potential to increase aerosol toxicity via vaporization, sometimes accompanied by chemical transformation.[36] In one survey a total of 126 flavour chemicals were detected in 103 bottles of e-cigarette refill fluid.[37] A total of 28 flavour chemicals were present at concentrations ≥1 mg/ml in at least one product, and six of these were present at concentrations ≥10 mg/ml. The levels of furaneol, benzyl alcohol, ethyl maltol, ethyl vanillin, corylone, and vanillin were significantly correlated with cytotoxicity.

Aldehydes originating from flavouring agents influenced the activity of cytochrome P450-dependent monooxygenases, in particular by inhibiting cytochrome P450 2A6, which is involved in the oxidative metabolism of nicotine. This inhibition can lead to disruption of nicotine metabolism and a putative increase of plasmatic nicotine levels with a worsening of the addiction state as well as toxicological aspects related to nicotine intake.[38]

Pulegone, a constituent of oil extracts prepared from mint plants, including peppermint, spearmint and pennyroyal, is a carcinogen that causes hepatic carcinomas, pulmonary metaplasia, and other neoplasms on oral administration in rodents. Analysis suggests that users of mint- and menthol- flavoured e-cigarettes are exposed to pulegone levels higher than smokers of menthol cigarettes.[39]

Despite recognition of the potential toxicity and carcinogenicity of flavouring agents or their derivatives, as indicated by publications cited in the three paragraphs above, there are very few, if any, investigations predicated on the identification of relevant chemicals or their metabolites in the biofluids of vapers.

The validity of comparisons with smoking

Assessing exposure data

As described in this section, the evidence that chemicals in e-cigarette aerosols as inhaled by vapers are detectable in tissues and biofluids is definitive. These findings are consistent with analyses of e-liquids and the corresponding aerosols as documented in respective publications.

The amount of vape-related toxins absorbed and/or metabolised may be anticipated to vary with the type of e-cigarette used and options taken by users.[40, 41] Such variation, and indeed variation between levels of relevant metabolites across various studies are acknowledged but are not relevant to a qualitative risk assessment.

Research discussed in this report does not include studies conducted by the tobacco industry. With that exclusion specified, none of the investigations referenced here suggest that the levels of exposure to toxins or carcinogens associated with vaping are trivial or inconsequential in relation to any cancer risk.

Studies of relevant biomarkers provide far greater insight regarding exposure than might be inferred from analyses of e-liquids and e-cigarette aerosol. While levels of PAHs (or relevant metabolites) were consistently higher in smokers, this was not necessarily the case for nicotine and its nitroso derivatives, heavy metals and some volatile organic compounds. Moreover, irrespective of this particular comparative assessment, none of the investigators cited here used the word ‘safe’ (or derivatives of it) to characterise e-cigarettes in light of their determinations. On the contrary, the investigators consistently called for research to identify possible adverse outcomes, specifically including cancer.

Proceeding from exposure to carcinogenic risk

As generated by combustion, tobacco smoke is a mixture of known carcinogens including PAHs and the nitroso-derivatives of nicotine recognised as causing cancer in smokers together with many others chemicals known to be carcinogenic in contexts apart from smoking.[42] Tobacco smoking remains the leading cause of cancer globally, far surpassing any other carcinogen in terms of its impact on human health.

In assessing the toxicology of aerosols from e-cigarettes, comparison with tobacco smoking has influenced research design, data collected and interpretations offered. Thus, for example, a study of urinary metabolites was titled *Comparison of systemic exposure to toxic and/or carcinogenic volatile organic compounds during vaping, smoking, and abstention*.[22] Likewise, an understanding of respiratory injury was framed with reference to *Biomarkers of potential harm in people switching from smoking tobacco to exclusive e-cigarette use, dual use or abstinence*.[43] When reviewing investigations of exposure biomarkers for e-cigarettes, Goniewicz[15] observed that when compared with smokers, exposure is significantly reduced in exclusive e-cigarette users.

The issue in question is not whether studies should reference data concerning smoking while assessing e-cigarettes. The issue is whether reference to data for smokers should be the primary criterion for evaluating the health impact of e-cigarettes. The issue is readily resolved. There is no basis in toxicology or public health for such a comparison to be the basis upon which any adverse health effects of e-cigarettes should be assessed.

The question of whether the carcinogenic impact of e-cigarettes should be assessed with reference to conventional cigarettes arises from investigations providing relevant findings. The answer is clear. There is no basis in biomedical science or public health for such a comparison to be an aspect of any determination of the adverse health effects of e-cigarettes.

In 2022 Vivarelli et al.[38] reviewed the toxicity of e-cigarette usage with a focus on pathological cellular mechanisms. They state “The problem is not whether or not e-cigarettes are as harmful as tobacco smoking; the key question is whether or not these new devices are harmful by themselves”.[38] Data presentation and analysis in this report is oriented toward this perspective. In particular, findings made about smoking are a secondary consideration in relation to whether e-cigarettes are carcinogenic.

Wrong risk assessments based on exposure

Decades ago, the ‘tar content’ of tobacco smoke according to cigarette brand name was published.[44] Though no quantitative relationship between tar content and risk of lung cancer was ever specified, lesser risk was imputed to low tar cigarettes, though such brands were never endorsed as ‘safer’. Even so, the inadequacy of this whole approach emerged,[45] particularly following the marketing of so-called ‘light’ or ‘mild’ cigarettes: a perception, that the amount of carcinogen inhaled is proportional to the carcinogenic risk, is wrong.[46, 47]

This intuitive, but flawed understanding has re-emerged. In a series of annual determinations, a 2015 assessment of e-cigarettes by Public Health England specified, as one of eight key messages that “the current expert estimate that using e-cigarettes is around 95% safer than smoking”.[48] This risk assessment was based on the level of smoking-related carcinogens anticipated to be inhaled by e-cigarette users in comparison with smokers. Subsequent Public Health England reports do not quantify the safety of e-cigarettes in this manner, but the damage has been done through the initial publicity.

The ‘95% safer’ determination received contemporary criticism in a Lancet editorial which addressed broad issues including lack of expertise and conflict of interest.[49]

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7. Tissue injury and case reports indicative of carcinogenic risk to humans

Inflammation and toxic injury to oral and respiratory tissue from vaping is evident histologically. A broad range of clinical studies have monitored biomarkers of harm in people using e-cigarettes. Molecular endpoints have included indicators of DNA damage, oxidative stress, epigenetic change, inflammation and immunosuppression. Corresponding reviews from 2018 to 2024 have involved an unequivocal expression of concern specifically in relation to carcinogenesis.

In 2020, under the title *The Evolving Landscape of e-Cigarettes: A Systematic Review of Recent Evidence*, a group of 10 investigators from Australia made a comprehensive assessment which was published in the journal *Chest*.[1] It included a determination of the question "Is there any direct evidence of harm or benefit to humans?", the question being primarily addressed in a table of clinical studies variously categorised as addressing general health, cardiovascular, oral health, poisonings, respiratory, neurological, vascular and blood marker, auditory, urine marker, infection and cancer markers. Such a listing is evocative of the categories of toxins inhaled by e-cigarette users.

While dealing with a wide spectrum of potential diseases which may be attributable to using e-cigarettes, the review under consideration was published with the by-line 'Thoracic Oncology'.[1]

In the same manner as the variety of toxins inhaled by vapers was acknowledged and then consideration was restricted to carcinogens in the context of exposure in section 5 of this report, the scope of disease outcomes implicated or anticipated by clinical studies of e-cigarette users is recognised here. However, nomination of those studies which are relevant to cancer is equivocal because cancer is often specified as one of multiple disease outcomes. Consequently, this section is presented in two parts: one to include description of tissue injury which may foreshadow cancer possibly together with other diseases and the second with reference to cancer-related biomarkers specifically.

Tissue Injury predisposing to cancer development

The scope of injury and disease attributable to e-cigarettes

In common with studies on exposure, investigations of tissue injury caused by vaping have often involved, and sometimes have been limited to, comparisons with smoking. In the assessments made in this section of available data, priority is given to comparing findings from e-cigarette users with corresponding data from never users, notwithstanding that findings for smokers are recorded in some of the relevant papers. Very few, if any, relevant studies distinguished between different types of e-cigarette design.

The scope of tissue injury caused by e-cigarettes is extremely broad. A 2022 review[2] notes that e-cigarettes contain alarmingly high levels of carcinogens and toxicants that may have long-lasting effects on the lung and other organ systems. The authors tabulate multiple studies variously implicating risk of neurological disorders, lung cancer, cardiovascular disease and tooth decay. Different components of e-cigarettes, including food-safe liquid solvents and flavourings, can cause health issues related to pneumonia, pulmonary injury and bronchiolitis.

When focusing on tissue injury that may predispose to malignant transformation almost all relevant studies are concerned with the lung and oral cavity.

EVALI: a complicated subject matter

The characteristic pattern of respiratory pathology identified by the USA Center for Disease Control (CDC) as 'E-cigarette and vaping associated lung injury' with the acronym EVALI is now widely-recognised.[3]

EVALI is characterised by a sterile exogenous pneumonitis like reaction with substantial involvement of innate immune mechanisms. In that assessment, EVALI was initially attributed to nicotine, flavour products and/or tetrahydrocannabinol (THC) oils. EVALI is described as involving (i) lipoid pneumonias, (ii) acute eosinophilic pneumonias, (iii) pneumonias with pleural effusion, (iv) acute pneumonitis, (v) respiratory bronchiolitis, (vi) interstitial lung disease, (vii) bronchiolitis obliterans organizing pneumonia and (viii) diffuse alveolar haemorrhages.

The majority of USA patients with EVALI report vaping with what are described as THC-containing counterfeit street e-cigarette products.[4] The term 'counterfeit street cartridges' is used to describe adulterations made by the use of cutting agents, such as medium-chain triglyceride oil, vitamin E (tocopherol) acetate. Thus CDC notification of 2,561 cases of confirmed and probable EVALI in the USA included a majority of male patients (67%) without prior medical problems and usage of THC was overwhelmingly prevalent.[5] Similar associations of THC vaping with EVALI have been made.[6]

In a landmark study, Blount et al.[7] established that EVALI is attributable to inhalation of vitamin E acetate in THC-containing vapes, noting that certain findings relating to respiratory injury by e-cigarettes may be caused by THC-containing aerosols rather than nicotine-containing products.

Accordingly, youth vaping and the THC-related EVALI cases may be identified as two separate epidemics.[8] Reviewing the toxicology of e-cigarettes in 2022, Gordon et al.[9] advise that lung injury associated with the EVALI epidemic should be evaluated separately from the adverse effects associated with vaping e-liquids containing nicotine and flavours. Consistent with that approach, studies focused on EVALI are not further addressed in this section.

Respiratory tissue injury attributable to e-cigarettes

In a study whose design was typical of many, Ghosh et al.[10] performed research bronchoscopies on healthy non-smokers, smokers and e-cigarette users and observed that vaper airways appeared friable and erythematous. Approximately 300 proteins were differentially expressed in smoker and vaper airways, with 113 uniquely altered in vapers, suggesting injury mediated in part by propylene glycol/vegetable glycerine.

Many relevant reviews of tissue injury caused by vaping have been published. Addressing the question of "What are the respiratory effects of e-cigarettes?" in 2019, Gotts et al.[11] concluded that current knowledge of these effects is insufficient to determine whether the respiratory health effects of e-cigarettes are less than those of combustible tobacco products. Likewise in 2020 Miyashita and Foley[12] assessed e-cigarettes and respiratory health by describing three aspects: disruption to lung function and gas exchange, impaired respiratory immunity and host defence systems, and respiratory inflammation and injury.

McDonough et al.[13] tabulate data from seven publications describing inflammation associated with e-cigarette use. Proteases, including neutrophil elastase and matrix metalloproteases, modulate cell signalling, inflammation, tissue remodelling, and leukocyte recruitment via cleavage of their target proteins. In a 2022 review, Park[6] notes that vaping results in the generation of increased reactive aldehyde species causing the cellular accumulation of 4-hydroxynonenal, which induces apoptosis, mitochondria dysfunction, and protein inactivation. Exposure to e-cigarettes induces secretion of proinflammatory cytokines, including interleukin (IL)-1β, IL-6, IL-8, and tumour necrosis factor alpha from epithelial cells and immune cells in the upper airway and lung parenchyma.

While a growing body of experimental and population-based evidence links vaping and e-cigarette components with adverse respiratory effects specifically including COPD and asthma, questions remain regarding the pulmonary toxicity profile of e-cigarettes relative to that seen in smokers.

Thus, for example, Ghosh et al.[14] report that neutrophil elastase, matrix metalloproteases activities and protein levels were equally elevated in both vapers' and smokers' bronchoalveolar lavage relative to non-smokers. They concluded that vaping induces nicotine-dependent protease release from resident pulmonary immune cells and hence the risk of developing chronic lung disease; vaping may not be safer than smoking.

Oral pathology

In 2023 Cameron et al.[15] aim to summarise the relevant evidence to better inform practitioners about the deleterious effects of vaping on oral health and the risks of oral cancer, so practitioners can better inform their patients. They conclude that e-cigarette use is not risk-free. Vaping can induce various patterns of tissue injury, including dysbiosis, inflammation, periodontal diseases, deterioration of dental and gingival health, and changes to the oral microbiome.

Biomarkers of malignant transformation

Background: Recognition of a subset of biomarkers of harm and how they have been assessed

With reference to tobacco products specifically, biomarkers of exposure may be distinguished from biomarkers of potential harm, which in respect of e-cigarettes may be recognised to implicate cardiovascular disease, chronic obstructive pulmonary disease and cancer. Such biomarkers are established or reasonably likely surrogate endpoints, for use in assessing pharmacodynamic responses, and reliability for predictive purposes.[16] Another term used in this context is 'biomarkers of systemic toxicity'.[13]

In this assessment, the focus is on biomarkers with an immediate relevance to malignant transformation. Such cancer-related biomarkers largely coincide with biological processes identified as key characteristics of carcinogens, and of chemical carcinogens in particular.[17] The key characteristics were proposed as a means of organising mechanistic data in the context of the IARC *Monographs* (monographs.iarc.who.int). These biological responses, depending on context, may be identified either as biomarkers of harm and/or key characteristics of carcinogens. They include evidence of, or the capacity to bring about DNA damage, oxidative stress, cellular immortality and altered cellular growth characteristics. When identified as a subset of currently-recognised biomarkers of harm, this subset is described here as biomarkers of malignant transformation.

This part of section 6 is concerned with biomarkers of malignant transformation as studied in humans, typically by epidemiology. In section 7, mechanisms of carcinogenesis will be examined with reference to all 10 of the key characteristics as subject to laboratory evaluation in respect of e-cigarette aerosols and with reference to particular chemicals in those aerosols.

As has been noted in other contexts, a major part of e-cigarette research involving biomarkers of malignant transformation is concerned with differentiating results for e-cigarette users from those for smokers and/or for dual users. Thus, one review of such findings concludes that switching from smoking to vaping or dual use appears to reduce levels determined in 12 out of 13 biomarkers of potential harm.[18] However in the present context, more important than any comparison with determinations in smokers, is whether biomarkers of harm assessed in vapers provide evidence of chronic biological change which may foreshadow disease.

Accordingly, the focus here will be on data differentiating the effects of e-cigarettes from determinations made in controls who are often identified as non-smokers. Data involving smokers who have switched to e-cigarettes, though sometimes included in studies under consideration, will not necessarily be recorded.

DNA damage

DNA damage, and specifically detection and monitoring of carcinogen metabolites covalently bound to the purine and pyrimidine bases in DNA ('adducts'), may be used as an indicator or biomarker of exposure as well as a biomarker of harm. The presence of DNA adducts is equally an indicator of harm, specifically in relation to genotoxicity. The following summation addresses all indicators of DNA damage apart from damage attributable to oxidative stress which is discussed separately.

Cheng et al.[19] analysed oral cell DNA adducts in samples provided by e-cigarette users and non-users over a three-month period, quantifying a specific adduct derived from acrolein. The median value in users was 179 fmol/µmol deoxyguanine (dG) while that in non-users was 21 fmol/µmol dG, the strongly significant difference demonstrating, according to the authors, that e-cigarette users have elevated levels of a carcinogen-DNA adduct in their oral cells.

In 2023 Guo and Hecht[20] reviewed DNA damage attributable to e-cigarettes. They tabulate 10 studies concerning the oral cavity, and variously focused on mucosa, saliva, oral epithelium, buccal cells and the products of cytobrushing. Indicators of DNA damage include specific adducts, apurinic sites (that is, loss of adenine or guanine) and micronuclei. The authors conclude that e-cigarette users have significantly higher levels of DNA adducts and meta-nuclear anomalies.

Oxidative stress

As noted while introducing a 2022 review of how smoking and vaping impact oxidative stress, the homeostasis of oxidation-reduction reactions is fundamental in biological processes and is maintained at a stable but non-equilibrium steady state.[21] A deviation from the metabolic steady state, which varies according to cellular location, leads to the concept of oxidative stress, as describing an imbalance between oxidants, such as reactive oxygen species. There are multiple indicators of such imbalance.

Toxins of various types enhance oxidative stress. Comparisons between tobacco smoke and e-cigarette aerosol shows that the latter releases lower levels of toxic compounds such as formaldehyde, acetaldehyde, acrolein and toluene, reactive oxygen species and non-negligible levels of potential carcinogens, heavy metals, tin, flavouring together with propylene oxide derived from heating propylene glycol. Concerning e-cigarettes, oxidative stress has been primarily investigated in relation to cardiovascular disease, with a specific focus on endothelial tissue. However, also recognised is a clear body of oxidative stress research undertaken with reference to carcinogenic risk.[21]

Most studies of oxidative stress in relation to e-cigarettes have involved laboratory-based systems (see next section), but some clinical investigations have been published. Almost all of these studies utilise the prostaglandin 8-oprostane (8-iso) which is a by-product of lipid peroxidation and a biomarker of oxidative stress and/or 8-hydroxy-2'-deoxyguanosine (8-OHdG), a well-established marker of oxidative DNA damage.

Singh et al.[22] found significantly elevated levels of urinary 8-iso for e-cigarette users compared to non-smokers. Likewise, Sakamaki-Ching et al.[23] reported a significant increase in 8-iso urinary levels for e-cigarette users (750.8 ± 433 pg/mg) when compared with non-smokers (411.2 ± 287.4 pg/mg, p=0.03). They also found that that 8-OHdG was significantly elevated in vapers on the same basis and increased e-cigarette usage (as measured by cotinine) was correlated with elevated urinary metal concentrations, which were correlated with oxidative DNA damage.

The differential effects of e-cigarette vehicles propylene glycol and glycerol, and nicotine in e-cigarettes was studied in a randomised blind crossover design study on multiple indicators including oxidative stress. In this regard, the principal finding was that vaping with nicotine raised plasma myeloperoxidase levels markedly; no differences between results for propylene glycol and glycerol were found.[24]

In review, McDonough et al.[13], referring to six tabulated studies in humans, concluded that when taken together, the results clearly show increased oxidative stress in e-cigarette users in comparison with non-smokers.

Epigenetic change

Delineation of findings concerning epigenetic changes in human tissue from vapers as described in this part involves specification of indicative studies.

DNA methylation

The commonest biomarker of epigenetic change evaluated in relation to the impact of e-cigarettes has been patterns of DNA methylation.

Because of its relative stability, methylation status at cg05575921 may serve as a biomarker for combusted tobacco smoke exposure. Some studies involve determinations in respect of a single specific DNA sequence. A study cited previously in respect of cotinine (a nicotine metabolite) determinations also reported that smoking was associated with a dose dependent demethylation of cg05575921, while e-cigarette usage did not.[25]

Patterns of methylation of the repetitive elements of *LINE-1* (*Long interspersed nuclear element-1*, a retrotransposon DNA sequence) were used to provide the first evidence of the association between vaping and DNA methylation loss in humans from peripheral blood.[26] The results showed a significant decrease in methylation of vapers by 18% and smokers by 13%, compared to control non-smokers, with no significant difference between vapers and smokers. Using the same basis for assessing loss of methylation, Camila et al.[27] differentiated vapers from controls, noting that the differences were reflected in representative RNA expression.

Herzog et al.[28] evaluated effects of tobacco or e-cigarette use on genome-wide DNA hypermethylation using over 3,500 buccal/saliva, cervical, or blood samples. The 535 identified smoking-related DNA methylation loci included detoxification or growth signalling, based on cell type and anatomical site. Hypermethylation of these sites taken together with other data predicted lung cancer development in buccal samples collected from smokers up to 22 years prior to diagnosis. Alarmingly as specified by the authors, these sites were also hypermethylated in e-cigarette users with a limited smoking history.

Altered transcription

As is typical of most research addressed in this report, many studies on the impact of vaping on gene expression are predicated on comparisons with smoking. Despite this transcriptional changes attributable to vaping by dint of determinations made in non-users can be discerned.

Thus, one clinical study involved collecting superficial nasal scrape biopsies, nasal lavage, urine, and serum from non-smokers (n=13), cigarette smokers (n=14), and e-cigarette users (n=12).[29] Smoking cigarettes or vaping e-cigarettes resulted in decreased expression of immune-related genes. Genes with decreased expression in smokers were also decreased in e-cigarette users. Additionally, vaping was associated with suppression of a large number of unique genes.

Following analyses of bronchial epithelial cells from groups of about 10 vapers, smokers and former smokers, e-cigarette usage was reported to have induced both distinct and shared patterns of gene expression relative to smoking.[30]

The tumour suppressor TP53 was significantly upregulated in buccal samples from vapers.[31] Song et al.[32] provided evidence that e-cigarette usage alters gene expression in a pattern similar to smoking. For 93% of these differentially expressed transcripts, gene expression levels in e-cigarette users were in between those of never-smokers and smokers, with certain transcriptional change being particular to e-cigarette users.

Any inference of a differential effect of e-cigarette usage on gene expression, either generally or with respect to specific genes must involve consideration of data from clinical studies as typified by those described above with, in general, more extensive and detailed findings from experimental systems as details in the next section.

Two reviews of epigenetic changes mediated by e-cigarettes published in 2021 indicate other endpoints that have been examined in this context.[33 34] Clearly, at the present state of knowledge, epigenetic change cannot be related to the likelihood of malignant transformation as can genotoxic effects. However, there is increasing evidence that carcinogens induce such effects and their role in carcinogenesis is gradually being elucidated.[35]

Inflammation

Endpoints addressed by molecular biomarkers described in this part of section 6 are typically molecular in character: DNA damage, indicators of oxidative stress, and epigenetic change indicated by DNA methylation patterns and altered transcription. The exception is inflammation which is primarily a cellular rather than a molecular phenomenon and relevant morphological change attributable to e-cigarettes has been delineated in the earlier part of this section addressing toxic injury. However, inflammation may be monitored by molecular indicators. Studies of the impact of e-cigarettes in humans using molecular markers of inflammation are outlined below.

As summarised in a review of the toxicological impact of e-cigarettes,[13] there are several pro-inflammatory molecules that are established biomarkers of inflammation and are also associated with smoking. These molecules include cytokines such as interleukins IL-6, IL-8 (also specified as CLCL8), IL-13 and IL-1β together with interferon, tumour necrosis factor (TNF)-γ, chemokines such as monocyte chemoattractant protein-1 and proteases, most commonly matrix metalloproteinases (MMPs). In common with the observation made concerning oxidative stress and its biomarkers, the inflammation biomarker variations are associated with multiple diseases apart from cancer including COPD and cardiovascular disease. Thus, some studies on inflammatory responses to e-cigarettes are focused, for example, on endothelial function.[36]

Singh et al.[22] found significantly higher levels of IL-6, IL-8, IL-13 and MMP-9 in the plasma of e-cigarette users in comparison with non-smokers. This study also found IFN-γ and IL-1β levels to be significantly higher in the urine and saliva respectively of e-cigarette users. Another study using bronchoalveolar lavage fluid from never smokers, e-cigarette and smokers found levels of IL-1β and IL-6 to be significantly higher in e-cigarette users than never-smokers.[32]

As distinct from studies involving adults, among non-smoking young people (mean age 28.7 years) acute exposure to e-cigarettes elevated C-reactive protein levels.[37]

Studies addressing inflammation biomarkers in vapers by comparison with corresponding data for smokers and related groupings have been undertaken. Christensen et al.[38] examined biomarkers of inflammation and oxidative stress across 3,712 adult participants. Biomarkers were similar between former smokers who currently use e-cigarettes and both former smokers who do not use any tobacco and never tobacco users, but among these groups most biomarkers were lower than those of current smokers. The authors concluded that exclusive e-cigarette users have biomarker concentrations that are similar to those of former smokers who do not currently use tobacco, and lower than those of smokers.

Introducing a 2024 review of inflammation biomarker changes in healthy adults secondary to e-cigarette use, Boss et al.[39] specify that to their knowledge, no review has summarised or categorised changes in inflammatory biomarkers after e-cigarettes. Their review is oriented toward cardiovascular disease, but the essential observations made are independent of that consideration.

Having assessed 37 studies that met the inclusion criteria, usage of e-cigarette containing nicotine for more than one month produced mixed results.[39] Two commonly measured inflammation biomarkers, matrixmetalloproteinase-9 and IL-6, were elevated in 75% and 60% of measured instances, respectively. These results aside, however, use of nicotine-containing e-cigarettes resulted in no significant changes in general inflammatory biomarker levels.

Immunosuppression

Multiple clinical studies have shown that use of e-cigarettes adversely affects the immune system. However, these investigations are not oriented or evaluated with reference to carcinogenic risk, but address susceptibility in infection[40] or asthma.[41] Beyond citing these two reviews, no attempt will be made to summarise relevant studies here.

Case reports

Case reports concerning e-cigarettes cover a broad spectrum. Issues frequently addressed include poisoning by e-liquids, exploding devices, burns, tissue injury and a variety of non-malignant diseases. A 2016 review categorised such case reports with reference to respiratory, gastrointestinal, cardiovascular, neurological or immune systems together with mechanical injury, nicotine poisoning and intentional misuse.[42] The following account of e-cigarette-related case reports is restricted to cancer.

Nguyen et al.[43] report two cases of oral carcinoma associated with chronic use of e-cigarettes. First, a 66-year-old male presented to the out-patient office (otolaryngology) with chief complaints of unintended weight loss, dysphagia and xerostomia. His past medical history was unremarkable other than a social history positive for use of e-cigarettes (20 times per day for past 13 years). After a comprehensive clinical investigation as detailed in the publication, a diagnosis of basaloid squamous cell carcinoma was made.

Second, a 59-year-old male complained of a nine-month history of a non-healing ulceration of the lower lip and, in the absence of any other relevant information, reported that he had smoked 30 e-cigarettes per day for the past 13 years. The patient's health history was otherwise unremarkable. Again, after a detailed investigation, a diagnosis of basaloid squamous cell carcinoma was made.

In describing these two cases, the authors concluded that patients and clinicians, including physicians, dentists and nurses, need to be aware that the use of e-cigarettes may be associated with an increased risk of oral cancer, as smoking is proven to be.

Klawinski et al.[44] describe the case of a 19-year-old male with an extensive history of nicotine-based vaping who developed an aggressive, poorly responsive HPV-negative squamous cell carcinoma of the oral cavity. There were no other carcinogenic risk factors. The authors observed that, given the rarity of this disease in patients of this age and the absence of HPV infection, this case suggests that e-cigarette use may pose a carcinogenic effect and may lead to the development of oral cavity cancer.

No comparable publications in respect of lung cancer were discovered. However, three cases in Germany of acute pulmonary illness that were considered to have been caused by the use of e-cigarettes have been documented. In the first two cases, acute pulmonary injury was very likely due to e-cigarette use, as all other possible causes were ruled out. A possible link to e-cigarette use was present in the third case.[45]

Studies in humans contributing to carcinogenic risk assessment

In the absence of definitive epidemiological data

When evaluating carcinogenicity data, studies in humans primarily involve determinations of increased risk of particular tumour type/s in populations known or determined to have been exposed to the agent

under consideration. Such findings are not available for e-cigarettes because of multiple considerations including the short passage of time since widespread usage was evident and difficulty in identifying vapers who have never smoked. Such limitations to the design of informative epidemiological investigations of putative carcinogenic hazards are not particular to e-cigarettes.

Because convincing demonstration of increased cancer risk associated with, and indeed, attributable to exposure to particular chemicals or living under particular circumstances takes decades to accrue, the notion of identifying and then using cancer biomarkers has been embraced. Studies of cancer biomarkers in vapers, as typified by those documented and/or included in reviews as described in this section may well be recognised as confirming the utility of cancer biomarkers, particularly when account is taken of the views now expressed by respective investigators.

Vaping compared to smoking

Even when the influence of the tobacco industry mediated by contributions to the medico-scientific literature is put aside, the notion of vaping as presenting less harm than smoking permeates much of the research cited in this section. The 'better than smoking' perspective may be understandable, since smoking is the major preventable cause of premature disease and death and any option that may avert such morbidity and mortality is worthy of note. However, presenting a positive aspect on vaping because the habit may not result in the greatest known burden of preventable disease and death is poor public health practice, to say the least.

In respect of e-cigarette-induced tissue injury, either as assessed morphologically or determined using biomarkers, there are multiple instances where determinations for vapers resulted in values less than those for smokers. There are also instances where differences between the respective outcomes were not evident, or even when the results for vapers were 'worse'. Against this background, examination of all the relevant publications indicates a reluctance of investigators to extrapolate their findings and speculate about the prospect of a decreased risk of smoking-related cancers through the advent of vaping. On the contrary, investigators indicate their awareness of tissue damage and biological changes evident in the vaper arm of the study and the need for caution. Rarely, if ever, do such assessments made of data delineated in this section include the word 'safer'.

Vaping considered a hazard in its own right

Many of the studies described in this section contrast determinations made in respect of vapers with those made for people who do not use e-cigarettes, the latter often identified as controls or non-smokers. This contrast is rarely addressed with reference to amounts of chemicals inhaled when vaping. However, tissue injury and perturbations in cancer biomarkers prompt investigators to note the need to assessing vaping as hazardous to health irrespective of any comparison with smoking.

Determining the carcinogenicity of e-cigarettes

As indicated in section 5, though focused on specific studies published since 2018, this report is not comprehensive to the extent of acknowledging every relevant publication. Rather, individual studies are summarised to indicate the character of investigations on hand and recourse is made to relevant reviews to offer a perspective based on the totality of available data, whether cited here or otherwise.

Evidence in humans of tissue injury and biological outcomes including DNA damage, oxidative stress and epigenetic change is one aspect of the qualitative risk assessment of e-cigarettes as carcinogenic to humans. In this, and any assessment of carcinogenicity, account must also be taken of relevant bioassays using experimental animals and results of laboratory investigations indicative of the mechanism/s of carcinogenesis that may be operative. These findings in respect of e-cigarettes are summarised in the next section.

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8. Animal testing and mechanistic evidence of carcinogenicity

E-cigarette aerosol is carcinogenic to experimental animals. In a single study of inhalation exposure for 54 weeks, about a quarter of exposed mice developed lung adenocarcinoma. Mechanistic data reviewed with reference to the 'key characteristics of carcinogens' reveal clear positive findings for electrophilicity, genotoxicity, DNA repair, inflammation and cellular immortalization, with some evidence for epigenetic change, oxidative stress and altered cellular behaviour.

Studies in experimental animals

From inception in 1972, versions of the Preamble to IARC *Monographs* have consistently included a statement to the effect that for practical purposes, agents demonstrated to be carcinogenic in animal bioassay may be regarded as carcinogenic to humans (monographs.iarc.who.int). Studies of e-cigarette aerosols and nicotine carcinogenicity using experimental animals include bioassay and experimental protocols having endpoints apart from cancer development.

Carcinogenicity bioassays

Tang et al.[1] tested the carcinogenicity of e-cigarette aerosol in mice. One group (n=45) was exposed to e-cigarette aerosol generated from e-liquid (nicotine [36 mg/mL] dissolved in a vehicle polypropylene glycol and vegetable glycerin at a 1:1 ratio]). The second group (n=20) was exposed to vehicle alone. Aerosols for both groups were generated using a 3-port e-cigarette aerosol generator set at a constant voltage, the same as used in commercial e-cigarettes. Mice were subjected to whole-body exposure. Mice were exposed for 4 h per day and 5 d per week for 54 weeks. The third group (n=20) were exposed to the ambient filtered air.

Upon examination, nine of 40 (22.5%) mice exposed to e-cigarette aerosol developed lung tumours. All lung tumours, subjected to histological examination by three pathologists, were identified as adenocarcinomas. Although no visible tumours were detected in the urinary bladders of any of the experimental groups, hyperplastic changes to the bladder urothelium were evident in mice exposed to e-cigarette aerosol (23 of 40 mice, 57.5%).

Several of the compounds present in e-cigarette aerosols have been shown to be carcinogenic in experimental animals. These chemicals include NNN, NNK, acrolein, benzene, 1,3-butadiene, formaldehyde, ethylene oxide, *o*-toluidine, trichloroethylene, acrylamide, N,N-dimethylformamide, styrene, acetaldehyde, acrylonitrile, 1-bromopropane, propylene oxide, nickel metal and some of its salts and certain chromium (VI) compounds. Details of all such bioassays are recorded in the respective IARC *Monographs* (monographs.iarc.who.int).

Nicotine has not been subject to a bioassay for carcinogenicity in experimental animals.

Other endpoints

Inhalation exposure of rats to e-cigarette aerosol for four weeks caused hyperplasia and metaplasia of the laryngeal mucosa, but this was not significant statistically.[2]

Glynos et al.[3] exposed mice for either three days or four weeks to ambient air, tobacco smoke, or different nicotine-containing e-cig aerosols and determined oxidative stress, inflammation, and pulmonary mechanics. In summary, exposure to e-cigarette aerosol can trigger inflammatory responses and adversely affect respiratory system mechanics, sometimes exacerbated by flavouring agents. The authors concluded that both vaping and smoking negatively impact lung biology.

Following inhalation of e-cigarette aerosol by rats exposed five days per week for four weeks, analysis of lung tissue indicated a booster effect on phase-I carcinogen-bioactivating enzymes and increase in oxygen free radical production and DNA oxidation to 8-hydroxy-2'-deoxyguanosine.[4]

Dobmane et al.[5] examined urothelial proliferative and cytotoxic effects after nicotine was administered in drinking water to mice and rats for four weeks. Histopathologically, seven of 10 rats and four of 10 mice showed simple hyperplasia following nicotine treatment compared to none in controls. The change was seen as possibly due to a mitogenic effect of nicotine and/or its metabolites.

Lee et al.[6] studied mice that were exposed to e-cigarette aerosol for three hours per day, 5 days per week, for 12 weeks. They directly measured DNA damage attributed to nitrosamines in different organs of e-cigarette aerosol exposed mice. Mutagenic O^6-methyldeoxyguanosines and γ-hydroxy-1,N2-propano-deoxyguanosines were detected in the lung, bladder and heart. DNA-repair activity and repair proteins XPC and OGG1/2 were significantly reduced in the lung. On the basis of these data and other *in vitro* studies, Lee et al.[6] propose that e-cigarettes, through damaging DNA and inhibiting DNA repair, might contribute to human lung and bladder cancer as well as to heart disease, although further studies are required to substantiate this proposal.

Examination has been made of the offspring from mouse dams exposed to e-cigarette aerosols during pregnancy.[7] The researchers concluded that in this mouse model, maternal exposure to e-cigarette aerosols resulted in epigenetic changes in offspring.

Carcinogenicity findings for e-cigarettes using experimental animals do not appear to have been subject to specific review, though a broader toxicological appraisal has been made. In 2020 under the title *How bad are e-cigarettes? What can we learn from animal exposure models?*, Marczylo[8] identifies a growing body of work has revealed toxicities after focusing on pulmonary, cardiovascular and central nervous systems. Observations noted include DNA damage and downregulation of DNA repair and antioxidant enzymes. However, the markers affected are often different between studies. He concludes that though vaping is much less hazardous than smoking on the basis of data considered, animal studies have identified that e-cigarettes are potentially hazardous and nicotine is integral to risk of health effects.

Laboratory data on mechanism organised using the 'key characteristics of carcinogens'

Evidence that e-cigarette aerosols, or specific compounds in these aerosols, may cause cancer is inherent in the monitoring of biomarkers of harm in people using e-cigarettes as previously described (section 7). In many instances, such evidence of molecular and cellular changes in human tissue have been complemented by laboratory studies to elucidate the nature of, for example, particular DNA damage, change in gene expression or the mediation of oxidative stress. These laboratory findings are presented in accordance with a recent (2019) innovation.

During the period 1970 to 2010, procedures for evaluating carcinogenicity data during the last fifty years have essentially involved making a distinction between agents which mutate DNA, sometimes referred to as 'genotoxicity' and those operating by some other mechanism. A more comprehensive approach to the organization of carcinogenicity data has been described on the basis of the 'key characteristics of carcinogens'.[9] These key characteristics recognize pathways to genotoxicity and also specify other pathways to cancer development including receptor-based effects and immunosuppression. These key characteristics are named with reference to properties that a particular carcinogen may exhibit, as follows:

1. Is electrophilic or can be metabolically activated
2. Is genotoxic
3. Alters DNA repair or causes genomic instability

4. Induces epigenetic alterations
5. Induces oxidative stress
6. Induces chronic inflammation
7. Is immunosuppressive
8. Modulates receptor-mediated effects
9. Causes immortalisation
10. Alters cell proliferation, cell death or nutrient supply

In respect of carcinogenicity specifically, there is some commonality between biological changes identified as biomarkers and as key characteristics. The use of one or the other of these descriptors is largely determined by context.

There is little to no prospect that a particular carcinogen will manifest all 10 key characteristics. On the contrary, these characteristics were identified to accommodate a range of recognised mechanisms of carcinogenesis. Operational incorporation of the key characteristics in the *Monograph* procedure was described in the 2019 revision of the *Monograph* Preamble. Likewise, the following presentation of mechanistic evidence for the carcinogenicity of e-cigarettes involves organisation of evidence using the key characteristics.

Publications cited below are restricted to studies involving e-cigarette aerosol or nicotine. With respect to particular chemical carcinogens present in e-cigarette aerosols, no attempt is made here to summarise findings indicative of the carcinogenicity of these particular compounds. Thus, in relation to the key characteristic involving metabolic activation to an electrophile, mammalian metabolism of the volatile organic carcinogens in e-cigarettes for example, has been widely studied prior to the production of, and/or without reference to, e-cigarettes. Such findings are not described here.

For information concerning the mechanism of action of specific carcinogenic compounds known to be present in e-cigarette aerosols, reference may be made to the 'Mechanistic and other relevant data' section of the corresponding IARC *Monograph* (monographs.iarc.who.int). In the vast majority of cases, such mechanistic data is not presented with reference to the 'key characteristics of carcinogens' since this approach to organization of these findings was not adopted in IARC *Monographs* until 2020.

1. Is electrophilic or can be metabolically activated

Indicators that a carcinogen may be metabolically activated to an electrophilic species include characterisation of a relevant metabolite as, for example, an epoxide or quinone, or the inference that such a reactive intermediate has been formed because certain DNA and protein adducts are evident. Most commonly, metabolic activation of chemical carcinogens is mediated by members of the cytochrome P-450 family of enzymes (designated by the prefix CYP).

Study of metabolic activation of carcinogens almost invariably involves investigation of individual chemicals rather than a characteristic exhibited by a complex mixture. Sun et al.[10] found that a condensate of e-cigarette aerosol enhanced metabolism of benzo[a]pyrene in a human oral keratinocyte cell line by inducing CYP1A1/1B1 mRNA and protein.

Nicotine is rapidly metabolized *in vivo* to cotinine and other metabolites, including a small portion of N-nitrosamines that may be further metabolized to methyl diazohydroxide and pyridyl-butyl derivatives.[11]

2. Is genotoxic

Genotoxicity is evidenced from DNA adducts, DNA damage (including strand breaks, DNA–protein cross-links, unscheduled DNA synthesis), intercalation, gene mutations, and cytogenetic changes such as chromosome aberrations or micronuclei.

Al-Saleh et al.[12] assayed DNA damage and chromosome breakage in human lymphoblastoid each treated with up to 33 different e-liquids. Though all the e-liquids induced DNA damage in TK6 cells, 20 resulted in cytotoxicity, the results being seen as indicative of genotoxic effects. A novel, automated, three-dimensional printed microfluidic array to demonstrate DNA reactivity related to relevant metabolites was used and showed that the genotoxic potential of tobacco and nicotine e-cigarettes was comparable when assayed by the same protocol.[13]

In vitro assessment of DNA damage attributable to e-cigarettes is exemplified by the analysis of normal epithelial and head and neck squamous cell carcinoma cell lines treated with e-cigarette aerosol extracts for 48 hours to eight weeks.[14] There was increased comet tail length and accumulation of c-H2AX foci, demonstrating increased DNA strand breaks.

Taking account of data for aerosols and also for specific chemical components therein, several reviews have assessed the genotoxicity of e-cigarettes. Armendariz-Castillo et al.[15] reviewed genotoxic and carcinogenic potential of compounds associated with e-cigarettes in 2019. Two years later, a review of cardiorespiratory and immunologic effects included the subheading 'Genotoxicity of e-cigarettes' to cover generation of DNA adducts, structural damage to DNA and impaired DNA repair, amongst others.[16] This review specifies that the genotoxic effects of e-cigarettes have been variously demonstrated, noting that this outcome may be attributable in part to nicotine.

As assessed by Guo and Hecht,[17] *in vitro* studies demonstrate that e-cigarette liquid or aerosol can induce DNA damage, oxidative stress, DNA double-stranded breaks, and genotoxicity in different types of oral cells. The corresponding clinical studies showed that e-cigarette users have significantly higher levels of N0-nitrosonornicotine, acrolein DNA adducts and metanuclear anomalies and significantly lower levels of apurinic/apyrimidinic sites than non-users.

In their review titled *DNA damage, DNA repair and carcinogenicity: tobacco smoke versus e-cigarette aerosol*, Tang et al.[18] distinguish the causative agents and DNA damage mediated by tobacco smoke from that attributable to nicotine, propylene glycol and vegetable glycerol as inhaled by vapers. Two major types of tobacco smoke DNA damage are induced by direct carcinogen aldehydes, cyclic-1,*N2*-hydroxy-deoxyguanosine (γ-OH-PdG) and α-methyl-1, *N2*-γ-OH-PdG, and these are different from adducts derived from PAHs and aromatic amines. In contrast, e-cigarette aerosols contain nicotine, propylene glycol and vegetable glycerine which result in O^6-methyl-deoxyguanosines (dG) and cyclic γ-hydroxy-1,*N2*–propano-dG.

Barhdadi et al.[19] describe *in silico* tools and literature data to identify potentially genotoxic e-liquid flavourings. Based on the analysis of 129 e-liquids collected on the Belgian market, 60 flavourings with positive *in silico* predictions for genotoxicity were identified.

3. Alters DNA repair or causes genomic instability

Indicators used in this context include alterations of DNA replication or repair as may be shown from reduced topoisomerase II, base-excision or double-strand break repair activity or of levels of the corresponding enzyme proteins.

Tang[20] notes that among other studies undertaken, nicotine and NNK reduced not only DNA repair activity, but also DNA repair proteins XPC and hOGG1/2.

4. Induces epigenetic alterations

The broad scope of epigenetic variation includes alterations in DNA methylation, histone modification and microRNA expression.

Dysregulation of 578 micro RNAs was identified following e-cigarette exposure in cultured human bronchial epithelial cells.[21] Nicotine-containing e-cigarette aerosol displayed the most profound effects upon micro RNA expression. The authors concluded that some e-cigarette-induced changes in gene expression are epigenetically programmed at the level of micro RNA regulation.

Nicotine-induced epigenetic change has been primarily addressed in relation to addiction, and that research has not been addressed here.

A relevant 2021 review concludes that by comparison with knowledge of tobacco smoke-induced epigenetic changes, very few studies have investigated vaping.[22]

5. Induces oxidative stress

Parameters of oxidative stress include the level of oxygen radicals, alternatively specified as reactive oxygen species (ROS) and various indicators of oxidative damage to macromolecules particularly including DNA where oxidative damage is most often assessed using oxidation to 8-hydorxy-2'-deoxyguanosine (8-oxo-dG).

Cells lining the oral cavity and respiratory tract are exposed to a range of environmental oxidants including ozone, nitrogen dioxide, diesel exhaust and tobacco smoke, and are therefore susceptible to oxidative injury. The extent to which oxidative stress is also induced by e-cigarette aerosols is the focus of a broad spectrum of research, in relation to COPD, cardiovascular disease and cancer.

In an *in vitro* study, human oral and lung epithelial cells were exposed to e-cigarette aerosol or mainstream tobacco smoke extracts.[23] The levels of oxidative DNA damage as indicated by 8-oxo-dG were similar, if not greater for e-cigarettes. On the basis of this and other findings, the authors concluded that e-cigarette aerosol extracts suppressed the cellular antioxidant defences and led to significant DNA damage.

In a 2022 review contrasting evidence of oxidative stress caused by smoking with that attributable to vaping products, Emma et al.[24] distinguished between effects of e-cigarettes on oxidative stress-related endothelial dysfunction and their impact on oxidative stress-related carcinogenesis. Concerning the latter, and noting that e-cigarette aerosols contain much lower carcinogenic and toxic substances compared to tobacco smoke, they referred to conflicting data in relation to oxidative stress-related carcinogenesis, suggesting the need for further studies, particularly clinical studies.

6. Induces chronic inflammation

The biomarkers of chronic inflammation include elevated white blood cells, myeloperoxidase activity together with altered cytokine and/or chemokine production. Though these effects are usually short-lived, if inflammatory signalling is activated long-term and becomes chronic, the effects may result in tumorigenesis as has been known for decades.[25] Oxidative stress and inflammation are intimately related and often investigated in the same study.

Treatment of human adult lung macrophages with e-liquid or condensate from vaporised e-liquid establish that the latter was significantly more toxic.[26] The investigators suggest that e-cigarette vapour may induce an inflammatory state in alveolar macrophages within the lung that is partly dependent on nicotine.

Inflammation is relevant to asthma, COPD and other non-malignant respiratory disease and most *in vitro* research on e-cigarettes is oriented toward these diseases, as exemplified by studies on endothelial cells, rather than cancer. Such research is not summarised here.

In an assessment of clinical studies as well as laboratory investigation, Kim et al.[27] noted that vegetable glycerine e-cigarette aerosols increased expression of IL6, IL8 and other inflammation markers in human bronchial epithelial cells after seven days of exposure.

Inflammation has been attributed to flavouring chemicals in e-cigarettes.[28]

Reviewing multiple studies using animal models and *in vitro* studies, in 2022 Park et al.[29] conclude that exposure to e-cigarettes induces secretion of proinflammatory cytokines, including interleukin(IL)-1β, IL-6, IL-8, and tumour necrosis factor alpha (TNF-α), from epithelial cells and immune cells in the upper airway and lung.

Addressing mechanisms of e-cigarette-induced epithelial cell damage, Auschwitz et al.[30] refer to studies showing e-cigarette aerosols decrease the section of cytokines in oral cells, and other research indicating increased levels of IL-8 and COX2. In respiratory tissue, e-cigarettes increase the pulmonary signalling for neutrophils and alveolar macrophages independent of whether the e-liquid contained nicotine.

7. Is immunosuppressive

Evidence of immunosuppression may include decreased immunosurveillance and demonstration of immune system dysfunction. As exemplified by studies on tobacco smoke, and subsequently on e-cigarette aerosol, immune function is rarely assessed in isolation, but rather is considered in the context of cellular behaviour and specifically in relation to inflammation and injury.

No laboratory studies of immunosuppression attributable by e-cigarette aerosols having a role in carcinogenesis were found.

8. Modulates receptor-mediated effects

Receptor-mediated effects are monitored directly by reference to altered binding or expression of particular receptors including the estrogen receptor, peroxisome proliferator-activated receptors, and the aryl hydrocarbon receptor. The role of such receptors in cancer causation by particular chemicals such as pharmacological steroids or dioxin-like substances, is seen as mechanistically distinct from genotoxic or mutation-based carcinogenesis.

While literature searches indicate some research addressing changes in expression of receptors associated with e-cigarettes, such studies are almost invariably, if not always, unrelated to any insight relating to tumorigenesis and involve cell populations not recognised in that context.

9. Causes immortalisation

Immortalisation is a phenomenon primarily identified at the cellular level and involving inhibition of senescence and cell transformation, with relevant molecular phenomena serving to clarify critical mechanisms. Describing a cellular transition from finite to infinite growth potential usually involves laboratory rather than clinical investigation. Malignant transformation of specifically-developed mammalian cell lines has long been recognised as a short-term test for carcinogenesis.

Tellez et al.[31] examined whether weekly exposure of human bronchial epithelial cell lines to e-cigarette aerosols would induce transformation and found a flavoured e-liquid aerosol transformed each of two lines, while an unflavoured one was less effective. When reporting their findings in 2023, the investigators observe that there have been no studies to address whether exposure to e-liquid aerosols can induce cell transformation, a process strongly associated with pre-malignancy.

10. Alters cell proliferation, cell death or nutrient supply

This key characteristic refers to a broad scope of biological endpoints which are mediated by all types of carcinogens and may be monitored with reference to growth factors, energetics and signalling pathways.

Malignant cellular characteristics may be induced by e-cigarette aerosols or their constituents. As noted previously, the studies are cited below are illustrative rather than comprehensive.

Exposure of human bronchial epithelial cells to e-cigarette aerosol affected oxidative and xenobiotic stress pathways among others. Moses et al.[32] suggest that the gene-expression alterations seen with the *in vitro* exposure system reflects the physiological effects experienced *in vivo* by e-cigarette users.

Tommasi et al.[33] report that in oral cells of vapers and smokers as compared to non-smokers, nearly 28% of the aberrantly expressed transcripts in vapers belonged to regulatory non-coding RNAs. Functional network analyses revealed that cancer was the top disease associated with the deregulated genes in both e-cigarette users and smokers, specifically identifying the 'Wnt/Ca+ pathway' in vapers.

E-cigarette exposure enhanced breast cancer cell growth in a mammary fat pad tumour model. Stimulated cell survival was mediated via direct interaction with infiltrated macrophages, and regulated by VCAM-1 and integrin α4β1. Pham et al.[34] argued that as shown for the first time, e-cigarettes promote breast cancer growth and metastasis.

Epithelial-to-mesenchymal transition enables metastasis. Exposure of human adenocarcinoma alveolar basal epithelial cells to e-cigarette liquids and aerosols for three to eight days induced epithelial-to-mesenchymal transition as characterised by acquisition of a fibroblast-like morphology, loss of cell-to-cell junctions, internalisation of E-cadherin, increased motility, and upregulation of relevant markers.[35]

Schaal et al.[36] show that nicotine can induce the expression of embryonic stem cell factor Sox2 which mediates stem cell properties in non-small cell lung adenocarcinoma cells while also reporting that e-cigarette extracts can induce expression of Sox2 as well as mesenchymal markers and enhance migration and stemness of these cells.

Concerning the impact of aerosol components, nicotine potentiates the growth of lung epithelial cells in a dose-response and interfered with p53 function triggered by sodium arsenite, prompting a warning about e-cigarettes.[37]

The impact of flavouring agents has been highlighted by a number of studies. These include demonstration that these agents may increase bronchial epithelial cell apoptosis while altering airway cytokines.[38] Jabba et al.[39] provide evidence that adducts of favour aldehydes formed in e-liquids are cytotoxic and inhibit mitochondrial function in respiratory epithelial cells.

Having identified 18 relevant publications, Wilson et al.[40] undertook a systematic review of the adverse effects of e-cigarettes on head, neck, and oral cells. Aberrant morphology, cytotoxicity, oxidative stress, reduced viability, delayed fibroblast migration, and genotoxicity were evident following relevant exposures. The authors suggest that future research must investigate chronic e-cigarette use and if it leads to periodontal disease and/or cancer.

Experimental data contributing to evidence of carcinogenicity

Findings from experimental animals

Causation of lung adenocarcinoma in mice by inhalation of e-cigarette aerosol is a compelling, but not definitive finding. Bioassay of chemicals for carcinogenicity in rodents is a complicated procedure requiring adherence to a wide variety of standards.[41] Confidence that such standards have been met is increased by the quality of this publication. Those considerations recognised, there is an inherent requirement for independent verification of this study and/or demonstration of carcinogenicity in another species and/or by a different route of administration.

A major assessment has been made of the extent to which site of tumour development in rodent bioassays is likely to be predictive of human cancer.[42] As a generalisation, concordance between tumour development in rodents and site of cancer in humans attributable to any particular carcinogen is not observed. Accordingly, the consideration that lung adenocarcinoma occurred in mice in the bioassay

under consideration does not mean that lung cancer in vapers is a more likely scenario that either oral cancer or bladder cancer.

As summarised by Marczylo[8] in 2020, rodent models have been used to assess the health impact of e-cigarettes. Despite differences between studies, observations include perturbations of pro-inflammatory and oxidative stress markers, sometimes together with DNA damage and downregulation of DNA repair and antioxidant enzymes.

Manifestation of the key characteristics

As originally proposed for the purposes of organising mechanistic data regarding carcinogenicity,[9] no ranking or method of integration was proposed as a means of determining confidence which might be vested in data concerning a particular agent/s. Similarly, for any particular characteristic, there are no criteria as to the type or amount of data which would be necessary to specify that a particular agent, and specifically a chemical or mixture, should be recognised as exhibiting that characteristic.

The characteristics of electrophilicity, genotoxicity and DNA damage are interdependent, specifically when manifested by particular chemicals or mixtures. However, for e-cigarette aerosol and its component chemicals, evidence for each of these often involves studies particular to one such characteristic rather than the same studies being cited three times over. In contrast to such breadth of evidence, data concerning receptor-based effects are relatively scant. To that extent, e-cigarette aerosol may be more readily identified with genotoxic carcinogens than receptor-based carcinogens.

The possible role of nicotine in the potential carcinogenicity of e-cigarette aerosols is not clarified by reference to the key characteristics. Given the multiple methods for assessing each characteristic, very few have been investigated in respect of nicotine. By comparison with all other components of e-cigarette aerosols, nicotine is deliberately present at a pharmacologically-effective level. The assessment now made provides a strong case for a more comprehensive exploration of the biological and pharmacological impact of this toxin.

E-cigarette aerosol has many components. That a mixture may manifest many of the key characteristics which have been formulated to embrace all known mechanisms of carcinogenesis is readably explicable with reference to the impact of various components. Equally such positive results in respect of so many key characteristics provides an inference of carcinogenicity of considerable weight.

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9. Qualitative carcinogenic risk for e-cigarettes

Reviews of e-cigarette carcinogenicity have transitioned from assertions of credibility to indications of likelihood for lung cancer and oral cancer in particular. Taking into account all findings concerning clinical studies, animal bioassay and mechanistic data presented in this report, the following assessment is made:

Nicotine-based e-cigarettes are likely to be carcinogenic to humans who use them.
E-cigarettes are likely to cause lung cancer and oral cancer.

This section draws from what is evident from the medico-scientific literature as presented earlier in this report so as to make a qualitative determination of the carcinogenicity to humans of e-cigarette aerosols. Particular attention is taken of assessments made by relevant authors who have addressed this matter – the carcinogenicity of e-cigarettes – as a primary consideration in reviews and commentaries variously addressing different types of investigation.

Though the potential carcinogenicity of e-cigarettes is subject to comment in the majority of publications cited in sections 5-7, the publications now discussed have focused on this matter as indicated by their respective titles, or at least of a specific section of a relevant review. That criterion specified, many such publications are constrained to particular tumour sites. These are summarised in the first instance followed by those reviews concerned with cancer at any anatomical site as a result of vaping. As previously, priority has been accorded to publications since 2018.

The potential to cause particular tumour types

Lung cancer

A 2021 publication *Vaping and lung cancer – A review of current data and recommendations*[1] concluded that although research remains somewhat equivocal, there is clear reason for concern regarding the potential oncogenicity of e-cigarettes/e-liquids with a strong basic and molecular science basis. The authors determined that further study in this field is strongly warranted and consideration should be made for tighter control and regulation of vaping products.

In the same year, Petrella[2] addressed the question "Electronic cigarettes, vaping-related lung injury and lung cancer: where do we stand?" and concluded that the long-term impact on human health of e-cigarettes is at present unknown, due to the lack of long-term follow-up of electronic cigarettes smokers.

Just two years later, Petrella et al.[3] presented a different outlook in *Clinical impact of vaping on cardiopulmonary function and lung cancer development: an update*. They concluded that although it was initially believed that vaping might have less harmful health consequences than those due to smoking, there is nowadays no robust evidence to recommend use of e-cigarettes as a less dangerous alternative.

Also in 2023, a comparison of smoking and vaping for lung cancer risk noted that smoking leads to chronic obstructive pulmonary disease, while vaping contributes to lung injury.[4] The authors observed that both tobacco smoke and e-cigarette aerosol increase proinflammatory cytokine expression in cells and affect protein regulation, leading to an increased lung cancer risk. They conclude that the toxic effects of vaping are essentially identical to that caused by smoking.

Head and neck cancer

In 2019 Flach et al.[5] outlined evidence for a role of e-cigarettes in the development of head and neck cancers. Key findings involved laboratory-based studies indicative of DNA damage and oxidative stress, followed by two case reports and clinical studies recording oral mucosal lesions, which, taken together, was assessed as low-level evidence.

An assessment in 2020[6] concluded that there is some evidence suggesting a potentially carcinogenic role of e-cigarettes in the pathogenesis of head and neck cancers. *In vitro* studies were considered and a lack of properly designed animal models was specified, noting a single *in vivo* study of e-cigarette aerosols on the laryngeal mucosa of rats. Overall, the evidence was characterised as postulative (sic) and not proven by epidemiological studies.

Oral cancer

The modest and dated reviews of head and neck cancer being associated with vaping may be contrasted with a high number and more recent reviews of oral cancer specifically. Indeed, while addressing head and neck cancer in relation to vaping, Flach et al.[5] note that 75% of relevant studies involved oral carcinogenesis, and only a minority of the reports looked into other head and neck cancers.

Concerning vaping and oral cancer, assessments made from 2018 to 2024 show a clear transition between the recognition of scant knowledge to a clear inference of concern; a scenario that was indicated earlier in relation to lung cancer. Thus Sultan et al.[7] in 2018 declare that there is no strong evidence suggesting a direct role for e-cigarettes in the pathogenesis of oral potentially-malignant disorders or oral cancer, while advocating caution until further evidence regarding long-term use and health complications is available.

A 2020 assessment[8] referred to DNA strand breaks on exposure of oral cell lines to e-cigarette aerosols as well as dysregulations of genes associated with carcinogenic pathways in oral tissues exposed to e-cigarette aerosols. The oral carcinogenic potential of e-cigarette was assessed as remaining unclear due to the lack of long-term prospective and large-scale case-control studies.

Some four or more years later, two reviews[9, 10] both refer to the presence of carcinogenic compounds in saliva and morphologic changes, DNA damage, and molecular pathways related to carcinogenesis in the oral cells of e-cigarette users. Multiple *in vitro* studies show that e-cigarettes induce DNA damage, oxidative stress, DNA double-stranded breaks, apoptosis, necrosis, and genotoxicity in different types of oral cells and promote aggressive phenotypes in pre-existing malignant cells. The reviews acknowledge a lack of definitive evidence that vaping may be described as a cause of oral cancer.

Bladder cancer

By comparison with a succession of reviews accessing if vaping causes lung cancer or oral cancer, few comparable publications are evident for bladder cancer, though concern has been raised. In 2017 Bourke et al.[11] observed that there are emerging data indicating that potentially harmful components of e-cigarettes such as tobacco-specific nitrosamines, polyaromatic hydrocarbons, and heavy metals could be linked to possible urologic health risks.

Five years later, consideration of urinary carcinogenic biomarkers in e-cigarette users, as they relate to the risk of bladder cancer, identified 40 such compounds and four metals.[12]

When assessing tumour development from vaping at various anatomical sites in 2023, Sahu et al.[13] include bladder cancer, making reference to e-liquids containing aromatic amines, aldehydes and PAHs, all of which have been found to cause bladder cancer in humans. They also note that e-cigarette users, compared to non-users, have higher levels in urine of agents that can be metabolised into compounds that can cause bladder cancer.

Trends in previously published assessments of carcinogenicity

E-cigarette aerosols

In 2018 a consensus study on public health implications of e-cigarettes by the National Academies of Sciences, Engineering, and Medicine, when addressing cancer, concluded that evidence in humans for an association is extremely sparse[14]. Likewise, a year later, Hawk and Maresso concluded that the long-term absolute risks of e-cigarettes, including any potential increased cancer risks, are poorly defined.[15]

Focusing on relevant mechanistic evidence available at that time, Mravec et al.[16] refer to 'direct carcinogenic effects' noting in this context, DNA damage and repair variously attributable to e-cigarette aerosols, and specifically to nicotine and its nitroso derivatives. Concerning 'indirect' 'hidden' tumour-promoting effects, the authors make reference to nicotine-induced activation of the sympathoadrenal system which may stimulate cancer initiation and progression.

Assessing the issue of carcinogenicity in 2022, Esteban-Lopez et al.[17] specify that e-cigarettes contain high levels of carcinogens and toxicants that may have long-lasting effects on other organ systems, including the development of neurological manifestations, lung cancer, cardiovascular disorders and tooth decay.

Also in 2022 review, Vivarelli et al.[18], having separately addressed 'Oxidative stress pre-inflammatory effect and lung tissue damage' are primarily concerned with 'Cancer risk and main oncogenic molecular mechanisms triggered by e-cigarettes'. They refer to growing evidence from basis studies on the carcinogenic potential of e-cigarette aerosol, nominating DNA damage and epithelial-mesenchymal transition, breast cancer progression and pulmonary metastasis promotion, modulation of the sympathoadrenal and related receptor systems and brain tumour cell growth through epidermal growth factor receptor.

Herbst et al.[19] specify policy on e-cigarettes in the name of the American Association for Cancer Research and the American Society of Clinical Oncology. They point out that while e-cigarettes emit fewer carcinogens than combustible tobacco, preliminary evidence links e-cigarette use to DNA damage and inflammation, key steps in cancer development.

Determination of priorities for IARC *Monographs* to be undertaken during 2025-29 specified e-cigarettes (listed as electronic nicotine delivery systems) as the most immediate need amongst agents not previously evaluated and accorded high priority on the basis of relevant animal cancer and mechanistic evidence.[20]

Aerosol components

Nicotine and its derivatives

Data suggesting nicotine may be carcinogenic in its own right[21, 22] have not been subject to systematic evaluation in this context. In relation to e-cigarettes specifically, a role in cancer development has been delineated with reference to the formation of N-nitroso derivatives[23] and via the nicotinic acetylcholine receptor.[24]

Volatile organic compounds

The overwhelming majority of publications describing toxins or carcinogens in e-cigarette aerosols present such findings in comparison to their levels in tobacco smoke. El-Hellani et al.[25] observe that such comparisons encouraged their proponents and some public health authorities to promote e-cigarettes as less lethal relative to their combustible counterparts. They offer a different perspective and, based on the mechanisms of formation of toxicants, submit that e-cigarettes may be described as chemical reactors: devices where mass transfer, diffusion, and heat transfer along with chemical reactions may occur.

E-cigarettes expose users to volatile organic compounds such as acrylamide, benzene, formaldehyde, acetaldehyde and propylene oxide.[26, 27] The source of these toxicants is both the direct distillation of

contaminants from e-liquids and chemical transformations of propylene glycol or vegetable glycerol and other constituents leading to the formation of new chemical compounds. Flavouring agents may markedly contribute to the wider variety of carbonyl compounds, which include formaldehyde, acetaldehyde and acrolein, inhaled by vapers.[27]

Metals

Metals that comprise the heating elements and tanks of e-cigarettes may be released into the liquids or the aerosols during use and increase the risk of cancer and non-cancer respiratory effects. Chromium and nickel, the key components of nichrome alloys commonly used in heating elements, appear to be the main contributors to the cancer and non-cancer risks from vaping. Also implicated in the potential carcinogenic risk are cadmium, lead, and arsenic.[28]

A 2024 assessment of carcinogenicity

The publications cited in this report provide the following insights concerning the parameters of carcinogenicity of e-cigarettes.

Production and use of nicotine-based e-cigarettes

Different types of e-cigarettes have been marketed, but all incorporate an electrical means of vaporizing e-liquids such that the user inhales an aerosol the composition of which varies qualitatively and quantitatively.[17, 18, 29] The assessment of carcinogenicity made here is concerned with nicotine-containing e-cigarettes, while recognising that agents possibly contributing to that risk (including e-liquid solvents, flavouring agents and products generated by the heating element) have implications concerning a possible risk from non-nicotine-containing e-cigarettes. The impact of e-cigarettes delivering illicit drugs has not been addressed.

Extensive research has described preferences regarding type and design of e-cigarettes as exercised by different groups, with particular emphasis on young people. However, design parameters and user preference are not of immediate importance in the present context because of two considerations. First, in a qualitative risk assessment, variation in, for example, the amount of vaporised nicotine inhaled is not a critical issue by comparison to whether inhalation of a physiologically-significant amount of material is evident. Second, in the overwhelming majority of studies involving biomarkers of harm (and in many other studies), individuals are identified simply as users of e-cigarettes with little reference to frequency of use and no information concerning different product design.

As underlying the requirement for an assessment of their carcinogenicity, there is no doubt that e-cigarettes have been and are marketed and used with the Australian community in common with what is documented for all high-income countries. Comparable knowledge regarding low- and middle-income countries is relatively lacking.

Physiological evidence of carcinogen exposure: absorption, metabolism and excretion

The broad scope and number of studies in which biomarkers of exposure have been monitored in users of e-cigarettes have been described (section 7). Some studies indicate vapers absorb equal or higher levels of nicotine than smokers. Endogenous formation of N-nitroso derivatives of nicotine has been described.

Accordingly, qualitative risk assessment of the carcinogenicity of e-cigarettes reasonably takes into account all such findings to fully assess studies relevant to e-cigarettes specifically. The results of investigations in respect of exposure for vapers in comparison with that experienced by smokers vary markedly (Table 1). However, in the context of qualitative risk assessment, the immediate issue is whether a particular exposure occurs, rather than its quantitation.

Table 1. Carcinogen exposure due to vaping

Carcinogen type	Carcinogen	Metabolite analysed	Vapers*	Smokers*
Tobacco-specific nitrosamines	NNK	NNAL	431%	21,996%
	NNN	N/A	80%	514%
	NNN (saliva)	N/A	5,740%	37,770%
Metals	Cadmium	N/A	30%	86%
	Lead	N/A	23%	36%
Volatile organic compounds	Acrylonitrile	N-Acetyl-S-(2-cyanoethyl)-L-cysteine	201%	9,322%
		N-Acetyl-S-(1-cyano-2-hydroxyethyl)-L-cysteine	30%	1,055%
	N,N-dimethyl-formamide	N-Acetyl-S-(N-methylcarb-amoyl)-L-cysteine	46%	359%
	Acrylamide	N-Acetyl-S-(2-carbamoyl-ethyl)-L-cysteine	95%	N/A
	Propylene oxide	2-Hydroxy-propyl methacrylate	89%	N/A
	Crotonaldehyde	N-Acetyl-S-(3-hydroxy-propyl-1-methyl)-L-cysteine	48%	N/A
	Acrolein	3-Hydroxypropyl mercapturic acid	32%	N/A
	Ortho-Toluidine	N/A	133%	

*Increase compared to non-users

Determinations involve urinary analysis except where otherwise stated. Table adapted from that shown in *Electronic Nicotine Delivery Systems: an updated Policy Statement from the American Association for Cancer Research and the American Society of Clinical Oncology.*[19]

Detection of multiple aerosol-related metabolites in urine, often specified as biomarkers of exposure provide unequivocal evidence of the absorption and excretion of these compounds most markedly including nicotine and a range of volatile organic compounds. Various of these, including tobacco-related N-nitroso compounds and aldehydes including formaldehyde, have the capacity to damage DNA, stemming from the production of recognised DNA adducts.[16, 18, 30]

Evidence of carcinogenicity in humans

There are a limited number of case reports concerning diagnosis of oral cancer in people who have vaped. The practitioners providing such reports allude to the possibility that usage of e-cigarettes may have contributed to the development of these particular malignancies.[31, 32]

There are no epidemiological data involving usage of e-cigarettes and associated cancer/s. This may be due to the short timeframe over which e-cigarettes have been widely used by comparison with the latent period for most relevant cancers exemplified by those cancers attributable to smoking. Moreover, identifying study populations through which cancer may associated with e-cigarettes is challenging when many vapers have also smoked tobacco before, during or after their use of e-cigarettes.

Evidence of carcinogenicity in experimental animals

In mice, following inhalation, e-cigarette aerosols are carcinogenic causing adenocarcinoma of the lung.[33] Concerning weight reasonably accorded to chemicals shown to be carcinogenic in experimental animals,

the Preamble to the IARC *Monographs* specifies that, in the absence of additional information such as a species-specific mechanism, chemicals which are carcinogenic in experimental animals should be regarded as presenting a carcinogenic hazard to humans.[34]

Mechanistic evidence of carcinogenicity

In this report, findings indicative of mechanism/s by which e-cigarette aerosols may be carcinogenic are presented in two groupings.

The first, and most persuasive, are clinical studies addressing molecular and cellular indicators of pathological change in vapers compared to non-users (section 7). These studies recognise biomarkers of harm including DNA damage, oxidative stress and inflammation. The nature and scope of such biological changes are immediately attributable to the impact of e-cigarettes in humans.

Review of *in vitro* studies of e-cigarette aerosols, and often more informatively, of individual chemicals found in such aerosols (section 8) has been limited to those studies specifically concerned with e-cigarettes, as distinct from the much wider literature addressing the carcinogenicity of these chemicals as is described in corresponding IARC *Monographs* which provide a range of relevant evaluations.

Of the chemicals in e-cigarette aerosols which are absorbed by vapers, the nicotine-derived N-nitroso compounds NNN and NNK, together with acrolein, benzene, 1,3-butadiene, formaldehyde, ethylene oxide, *o*-toluidine and trichloroethylene, are all evaluated as *carcinogenic to humans (Group 1)* in respective IARC *Monographs* (monographs.iarc.who.int). In this context, acrylamide, N.N-dimethylformamide and styrene are *probably carcinogenic to humans (Group 2A)* and acetaldehyde, acrylonitrile, 1-bromopropane, crotonaldehyde and propylene oxide are *possibly carcinogenic to humans (Group 2B)*.

The relevant *in vitro* studies are organised with reference to the 'key characteristics of carcinogens'.[35] These data may be combined with relevant findings made in clinical studies to assess evidence for manifestation of each of the key characteristics in respect of e-cigarette aerosols (Table 2).

The strength of overall evidence shown in Table 2 takes account of both evidence in humans and experimental evidence together, if warranted, of carcinogenicity data for particular aerosol components. The assessments are at best indicative, and the highest rating is indicative of clear evidence rather than fulfilling any notional requirement of certainty. Though a few carcinogens are recognised as acting via more than a single reaction pathway, the wide spectrum of characteristics manifested by aerosols is immediately indicative of a complex mixture. The scope of findings also indicates that no single biological process or type of investigation predominantly accounts for the comprehensive positive determinations that have been made.

The data presented in Table 2 indicate that the mechanistic evidence according carcinogenic character to e-cigarette aerosols is manifestly clear.

Table 2. Evidence for manifestation of the 'key characteristics' of carcinogens by e-cigarette aerosols[35]

Key characteristic	Clinical evidence	Animal & *in vitro*	Overall result	Remarks
Is electrophilic	+++	+++	+++	Metabolites detected and cytochrome-P450 role
Is genotoxic	+++	+++	+++	Comprehensive data available and specifically reviewed
Alters DNA repair	+	+	+	Positive findings from a limited number of studies
Induces epigenetic change	+++	++	+++	DNA methylation and altered transcription
Induces oxidative stress	+++	++	+++	Multiplicity of clinical and *in vitro* studies
Induces chronic inflammation	+++	+++	+++	Predominantly clinical evidence
Is immunosuppressive	++	-	++	Inference from susceptibility to infection
Modulates receptor-mediated effects	+	-	+	Clinical evidence re nicotine receptors
Causes immortalisation	+	+++	+++	Tumorigenicity in mice shows implicit immortalisation
Alters cell behaviours or nutrient supply	++	++	++	Includes histopathological evidence from vapers

In the Table, confidence in positive findings is indicated with reference to a range extending from a reasonable inference (+) to confirmatory evidence (+++); inadequate evidence is indicated by (-). The 'overall result' takes account of human (clinical) evidence, laboratory-based evidence (animal & in vitro) together with findings (any type of data) made concerning individual chemical components of e-cigarette aerosols.

Overall assessment

Nicotine-based e-cigarettes are likely to be carcinogenic to humans who use them. E-cigarettes are likely to cause lung cancer and oral cancer.

This determination is primarily based on carcinogenicity of e-cigarette aerosol in mice and a broad spectrum of mechanistic findings, as determined from both studies in humans and from laboratory investigation. Account has also been taken where relevant of the known carcinogenicity for particular components of e-cigarette aerosols.

This assessment has not addressed any qualitative risk attributable to passive or second-hand inhalation of e-cigarette aerosols.

The overall assessment specified above is a qualitative determination. Such a qualitative finding does not provide for differentiation between different types of e-cigarettes or consumer-determined variation in the manner that e-cigarettes are used.

The burden of cancer attributable to e-cigarettes cannot be specified either in relation to the present time or with reference to some future time for lack of relevant epidemiological data.

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E-cigarettes and Cancer Research Report Working Group Members

Professor Bernard Stewart AM - Chair

BSc, MSc, PhD, FRACI, DipLaw, DipLegalPract

Discipline of Paediatrics and Child Health, University of New South Wales

Professor Billie Bonevski - Public Health (Tobacco)

BA(Hons1), PhD, FSRNT

Dean (Research) and Director, Flinders Health and Medical Research Institute, Matthew Flinders Professor of Public Health, College of Medicine and Public Health, Flinders University

Professor Camile Farah - Head and Neck Cancer

BDSc, MDSc(OralMed OralPath), PhD, GCEd(HE), GCExLead, FRACDS(OralMed), FOMAA, FIAOO, FICD, FPFA

Specialist in Oral Medicine and Oral Pathology, Hollywood Medical Centre

Adjunct Professor, Central Queensland University

Emeritus Professor Lin Fritschi - Cancer Epidemiology

MBBS, PhD, FAFPHM

Professor of Epidemiology, Curtin School of Population Health, Faculty of Health Sciences, Curtin University

Associate Professor Ashley Hopkins - COSA Epidemiology Group Chair

BPharm(Hons), PhD

Associate Professor and NHMRC Investigator Fellow, College of Medicine and Public Health, Flinders University

Dr Malinda Itchins - COSA Lung Cancer Group Chair

BMedSc, MBBS(Hons1), PhD, FRACP

Staff Specialist Medical Oncologist, Royal North Shore Hospital

Visiting Medical Officer Thoracic Cancers, Chris O'Brien Lifehouse

Clinical Senior Lecturer, Faculty of Medicine and Health, University of Sydney

Dr Laura Kirsten - COSA Psycho-Oncology Group Chair

DPsyc(Clinical), PhD

Principal Psychologist, Nepean Cancer and Wellness Centre

Associate Professor Henry Marshall - Thoracic Medicine

BSc(Hons), BM(Soton), MRCP(Lond), PhD, FRACP

Thoracic Physician, University of Queensland Thoracic Research Centre and Department of Thoracic Medicine, The Prince Charles Hospital

Cassandra Mazza - Cancer Nursing

BNg, MPH, MCaHaemN, MNP

Oncology Nurse Practitioner, Sunshine Coast Hospital and Health Service

Dr Natansh Modi - COSA Epidemiology Group Chair (Acting)

BPharmSc, BPharm(Hons1), PhD

Lecturer, Clinical and Health Sciences, University of South Australia

Associate Professor Marissa Ryan - COSA Cancer Pharmacists Group Chair

BPharm, GradDipClinPharm, MHlthMgt, PhD, FANZCAP (OncHaem, Lead&Mgt)

A/Consultant Pharmacist (Cancer), Princess Alexandra Hospital

Adjunct Associate Professor, School of Pharmacy and Pharmaceutical Sciences, University of Queensland

Associate Professor Freddy Sitas - Cancer Epidemiology

BSc, MSc(Med), MSc(Epidemiology), DPhil(Epidemiology), FAEA

Chief Operating Officer, International Centre for Future Health Systems, University of New South Wales

Adjunct Associate Professor, School of Population Health, University of New South Wales

Editor-in-Chief, Cancer Epidemiology (Elsevier)

Megan Varlow - Public Health (Tobacco)

BA, PGDip, MPsych, EMPA, MAICD

Director of Cancer Control Policy, Cancer Council Australia

E-cigarettes and Cancer Research Report Support and Editorial Team

Dr Hayley Griffin

BMedSc, MNutrDiet, PhD

Project Manager, Clinical Oncology Society of Australia