Australian Government
Department of Health and Aged Care
Australian Industrial Chemicals Introduction Scheme

Acetoin, cinnamaldehyde, diethylene glycol and ethylene glycol with end use in non-nicotine e-cigarettes (vaping products)

Evaluation statement

25 September 2023

Draft

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AICIS evaluation statement

Subject of the evaluation

Acetoin, cinnamaldehyde, diethylene glycol and ethylene glycol with end use in non-nicotine e-cigarettes (vaping products)

Chemicals in this evaluation

Name	CAS registry number
2-Propenal, 3-phenyl-	104-55-2
1,2-Ethanediol	107-21-1
Ethanol, 2,2'-oxybis-	111-46-6
2-Butanone, 3-hydroxy-	513-86-0

Reason for the evaluation

Evaluation Selection Analysis indicated a potential human health risk.

Parameters of evaluation

These chemicals are listed on the Australian Inventory of Industrial Chemicals (the Inventory). This evaluation focuses on the risk to public health from the use of the chemicals in non-nicotine e-cigarettes (vaping products). It does not consider risks to worker health and safety.

2-Propenal, 3-phenyl- (CAS No. 104-55-2), 1,2-ethanediol (CAS No. 107-21-1) and ethanol, 2,2'-oxybis- (CAS No. 111-46-6) have previously been assessed under the National Industrial Chemicals Introduction and Assessment Scheme (NICNAS 2013; NICNAS 2016a; NICNAS 2016). These assessments focused on the risks to human health from other industrial uses of the chemicals.

Throughout this report, chemical group members will be referred to by their synonyms, as follows:

- cinnamaldehyde (2-propenal, 3-phenyl- (CAS No. 104-55-2))
- ethylene glycol (1,2-ethanediol (CAS No. 107-21-1))
- diethylene glycol (ethanol, 2,2'-oxybis- (CAS No. 111-46-6))
- acetoin (2-butanone, 3-hydroxy- (CAS No. 513-86-0)).

Summary of evaluation

Summary of introduction, use and end use

The chemicals in this group have all been identified as being present in e-cigarette liquids in Australia and internationally. Cinnamaldehyde and acetoin have been identified as flavouring components of e-cigarettes, at concentrations of 142.5 mg/mL and 529 µg/e-cigarette, respectively. Ethylene glycol has been identified as a humectant at concentrations as high as 74%. Diethylene glycol has been identified as a contaminant at 4 µg/g.

Human health

Summary of health hazards

The identified health hazards are based on available data for the chemicals in this group. The toxicological endpoints of focus in this report are those considered to be relevant to the use of these chemicals in non-nicotine e-cigarettes (vaping products).

Based on the available data, these chemicals are not expected to:

- cause acute inhalation toxicity
- have genotoxic potential
- cause reproductive or developmental toxicity (although no data are available for acetoin)

Cinnamaldehyde and ethylene glycol have existing classifications on Safe Work Australia's (SWA) Hazardous Chemicals Information System (HCIS), including for Specific target organ toxicity (single exposure) Category 3, H335: May cause respiratory irritation (SWA n.d.). The available data support these classifications.

Cinnamaldehyde has been shown to depress the respiratory rate of CF-1 mice when exposed via nose-only inhalation to the chemical for 1 minute. The ED25 (dose providing a 25% reduction in respiratory rate) was calculated to be 241 µg/L. Cinnamaldehyde also induced coughing in human volunteers following inhalational exposure with a distinct dose-response (cough) relationship.

In a repeated inhalational exposure tolerance study, human subjects reported upper respiratory and/or nasal irritation in response to ethylene glycol exposure at concentrations of 140 mg/m^3 and higher. As concentrations increased, tolerance decreased. At the highest dose tested (380 mg/m^3), subjects could only tolerate breathing in the chemical for 'a couple of breaths'.

There were no data to evaluate respiratory sensitisation for any of the chemicals. Limited data are available to determine adverse health effects following repeated inhalation exposure.

Cinnamaldehyde and acetoin may have immunotoxic potential. Cinnamaldehyde has been shown to exert effects on epithelial cells, fibroblasts and macrophages in the lungs. Cinnamaldehyde may also adversely affect mucociliary clearance in the lungs. Acetoin and cinnamaldehyde have been shown to impair epithelial barrier function. These chemicals may increase the risk of respiratory infections in e-cigarette users. Cinnamaldehyde may have the potential to cause cardiotoxicity. However, supporting data are limited to in vitro studies.

Repeated oral exposure to ethylene glycol and diethylene glycol is associated with adverse effects on the kidney. No data are available to examine whether these effects may occur following inhalation exposure. The workplace exposure standard for diethylene glycol is set based on concerns for kidney toxicity.

No adverse effects were reported in a 3-month inhalation study with acetoin. However, in e-cigarette liquids, acetoin degrades to diacetyl, which is the oxidised form of acetoin. Diacetyl is reported to cause adverse respiratory effects in humans following repeated inhalational exposure, and vaping-associated pulmonary illness (VAPI). In addition, based on the weight of evidence, diacetyl may be carcinogenic following inhalation exposure.

Chemicals in this group have other hazards (see **Existing Australia Regulatory Controls**) that are outside the scope of this evaluation.

Summary of health risk

Public

The chemicals in this evaluation have been identified as ingredients in non-nicotine containing e-cigarettes (vaping products). Based on the available use information, the public may be exposed to these chemicals:

- by directly inhaling vapours and aerosols during use of non-nicotine liquids in e-cigarette devices
- by second-hand inhalational exposure to e-cigarette vapours.

The proportion of people in Australia who had ever used e-cigarettes between 2016 and 2019, rose from 8.8% to 11.3%. Australian e-cigarette use has increased across all age groups, with a particularly notable increase in young adults (AIHW 2019; NHMRC n.d.). As of early 2023, a largescale Australian survey study estimated that 8.9% of the Australian population aged 14 and over were ‘current vapers’. The authors noted that this represented a significant increase compared with previous years and that this increase was particularly apparent in the 14–34 age group (CBRC 2023).

E-cigarette devices heat e-cigarette liquids to create an inhalable aerosol. The liquids typically contain flavouring compounds (often more than one) dissolved in a humectant or vehicle, such as propylene glycol or glycerol (or ethylene glycol, less frequently). The liquids also often contain nicotine (even when nicotine is omitted from the label). The aerosol created through heating penetrates deeply into the lungs when inhaled.

Given the short period that these products have been available on the market, the long term safety and health effects associated with e-cigarette use are unknown. However, the available evidence suggests that regular use of e-cigarettes is likely to have adverse health consequences (AICIS 2022; AICIS 2023a; Clapp and Jaspers 2017; CSIRO 2018; NHMRC n.d.; NICNAS 2019; TGA 2021b). As of February 2020, 2807 cases of VAPI and 68 associated deaths were reported in the US (Krishnasamy et al. 2020). This is further supported by numerous case reports detailing VAPI and include recently reported cases of vaping-associated bronchiolitis obliterans, also referred to as ‘popcorn’ lung (Landman et al. 2019). Despite this, further investigation into the risk of other e-cigarette ingredients is ongoing.

Although many of the flavouring compounds used in e-cigarettes are ‘generally recognised as safe’ for use in food, including cinnamaldehyde and acetoin, inhalation data are limited and not considered as part of these food use risk assessments. Limited data are available

regarding the adverse effects of directly inhaling the chemicals due to their presence in e-cigarettes. Cinnamaldehyde and ethylene glycol are known respiratory irritants and there are associations with potential adverse effects for all chemicals in this group (see **summary of health effects**).

Exposure to e-cigarette ingredients may produce adverse effects in people with pre-existing respiratory illness (Clapp and Jaspers 2017). Cumulative effects resulting from combined exposure to multiple different e-cigarette ingredients may also occur.

These chemicals are currently risk managed for use in nicotine vaping products. No specific controls are currently available for use in non-nicotine vaping products. Overall, these chemicals may pose a risk to the public that requires management (see **Proposed means for managing risk** section).

Proposed means for managing risk

Public health

Recommendation to Department of Health and Aged Care

It is recommended that the delegate of the Secretary for Poisons Scheduling list these chemicals in the *Poisons Standard (the Standard for the Uniform Scheduling of Medicines and Poisons)* (SUSMP).

It is recommended that to manage the potential risks associated with the use of the chemicals, the entry prohibits their use in products intended to be inhaled (e.g. non-nicotine e-cigarette fluids).

Consideration should be given to the following:

- the potential widespread use of these chemicals in e-cigarette products available in Australia
- these chemicals are prohibited ingredients in nicotine vaping products in Australia (TGA 2021a)
- 2 of the chemicals, cinnamaldehyde and ethylene glycol, are known respiratory irritants
- diethylene glycol has regulatory controls to protect workers from inhalation based on kidney toxicity
- in e-cigarette liquids, acetoin degrades to diacetyl, which is the oxidised form of acetoin. Diacetyl is reported to cause adverse respiratory effects in humans following repeated inhalational exposure, and VAPI.

These chemicals are prohibited ingredients in nicotine-containing e-cigarettes in Australia under the *Therapeutic Goods (Standard for Nicotine Vaping Products)* (TGO 110) *Order 2021*. Therefore, the SUSMP entry should align with this prohibition so that the use of these 4 chemicals is effectively prohibited in all e-cigarettes (vaping products) in Australia.

Conclusions

The conclusions of this evaluation are based on the information described in this Evaluation Statement.

Considering the proposed means of managing risks, the Executive Director proposes to be satisfied that the identified human health risks can be managed within existing risk management frameworks. This is provided that:

- all requirements are met under environmental, workplace health and safety and poisons legislation as adopted by the relevant state or territory
- the proposed means of managing the risks identified during this evaluation are implemented.

Note: Obligations to report additional information about hazards under *Section 100 of the Industrial Chemicals Act 2019* apply.

Supporting information

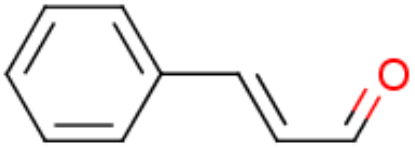
Grouping rationale

These chemicals have been grouped together based on their potential use in non-nicotine containing e-cigarettes (vaping products). Their structural similarities and toxicological profiles are not the main drivers for grouping.

Chemical identity

Chemical name	2-Propenal, 3-phenyl-
CAS No.	104-55-2
Synonyms	cinnamaldehyde; 3-phenylpropenal; cinnamic aldehyde; cinnamyl aldehyde; (E)-3-phenylprop-2-enal
Molecular formula	C9H8O
Molecular weight (g/mol)	132.16
SMILES	C1=CC=C(C=C1)/C=C/C=O
Chemical description	Cinnamaldehyde predominantly consists of the thermodynamically stable trans-isomer, >97% trans-cinnamaldehyde (CAS No. 14371-10-9), with up to 1 % cis-cinnamaldehyde (CAS No. 57194-69-1) (NTP 2004; EFSA 2015). Therefore, the isomeric mixture is expected to have a nearly identical profile to the pure trans isomer.

Structural formula:

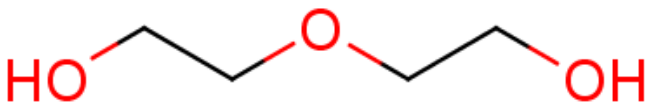


Chemical name	1,2-Ethanediol
CAS No.	107-21-1
Synonyms	ethylene glycol; glycol; monoethylene glycol
Molecular formula	C2H6O2
Molecular weight (g/mol)	62.07
SMILES	C(CO)O
Chemical description	-

Structural formula:

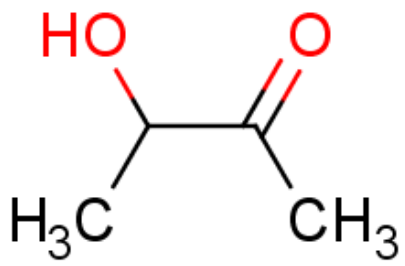
Chemical name	Ethanol, 2,2'-oxybis-
CAS No.	111-46-6
Synonyms	diethylene glycol; 2,2'-oxybis[ethanol
Molecular formula	C4H10O3
Molecular weight (g/mol)	106.12
SMILES	C(COCCO)O
Chemical description	-

Structural formula:



Chemical name	2-Butanone, 3-hydroxy-
CAS No.	513-86-0
Synonyms	acetoin; .gamma.-hydroxy-.beta.-oxobutane; 3-hydroxy-2-butanone; acetylmethyl carbinol
Molecular formula	C4H8O2
Molecular weight (g/mol)	88.11 g/mol
SMILES	CC(C(=O)C)O
Chemical description	-

Structural formula:



Introduction and use

There is increasing evidence that many non-nicotine containing e-cigarettes available in Australia contain nicotine despite labelling to the contrary. Testing undertaken by the TGA found that 168 of 296 e-cigarette products tested (57%) contained undeclared nicotine (TGA 2022). As a result, use data for all e-cigarettes have been considered in this evaluation.

Australia

No comprehensive information is available on the introduction and use of the chemicals in e-cigarettes in Australia. Isomers of cinnamaldehyde, including trans-cinnamaldehyde (CAS No. 14371-10-9) have been identified in e-cigarettes in Australia and abroad (Larcombe 2022). In this study, trans-cinnamaldehyde was identified in 48 'fresh' (maximum concentration, 97.9 mg/L) and 38 'aged' e-liquids (maximum concentration, 142.5 mg/L) from a total of 65 products tested. 'Fresh' e-cigarette liquids refers to previously unused products. 'Aged' e-cigarette liquids refer to those which have undergone repeated heating and cooling, a process which mimics e-cigarette use. This process may result in altered chemical profiles (through oxidation, polymerisation and promoting reactivity between chemical components and the e-cigarette device itself) (Larcombe et al 2022).

It is expected that the vast majority, if not all of the e-cigarettes containing chemicals included in this report are imported into Australia. Australian manufacture of e-cigarette liquids is expected to be minimal.

Other industrial uses have been identified for these chemicals in Australia, including cosmetic, domestic and commercial uses (NICNAS 2013, NICNAS 2016a, NICNAS 2016b). These uses are not in the scope of this evaluation.

International

Internationally, these chemicals have been detected in e-cigarette liquids either as an ingredient or contaminant (NICNAS 2019). Acetoin and cinnamaldehyde have also been identified in e-cigarette emissions (NICNAS 2019).

Cinnamaldehyde is used to create a cinnamon flavour in e-cigarettes and is often used in conjunction with other flavour ingredients. The chemical has been found to be present in e-cigarette liquids at 140 mg/mL (Behar et al. 2016).

Ethylene glycol has been identified as a humectant in e-cigarette liquids (NICNAS 2019). It has been used in e-cigarettes in place of more commonly used humectants glycerol and polyethylene glycol. It has been reported to be used at concentrations as high as 74% (Hutzler et al. 2014). Humectants are used to produce the characteristic exhaled vape 'cloud' or 'smoke' produced after 'puffing' on an e-cigarette (which is intended to mimic that produced through tobacco smoking). Ethylene glycol is expected to be used either in place of, or in conjunction with more commonly used humectants propylene glycol and glycerol.

Diethylene glycol has been identified as a contaminant in e-cigarette liquids (NICNAS 2019; Peace et al. 2016). The chemical has been found to be present in e-cigarette liquids at 4 µg/g (Varlet et al 2015). Glycerol, or glycerin, a commonly used e-cigarette ingredient can be contaminated with diethylene glycol (Lang 2007).

Acetoin has been identified as a flavouring component of e-cigarette liquids (NICNAS 2019). In one study, acetoin was detected in 46 of 51 e-cigarette flavours tested at concentrations up to 529 µg/e-cigarette (Allen et al. 2016). In another study, it was detected at 16 µg/g (Varlet et al. 2015). Acetoin is often detected in conjunction with the flavouring compound diacetyl. Several other industrial uses have been identified for cinnamaldehyde, ethylene glycol and diethylene glycol in former NICNAS Tier II Human Health IMAP assessment reports (NICNAS 2016a; NICNAS 2016b; NICNAS 2016c). These uses are not in the scope of this evaluation.

Existing Australian regulatory controls

Public

Therapeutic Goods (Standard for Nicotine Vaping Products)

Cinnamaldehyde, ethylene glycol, diethylene glycol and acetoin are listed in Schedule 1 of the Therapeutic Goods (Standard for Nicotine Vaping Products) (TGO 110) Order 2021. Schedule 1 substances must not be added as ingredients to nicotine vaping products (TGA 2021a).

Poisons Standard – The Standard for the Uniform Scheduling of Medicines and Poisons

Diethylene glycol and ethylene glycol are listed in the *Poisons Standard – The Standard for the Uniform Scheduling of Medicines and Poisons (SUSMP)*, as follows (TGA 2023).

'Schedule 5:

DIETHYLENE GLYCOL (excluding its salts and derivatives) in preparations containing not less than 10 mg/kg of denatonium benzoate as a bittering agent except:

(a) in paints or paint tinters; or
(b) in toothpastes or mouthwashes containing more than 0.25% of diethylene glycol; or
(c) in other preparations containing 2.5% or less of diethylene glycol.

ETHYLENE GLYCOL (excluding its salts and derivatives) in preparations containing not less than 10 mg/kg of denatonium benzoate as a bittering agent except:

(a) in paints or paint tinters; or
(b) in toothpastes or mouthwashes containing more than 0.25% of ethylene glycol; or
(c) in other preparations containing 2.5% or less of ethylene glycol.

Schedule 6:

DIETHYLENE GLYCOL (excluding its salts and derivatives) except:

(a) when included in Schedule 5; or
(b) in paints or paint tinters; or
(c) in toothpastes or mouthwashes containing more than 0.25% of diethylene glycol; or
(d) in other preparations containing 2.5% or less of diethylene glycol.

ETHYLENE GLYCOL (excluding its salts and derivatives) except:

(a) when included in Schedule 5; or
(b) in paints or paint tinters; or
(c) in toothpastes or mouthwashes containing more than 0.25% of ethylene glycol; or
(d) in other preparations containing 2.5% or less of ethylene glycol.

Schedule 10:

DIETHYLENE GLYCOL for use in toothpastes or mouthwashes except in preparations containing 0.25% or less of diethylene glycol.

ETHYLENE GLYCOL for use in toothpastes or mouthwashes except in preparations containing 0.25% or less of ethylene glycol.'

Workers

The following HCIS health hazard classifications apply (SWA n.d.):

Cinnamaldehyde (CAS No. 104-55-2):

Health hazards	Hazard category	Hazard statement
Acute toxicity	Acute Tox. 4	H312: Harmful in contact with skin
Skin corrosion/irritation	Skin Irrit. 2	H315: Causes skin irritation
Serious eye damage/eye irritation	Eye Irrit. 2A	H319: Causes serious eye irritation
Skin Sensitisation	Skin Sens. 1	H317: May cause an allergic skin reaction
Specific target organ toxicity (single exposure)	STOT Single Exp. 3	H335: May cause respiratory irritation

Ethylene glycol (CAS No. 107-21-1):

Health hazards	Hazard category	Hazard statement
Acute toxicity	Acute Tox. 4	H302: Harmful if swallowed
Specific target organ toxicity (single exposure)	STOT Single Exp. 3	H335: May cause respiratory irritation

Diethylene glycol (CAS No. 111-46-6):

Health hazards	Hazard category	Hazard statement
Acute toxicity	Acute Tox. 4	H302: Harmful if swallowed

There are no existing health hazard classifications for acetoin (CAS No. 513-86-0).

Safe Work Australia's Workplace Exposure Standards for Airborne Contaminants list recommends workplace exposure standards (WES) for airborne contaminants in the workplace (SWA 2023).

Ethylene glycol and diethylene glycol have the following WES in Australia (SWA n.d). Ethylene glycol (vapour) has a time weighted average (TWA) of 52 mg/m^3 (20 ppm) and a STEL of 104 mg/m^3 (40 ppm). Ethylene glycol as a particulate has a TWA of 10 mg/m^3. Diethylene glycol has as TWA of 100 mg/m^3 (23 ppm).

Safe Work Australia is currently reviewing WESs, including for ethylene glycol and diethylene glycol. Further information about the review of WES is available on the SWA website (SWA 2023).

There is no WES in place for cinnamaldehyde or acetoin in Australia (SWA n.d.).

International regulatory status

Exposure standards

Cinnamaldehyde has a reported TWA of 3 mg/m^3 in Russia. The USA Temporary Emergency Exposure Limits (TEELs) for 1 hour, 2 hours and 3 hours for cinnamaldehyde are 4, 30 and 500 mg/m^3, respectively (Galleria Chemica).

Ethylene glycol (aerosol) has a TWA of 10 mg/m^3 in different countries such as the UK, Canada and the Netherlands. It has STELs of 10 and 20 mg/m^3 in Canada and the UK, respectively. Ethylene glycol as a vapour has TWAs ranging between 52–250 mg/m^3 in different countries such as the UK, Canada and Taiwan. Ethylene glycol has STELs of 4, 104 and 325 mg/m^3 in the UK, Ireland and Canada, respectively.

Diethylene glycol has a TWA ranging from 10–100 mg/m^3 (44–23 ppm) in different countries including Austria, Denmark, New Zealand, Iceland, Germany South Africa, Sweden, Switzerland, the UK and USA. Diethylene glycol has STELs ranging between 22–176 mg/m^3 (5-40 ppm) in Austria, Estonia, Denmark, New Zealand, Germany, Sweden and Switzerland (Galleria n.d.; MAK 1998).

No international exposure standards are available for acetoin.

Canada

Diethylene glycol is listed on the Health Canada List of Prohibited and Restricted Cosmetic Ingredients (Hotlist). It is 'not permitted in oral or leave-on products'.

Ethylene glycol is not permitted to be used as a diluent in e-cigarettes in Canada, as per the *Canada Consumer Product Safety Act* (Government of Canada 2022).

European Union

In 2022, the European Union proposed a ban on the availability of certain flavoured e-cigarettes which contain tobacco products (EC 2022). The ban will apply from October 2023.

Cinnamaldehyde has the following restrictions in the EU (Galleria):

- European Commission (EC) Toy Safety Directive 2009/48/EC: Allergenic fragrances toys shall not contain
- EC Cosmetics Regulation Annex III (List of substances with restricted use in cosmetic products): The presence of the substance must be indicated in the list of ingredients when its concentration exceeds 0.001 % in leave-on products and 0.01 % in rinse-off products.

Diethylene glycol has the following restrictions in the EU (Galleria):

- European Commission Cosmetics Directive Annex II (List of Prohibited Substances)

- European Commission Cosmetics Directive Annex III (List of Restricted Substances) – the chemical (as traces in ingredients) is restricted to 0.1 % maximum in the finished cosmetic products.

New Zealand

New Zealand has limited the sale of vaping products to those that are mint, menthol or tobacco flavoured. Specialist vaping product retailers are permitted to sell other flavours (Government of New Zealand 2021).

Cinnamaldehyde is listed in the New Zealand *Cosmetic Products Group Standard in Schedule 5—Components cosmetic products must not contain except subject to the restrictions and conditions laid down*. The presence of the substance must be indicated in the list of ingredients when its concentration exceeds 0.001% in leave-on products and 0.01% in rinse-off products (Government of New Zealand 2020).

United Kingdom

Though not explicitly listed, the chemicals in this evaluation are expected to be covered by 'Advice on ingredients in nicotine containing liquids in electronic cigarettes and refill containers' as substances not permitted as ingredients in e-cigarette liquids as they "pose a risk to human health in heated or unheated form" (UK MHRA n.d.).

United States of America

Ethylene glycol (and mixtures containing 10% or more by weight) is considered a hazardous substance under Section 3(b) of the *United States Federal Hazardous Substances Act* as designated by the Consumer Product Safety Commission. Ethylene glycol and mixtures containing 10% or more by weight require labelling with the signal word "Warning" and the statement "Harmful or fatal if swallowed".

Other

An International Fragrance Association (IFRA) Standard applies to cinnamaldehyde, under which, use of the chemical as a fragrance ingredient in finished products is restricted to a concentration limit of up to 1.8%, depending on the product category. IFRA states that the Standards are compulsory for all its members (IFRA 2023).

Human exposure

Public

Repeated inhalational exposure to these chemicals may occur in people using e-cigarettes. The levels of exposure are difficult to determine as there is a lack information on e-cigarette ingredients, as well as a lack of accurate labelling of these products. Even when ingredients are listed on e-cigarette packaging, relative concentrations of chemicals are not.

Second-hand, or indirect inhalational exposure to e-cigarette vapours and aerosols are also expected to occur. Adverse respiratory effects in persons following indirect exposure to vaping has been reported (Islam et al. 2022). This route of exposure cannot be excluded as a

potentially significant exposure pathway for these chemicals, in all demographics of the Australian public (Czogala et al. 2013; Islam et al. 2022).

Health hazard information

Cinnamaldehyde, ethylene glycol and diethylene glycol have previously been assessed under the former scheme, NICNAS. The information presented below includes previously assessed toxicity data relating to inhalation exposure and effects on the respiratory system and any newly identified toxicity data. Human data and studies with equivocal results have been preferentially included. This section also includes relevant toxicity data for acetoin.

More information on data used to draw conclusions for genotoxicity, carcinogenicity and reproductive toxicity is available in the Tier II Human Health IMAP assessment reports (NICNAS 2013; NICNAS 2016a; NICNAS 2016b).

Toxicokinetics

Cinnamaldehyde

Cinnamaldehyde is rapidly absorbed via the gastrointestinal tract based on excretion data in rats and mice (~80% and >90% of administered dose excreted in urine and faeces at 24 and 72 hours after exposure, respectively) (Bickers et al. 2005; FFHPVC 2005). Absorption is also expected to occur via the dermal route (24% and 52% of cinnamaldehyde was absorbed within 72 hours following exposure under non-occlusive and occlusive conditions, respectively) (Bickers et al. 2005).

After oral exposure, cinnamaldehyde is distributed primarily to the gastrointestinal tract, kidney and liver, with a small amount distributed to fat (FFHPVC 2005).

Cinnamaldehyde undergoes extensive oxidation and conjugation to produce a variety of metabolites. The major urinary metabolite of cinnamaldehyde is hippuric acid (glycine conjugate of benzoic acid). After repeated treatment with high doses in rats, benzoic acid was found to be the major metabolite (Bickers et al. 2005; FFHPVC 2005).

Cinnamaldehyde is expected to be excreted as polar metabolites mainly in the urine and to a lesser extent, in the faeces (~77–90% vs 7–16% at 24 hours). The excretion is independent of dose (up to 250 mg/kg body weight (bw)), species (rats vs mice), sex or route of exposure (oral vs intraperitoneal (i.p.)) (Adams et al. 2004; Bickers et al. 2005; FFHPVC 2005).

Ethylene glycol

Ethylene glycol has been shown to be rapidly absorbed following oral exposure in a number of non-human mammal species including rats, mice, monkeys, dogs and gravid rabbits. Peak blood levels are observed in the 1–3 hour range. Blood concentrations following ingestion of a known dose suggests near complete absorption of ethylene glycol occurs in rats and mice (ATSDR 2010; NTP 2004). Dermal absorption of ethylene glycol has been shown to occur (up to 36% of the administered dose in Sprague Dawley (SD) rats). Higher absorption rates have been documented in CD-1 mice (up to 84% of administered dose). In rats, absorption via the inhalation route has been estimated to be 60–90 % of the inhaled dose (ATSDR 2010; NTP 2004).

Analysis of tissue, blood, and urine in humans, rats, mice, monkeys and dogs showed that ethylene glycol is readily distributed to these compartments following oral, dermal or

inhalation exposure and also crosses the placenta in pregnant rabbits following oral exposure (ATSDR 2010; NTP 2004).

The metabolic pathway for ethylene glycol is similar in humans, monkeys, dogs, rabbits, rats and mice (ATSDR 2010). The first major metabolic step is the conversion of ethylene glycol to glycoaldehyde. Glycoaldehyde has a very short half-life and is rapidly converted to glycolic acid and to a lesser extent, glyoxal. Glycolic acid is a major metabolite in humans. The second major metabolic step is the oxidation of glycolic acid to glyoxylic acid in a rate-limiting reaction. Further metabolism of glyoxylic acid leads to the formation of formic acid, glycine, and oxalic acid. The toxicity of ethylene glycol is attributed to glycolic acid and oxalic acid. They can accumulate in the body due to their rate-limiting metabolism. The accumulation of these metabolic products results in acidaemia (increased blood pH), oxalosis (due to the metabolite oxalic acid) and renal interstitial oedema (NICNAS 2016b).

The elimination of ethylene glycol from plasma in humans and laboratory animals was reported to be rapid following oral exposure. The elimination half-lives in blood ranged from 1–4 hours in rats, mice, monkeys and dogs, and the primary excretion pathways were via exhaled air and urine, independent of the exposure route. Human elimination data of the chemical, mostly sourced from cases of accidental poisonings, indicate half-lives in blood ranges from 2.5–8.4 hours. Minimal concentrations of the chemical can be detected in urine or tissue after 24–48 hours (ATSDR 2010; NTP 2004).

Diethylene glycol

Diethylene glycol is rapidly and almost completely absorbed via the oral route in laboratory animals. Up to 96% of diethylene glycol was absorbed within 2 hours in rats after single gavage doses at 1120 and 5600 mg/kg bw. Limited information on absorption of diethylene glycol via dermal and inhalation routes are available. However, the chemical in vapour or aerosol form is likely to be absorbed soon after it enters the upper respiratory passages due to its polar and hygroscopic characteristics. More generally, dermal and inhalation absorption of glycol ethers as a class is expected (Singh et al. 2023).

Due to its high water solubility and low partition coefficient, diethylene glycol is rapidly distributed from the blood throughout the following tissues in the order: kidneys > brain > spleen > liver > muscle > fat (i.e. the same order as the blood flow).

In animals, the metabolic pathway for diethylene glycol is oxidation via alcohol dehydrogenases and aldehyde dehydrogenases (ADH/ALD). Identified diethylene glycol metabolites include CO_2, 2-(hydroxyethoxy)acetic acid (2-HEAA), and oxalic acid.

Diethylene glycol and its metabolites are readily cleared from the blood and excreted in the urine. Depending on the administered dose, approximately 45–70% of the total oral dose is excreted unchanged in the urine within 48 hours, and 11–37% as 2-HEAA after oxidative metabolism (NICNAS 2013).

Acetoin

Limited information on the toxicokinetics of acetoin are available. Acetoin is a water-soluble methyl ketone and is expected to be to be well absorbed via oral and inhalational routes. In rats and mice, acetoin has been shown to be rapidly absorbed from the gastrointestinal tract following oral administration.

Acetoin is metabolised primarily via oxidation at low concentrations in vivo and by reduction to 2,3-butanediol at high concentrations (WHO 1999).

In a study in a dog, a total of 78 g of acetoin was orally administered over a 2 month period. Butane-2,3-diol was identified as the major urinary excretion product. This product represented 5–25% of the administered dose. The remainder of the dose was completely metabolised (WHO 1999).

Reduction of ketones is mediated by alcohol dehydrogenase and NADPH-dependent cytosolic carbonyl reductases. Reduction of acetoin is catalysed by the substrate-specific enzyme acetoin reductase (WHO 1999).

Acute Toxicity

Inhalation

There are limited acute inhalation toxicity data for the chemicals in this group. The chemicals are not considered to cause acute toxicity via inhalation.

There are no acute inhalation toxicity data for cinnamaldehyde. However, a median lethal concentration (LC50) of 68.88 mg/L was predicted for cinnamaldehyde using the Organisation for Economic Co-operation and Development (OECD) toolbox. This was calculated for a 4 hour exposure to cinnamaldehyde vapour in Wistar rats of both sexes (REACH n.d.-b).

Ethylene glycol is expected to have low acute toxicity via the inhalation route. The LC50 in rats and mice has been reported to be >200 mg/m^3 (2-hour inhalation exposure). No further details were reported. In another study, LC50s of >2.5 mg/L were reported in CD rats and mice (6-hour inhalation exposure). No adverse effects were reported (NICNAS 2016b).

Diethylene glycol is expected to have low acute toxicity via the inhalation route. LC50s of >4600 mg/m^3 (4-hour inhalation exposure) in rats and LC50 >130 mg/m^3 (2-hour inhalational exposure) in mice were reported for the chemical (as an aerosol). No mortalities or toxic effects were observed in these studies (NICNAS 2013).

There are no acute inhalation toxicity studies available for acetoin.

Observation in humans

Mortality has been observed in humans following intentional or accidental ingestion of ethylene glycol, with the lethal oral dose estimated to be 1400–1600 mg/kg bw (NICNAS 2016b). Toxic effects have been characterised by (potentially overlapping) stages. During the first stage (0.5–12 hours after intake), signs and symptoms include central nervous system depression with ataxia, slurred speech, somnolence, convulsions and gastrointestinal upset. During the second stage (12–72 hours after intake), signs and symptoms include metabolic acidosis with reductions in blood pH and bicarbonate levels and cardiopulmonary effects such as tachypnoea, hyperpnoea, tachycardia, cyanosis, pulmonary oedema and cardiac failure. During the third stage (24–72 hours after intake) renal toxicity is observed possibly from deposition of calcium oxalate crystals in the kidney. Histological investigation of the kidneys has shown tubular necrosis and the presence of oxalate crystals. During a possible 4th stage (6 or more days after intake) signs and symptoms include hearing loss, facial paralysis and other neurologic effects (NTP 2004).

Diethylene glycol toxicity has been observed in humans following ingestion. Typical features of acute toxicity include neurological impairment, metabolic acidosis and acute renal failure. Mortality and morbidity are high (most deaths occur within the first 2 weeks following DEG exposure). Humans appear to be 10 times more susceptible to acute oral toxic effects of DEG

compared with experimental animals, with median lethal dose of 1490 mg/kg bw in humans compared with >15000 mg/kg bw in rats (NICNAS 2013).

Corrosion/Irritation

Respiratory

Cinnamaldehyde is classified for specific target organ toxicity (single exposure) – category 3; H335 (May cause respiratory irritation). In a study designed to investigate the potential for cinnamaldehyde to cause respiratory irritation, the respiratory rate of CF-1 female mice was assessed following exposure to nebulised cinnamaldehyde for 1 minute, either through nose-only breathing or via a tracheal cannulation. Marked respiratory depression with nose-only inhalation was observed. The ED25 was calculated to be 241 µg/L. No significant effects were observed when inhalation occurred via tracheal cannula (Cocchiara et al. 2005). Inhalation of nebulised cinnamaldehyde produced irritating effects in the upper airways of humans in a single study (see **observation in humans**). Cinnamaldehyde and cinnamaldehyde propylene glycol acetal (potential reaction product in e-cigarette liquids) activated transient receptor potential (TRP) irritant receptors in HEK-293T cells, suggesting they may act as airway irritants in e-cigarette users (Erythropel et al. 2018).

Ethylene glycol is classified for specific target organ toxicity (single exposure) – category 3; H335 (May cause respiratory irritation). This is supported by human data (see **Repeated dose toxicity— observation in humans**).

Diethylene glycol and acetoin are not classified for respiratory irritation. No respiratory irritation data are available for these chemicals.

Observation in humans

Cinnamaldehyde induced coughing in all 10 human subjects following inhalation of nebulised chemical (dose levels from 125–800 mM), with a distinct dose-response relationship observed. The response was the number of coughs recorded after exposure to the chemical. The chemical was found to be a specific agonist of the TRP ankyrin-1 receptor, and induced cough due to chemaesthesis of the airways (Birrell et al. 2009).

Mucous membranes may also become irritated following exposure to high concentrations of inhaled cinnamaldehyde which may lead to coughing. No further information is available (Garcia and Harbison 2015).

Ethylene glycol has been shown to cause respiratory irritation in a respiratory tolerance study in 19 human volunteers (see **Repeat dose toxicity – observation in humans**).

Sensitisation

Respiratory sensitisation

No data are available to evaluate the respiratory sensitisation potential for the chemicals in this group.

There were no alerts for respiratory sensitisation for the chemicals in this group, based on the mechanistic profiling functionality of the OECD Quantitative Structure-Activity Relationship (QSAR) Application Toolbox (OECD QSAR Toolbox n.d.).

Repeat dose toxicity

Inhalation

Limited data are available to determine health effects following repeated inhalation exposure.

No data are available to evaluate repeat dose inhalation toxicity for cinnamaldehyde. No toxicologically significant treatment related effects have been reported in various studies investigating repeated oral exposure (NICNAS 2016a).

Repeated oral exposure to ethylene glycol is consistently associated with adverse effects on the kidney such as crystal nephropathy in rodents (NICNAS 2016b). A no observed adverse effect concentration of 355–400 mg/m^3 (140–160 ppm) (rats and mice, 8 hour/day, 16 week) has been reported based on renal toxicity (SWA 2021a). No study details are available.

In non-guideline repeat dose inhalation toxicity studies, mortality was reported in 1/15 rats, 3/15 guinea pigs, 1/3 rabbits, 0/3 dogs and 0/3 monkeys exposed (whole-body) to 12 mg/m^3 of ethylene glycol aerosol for 90 days. Results were likely confounded due to oral exposure to ethylene glycol aerosol deposited on fur. No sub-lethal signs of toxicity were reported (ATSDR 2010).

In another study, exposure to an aerosol of ethylene glycol to 10 or 57 mg/m^3 for 8 hours/day, 5 days/week for 6 weeks caused no mortality in rats (15 animals/dose), guinea pigs (15 animals/dose), rabbits (3 animals/dose), dogs (2 animals/dose), or monkeys (2 animals/dose) (ATSDR 2010).

No repeat dose inhalation toxicity studies are available for diethylene glycol. Available animal data suggest that repeated oral exposure to the chemical is associated with adverse health effects, mainly in the kidneys (oxalate crystalluria, increased urine volumes, hydropic degeneration and tubular necrosis) and, to a lesser extent, the liver (vacuolar degeneration) (NICNAS 2016b).

Acetoin has been assessed in 2-week and 3-month repeat dose inhalation toxicity studies in Wistar rats and B6C3F1/N mice (NTP 2023).

In the 2-week studies, Wistar rats (5/sex/dose) and B6C3F1/N mice (5/sex/dose) were exposed via whole body inhalation to acetoin vapours at concentrations up to 800 ppm for 6 hours per day, 5 days per week, for 2 weeks. There were no significant exposure-related adverse effects in rats or mice reported in the 2-week studies (NTP 2023).

In a 3-month repeat dose inhalation toxicity study of acetoin, Wistar rats (10/sex/dose) and B6C3F1/N mice (10/sex/dose) were exposed via whole body inhalation to acetoin vapour at concentrations up to 800 ppm for 6 hours per day, 5 days per week, for 13–14 weeks. There were no significant exposure related adverse effects in rats or mice reported in this study (NTP 2023).

In e-cigarette liquids, acetoin degrades to diacetyl, which is the oxidised form of acetoin (Vas et al. 2019). Diacetyl is reported to cause adverse respiratory effects in humans following repeated inhalational exposure, and VAPI (AICIS 2022).

Observation in humans

Ethylene glycol was assessed in a repeated exposure, inhalational tolerance study wherein 19 human volunteers were exposed to the chemical as an aerosol (on average 23–30 mg/m^3) for 20–22 hours/day for 30 days. During the last 10 days of the study, the concentration of aerosol was gradually increased to determine the threshold of discomfort. At 140 mg/m^3 most of the volunteers reported upper respiratory and/or nasal irritation. At higher doses, the exposure was only tolerable for 15 minutes at 188 mg/m^3, 2 minutes at 244 mg/m^3 and a 'couple of breaths' at 308 mg/m^3. At the highest exposure dose, volunteers reported intense respiratory discomfort with a burning sensation in the trachea and a burning cough. While some volunteers reported having a headache, no further adverse effects were reported (ATSDR 2010). Inhalation of acetoin has been associated with the induction of bronchiolitis obliterans (Clapp and Jaspers 2017); however, this association has not been fully elucidated.

Genotoxicity

Based on the weight of evidence, including in silico modelling results, the chemicals in this group are not expected to be mutagenic or genotoxic.

Cinnamaldehyde contains an α,β-unsaturated aldehyde group, which is a structural alert for DNA and protein binding in the OECD Toolbox (OECD 2023) and Ames mutagenicity in the expert rule based system, DEREK (Deductive Estimation of Risk from Existing Knowledge) Nexus (Lhasa Limited n.d.). Additional alerts for genotoxicity were identified using metabolic and autooxidation simulators (OECD 2023). The chemical was predicted to be in vitro Ames negative and in vitro chromosomal aberration positive in OASIS TIMES (OASIS LMC n.d.). The predictions were within the applicability domain of the models. The chemical has produced some positive results in in vitro genotoxicity tests, and mostly negative tests in in vivo genotoxicity tests. In a sex linked recessive lethal mutation test in *Drosophila melanogaster* males, cinnamaldehyde was found to induce sex linked recessive lethal mutations (but not reciprocal translocation mutations) following injection of cinnamaldehyde (20,000 ppm) (Bickers et al. 2005). Based on the available data and weight of evidence, the chemical is not considered to be genotoxic (NICNAS 2016a).

Ethylene glycol and diethylene glycol have no structural alerts for genotoxicity using the OECD QSAR Application Toolbox (OECD 2023) and the expert rule-based system, DEREK (Deductive Estimation of Risk from Existing Knowledge) Nexus (Lhasa Limited n.d.). Some alerts were identified using metabolic and autooxidation simulators (OECD 2023). They were predicted to be in vitro Ames negative, in vitro chromosomal aberration negative and in vitro mouse lymphoma negative in OASIS TIMES (OASIS LMC n.d.). The predictions were within the applicability domain of the model. Based on the weight of evidence from the available genotoxicity studies, ethylene glycol and diethylene glycol are not considered to be genotoxic (NICNAS 2016b; NICNAS 2013).

Limited data are available for acetoin. The chemical has no structural alerts for genotoxicity using the OECD QSAR Application Toolbox (OECD 2023) and the expert rule based system, DEREK (Deductive Estimation of Risk from Existing Knowledge) Nexus (Lhasa Limited n.d.). Some alerts were identified using metabolic and autooxidation simulators (OECD 2023). The chemical was predicted to be in vitro Ames negative, in vitro chromosomal aberration negative and in vitro mouse lymphoma negative in OASIS TIMES (OASIS LMC n.d.). The predictions were outside the applicability domain of the model. The chemical was negative in an OECD Test Guideline (TG) 471 assay when tested in *Salmonella typhimurium* strains TA98, TA100 and TA102, at concentrations up to 5 µL/plate, in the absence or presence metabolic activation (REACH n.d.-b). No other genotoxicity studies were available in the literature. Although the oxidised form of acetoin, diacetyl, is considered to have genotoxic potential in vitro, it is not

expected to be genotoxic in vivo. Diacetyl gave mixed results in in vitro genetic toxicity studies and negative results in the only available in vivo mammalian erythrocyte micronucleus test (AICIS 2022).

Carcinogenicity

Based on the available data, cinnamaldehyde is not considered to be a carcinogen. In 2 year carcinogenicity studies in Fischer (F)344 rats and B6C3F1 mice (50/animals/sex/dose) fed trans-cinnamaldehyde at doses up to 200 mg/kg bw/day, it was reported that:

- rats showed increased incidences of preputial and prostate gland adenomas and mononuclear cell leukaemia
- mice showed forestomach hyperplasia.

No other treatment-related neoplasms or non-neoplastic lesions were reported in either species. These effects were considered to be within the historical range in controls, or likely to represent biological variations unrelated to exposure to the chemical (NICNAS 2016a; REACH n.d.-b).

Based on the available data, ethylene glycol is not considered to be a carcinogen. There was no evidence of carcinogenicity in studies conducted in various rodent species. No tumours were reported in:

- SD rats administered the chemical at up to 3000 mg/kg bw/day in the diet for 2 years
- F344 rats administered 1000 mg/kg bw/day in the diet for one year
- B6C3F1 mice administered up to 12,000 mg/kg bw/day in the diet for 2 years
- CD-1 mice administered up to 1000 mg/kg bw/day in the diet for 2 years (NICNAS 2016b; NTP 2004).

Based on the available data, diethylene glycol is not considered to be a carcinogen. Urinary bladder calculus and tumour responses were recorded in some long term oral carcinogenicity studies in rats. Bladder tumours were found to be associated with the formation of oxalate-containing bladder stones in a 2 year feeding study (Fitzhugh and Nelson 1946). In other studies, the chemical only induced bladder tumours when a foreign body or lesion was present, such as an oxalate-containing bladder stone or a surgery-induced bladder lesion. These studies concluded that the observed bladder tumours were due to mechanical irritation by oxalate-containing bladder stones, rather than a true carcinogenic response to diethylene glycol. In more recent studies, DEG did not demonstrate any evidence of carcinogenic effects after oral administration. Several studies in mice also showed that diethylene glycol is not carcinogenic following dermal application (NICNAS 2013).

No data are available to evaluate carcinogenicity for acetoin. Diacetyl, the oxidised form of acetoin is considered to have carcinogenic potential. Sustained cytotoxicity and cell proliferation resulting from chronic diacetyl exposure in combination with the DNA binding potential may contribute to the induction of respiratory tumours. However, data are not sufficient for hazard classification of diacetyl (AICIS 2022).

Observation in humans

A limited number of human epidemiological studies have reported that exposure to ethylene glycol does not increase the risk of cancer. Exposure (inhalation) in 1666 chemical plant employees was not found to increase the odds ratio for any type of cancer (ATSDR 2010).

No information is available in the literature concerning the occurrence of bladder stones in humans after ingesting diethylene glycol. Overall, although some human carcinogenicity data are available, the data are insufficient to evaluate the carcinogenic potential of diethylene glycol (NICNAS 2013).

Reproductive and development toxicity

Based on the available data, cinnamaldehyde does not show specific reproductive or developmental toxicity (NICNAS 2016a). Developmental effects observed in rodent studies only occurred secondary to maternal toxicity. In a study with SD rats, animals were administered cinnamaldehyde via oral gavage at doses of 0, 5, 25 or 250 mg/kg bw/day on gestation days (GD) 7–17. Treatment related increases in the incidence of defective cranial ossification in all dose groups were observed. Renal abnormalities including dilated pelvis and reduced papilla and dilated ureters were observed at the lowest and middle doses, but not at the highest dose. Offspring at 25 mg/kg bw/day had significantly increased instances of reduced ossification of the tympanic bulla. An increase in the incidence of abnormal sternebrae was also reported in the 25 mg/kg bw/day group. A lowest observed adverse effect level (LOAEL) of 5 mg/kg bw/day for developmental toxicity was reported based on the reduced cranial ossification and kidney variations. However, these effects were not found to be dose related and may be attributed to a decrease in maternal weight gain that was noted in the mid- and high-dose groups (NICNAS 2016a). No signs of toxicity were reported in the dams or in the offspring of CD-1 mice after exposure to 1200 mg/kg bw/day during GD 6–13 (cinnamaldehyde) or GD 7–14 (trans-cinnamaldehyde) (NICNAS 2016a).

Based on the available data, ethylene glycol does not show specific reproductive or developmental toxicity. The available data from rat studies suggest that developmental effects were only observed secondary to maternal toxicity. The chemical is not toxic to reproduction (NICNAS 2016b). In one study, CD-1 mice and SD rats were orally administered ethylene glycol at high doses (500 mg/kg bw/day, and 1000 mg/kg bw/day, respectively) on GD 6–15. Developmental effects in mice and rats such as axial skeletal malformations, external malformations, reduced body weights and increased post-implantation loss were observed (NTP 2004). However, the NTP Centre for the Evaluation of Risks to Human Reproduction (CERHR) concluded that developmental toxicity may not be attributed directly to the chemical but from the accumulation of glycolic acid, which is a metabolic breakdown product of ethylene glycol. The panel also indicated that developmental effects were seen at doses that exceed saturation of glycolic acid metabolism. Observations from rat studies suggest that oral doses resulting in developmental toxicity (1000 mg/kg bw/d) are greater than those associated with maternal and renal toxicity at 500 mg/kg bw/day (NTP 2004).

Available animal studies indicate that diethylene glycol induces adverse effects on fertility and development, but only at doses higher than those associated with repeated dose effects and in the presence of maternal toxicity. Observed effects include reduced litter numbers, litter sizes and live pup weights in mice, and foetal abnormalities in rats and mice such as reduced foetal body weights, skeletal variations and/or malformations, and related mortality at high, maternally toxic doses (NICNAS 2013).

There are no reproductive or developmental toxicity data available for acetoin.

Immunotoxicity

Cinnamaldehyde has the potential to impair respiratory immune cell function. Both cinnamaldehyde containing e-cigarette liquids, and cinnamaldehyde alone was able to suppress macrophage phagocytosis (Clapp et al. 2017). Conversely, cinnamaldehyde and acetoin have also been associated with pro-inflammatory responses in airway epithelial cells

and lung fibroblasts. These chemicals have also been associated with the impairment of epithelial barrier function (Clapp et al. 2017; Gerloff et al. 2017). Cinnamaldehyde has also been shown to disrupt mitochondrial function, inhibit bioenergetic processes, and reduce ATP levels, which correlates with impaired ciliary beat frequency and diminish mucociliary clearance in the airways (Clapp et al. 2019). Taken together, inhalation of cinnamaldehyde may increase the risk of respiratory infections in e-cigarette users.

Other

Cinnamaldehyde has been shown to alter contraction-dependent signal amplitude, beating rate, and cell morphology in spontaneously beating human induced pluripotent stem cell (IPSC)-derived cardiomyocytes in vitro. Heating of cinnamaldehyde was found to attenuate these effects. Collectively, these results suggest that cinnamaldehyde may alter cardiac excitability, in part by impairing the processes that regulate membrane potential and depolarisation (Nystoriak et al. 2019).

A study showed that e-cigarette vapours containing cinnamaldehyde were more toxic to HL-1 (mouse atrial) cardiomyocytes than fruit flavoured e-cigarettes vapours (Abouassali et al. 2021). Exposure of human IPSC-derived cardiomyocytes to cinnamaldehyde affected the beat frequency and prolonged the field potential duration of these cells more than fruit flavoured e-cigarette vapour.

In an in vitro study, e-cigarette liquids were tested on the human cell line HEK293T (Correia-Álvarez et al 2020). Investigators measured toxicity, mitochondrial membrane potential ($\Delta\Psi$ m), reactive oxygen species production (ROS), and cellular membrane potential (Vm) using high throughput screening (HTS) approaches. The study showed that some e-liquids , including those containing acetoin and cinnamaldehyde, caused a decrease in $\Delta\Psi$ m and Vm and an increase in ROS production and toxicity in a dose-dependent fashion (Correia-Álvarez et al 2020). These changes may be associated with cell damage or cell death in the airways of users of e-cigarettes containing acetoin and cinnamaldehyde.

In another in vitro study, 2 monocytic cell lines (MM6 and U937) were treated with commonly used e-cigarette flavouring chemicals, including cinnamaldehyde and acetoin at different doses between 10 and 1000 µM (Muthumalage et al. 2018). Cinnamaldehyde induced dose dependent cytotoxicity and increased ROS production (measured by H_2O_2 equivalents), in a dose dependent manner in both cell lines. At low doses, cinnamaldehyde caused significantly increased interleukin (IL)-8 production (U937 cells); however, higher doses caused a reduction in IL-8 production compared with controls. Cinnamaldehyde caused increased IL-8 production (MM6 cells). Acetoin did not affect cell viability in either cell line. Acetoin induced dose dependent cytotoxicity and increased ROS production. Acetoin caused a dose dependent increase in IL-8 production (MM6 cells) and a dose dependent reduction in IL-8 production (U937 cells). Collectively, these findings highlight the potential for cinnamaldehyde and acetoin to impair normal cell function in an in vitro setting (Muthumalage et al. 2018).

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