# ELECTRONIC CIGARETTES: A SYSTEMATIC LITERATURE REVIEW ON THE EFFECTS OF FLAVOR CHEMICALS ON EARLY EMBRYONIC DEVELOPMENT

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ABSTRACT

Pregnant women who cannot quit smoking tobacco cigarettes have sometimes switched to electronic cigarettes (ECs) due to perceptions that ECs are "safer". In fact, 7.0% of women report using ECs at some point in their pregnancy, while 1.4% report using ECs during the last 3 months of pregnancy (Kapaya et. al, 2019). ECs aerosols contain high concentrations of flavor chemicals, such as cinnamaldehyde, vanillin, and menthol, which have the potential of binding to transient receptor potential (TRP) channels. Seventeen TRP channels were expressed at various stages of early human development including: TRPA1, TRPV1, TRPV3, TRPV4, TRPV6, TRPM2, TRPM5, TRPM4, TRPM7, TRPM8, TRPC3, TRPC6, TRPC7, TRPML1, TRPML2, TRPP1, and TRPP3 (Toxicology). This review evaluates the TRP channels and their roles in the early stages of human development and the potential harm that flavor chemicals may cause by activating TRP channels in human embryos. This will help pregnant women understand the potential risks of using ECs and will enable better regulation of their manufacture and sales.

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Introduction

Pregnant women who cannot quit smoking tobacco cigarettes have sometimes switched to electronic cigarettes (ECs), due to perceptions that ECs are "safer" (McCubbin et. al, 2017). In fact, 7% of women report using ECs at some point in their pregnancy, while 1.4% report using ECs during the last 3 months of pregnancy (Kapaya et. al, 2019). A study that examined associations between EC use and participant characteristics found that the use of ECs to quit smoking may be common in women of reproductive age, including those who are pregnant (Oncken et. al, 2017). In this study, 53% of the participants had previously tried ECs, 14% of which reported EC use during pregnancy (Oncken et. al, 2017). ECs are popular devices designed to heat a liquid solution that generates an inhaled aerosol or vapor (Klein et. al, 2019). The liquid solution in most ECs contains nicotine and is available in a variety of flavors (Klein et. al, 2019). ECs contain ingredients such as propylene glycol, glycerol, ethylene glycol and polyethylene glycol mixed with concentrated flavors, and variable percentages of nicotine (Hahn

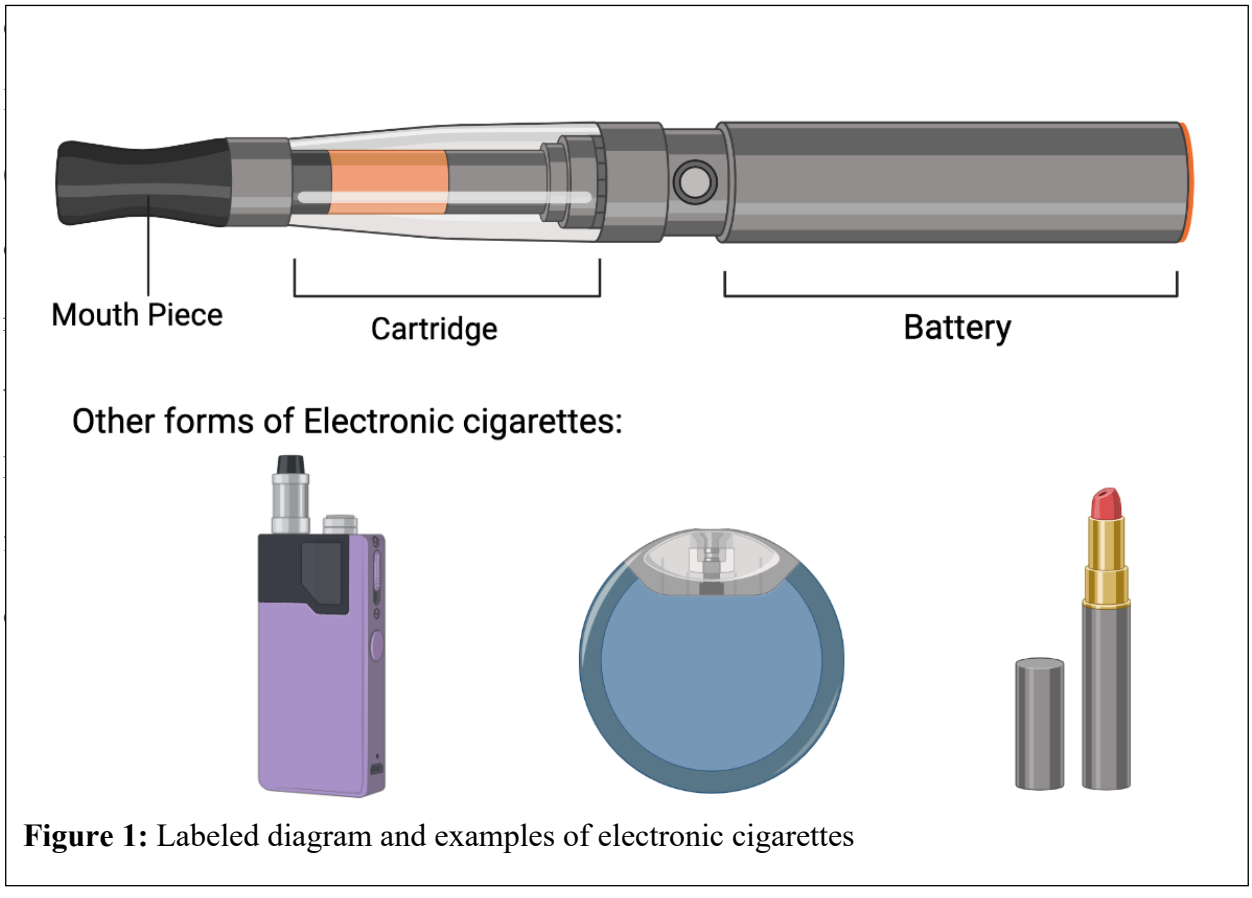


**Figure 1:** Labeled diagram and examples of electronic cigarettes

This review will explore the current knowledge on how flavor chemicals in EC aerosols interact with TRP channels to affect early embryonic development and will focus on the effects of the most used flavor chemicals, including cinnamaldehyde, vanillin, menthol, and nicotine, on early embryonic development. There are at least 17 TRP channels in early stages of human development (oocyte to epiblast) (Toxicology). TRP channels are a diverse family of ion channels that can sense intracellular calcium and magnesium concentrations (De Clercq et al. 2018). TRP channels can detect signals and convert those signals into cellular responses that are extremely important for embryonic development (De Clercq et al. 2018 and Vrenken et al. 2016). For example, TRPM7 was required in early embryonic development in mice (Komiya et al. 2015). TRP channels are important in the discussion of the effects of vaping ECs while pregnant on embryonic development because research has indicated that TRP channels may be affected by EC flavor chemicals. TRPA1 channels are triggered by menthol, reactive oxygen species (ROS), acrolein, formaldehyde, cinnamaldehyde, benzaldehyde, icilin, gingerol, cannabis, nicotine, and acetaldehyde (Omaiye et al. 2019). TRPV1-V6 channels are primarily triggered by vanillin (Omaiye et al. 2019). TRPV1 is also triggered by nicotine (DeVito et al. 2018); TRPV2 and TRPV4 are triggered by cannabidiol (Greene et al. 2019); finally, TRPV3 is triggered by menthol and eugenol (Lumpkin et al. 2002). The TRPM8 channel is triggered by icilin, cannabidiol, menthol, WS-3 (a cooling agent), and eucalyptol (Premkumar et al. 2014 and Rosbrook et al. 2016). This review will summarize the current knowledge of the effects of EC flavor chemicals on early embryonic development and inform pregnant women on the effects of EC vaping during pregnancy.

## Methods

This review follows the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-analysis) guidelines for conducting systematic reviews. The flowchart for the systematic review is represented in Figure 2.

Search Strategy

An Internet search using the keywords or phrases: “embryonic stem cells”, “electronic cigarettes”, “e-cigarettes”, “toxicity”, “pregnancy”, “adverse effects”, “embryo”, “fetus”, “development”, “prenatal development”, “disease”, “congenital defects”, “vanillin”, “menthol”, “ethyl maltol”, “cinnamaldehyde”, “triacetin”, and “nicotine” was carried out in PUBMED and general internet search engines such as Google Scholar. References in case reports or other related peer- reviewed literature also contributed to gathering literature.

Inclusion criteria

Case reports and reviewed abstracts dealing with developmental effects attributed to ECs with and without flavor chemical use or exposure on embryos were included. At a minimum, the cases reporting systemic effects needed to: (1) clearly discuss the effects of flavor chemicals and/or EC liquid on embryonic development (2) examine the mechanisms which cause these effects.

Exclusion criteria

Case reports or abstracts that reported on EC use or EC products without direct correlation to embryonic development were excluded from the study. Reports that did not examine any of the flavor chemicals included in the keywords were not included.

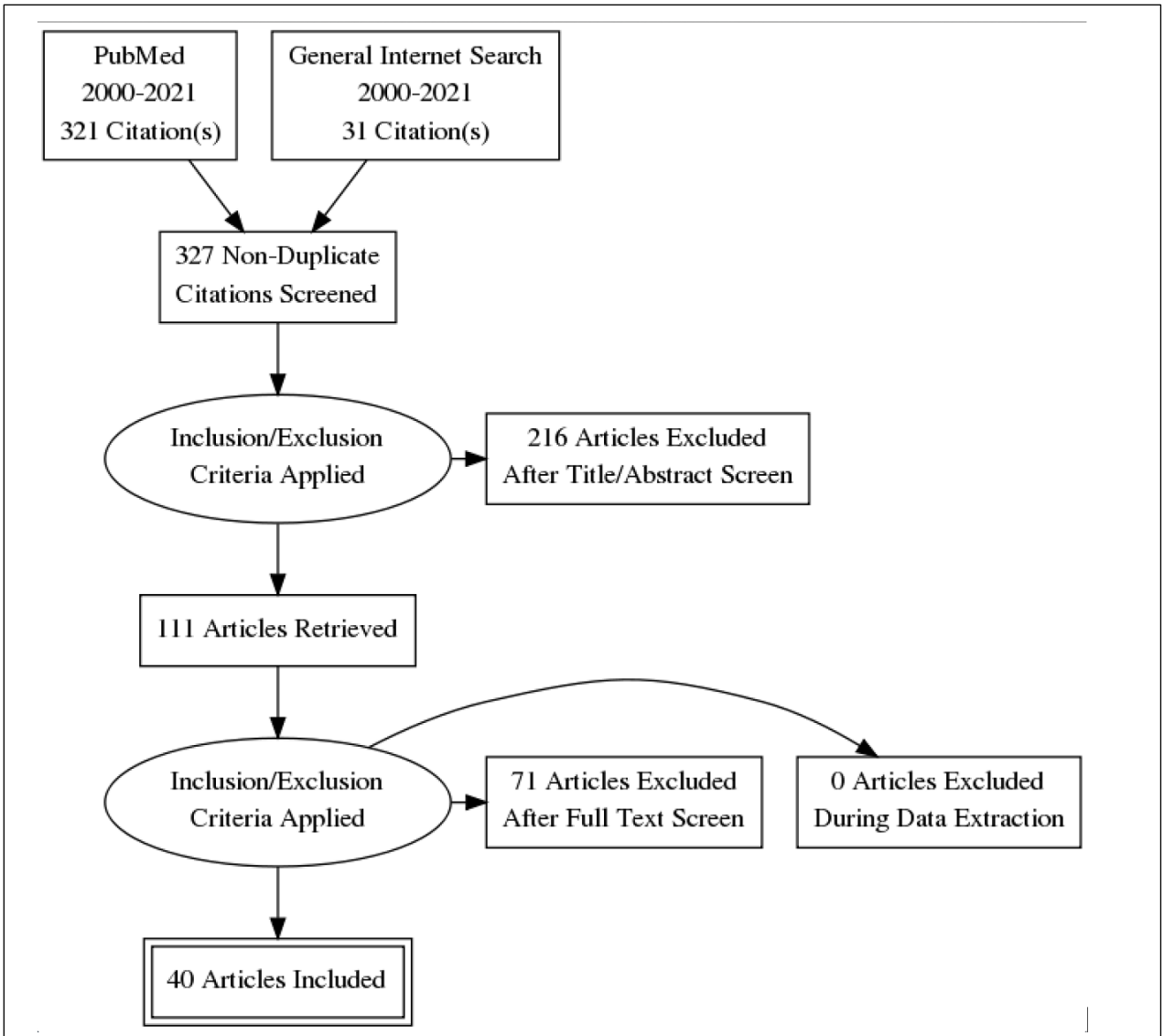


**Figure 2**: PRISMA Flow Chart of PubMed and General Internet search findings illustrating the quantity of literature initially found and then finally literature used in this review after eliminating duplicate documents and those that did not pass the inclusion criteria.

Functions of TRP channels in embryonic development

In mammals, the TRP superfamily contains 28 members, which are divided into six subgroups: ankyrin (TRPA), vanilloid (TRPV), canonical (TRPC), melastatin (TRPM), polycystin (TRPP), and mucolipins (TRPML) (De Clerq et. al, 2018). TRPA1 functions as an irritating-receptor, and pungent compounds from mustard, garlic, and environmental irritants, such as formaldehyde and acrolein, can activate the channel (Nilius et. al, 2011 and Kunkler et.

al, 2011). The TRPV family contains the TRPV1 channel, which is activated by heat and capsaicin, while TRPV2-4 channels are sensitive to high temperatures (Vay et. al, 2012 and Vriens et. al, 2014). TRPV5 and TRPV6 are activated by vanillin, and they are the most highly calcium selective (Den Dekker et. al, 2003 and Mulier et. al, 2017). TRPC family channels are a group of receptor-operated calcium permeable nonselective cation channels that use osmoreceptors to regulate their signaling activities (Weick et. al, 2009 and Wang et. al, 2020). Of the TRPM family, TRPM 3-5 channels are only permeable to calcium, while TRPM 6-7 are permeable to both calcium and magnesium and can be triggered by menthol (De Clerq et. al, 2018). The TRPP family consists of three members that are also calcium permeable (De Clerq et. al, 2018). TRPP2, is also known as PKD2, while TRPP3 and TRPP5 are known as polycystin 2 (PC-2) (De Clerq et. al, 2018). PKD2 functions as a calcium-permeable TRP-like channel (DeClercq et. al, 2018). Finally, the TRPML family is mostly restricted to performing functions within intracellular vesicles such as lysosomes (De Clerq et. al, 2018). The TRPML family consists of the three members TRPML1, TRPML2, and TRPML3 (Venkatachalam et. al, 2015). TRPML has roles in vesicular trafficking and biogenesis, maintenance of neuronal development, function, and viability, and regulation of intracellular and organellar ionic homeostasis (Venkatachalam et. al, 2016). Of all the TRP channels, TRPML channels are the least selective cation channels that have variable permeability to a variety of cations including $Na^{+}$, $Ca^{2+}$, $Fe^{2+}$, and $Zn^{2+}$ (Venkatachalam et. al, 2015). TRP channels generally permit influx of calcium and other ions (magnesium), allowing TRP channels to aid in embryonic development (Vrenken et. al, 2016).

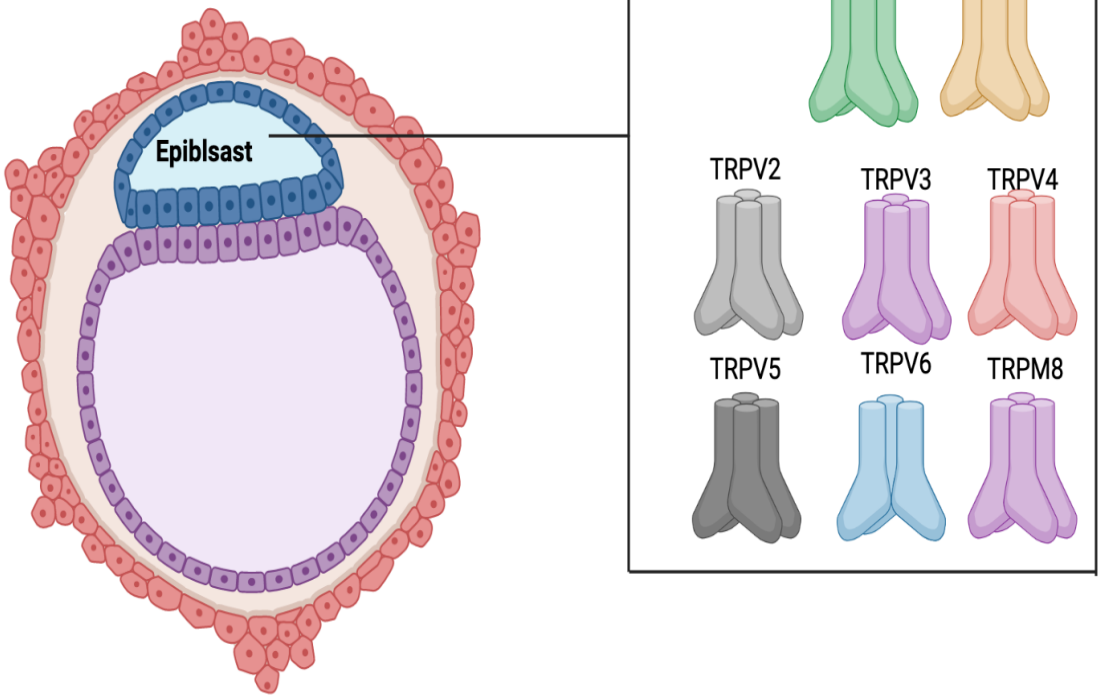


**Figure 3a:** TRP channels present in early embryonic development. TRPA1, TRPV1, 2, 3, 4, 5, 6 and TRPM8 are highly expressed in early development

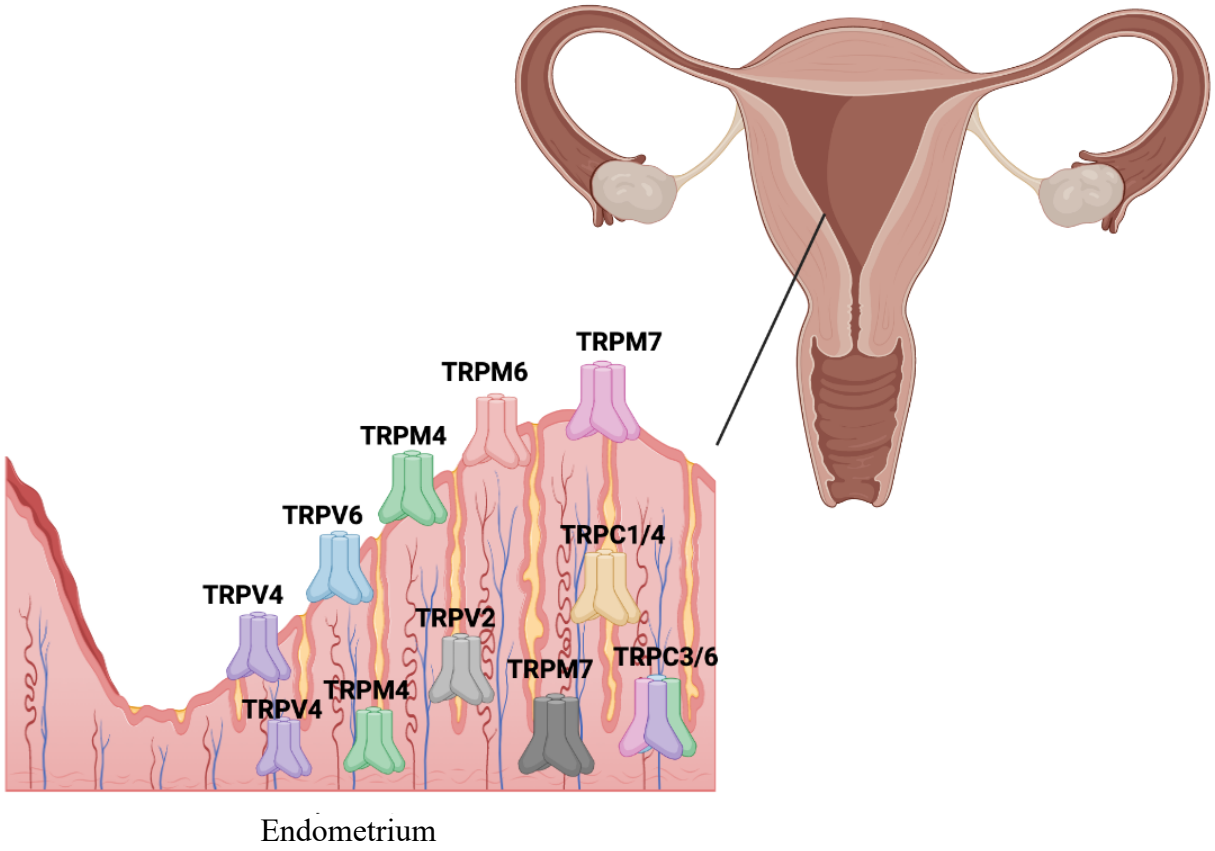


**Figure 3b:** TRP channel expression in female reproductive organs of mice. TRPV 2,4,6; TRPM 4,6,7; and TRPC1,3,4,6 is found in the uterus on the endometrial line in mice.

**Table 1: Summary of TRP Channels and their Functions**

| TRP Channel | Function/Significance | Source |
|---|---|---|
| TRPA1 | Operates as an irritating-receptor since pungent compounds from mustard, garlic, and environmental irritants such as formaldehyde and acrolein can activate the channel | DeClercq et al., 2016, Establishing life is a TriP- TRP channels in reproduction |
| TRPV1 | Activated by heat and capsaicin | DeClercq et al., 2016, Establishing life is a TriP- TRP channels in reproduction |

| | | |
|---|---|---|
| TRPV2 | SKF-96365 (inhibitor) does not only inhibit TRPV2, but it can also induce a reverse operation of the Na+/Ca2+ exchanger within the same concentration range or activate cation influx<br><br>Exhibits high temperature sensitivities | DeClercq et al., 2016, Establishing life is a TriP- TRP channels in reproduction |
| TRPV3 | Exhibit high temperature sensitivities<br><br>Channel found to be progressively expressed during oocyte maturation in mice<br><br>Activation of TRPV3 provokes egg activation by inducing a robust calcium entry | DeClercq et al., 2016, Establishing life is a TriP- TRP channels in reproduction |
| TRPV4 | Exhibit high temperature sensitivities<br><br>Transcripts and protein have been identified in ciliated epithelial cells of the mouse female reproductive organs, where it plays a role in mechanotransduction process<br><br>Channels were found to be stimulated by changes in mucus viscosity, leading to increased intracellular calcium levels<br><br>Could play a role in controlling myometrial calcium concentrations and may be important transducers of agonist-mediated signals that increase at the time of parturition and labor | DeClercq et al., 2016, Establishing life is a TriP- TRP channels in reproduction |
| TRPV5 | Insensitive to heat<br><br>High calcium-selectivity<br><br>Exclusive expression in epithelial cells further suggests an important role in calcium uptake and homeostasis<br><br>TRPV5 and TRPV6 channels are described to be co-expressed in the human placenta | DeClercq et al., 2016, Establishing life is a TriP- TRP channels in reproduction |

| | | |
|---|---|---|
| TRPV6 | Insensitive to heat<br><br>High calcium-selectivity<br><br>Exclusive expression in epithelial cells further suggests an important role in calcium uptake and homeostasis<br><br>TRPV5 and TRPV6 channels are described to be co-expressed in the human placenta | DeClercq et al., 2016, Establishing life is a TriP- TRP channels in reproduction |
| TRPM8 | Specifically expressed in a subset of pain- and temperature-sensing neurons.<br><br>Cells overexpressing the TRPM8 channel can be activated by cold temperatures and by a cooling agent, menthol.<br><br>TRPM8 neurons thus appear to belong to a subset of nociceptive thermoreceptive neurons that express trkA, an NGF receptor, during development.<br><br>TRPM8 is expressed in a subpopulation of thermoreceptive/nociceptive neurons distinct from the well-characterized heat- and pain-sensing neurons<br><br>When the temperature was lowered from 25C to 15C, an increase in intracellular calcium was observed in TRPM8-expressing cells<br><br>TRPM8 responds to menthol at 25C. Intensity of the TRPM8 response is dependent on menthol concentrations. | Peier et al., 2002, A TRP Channel that Senses Cold Stimuli and menthol |

Animal Studies

EC experiments on pregnant animals have shown numerous short- and long-term effects on the development of their offspring. An experiment done on *Xenopus laevis* embryos and mammalian neural crest cells showed that aerosolized EC liquids containing 24 mg/mL of nicotine exhibited significant shifts in the position of the mouth and ventral facial landmarks, as well as warping of the transformation grid in the lower portion of the face (Kennedy et. al, 2017).

Further experimentation on the presence or lack of nicotine with aerosolized ECs showed that higher concentrations of nicotine exacerbated the defects (Kennedy et. al 2017). The authors then tested the following EC flavors to determine if they influenced craniofacial/orofacial defects: red tobacco, dark chocolate, milk chocolate, melon, candy, menthol, strawberry, almond, caramel, vanilla, biscuit, vienna cream, cereal, berries, cream, and citrus. Overall, embryos exposed to strawberry, almond, caramel, vanilla, biscuit, vienna cream, cereal, berries, cream, and citrus flavors had dramatic facial defects including narrower face shape, protruding lenses, rounder mouths, and median oral clefts in the upper lip region, while other flavors had minor or negligible effects in the orofacial size and shape (Kennedy et. al, 2017). Exposure to ECs, with or without nicotine, on *Xenopus* embryos had adverse effects on craniofacial development with certain EC flavors exacerbating these defects. Dickinson et. al 2022 explored the role that retinoic acid signaling has in causing craniofacial defects in *Xenopus* embryos exposed to vanilla EC flavors. In early embryonic development, the retinoic acid signaling pathway regulates germ layer formation. Analysis of differentially expressed genes in treated embryos revealed several genes associated with retinoic acid metabolism (Dickinson et. al, 2022). Variants of retinoic acid were associated with craniofacial defects in humans (Johnston et. al, 1995). Retinoic acid signaling was investigated because the types of craniofacial defects seen in EC liquid treated animals are like those produced when retinoic acid signaling is blocked. Exposure to EC liquid resulted in similar craniofacial malformations as a retinoic acid receptor antagonist treatment (Dickinson et. al, 2022). In another experiment, exposure to EC liquid significantly altered six retinoic acid associated genes (Dickinson et. al, 2022). 13-cis-retinoic acid was used as a treatment for embryos whose retinoic acid signaling was inhibited, and this treatment significantly reduced the EC liquid-induced malformations in the midface (Dickinson et. al,

2022). This suggests that there is possibly an interaction between retinoic acid signaling and EC liquid exposure (Dickinson et. al, 2022). When these experiments were done with vanillin, embryos had craniofacial defects mimicking the embryos with retinoic acid deficiency (Dickinson et. al, 2022). Overall, EC liquid exposure induced craniofacial defects and dysregulated retinoic acid signaling, and these effects were exacerbated by vanillin and other flavor chemical exposure.

EC liquid and cinnamaldehyde have also been shown to disturb bone, cartilage, and vasculature development in zebrafish embryos (Bhattacharya et. al, 2021). Bhattacharya et. al 2021 examined zebrafish embryonic development after being exposed to cinnamaldehyde and nicotine or cinnamaldehyde alone (both with VG/PG) (Bhattacharya et. al, 2021). They found that exposures to both EC groups disturbed the development of the cleithrum, craniofacial cartilage, and reduced hatching success (Bhattacharya et. al, 2021). The effects of cinnamaldehyde were exacerbated by nicotine (Bhattacharya et. al, 2021). These studies show that EC liquids alone can disturb craniofacial development in embryos and two of the most popular EC flavors, vanillin and cinnamaldehyde, exacerbate these effects.

In addition to craniofacial defects, ECs also affected lung development in mouse embryos. One study assigned pregnant mice to one of three groups: room air (sham), EC liquid without nicotine, and EC liquid with 18 mg/mL of nicotine to assess the effect of ECs on respiratory health (Chen et. al 2018). Mother mice were exposed for 6 weeks before gestation, during gestation, and during lactation (Chen et. al 2018). Offspring of mothers exposed to ECs containing nicotine had increased DNA methylation in the lungs (Chen et. al 2018). This is significant because it reveals an epigenetic effect that can potentially cause detrimental health outcomes in terms of anatomical development, protein kinase activity, and gene expression of

proinflammatory cytokines in the offspring. This experiment did not specify which of these possible epigenetic effects could occur but suggested future studies to identify site-specific methylation changes because of EC exposure. In a study on mice, Noel et. al, 2020 showed that in utero exposure to EC aerosols impaired *Wnt* signaling during mouse lung development (Noel et. al, 2020). This study separated the female mice into four groups: preconception female Air, preconception female EC, prenatal female Air, and prenatal female EC (Noel et. al 2020). The preconception exposure groups were exposed to 36 mg/mL cinnamon and nicotine flavored EC aerosol or high- efficiency particulate air (HEPA) for 2 hours a day, for 12 days, before mating. Prenatal mice were exposed from days 6–19 of gestation to ensure exposure of cinnamon and nicotine flavored EC aerosols was during the critical and sensitive window of lung organogenesis (Noel et. al 2020). The cinnamaldehyde concentration of 36 mg/mL was used to mimic the behavior of heavy vapers. This exposure method more accurately illustrates human behavior for two reasons. First, cinnamaldehyde is a popular flavor chemical used in ECs which makes understanding the effects of this flavor particularly useful. Second, the mice in this study were exposed to ECs before and during pregnancy, which simulates behavior of some humans who use EC during pregnancy. Most other animal studies only expose mothers during gestation. This study reveals that preconception and prenatal exposures to EC aerosols decrease birth weight, body length, and impair lung development in mice (Noel et. al 2020). In utero EC aerosol exposure in postnatal day 0 offspring downregulated the expression of *Shh* and 75 *Wnt*-related lung genes (Noel et. al, 2020). The downregulation of *Wnt* and the resulting lung impairments of the offspring implies that *Wnt* signaling plays a key role in lung development. Overall, this study found that preconception and prenatal exposure to cinnamon and nicotine

flavored ECs downregulate *Wnt* signaling pathways leading to lung impairment in EC exposed dams.

ECs also have the potential of causing genetic and developmental defects in embryos. Another study investigated the effects of maternal EC aerosol exposure on offspring in a mouse model to determine if ECs affect global DNA methylation (Nguyen et. al, 2018). The mice were randomly split into three groups 6 weeks before pregnancy, during pregnancy, and during lactation with full body exposure: ambient air, EC aerosol with nicotine, and EC aerosol without nicotine (Nguyen et. al, 2018). Note that this study, like the one by Noel et. al, also exposed mothers to EC aerosol before pregnancy, which models' human behavior well. DNA methyltransferases (Dnmt3a and Dnmt3b) were shown to play a key role in neurogenesis and are present in different regions of the brain (Nguyen et. al, 2018). Maternal exposure to EC aerosols, with or without nicotine, resulted in reduced Dnmt3a/Dnmt3b gene expression, particularly in adulthood (Nguyen et. al, 2018). Dnmt3a/Dnmt3b are de novo methyltransferases, meaning they establish DNA methylation patterns during embryogenesis and set up genomic imprints during germ cell development (Zhang et. al, 2017). Since Dnmt3a/Dnmt3b are extremely important genes in embryonic development, the finding that these genes are decreased because of EC aerosol (with or without nicotine) exposure, indicates that EC aerosol disrupts DNA methylation. Therefore, disruption in global DNA methylation is associated with significant changes in chromatin modification enzymes in the brains of offspring, but more research is needed to determine if these epigenetic changes can cause developmental delay and cognitive deficits in offspring (Nguyen et. al, 2018).

A subsequent study by Nguyen et. al 2019, revealed further neurological effects in the offspring of mice after switching mothers from tobacco cigarettes to ECs during pregnancy

(Nguyen et. al, 2019). Mice were divided into three treatment groups: ambient air, tobacco cigarette smoke exposure (SE), and SE prior to gestation followed by EC aerosol exposure during gestation and lactation (Nguyen et. al 2019). This study attempts to mimic human behavior as pregnant women who vape ECs during pregnancy are often switching from tobacco cigarettes because of the narrative that ECs are a safer alternative during pregnancy. Significant changes in gene expression levels were mainly observed in offspring from mothers exposed solely to tobacco cigarette smoke. However, *Aurora kinase* (Aurk)A and AurkB (which are genes that are involved in mitotic division) were significantly decreased in offspring from mothers switching to ECs, and significantly increased in offspring from mothers exposed to cigarette smoke only (Nguyen et. al, 2019). This suggests that switching from tobacco cigarettes to ECs during pregnancy alters epigenetic gene expression levels compared to continued tobacco smoke exposure. However, the specific consequences of these altered genes on embryonic and offspring development still require investigation. The study had difficulty differentiating the effects of tobacco chemicals, nicotine, and the chemicals in the e-fluids and how they may alter neurological function (Nguyen et. al, 2019).

EC experiments on mice have also shown brain injury in offspring after birth and even into adulthood. A study by Sifat et. al 2020 looked at the effects of prenatal EC exposure on hypoxic ischemic brain injury and brain glucose utilization, which are critical determinants of the severity of hypoxic ischemic injury. After exposing mice via direct inhalation to 111 ng/ml of cotinine EC and oxygenated air or oxygenated air alone, EC exposed offspring had reduced neuronal viability in ischemic conditions with an upregulation of DNA damage markers, indicating increased neuronal death (Sifat et. al, 2020). The dose of cotinine was used to mimic heavy vapers. Furthermore, in vivo studies revealed that EC exposed offspring had increased

brain injury (Sifat et. al, 2020). The long-term locomotor, cognitive, and motor function effects of EC exposed offspring after neonatal hypoxic ischemic (HI) brain injury were investigated by conducting a novel object recognition test (which evaluated short term memory function) (Sifat et. al, 2020). Offspring exposed to EC vapor had decreased memory (Sifat et. al, 2020). Using a Morris water maze test, which evaluates learning and reference memory, EC exposed offspring had impaired reference memory and worsened spatial acquisition (Sifat et. al, 2020). These cognitive effects reveal that prenatal EC exposure has negative, long- term effects on adolescents' offspring with hypoxic ischemic brain injuries. These findings are significant because they are one of the first to demonstrate long-term effects on the offspring of mothers exposed to EC vapors. Therefore, in addition to the immediate effects discussed thus far, there is evidence that using ECs during pregnancy can also have long term effects on the offspring. Offspring of the EC vapor exposed mothers had decreased expression of glucose transporters suggesting that offspring of EC exposed mothers are more susceptible to HI brain injury.

Another study from Church et. al, 2020 randomly split mother mice into three groups during gestation (filtered air, EC, and EC + 16 mg/mL nicotine) and hypothesized that maternal exposure to EC aerosols with and without nicotine had behavioral and neuroimmune developmental effects persisting into adulthood of the offspring (Church et. al, 2020). Brain cytokine analysis revealed that IFNc and IL-4 levels in the offspring of EC + nicotine were lower, while offspring of EC exposed mothers showed elevated IL-6 in the cerebellum (Church et. al, 2020). These findings suggest neuroinflammatory consequences of gestational EC exposure with or without nicotine (Church et. al, 2020). However, it is important to note that the analysis of the cortex did not differentiate between frontal, occipital, or temporo-parietal regions of the brain. This is significant because the differences in cytokine expression in a subregion of

the cortex may be masked or diluted by the inclusion of other areas; so, these results cannot with certainty attribute altered cytokine expression to EC exposure (Church et. al, 2020). The results of the elevated plus maze and the swim time in the forced swim tests illustrated that EC aerosol and nicotine exposed offspring have their stress coping strategies hindered (Church et. al, 2020). Interestingly, males were more vulnerable to having their stress coping strategies hindered by the EC aerosol and nicotine mixture, which may indicate sex- specific differences on the effects of EC aerosol exposed offspring. Furthermore, adult male and female offspring of mice exposed to EC aerosol (with and without nicotine) had declined hippocampal-dependent novel object recognition scores (Church et. al, 2020). These behavioral differences between control and EC exposed offspring, suggest that exposure to EC aerosol alone during development could have lasting consequences on the brain.

EC experiments on pregnant mice have also demonstrated sex-specific effects on offspring exposed to EC aerosols and flavors. Wetendorf et. al (2019), conducted a study where mice were exposed through a whole-body inhalation system to: EC with 24 mg/mL nicotine or room air (Wetendorf et. al, 2019). This paper examined whether EC exposure impairs implantation and offspring health. After exposing mice to ECs for 4 months, ECs delayed the onset of the first litter and slightly reduced the total pup and litter number (Wetendorf et. al, 2019). According to these findings, EC exposure can potentially delay first time pregnancy, however further investigation is needed to determine if ECs can affect pregnancy initiation in women. This study is interesting because it investigates not only fetal effects, but whether pregnancy itself is affected. As mentioned earlier, women who vape ECs are generally vapers of ECs or smokers of tobacco cigarettes prior to pregnancy so this study provides insight on the effect using ECs can have prior to pregnancy. In addition, the authors found sex-specific effects

on the offspring of EC exposed mothers. Male offspring of EC exposed mothers had impaired reproductive fitness, with one male failing to produce offspring until 35 days after mating (Wetendorf et. al, 2019). Female offspring did not seem to have any weight differences compared to the control after birth but were significantly smaller than controls at 8.5 months (Wetendorf et. al, 2019). This suggests that ECs are causing metabolic dysregulation to offspring exposed in utero. Overall, EC exposure before pregnancy can delay implantation and the offspring of EC exposed mothers show evidence of metabolic dysregulation affecting weight and size later in life (Wetendorf et. al, 2019). This study provides some insights into sex- specific effects on offspring exposed to EC aerosols in utero, but further investigation is required to determine the details of these effects.

EC exposure can damage the liver and alter nutrient metabolism in pregnant mice and their offspring. Therefore, there may be metabolic dysregulation in the offspring of EC exposed mothers. Li et. al (2020) investigated the impact of maternal e-vapor exposure before gestation and during pregnancy on hepatic metabolic markers, oxidative stress (OS), inflammation, and mitochondrial health in both the mothers and their male offspring (Li et. al, 2020). After randomly dividing mice into three groups (room air, EC vapor and 18 mg/mL nicotine, and EC vapor without nicotine) mothers and offspring exposed to EC vapor with and without nicotine showed adverse effects (Li et. al, 2020). The 18 mg/mL of nicotine used in EC exposure is equivalent to a light vaper. EC vapor exposure was detrimental to liver health in both mothers and offspring because the EC vapor induced OS, inflammation, and liver injury (Li et. al, 2020). However, exposure to EC vapor containing nicotine did not cause liver damage, but rather promoted hepatic steatosis (build-up of fat in the liver) in the adult offspring (Li et. al, 2020). This is significant because it suggests that nicotine may be providing a protective effect against

EC vapor to the mothers and offspring. EC vapor-exposed offspring showed increased gluconeogenesis, which was linked to impaired systemic glucose clearance, but this was not because of insulin resistance (Li et. al, 2020). The authors stipulated that the EC exposed offspring had reduced mitochondrial numbers because the glucose transporters were increased in the liver meaning less energy was being allocated to increasing mitochondrial numbers (Li et. al, 2020). Therefore, the liver damage exhibited in EC exposed offspring was the result of increased glucose uptake revealing that exposure to nicotine free EC vapor promoted liver disorder and altered nutrient metabolism in both mothers and offspring exposed (Li et. al, 2020).

**Table 2: Animal Studies**

| Source | *Species* | Exposure method | E-liquid/ flavor tested | Summary |
|---|---|---|---|---|
| Kennedy et. al 2017 | *Xenopus laevis* | Embryos were exposed to lab grade aerosolized EC from the 2-cell stage | nicotine, PG, VG, red tobacco, dark chocolate, milk chocolate, melon, candy, menthol, strawberry, almond, caramel, vanilla, biscuit, vienna cream, cereal, berries, cream, citrus | Aerosol from commercial flavored fluids produced orofacial defects such as cranial cartilage, muscle and blood distribution in the absence of nicotine<br><br>High nicotine in the same aerosols produced more severe effects |
| Chen et. al 2018 | *Balb/C mice* | full body exposure | EC liquid, nicotine | EC exposure during pregnancy induced an inflammatory environment in the lungs of both the mothers and offspring<br><br>Exposure to ECs induces immune dysregulation within the lung of offspring<br><br>Male offspring are more susceptible to the effect of maternal smoking |

| | | | | |
|---|---|---|---|---|
| Nguyen et. al 2018 | *BALB/c mice* | Full body exposure | nicotine | EC exposure results in changes in global DNA methylation, histone-lysine demethylases, histone acetyltransferases, and histone phosphorylation in offspring brains lead to developmental changes in the offspring |
| Nguyen et. al 2019 | *Balb/c mice* | Full body exposure | EC aerosol and tobacco cigarette | Exposure to EC aerosols after tobacco smoke exposure did not have any effect on number of offspring produced<br><br>Offspring from mothers that switched to EC aerosol or were exposed to continuous cigarette smoke during pregnancy had a low birth weight<br><br>Switching to ECs during pregnancy reduced DNA methylation in the offspring |
| Wetendorf et. al 2019 | *C57BL/6J mice* | Whole body inhalation | EC aerosol and nicotine | EC exposed mice exhibit a delay in embryo attachment<br><br>EC exposed males exhibited a slight reduction in fertility, pup number, and weight<br><br>ECs negatively influence implantation success and the future health of the in utero exposed fetus, resulting in abnormal pregnancy outcomes. |
| Sifat et. al 2020 | *CD1 mice* | Direct inhalation | Nicotine and EC vapor | Maternal EC exposure increased neuronal death in dams<br><br>Reduction in neuronal GLUT3 expression (a major |

| | | | | |
|---|---|---|---|---|
| | | | | glucose transporter in neurons) in EC offspring<br><br>Mitochondrial membrane potential was significantly decreased in EC exposed neurons that could lead to decreased ATP production |
| Church et. al 2020 | *CD1 mice* | Whole body exposure | Nicotine, PG, VG EC liquid | Offspring of dams exposed to PG/VG alone expressed elevated IL-6 in the cerebellum.<br><br>Adult offspring of both sexes exposed to nicotine exhibited elevated locomotor activity<br><br>Daily exposure to PG/VG throughout gestation disrupted learning and memory performance in offspring and increased neuroinflammation. |
| Li et. al 2020 | *Balb/c mice* | Aerosol exposure | nicotine and EC liquid | Exposure to EC aerosol induced insulin resistance and impaired insulin receptor pathway activation<br><br>Exposure to EC aerosol promotes liver disorders and alters nutrient metabolism in both the dams and their offspring. |
| Noel et. al 2020 | *BALB/c mice* | Full body exposure | cinnamaldehyde, nicotine | EC exposure decreased offspring measurements at birth<br><br>EC exposure during pregnancy disturbs fetal development<br><br>EC aerosol exposures were related to decreased growth and proliferation of lung cells |

| | | | | |
|---|---|---|---|---|
| Dickinson et. al, 2021 | *Xenopus laevis* | Embryos were exposed to lab grade aerosolized EC liquids (ecigAM) from the 2-cell stage | Vanillin | Potential interaction between retinoic acid signaling and EC liquid exposure<br><br>EC liquid and vanillin had craniofacial defects mimicking embryos with retinoic acid deficiency |

Human Studies

EC studies done on human stem cells have illustrated the effects EC's and their flavored chemicals have on development. Bahl et. al 2012 compared EC refill fluid cytotoxicity using embryonic and adult models. 35 refill products were evaluated for cytotoxicity in 96-well plates using the MTT assay with human embryonic stem cells (hESC), mouse neural stem cells (mNSC), and human pulmonary fibroblasts (hPF) (Bahl et. al, 2012). The 96-well plates were laid out to have various concentrations of refill solution (0.001%, 0.01%, 0.03%, 0.1%, 0.3%, and 1%) in ascending order from left to right, with negative controls in columns 1,2, 10 and 11 (Bahl et. al, 2012). In general, hESC and mNSC were more sensitive to refill solutions/flavors than the adult lung fibroblasts, which concurs with the fact that embryos and newborns are more sensitive to environmental chemicals than adults (Bahl et. al, 2012). Of the flavors tested, "Cinnamon Ceylon (#22)" was the most potent and strongly effected all three cell types (Bahl et. al, 2012). However, cytotoxicity among all flavors was highly variable, even when from a single manufacturer (Bahl et. al, 2012). These results probably underestimated the cytotoxicity of refill fluids to lung cells because the exposure method, in order to avoid a vapor effect, only allowed for a maximum of 1% concentration, when in reality someone vaping ECs would receive 100 times more than this concentration. These data illustrate the importance of using multiple cell

types when evaluating EC products, especially when studying embryos because there were clearly more adverse effects on embryonic and newborn cell types than adult cell types.

Raez-Villanueva et. al 2018 determined the effects of EC vapor on placental trophoblast cell function. Extra villous trophoblast cells (HTR-8/SVneo) were exposed to unflavored EC vapor-conditioned media with and without nicotine to assess cell viability, proliferation, migration (wound healing assay), invasion (transwell extracellular matrix invasion assay), and tube formation (a surrogate for studying angiogenesis) (Raez-Villanueva et. al, 2018). This is an important study because early placentation requires extensive angiogenesis and trophoblast cell invasion of the maternal decidua to establish the feto-maternal circulation (Raez-Villanueva et. al, 2018). Therefore, any negative impact on these functions can negatively impact fetal development and growth (Raez-Villanueva et. al, 2018). Treatment of HTR-8/SVneo cells with EC vapor-conditioned media with or without nicotine at concentrations up to 10% did not significantly affect cell viability, proliferation, or migration (Raez-Villanueva et. al, 2018). However, after incubation with 1% EC conditioned media, there was a significant decrease in the complexity of the tubular network with decreases in total tube length, segment length and a lower number of branching points in treated cells (Raez-Villanueva et. al, 2018). In addition to this, treatment of HTR-8/SVneo cells with EC vapor conditioned media with and without nicotine significantly inhibited the invasive capacity of these cells (Raez-Villanueva et. al, 2018). None of the deficits in invasion and angiogenesis were dependent on the presence of nicotine, which is interesting because studies done in rats showed that exposure to nicotine results in reduced trophoblast invasion and placental vascularization (Raez-Villanueva et. al, 2018). This discrepancy could be explained by the fact that the cells were only a single cell type (trophoblasts) used to model complex processes in placentation that in vivo involve multiple cell

types (Raez-Villanueva et. al, 2018). Since the results were not dependent on the presence of nicotine, other components of EC vapor likely contribute to the results in these experiments. Although the number of studies experimenting with the direct effects ECs have on embryonic development is limited, the human embryonic studies that currently exist show that there are potentially adverse effects and future studies are needed to determine the mechanisms and outcomes these effects can potentially have on human embryonic development.

**Table 3: Human Studies Table**

| Source | *Species* | Exposure method | E-liquid/ flavor tested | Summary |
|---|---|---|---|---|
| Bahl et. al, 2012 | Human embryonic stem cells (hESC), mouse neural stem cells (mNSC), and human pulmonary fibroblasts (hPF) | 35 refill products were evaluated for cytotoxicity in 96-well plates using the MTT assay, with various doses of refill solution (0.001%, 0.01%, 0.03%, 0.1%, 0.3%, and 1%). | VG, caramel, butterscotch, cinnamon ceylon, menthol arctic, vanilla tahiti, bubblegum, and butterfinger | Stem cells from embryos (hESC) and newborns (mNSC) were more sensitive to refill solutions than differentiated adult lung fibroblasts<br><br>Cinnamon Ceylon (#22) was the most potent of the refill fluids tested and strongly inhibited survival of all cell types<br><br>Flavor chemicals cytotoxicity was highly variable |
| Raez-Villanueva et. al, 2018 | HTR-8/SVneo cells | Cells were allowed to attach for 24 h. After 24 h, media was removed and cells were treated with | EC liquid and nicotine | EC vapor with and without nicotine impaired tube formation |

| | | | | |
|---|---|---|---|---|
| | | control or vapor conditioned media | | EC vapor with and without nicotine inhibited invasion<br><br>There were significant deficits in trophoblast function following exposure to an unflavored formulation<br><br>Exposure to EC conditioned media generated from unflavored e-liquids impairs placental trophoblast cell function in vitro |

Discussion

This review explored the current knowledge on how flavor chemicals in EC aerosols affect early embryonic development. The chemical characteristics and presence of potentially toxic chemicals in EC flavors remain largely unexplored with potentially adverse effects on embryonic development through their interactions with TRP channels. TRP channels are known to bind to an array of flavor chemicals including vanillin, nicotine, cinnamaldehyde, menthol, etc. Although there has been some groundwork on the effects of ECs on embryonic development, there are still areas that need to be addressed regarding their interactions with TRP channels. The most used flavor chemicals by EC vapers and in the reviewed literature included cinnamaldehyde and vanillin. All the studies that investigated the effects of flavor chemicals found that, regardless of the flavor, the effects studied were exacerbated when EC aerosol contained flavor chemicals versus when they did not.

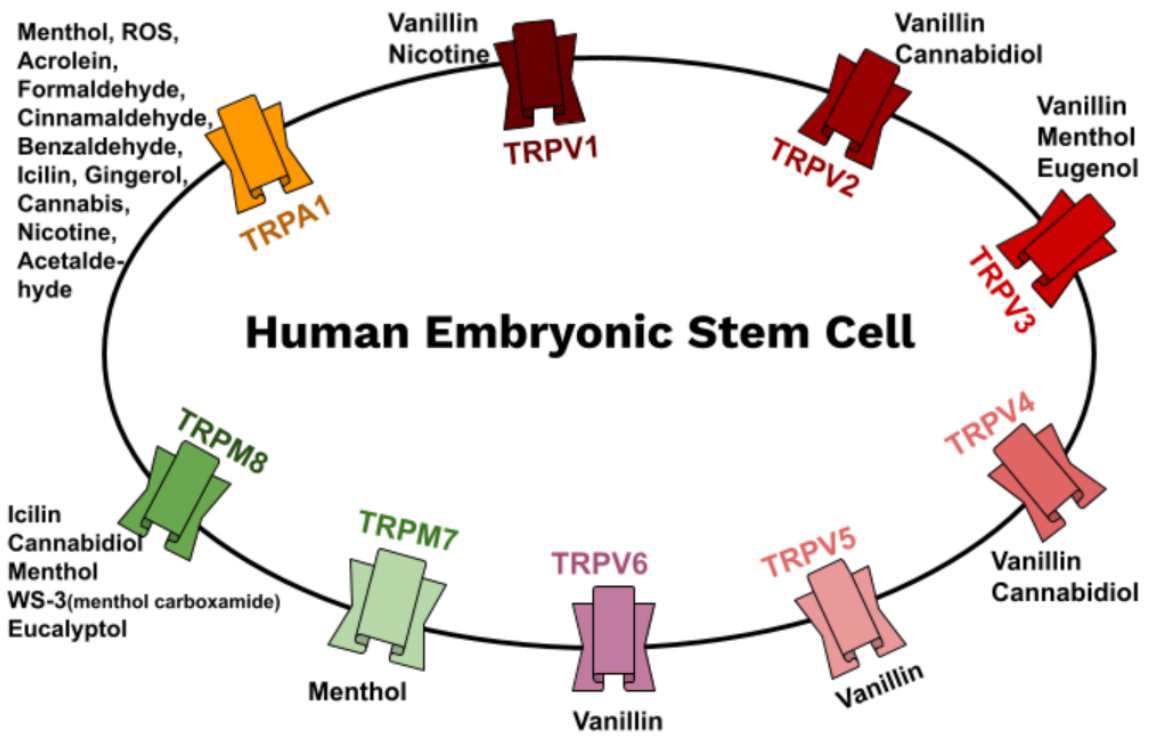


**Figure 4**. The Major TRP Channels and Respective EC Flavor Ligands. This image provides a visual summary of the flavored aerosols and other ligands that bind to their respective TRP channels.

Flavor chemicals in ECs are often used in much higher concentrations than they would be in other consumer products such as food (Omaiye et. al, 2019). However, it is not currently understood what concentrations embryos are receiving to trigger interactions with TRP channels. Determining the concentration reaching embryos should be studied in greater detail because by understanding the concentration levels reaching an embryo, we can better determine the effects of those EC flavors. Studies also need to be conscious of whether their exposure methods are acute or chronic. Some pregnant women have stated they continue vaping up until their third trimester or vape while breastfeeding, however most studies only expose embryos to EC flavors at the beginning of their development. To create a more comprehensive understanding of the effects of EC flavor chemicals on TRP channels and early embryonic development, future

studies must be designed to reflect human behavior as closely as possible both in their concentrations tested and in the exposure method.

Studies on secondhand smoke found that embryos receive large amounts of toxins from their exposed mothers through both the amniotic fluid (Smeester et. al, 2017) and the umbilical cord (Ramadani et. al, 2019). A study done by Khachatoorian et. al, 2022 separated two groups of EC users by “mouth inhalers” and “lung inhalers”. “Lung inhalers” referred to the group that retained aerosol chemicals and exhaled little into the environment (Khachatoorian et. al, 2022). Lung inhalers had ~100% retention for flavor chemicals and nicotine (Khachatoorian et. al, 2022). Based on our knowledge of the damage nicotine can do to the mother, it is likely that high concentrations in an embryo also causes damage. The exact concentration of flavor chemicals that reach an embryo from its pregnant mother is not yet clear, but it can be estimated based on our understanding of the path oxygen takes from the mothers’ lungs to the embryo. First the oxygen enters the mother’s lungs, then goes to the heart, then the dorsal aorta and then the vasculature. From there it travels to the uterus then to the placenta, then the umbilical cord finally reaching the fetus.

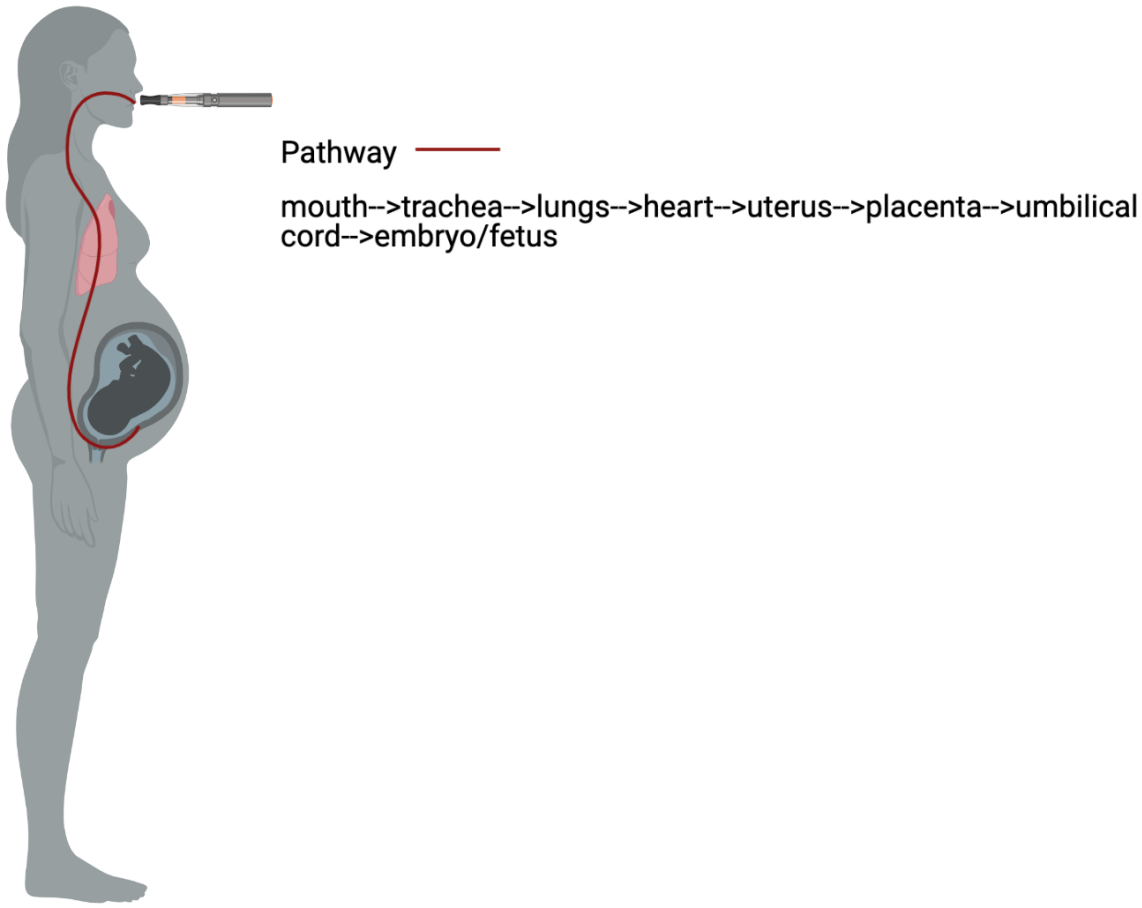


**Figure 5:** Pathway of oxygen from mothers' mouth through the trachea down to the lungs. From the lungs, the oxygen inhaled goes to the heart then to the uterus where it is picked up by the placenta and umbilical cord where it reaches the embryo/fetus.

The literature illustrates that flavor chemicals with a variety of concentrations can have negative effects on embryonic development. Although there is not a complete understanding of the concentrations reaching an embryo, there seems to be enough reaching an embryo to react with TRP channels and cause a variety of effects. To estimate the concentration of flavor chemicals reaching human embryos, we created a fetal exposure model. Behar et. al (2018) analyzed the concentrations of flavor chemicals in EC fluids The concentration of

cinnamaldehyde was 155 mg/mL, menthol was 84 mg/mL, and vanillin was 31 mg/mL (Behar et. al, 2018). Omaiye et. al (2019) examined nicotine concentrations in ECs and discovered an average concentration of 60.9 mg/mL in JUUL products. Khachatoorian et. al (2022) showed that 100% of the inhaled chemicals in EC aerosols are retimed in lung inhalers. Knowing a pregnant woman has about 5L of blood in her body, we can estimate what concentration is reaching an embryo if she inhales the aerosol from 1 mL of fluid. (Hughey, 2010). For each 1 mL of cinnamaldehyde inhaled approximately 0.031 mg of cinnamaldehyde may each an embryo (155 mg/mL/ 5000 mL = 0.031 mg/mL). For menthol, 0.017 mg/mL of menthol could reach the embryo (84 mg/mL / 5000 L = 0.017 mg/mL). For vanillin, 0.006 mg/mL will reach the embryo (31 mg/mL / 5000 mL = 0.006 mg). For nicotine, 0.012 mg/mL will reach the embryo (60.9 mg/ mL / 5000 mL = 0.012mg).

Behar et. al (2018) determined that in the MTT assay the $IC_{50}$ values for cinnamaldehyde and vanillin to be $4*10^{-5}$ mg and $4*10^{-3}$ mg respectively (Table 4). Nair et. al (2020) studied menthol in ECs on human bronchial epithelium. The menthol $IC_{50}$ value on this cell type was 0.87 mg/mL. Song et. al (2020) evaluated nicotine in human middle ear epithelial cell lines and the nicotine $IC_{50}$ value on this cell type was 0.07 mg/mL. However, it is important to note that this $IC_{50}$ values for menthol and nicotine were found on human bronchial epithelium and human middle ear epithelial cell lines and not embryonic stem cells. We would anticipate human embryonic stem cells to be more sensitive than these cell types and so the $IC_{50}$ value for menthol and nicotine in an embryonic cell is likely lower than this value.

From our fetal exposure model, we see that approximately 0.031 mg/mL of cinnamaldehyde, 0.017 mg/mL of menthol, 0.006 mg/mL of vanillin, and 0.012 mg/mL of nicotine are reaching an embryo in a mother who vapes these flavor chemicals. For

cinnamaldehyde and vanillin, the $IC_{50}$ is much lower than the concentrations reaching the embryo. Therefore, it is likely that the estimated flavor chemicals reach the embryo at a concentration sufficiently high to activate TRP channels. Menthol and nicotine, the IC50 values were slightly higher than the concentrations reaching the embryo. However, because these IC50 values were obtained from different cell lines we would expect human embryonic stem cells to be more sensitive and have lower IC50 values. Therefore, it is probable that the estimated flavor chemicals reach the embryo at a concentration sufficiently high to activate TRP channels. Overall, all the flavor chemicals are most likely able to trigger TRP channels after being diluted from the mother's blood and affect embryonic development.

**Table 4:** Embryo/Fetal Exposure Model: Concentrations of Flavor Chemicals and Nicotine Likely Reaching the Embryo/Fetus

| **Flavor chemical** | **Concentration found in EC fluids** | **Value after dilution in mother's blood** | **IC50 value** | **Potential Effect** |
|---|---|---|---|---|
| Cinnamaldehyde | 155 mg/mL | 0.031 mg/mL | 4*10^-5 mg/mL | Since the concentration potentially reaching the embryo is higher than the MTT $IC_{50,}$ there is likely an effect on the embryo. |
| Menthol | 84 mg/mL | 0.017 mg/mL | 0.87 mg/mL | Since the concentration potentially reaching the embryo is slightly lower than the MTT $IC_{50,}$ there may be an effect on the embryo. |
| Vanillin | 31 mg/mL | 0.006 mg/mL | 4*10^-3 m/mL | Since the concentration potentially reaching the embryo is higher than the MTT $IC_{50}$, there is likely an effect on the embryo. |
| Nicotine | 60.9 mg/mL | 0.012 mg/mL | 0.07 mg/mL | Since the concentration potentially reaching the embryo is slightly lower than the MTT $IC_{50,}$ there may be an effect on the embryo. |

Embryos exposed to higher doses of nicotine had exacerbated defects compared to those exposed to only EC refill fluid (Kennedy et. al, 2017). Although it is shown that higher doses lead to more exacerbated effects, it is important to take into consideration how relevant this is to human behavior. This study exposed embryos to 18 and 24 mg/mL of nicotine and our fetal exposure model shows that up to 60.9 mg/mL of nicotine can be present in ECs. Therefore, the effects on embryos seen in this paper would be even more exacerbated if the concentrations of nicotine reflected those seen in ECs. EC liquid and cinnamaldehyde have also been shown to disturb bone, cartilage, vasculature development, and reduce hatching success in zebrafish embryos (Bhattacharya et. al, 2021). The flavor of both vaping liquids was cinnamon, containing either 12 mg/ml nicotine or 0 mg/mL of nicotine (Bhattacharya et. al, 2021). A significant increase in craniofacial defects in embryos that have been exposed with higher defects in those exposed to higher doses. Bhattacharya et. al (2021) defends its concentration use by citing the popularity of the specific vape flavor that was used in experimentation. Despite the different concentrations tested, both Bhattacharya et. al (2021) and Kennedy et. al (2015) agreed that a variety of doses of ECs exposed to embryos resulted in craniofacial defects. Both studies were acute in exposure meaning that experimental groups were only exposed for a relatively short time during development. Human behavior can be different as some mothers have reported vaping up until their third trimester of pregnancy (Kapaya et. al, 2015). Overall, these results are reliable but more studies that take chronic use into consideration need to be done.

An issue in current studies regarding effects of ECs on embryonic development is the experimental design not reflecting human behavior. Chen et. al (2017) study accounts for human behavior in its exposure method. The mother mice were exposed before, during, and after

pregnancy while breastfeeding. This is like the behavior seen in pregnant human mothers. This study used full body exposure of the female mice; however, an inhalation exposure method may have proven more reliable in mimicking the effects on human embryos as it more accurately illustrates human behavior. In Noel et. al (2020) study on mice, inhalation to 36 mg/mL of nicotine cinnamon-flavored EC aerosols for 14 –31 days impaired *Wnt* signaling during mouse lung development (Noel et. al, 2020). This exposure method more accurately illustrates human behavior for two reasons. First, cinnamaldehyde is a popular flavored chemical used by EC vapers, and this animal study incorporating it reveals important information for the many who vape this flavor. Second, the mouse models were exposed to ECs before and during pregnancy, which is accurate because most mothers who vape ECs during pregnancy were either smoking tobacco cigarettes or vaping ECs before getting pregnant. However, Noel et. al (2020) does not explore a more chronic exposure to EC liquid. In Noel et. al (2020) mice were exposed for 14-31 days while women may be vaping longer than 14-31 days after implantation. In Nguyen et. al (2018) study, mice were randomly split into three groups 6 weeks before pregnancy, during pregnancy, and during lactation with full body exposure: ambient air, EC aerosol with 18 mg nicotine, and EC aerosol without nicotine (Nguyen et. al, 2018). This study, like the one by Noel et. al (2020), also exposed mothers to EC aerosol before pregnancy which models' human behavior well.

EC experiments from Sifat et. al (2019) and Church et. al (2020), on mice have shown brain injury in offspring after their birth and even into adulthood. These studies are a good reflection on human behavior because the mice were exposed by direct inhalation, which is how pregnant women vaping ECs would expose their embryos while pregnant. Furthermore, pregnant mice were exposed from gestational day 5 until 7 days after delivery. This is a nice set up because it

follows the effects of EC exposure on an embryo throughout the entire pregnancy. One thing that this study did not consider is exposing mice to EC vapor before getting pregnant and exploring what effect that would have. When we look at human behavior, we see that women who vape ECs while pregnant were either tobacco cigarette smokers or EC vapers before finding out they were pregnant. The findings of this study did not evaluate the roles of TRP channels on these effects however, TRPA1 and TRPV1 are triggered by nicotine, which was used in this study, so these channels are most likely related to these effects on development.

Wetendorf et. al (2019) hypothesized epigenetic and metabolic dysregulations in the offspring of EC exposed offspring and Li et. al (2020) was able to experimentally determine this was the case in terms of liver health and glucose uptake. Both studies utilized nicotine and EC fluid where Wetendorf et. al (2019) exposed their experimental groups to 24mg/mL nicotine while Li et. al (2020) exposed their experimental groups to 18 mg/mL nicotine. This difference in nicotine dose is important because Li et. al (2020) was able to confirm the findings of Wetendorf et. al (2019) despite using a lower exposure dose of nicotine. Therefore, although the fetal exposure model indicates nicotine concentration in ECs can be up to 60.9 mg/mL, 24mg/mL is enough to elicit physiological problems in embryos or offspring. These studies should also experimentally determine the role of TRP channels however since these studies tested nicotine TRPV1 and TRPA1 are most likely involved in the effects.

Fetal tobacco syndrome is associated with low birth weight, smaller size, premature labor and preterm delivery, miscarriage, increased risk of sudden infant death syndrome, neurological damage, and behavioral problems (Nieburg et. al, 1985). The literature reviewed in this paper identified neural, behavioral, and/or lower size ad birth weights with exposure to EC aerosols and flavors in both human and animal studies. It is important to note that regardless of the flavor

chemical concentrations, there is clearly an array of effects of EC exposure on embryonic development; all of which are exacerbated when flavor chemicals are added to EC refill fluids.

In summary, EC aerosols seem to contribute to many harmful effects on embryonic development, which are exacerbated by the presence of flavor chemicals. TRPA1, TRPV1,2,3,4,5,6, and TRPM8 are expressed in early embryonic development, and all interact with one or more of the following flavors: vanillin, menthol, cinnamaldehyde, nicotine, etc. Future studies on the effects of EC flavor chemicals on embryonic development should focus on understanding the interactions between TRP channels and flavor chemicals and how these interactions affect how these pathways can result in pathological problems. This literature review provides the public access to scientific evidence that can inform their decisions about EC use, especially while pregnant.

## Conclusion

Although this area of research is still relatively new, it is important that answers can be found soon. There is an urgency to find answers because ECs are sometimes considered "safer" than tobacco cigarettes. As a result, pregnant women who cannot quit smoking tobacco cigarettes have switched to EC. In fact, 7.0% of women report using ECs at some point in their pregnancy, while 1.4% report using ECs during the last 3 months of pregnancy (Greene, 2019).

ECs and their flavor chemicals (especially menthol, cinnamaldehyde, vanillin, and nicotine) caused orofacial defects, disrupted the retinoic acid signaling pathway, induced inflammatory responses in lungs of exposed dams, induced immune dysregulation, decreased litter size, birth weight, and birth size, affected brain development, increased oxidative stress, and altered nutrient metabolism. TRP channels that are highly expressed in early embryonic development including TRPA1, TRPV1,2,3,4,5,6, and TRPM8 are triggered by many flavor chemicals used in ECs. In all the studies examined, EC refill fluid exposure resulted in one of the

above conditions, and all papers that used cinnamaldehyde, vanillin, and/or nicotine showed that all effects were exacerbated in the experimental group that was exposed to flavor chemicals versus those exposed to only EC refill fluid. Being conscious of the concentrations being used in experiments is not only important in mimicking human behavior but also to assess the extent of the damages more accurately on exposed embryos and dams. It is crucial that a literature review becomes available in this area of research because the public needs access to scientific evidence that can inform their decisions. In addition, this review may encourage pregnant women not to vape ECs until the effects of their flavor chemicals on human embryos are fully understood.

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