TOX/2018/45

COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COT)

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes). Paper 6: A review of data relating to developmental toxicity in offspring following parental exposure to nicotine.

1 Background

1. The COT is reviewing the potential toxicity of electronic nicotine delivery systems (ENDS) and electronic non-nicotine delivery systems (ENNDS) (also referred to as E(N)NDS). As part of this review, at the July 2018 COT meeting an overview paper was presented on the toxicity of the ENDS constituent, nicotine, (TOX/2018/25). Members requested further information on developmental toxicity of nicotine. This paper provides more information on this topic, comprising a general review of the literature of relevance to developmental toxicity to offspring from parental (principally maternal) exposure to nicotine in humans. Developmental toxicity relating to exposure during adolescence is not included.

2 Introduction

2. E(N)NDS are battery-powered devices containing a liquid (E(N)NDS liquid or ‘e-liquid’). The E(N)NDS liquid is heated on use to produce an aerosol that is inhaled by the user (‘puffing’, ‘vaping’). E(N)NDS were first introduced commercially in China in 2004 and subsequently in the EU (2005) and USA (2007) as nicotine-delivery devices. The main constituent parts of an E(N)NDS device are a mouthpiece, cartridge (tank) containing E(N)NDS liquid, a heating element/atomizer, a microprocessor, a battery, and sometimes an LED light. Commercially available devices are sometimes categorised as first, second, or third generation. First-generation devices look like conventional cigarettes (CC) and thus are termed ‘cigalikes’. Initial models comprised three principal parts; a lithium-ion battery, a cartridge and an atomizer. However, more recent models mostly consist of a battery connected to a ‘cartomizer’ (cartridge/atomizer combined), which may be replaceable, but is not refillable. Second-generation E(N)NDS are larger and have less resemblance to tobacco cigarettes. They often resemble pens or laser pointers (hence the name, ‘vape pens’). They have a high-capacity rechargeable lithium-ion battery and a refillable atomizer (sometimes referred to as a ‘clearomizer’). Third-generation models (‘advanced personal vapers’, ‘mods’) are also refillable, have very-high-capacity lithium-ion batteries and are highly customisable (different coil options, power settings, tank sizes). In addition, highly advanced ‘fourth generation’ E(N)NDS (innovative regulated mods) are now being described.

3. Constituents that have been identified in E(N)NDS liquids and/or aerosols include propylene glycol (PG), vegetable glycerine (VG, glycerol), water, nicotine, carbonyls, volatile organic compound (VOCs), tobacco-specific nitrosamines (TSNAs), polycyclic aromatic hydrocarbons (PAHs), metals, ethanol, ethylene glycol, di-ethylene glycol, flavouring compounds, flavour enhancers, sweeteners, and phenolics. Data on reported levels of some of these constituents were summarised in discussion paper, TOX/2018/16, presented at the February 2018 COT meeting. TOX/2018/16 noted that nicotine concentrations stated on product labels are generally in the range of up to 20 mg/mL, although products with higher nicotine concentrations may be available in some countries. In the UK, the Tobacco and Related Products Regulations 2016 states that "nicotine-containing liquid which is presented for retail sale in an electronic cigarette or refill container must not contain nicotine in excess of 20 milligrams per millilitre" (Part 6, section 36(4)). Several investigations found that nicotine concentrations do not always correlate well with levels stated on the label. Reported proportions of nicotine emitted from ENDS liquids to aerosols on puffing vary, and this also likely depends on a combination of device characteristics, puffing behaviour, and the overall composition of the ENDS liquid.

4. Discussion paper, TOX/2018/25, which overviewed nicotine toxicity, described factors that affect the amount of aerosolized nicotine available for inhalation, as summarised by the U.S. Department of Health and Human Services report 'E-cigarette use among youth and young adults. A report of the Surgeon General' (U.S. Department of Health and Human Services 2016). These factors include the nicotine concentration in the e-liquid, power (voltage and resistance) of the ENDS device, and user behaviour (puffing topography). Plasma nicotine concentrations measured after ENDS use can vary substantially, with levels observed ranging from lower to higher than those achieved by CC smoking. There is variation across products, with cigalikes generally delivering less nicotine than tank systems, and low resistance, dual-coil cartomizers being able to deliver nicotine at lesser or greater levels than CC, depending on the e-liquid nicotine concentration. In controlled sessions using equivalent types of devices and e-liquid nicotine concentrations, user puffing topography has been shown to affect nicotine delivery. One study showed a 10-fold variation in the plasma nicotine concentration (0.8–8.5 ng/mL) achieved across participants under equivalent conditions. ENDS users are reported to develop the capability to extract more nicotine from the devices as they become more experienced with use.

5. TOX/2018/25 also noted that the US National Academy of Sciences (NAS) report 'Public health consequences of e-cigarettes' had reviewed knowledge of nicotine exposure profiles from ENDS use. Twenty-seven clinical studies that evaluated nicotine exposure profiles from ENDS use had been reviewed, of which 17 studies looked at inexperienced ENDS users and 12 focussed on experienced users, the NAS concluding that "These studies suggest that e-cigarettes deliver lower levels of nicotine when used by e-cigarette-naïve smokers compared with levels delivered by combustible cigarettes" (around 1 mg), but also that "studies of nicotine delivery

and systemic retention in experienced users suggest that e-cigarettes can deliver nicotine in the range of a typical combustible tobacco cigarette, and most of the nicotine is systemically retained under experimental conditions" (NAS 2018).

3 Literature searches and scope of the review

6. This discussion paper has been compiled from a combination of original literature identified from literature searches, from reviews and systematic reviews, and examination of reference lists from these sources for further relevant citations. The aim was to compile a literature base comprising an overview of:

- Data from studies in humans, including an overview of adverse effects associated with maternal or paternal tobacco exposure and a more detailed review of all identified studies on nicotine replacement therapy (NRT) in pregnancy.

- Studies in animal models of relevance to the potential developmental toxicity associated with parental exposure to nicotine in humans (includes studies of exposure *in utero*, via breast milk, direct exposure of offspring during the early postnatal period, and a very limited literature set relating to paternal exposure).

7. Full details of literature searches are given in Annex A. Briefly, PubMed and Scopus databases were searched on Title/Abstract with terms relating to nicotine exposure and developmental toxicity, with restriction to English-language publications, and no date limit. Due to the large amount of relevant publications obtained (around 1000), recent reviews of the topic that were identified from these searches were taken as the starting point for structuring this discussion paper. Publications that were considered to report key studies in the development of the field were obtained and reviewed in full. In addition, reference lists of publications and reviews were examined for further citations of interest. However, as this is a very extensive and active field of research, it was not possible to review all published studies. A final search of PubMed to update for newly published literature of particular relevance was carried out on 23/10/2018.

8. A substantial database of literature also exists relating to adverse effects on neurodevelopment associated with nicotine exposure during adolescence. This area is not covered in the present discussion paper.

9. A small number of studies in mice have evaluated developmental effects of exposure to E(N)NDS aerosols, both with and without nicotine. These studies are not included as they are reviewed in the accompanying discussion paper, TOX/2018/46.

4 Toxicokinetics

10. A detailed review of nicotine chemistry, metabolism, kinetics, and biomarkers can be found in Benowitz, Hukkanen and Jacob (2009), and this area was also

overviewed in the COT discussion paper on nicotine toxicity, TOX/2018/25. An overview of information of relevance to the situation in pregnancy and lactation is given below.

11. Nicotine in maternal circulation readily crosses the placenta and enters the fetal circulation. Subsequently, much of the fetal nicotine returns to the maternal circulation for elimination, but some enters the amniotic fluid via fetal urine, from where it can be absorbed by the fetus dermally. Concentrations of nicotine in fetal serum and amniotic fluid are reported to be slightly higher than in maternal serum. Nicotine also passes into breast milk, with concentrations reported to be 2- to 3-fold those in maternal plasma (Benowitz et al. 2009, Maritz and Harding 2011).

12. The metabolism of nicotine and cotinine is substantially increased in pregnant women (Benowitz et al. 2009, Bowker et al. 2015). However, fetal metabolism is slow, leading to higher concentrations of nicotine in fetal, compared with maternal, tissues and fluids (Maritz and Harding 2011, U.S. Department of Health and Human Services 2016, NAS 2018). Very high nicotine concentrations have been detected in dried blood spots of neonates from mothers who smoked CC during pregnancy (NAS 2018). Neonates have diminished nicotine metabolism, with a half-life of 3- to 4-fold in newborns exposed to tobacco smoke compared with adults, although cotinine metabolism is not different to that in adults (Benowitz et al. 2009).

13. Nicotinic acetylcholine receptors (nAChRs) are present in fetal brain (Smith et al. 2010) and lung (Wang et al. 2001) and acetylcholine (ACh) acts on these receptors to modulate functional connections during development (Dwyer, Broide and Leslie 2008). The presence of nAChRs in fetal brain has been shown from as early as 5 weeks of gestation (Hellström-Lindahl et al. 1998, *cited in* U.S. Department of Health and Human Services 2016). Nicotine has been shown to bind to these receptors in the fetus (Pentel et al. 2006, Wong et al. 2015, *cited in* U.S. Department of Health and Human Services 2016), and thus has the potential to cause disruption of normal development.

5 Effects in humans

5.1 Overview of knowledge on adverse outcomes associated with maternal use of tobacco products during pregnancy

14. Reported rates of maternal use of combustible tobacco products (‘conventional cigarettes’, CC) during pregnancy across Europe vary from around 5% in Sweden, Austria, and Switzerland to 40% in Greece, with a rate of around 20% in England (*data cited by* McEvoy and Spindel 2017). Smokeless tobacco products, for example ‘Swedish snus’, are also used by some pregnant women. *In utero e*xposure via maternal use of tobacco products during pregnancy has been associated with adverse health outcomes in the offspring. To provide a background context for discussion of the potential role of nicotine, knowledge is summarised briefly below. More extensive reviews of literature on adverse effects of *in utero*

exposure to tobacco products can be found in the publications by the U.S. Surgeon General (HHS 2014), England et al. (2017), and Peterson and Hecht (2017).

5.1.1 Neurodevelopment

15. Acetylcholine plays a key role in the regulation of fetal brain and central nervous system (CNS) development through its interaction with nAChRs. Regional expression of receptor nAChR subtypes during development has been shown to be altered by maternal CC smoking during pregnancy (*reviewed by* England et al. 2015, England et al. 2017.

16. England et al. (2017) noted that although the effects of nicotine on human fetal brain development have not been studied directly, imaging studies have indicated that maternal CC smoking during pregnancy is associated with effects on fetal brain structure that are consistent with animal studies of fetal nicotine exposure, notably disrupted brain development independent of effects on fetal growth (Ekblad et al. 2010, Haghighi et al. 2013, El Marroun et al. 2014 *cited in* England et al. 2017). Maternal CC smoking during pregnancy was associated with decreased transcerebellar and lateral ventricle diameter/width on ultrasound and decreased overall brain volume on magnetic resonance imaging (MRI) in the fetus, and with smaller frontal lobe and cerebellar volumes in infancy when compared with no CC smoking (Roza et al. 2007, Ekblad et al. 2010, Anblagen et al. 2013 *cited in* England et al. 2017). In addition, maternal CC smoking during pregnancy has been associated with structural brain differences later in life, in children (Rivkin et al. 2008, El Marroun et al. 2014 *cited in* England et al. 2017)) and adolescents (Paus et al. 2008, Toro et al. 2008, Lotfipour et al. 2009, Haghighi et al. 2013, Liu et al. 2013 *cited in* England et al. 2017), including reduced cerebral cortical grey matter, reduced subcortical grey matter volumes in the amygdala, thalamus, and palladium, and reduced volume in the corpus callosum.

17. These structural effects have been linked by real-time fetal monitoring with functional alterations in the fetus, including reduced heart rate variability, increased mouth and self-touch movements, and impaired recognition of maternal voice (Oncken et al. 2002, Zeskind & Gingras 2006, Cowperthwaite et al. 2007, Reissland et al. 2015 *cited in* England et al. 2017).

18. Maternal CC smoking or tobacco use during pregnancy have been associated with alterations in behaviour and stress response in newborns (e.g. abstinence/withdrawal, hypertonicity, irritability and excitability) and in the later neonatal period (self-regulation, attention, requirement for soothing). A dose–response relationship to nicotine exposure has been identified for effects in newborns (Law et al. 2003, Godding et al. 2004, Hurt et al. 2005, Mansi et al. (2007), Stroud et al. 2009, *cited in* England et al. 2017). Altered cortisol response to stress has also been observed in newborns and infants of smoking mothers.

19. Smoking CC has also been associated with effects on longer term behavioural outcomes during childhood and adolescence, particularly externalizing

and disruptive behaviours, but also internalizing behaviours such as anxiety and depression (Ramsay et al. 1996, Wakschlag et al. 2002, D'Onofrio et al. 2008, Schuetze et al. 2008, Stroud et al. 2009, Yolton et al. 2009, Espy et al. 2011, Gaysina et al. 2013, Stroud et al. 2014, Estabrook et al. 2015, Wiebe et al. 2015 *cited in* England et al. (2017)). The latest report on 'The Health Consequences of Smoking' by the US Surgeon General noted that causal relationships between smoking and long term cognitive and behavioural outcomes in humans are difficult to establish due to the multiple potential confounding factors. The report concluded that the evidence is suggestive, but not sufficient, to infer a causal relationship between maternal prenatal smoking and disruptive behavioural disorders, particularly attention deficit hyperactivity disorder (ADHD), but insufficient to infer the presence or absence of a causal relationship between maternal prenatal smoking and anxiety and depression, Tourette syndrome, schizophrenia, or intellectual disability (HHS 2014). Subsequently, adoption and sibling studies have further supported an association of maternal smoking with externalising behaviours and oppositional defiant disorder behaviours (Gaysina et al. 2013, Estabrook et al. 2015 *cited in* England et al. 2017).

20. Maternal CC smoking has been associated with obesity in children, but it is not clear to what extent potential confounding factors have been evaluated (*reviewed by* Behl et al. 2013 *and discussed in* U.S. Department of Health and Human Services 2016, page 111).

21. Maternal tobacco use during pregnancy has been established as a risk factor for tobacco use and dependence in offspring, independent of subsequent parental tobacco use, (*see the review by* U.S. Department of Health and Human Services 2016, page 110 *and refs therein*). It has been suggested that this may be due to abnormal *in utero* development of neural and dopamine systems that promote sensitivity to nicotine dependence.

5.1.2 Fetal growth, pregnancy outcomes, perinatal mortality, sudden infant death syndrome

22. Maternal CC smoking is causally associated with adverse pregnancy outcomes including ectopic pregnancy, fetal growth restriction, increased risk of preterm delivery, placental abruption, perinatal mortality (stillbirth and neonatal death) and sudden infant death syndrome (SIDS) (HHS 2014, England et al. 2017, Peterson and Hecht 2017). Increased risk of stillbirth, neonatal death and SIDS is postulated to be mediated in part through disruption of fetal and infant stress responses which regulate cardiovascular adaptation during periods of hypoxia, and cardiorespiratory control and arousal (*discussed by* England et al. 2017; *reviewed in detail in* Duncan 2018). A dose–response relationship between cotinine and altered arousal pattern has been observed in preterm infants (Richardson et al. 2009, *cited in* U.S. Department of Health and Human Services 2016).

23. Maternal smoking has also been associated with increased risk of major birth defects. The latest update on the health consequences of smoking by the US

Surgeon General concluded that there is sufficient evidence to infer a causal relationship between maternal smoking in early pregnancy and orofacial clefts, and suggestive but not sufficient evidence to infer a causal relationship between maternal smoking in early pregnancy and clubfoot, gastroschisis, and atrial septal heart defects (HHS 2014).

24. Use of smokeless tobacco during pregnancy has also been associated with increased risk of preterm delivery, stillbirth, and orofacial cleft defects, but with no or only a small effect on fetal growth restriction (HHS 2014, England et al. 2017). The US Surgeon General concluded that the evidence supports the hypothesis that nicotine plays a key role in mediating adverse effects of smoking on preterm delivery and stillbirth (HHS 2014).

5.1.3 Pulmonary development

Timeline of pulmonary development in humans

25. Pulmonary development[1] in humans occurs during the prenatal and postnatal periods. During prenatal development, lung buds develop during embryogenesis (weeks 0-6), with lobar and subsegmental branching leading to formation of the bronchopulmonary segments. During the pseudoglandular stage (weeks 7-16), the conducting airways up to the terminal bronchioli and preacinar pulmonary vessels form by repeated branching. Further growth after this occurs only by elongation and widening of existing airways. Airway cartilage begins to form, respiratory epithelium begins to differentiate, and cilia appear in the proximal airways. During the canalicular stage (weeks 16-24) terminal respiratory units form and are vascularized. Differentiation of type I and type II epithelial cells occurs. By the end of this period, gas exchange is possible. The terminal, saccular stage of lung development (weeks 24-40) is characterized by decreased interstitial tissue and thinning of airspace walls, with increasing surface area of the gas-exchanging units, formation of true alveoli, and maturation of surfactant in type II epithelial cells. The alveolar period of lung development occurs postnatally. Mature alveoli are present from approximately 5 weeks after birth, and the majority of subsequent postnatal alveolar growth occurs during the first few years of life, with slower growth into adolescence.

Effects of maternal CC smoking on pulmonary development

26. Exposure to CC smoke *in utero* via maternal smoking during pregnancy has been associated with adverse effects on pulmonary development and infant/childhood respiratory health (*reviewed by* Gibbs, Collacoand McGrath-Morrow 2016, Spindel and McEvoy 2016, McEvoy and Spindel 2017).

27. Some children from mothers who smoked CC during pregnancy have altered pulmonary function test outcomes, including reduced forced expiratory flow (FEF) and respiratory compliance and altered tidal breathing patterns, and these outcomes

[1] http://www.clevelandclinic.org/pediatrics/pdf/difiore_lung_dev.pdf; https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5320013/

are observed soon after birth, before postnatal CC smoke exposure (Hanrahan et al. 1992, Hoo et al. 1998, McEvoy et al. 2014 *cited in* McEvoy and Spindel 2017). The observation of altered pulmonary function test outcomes in preterm infants with a mean gestation of 32 weeks, when tested at 36 weeks after last menstrual period, suggests that effects are not limited to the end of gestation (Hoo et al.,1998 *cited in* McEvoy and Spindel 2017). Studies have indicated that such altered pulmonary function persists into childhood and early adulthood, in the absence of a likely association with postnatal CC smoke exposure (Cunningham et al.,1994, Hayatbakhsh et al., 2009 *cited in* McEvoy and Spindel 2017). Infants born to mothers who smoked CC during pregnancy also show increased rates of bronchitis, lower respiratory tract infections, wheezing and asthma, and such effects have been shown to be associated with prenatal exposure independently of postnatal exposure *(reviewed by* England et al. 2017, McEvoy and Spindel, 2017).

28. Correlating with findings from animal studies of prenatal nicotine exposure (see section 6.3), prenatal exposure to CC smoke in humans has been associated with thickened walls surrounding airways and pulmonary vessels, and increased connective tissue and α7nAChR expression in infants with unexplained perinatal death or SIDS (Elliot et al. 1998, Lavezzi et al. 2014 *cited in* England et al. 2017).

Genetic, epigenetic, and transgenerational effects

29. Decreased lung function tests or respiratory problems are not observed in all children whose mothers smoked CC during pregnancy and studies have indicated that genetic and epigenetic factors may be involved in the development of altered pulmonary function associated with *in utero* CC smoke exposure (*discussed by* Gibbs et al. 2016, McEvoy and Spindel, 2017).

30. Prenatal CC smoke exposure has been reported to cause changes in DNA methylation that are associated with increased risk of asthma. Studies in humans have indicated methylation changes in infants born to mothers who smoked CC during pregnancy, with the changes persisting into childhood. This includes a number of genes involved in detoxification and immune regulation (Suter et al. 2010, Hinz et al. 2012, Joubert et al. 2012 *cited in* Gibbs et al. 2016), including lung-specific altered methylation pattern in an asthma-associated gene, *DPP10* (Chhabra et al. 2014, *cited in* Gibbs et al. 2016). The commentary on this area by Gibbs et al. (2016) pointed out that knowledge is currently limited and further work is needed to correlate epigenetic findings in specific tissues with functional measures of lung disease. One study showed altered lung development in the children of non-CC-smoking women whose own mothers smoked CC during pregnancy (Li et al. 2000 *cited in* McEvoy and Spindel 2017). Some, but not all, epidemiological studies have observed grandmaternal effects of CC smoking on asthma risk (Li et al. 2005, Miller et al. 2014, Magnus et al. 2015 *cited in* Gibbs et al. 2016).

5.2 Paternal CC smoking

31. A review of potential effects of paternal CC smoking on offspring noted that studies have reported possible association with childhood cancers and with birth defects, including anorectal malformations, cardiovascular anomalies, congenital heart disease, cleft palate, hydrocephalus, urethral stenosis, spina bifida, and reduced kidney volume. However, the review noted that studies to date have yielded mixed results. Chronic CC smoking in men leads to reduced fertility, with abnormalities in sperm count and characteristics (reviewed by Esakky and Moley 2016). Adolescent sons of fathers who started smoking before puberty have been reported to be at increased risk of obesity (reviewed by Soubry, Verbeke and Hoyo 2014).

5.3 Studies of exposure to nicotine replacement therapy during pregnancy

32. NRT products are products that are regulated as drugs or medicines to aid smoking cessation. They are available in different forms including chewing gum, lozenge, oral tablet, skin patch, nasal spray, and inhaler, delivering nicotine at a range of doses and pharmacokinetic profiles (*reviewed by* Royal College of Physicians 2016, Lee and Fariss 2017).

33. The review of nicotine kinetics by Benowitz et al. (2009) notes that plasma nicotine levels from NRTs tend to be in the range of those in low-level CC smokers. Blood plasma nicotine levels in CC smokers generally range from 10 to 50 ng/mL, with typical daily trough concentrations of 10 to 37 ng/mL and peaks of 19 to 50 ng/mL, and a mean nicotine boost per 1 CC smoked of 10.9 ng/mL. *Ad libitum* use of NRT products generally provides a plasma nicotine concentration approximately one-third to two-thirds of that achieved by CC smoking. Steady-state plasma nicotine concentrations from transdermal patches are in the range of 10-20 ng/mL, with a range of 5-15 ng/mL from gum, inhaler, sublingual tablet, and nasal spray. Systemic doses delivered from different nicotine delivery systems are reported as follows: smoking 1 CC, 1–1.5 mg; nicotine gum, 2 mg from one 4-mg gum; transdermal patch, 5–21 mg per day; nasal spray, 0.7 mg per 1-mg dose of 1 spray in each nostril; inhaler, 2 mg for a 4-mg dose released from the 10-mg inhaler; lozenge, 1 mg for a 2 mg lozenge; oral snuff, 3.6 mg for 2.5 g held in the mouth for 30 min; chewing tobacco, 4.5 mg for 7.9 g chewed for 30 min (Benowitz et al. 2009) *and refs therein*).

34. In the UK, NICE Guidance recommends that NRT should be offered for use by pregnant women only if they have not managed to quit smoking without medication. NRT should only be prescribed to women once they stop smoking, initially to cover a period of 2 weeks from the date of cessation, and with subsequent prescriptions only given to women who are abstinent from smoking at reassessment (NICE 2010).

35. Studies of the potential effects of NRT use during pregnancy can be grouped into clinical trials and population-based epidemiological studies.

5.3.1 Randomised clinical trials

36. Randomised clinical trials (RCTs) of NRT use during pregnancy have mostly focussed on the primary outcome of efficacy for smoking cessation, but some studies have also recorded other effects, including potential beneficial and/or adverse effects.

UK – Smoking, Nicotine, and Pregnancy (SNAP)

37. The 'Smoking, Nicotine, and Pregnancy' (SNAP) trial[2] was a randomised, double-blind, placebo-controlled trial of NRT for smoking cessation during pregnancy conducted by researchers at the University of Nottingham (Coleman et al. 2012b). A total of 1050 women aged 16–50 years who were between 12 and 24 weeks pregnant and attending ultrasonography appointments at clinics in the East Midlands, currently smoking ≥ 5 cigarettes per day (median, 14 cigarettes per day), and had smoked ≥ 10 cigarettes per day before pregnancy (median, 20 cigarettes per day), were randomised between May 2007 and February 2010 to either NRT or placebo treatment. The NRT intervention (n = 521) was an 8-week course of 15 mg per 16 hours nicotine as a transdermal patch. This was split into an initial 4-week treatment, which was renewed for a further 4 weeks if required for women who were not smoking at the 4-week point. Behavioural support was also given. The placebo group (n = 529) underwent the same protocol, except that the transdermal patches did not contain nicotine. The primary outcome measure was self-reported abstinence from smoking from the quit date (within 2 weeks of randomisation) and immediately prior to childbirth, validated by exhaled carbon monoxide (CO) and salivary cotinine measurements. Secondary endpoints were indices of 1] abstinence from smoking at various time points during and after pregnancy, 2] fetal loss and morbidity, 3] maternal morbidity and mortality, 4] early childhood outcomes (behaviour and development, disability and respiratory symptoms at 2 years), 4] health economic data. Abstinence from smoking rates at 1 month after quit date were 21.3% (NRT) and 11.7% (placebo) (odds ratio (OR) = 2.05, 95% CI 1.46 to 2.88). However, overall compliance with patch use was low (7.2% in the NRT group and 2.8% in the placebo group, for > 1 month). At delivery, randomisation to NRT was not associated with a significantly increased rate of abstinence from smoking compared with placebo (9.4% in the NRT group, 7.6% in the placebo group; OR = 1.26, 95% CI 0.82 to 1.96). The authors commented that for singleton births (n = 1038), birth weight and rates of preterm birth, low birth weight, and congenital abnormalities were similar in the NRT and placebo groups, except that there were significantly more deliveries by caesarean section in the NRT compared with placebo group, and that rates of other adverse events were similar between the two groups (Coleman et al. 2012b).Data covering these aspects that were presented in Table 3 (Birth Outcomes) and Table 4 (Adverse Events) of the report by Coleman et al. (2012b) are summarised in Table 1**.**

[2] https://www.ncbi.nlm.nih.gov/books/NBK262437/, accessed 04/09/2018

Table 1. Birth outcomes and adverse events according to study group in the UK 'SNAP' trial of NRT for smoking cessation in pregnancy. Data are summarised from Tables 3 and 4 of Coleman et al. (2012b).

Outcome	NRT	placebo	Comparison NRT vs. placebo
	n (%)	n (%)	
Birth outcomes			
Total available for evaluation	*515*	*521*	
			OR (95% CI)
Miscarriage	3 (0.6%)	2 (0.4)	1.52 (0.25-9.13)
Still birth	5 (1.0%)	2 (0.4)	2.59 (0.50-13.4)
Neonatal death	0 (0%)	2 (0.4)	
Preterm birth	40 (7.9)	45 (8.7)	0.90 (0.58-1.41)
Low birth weight	56 (11.0)	43 (8.3)	1.38 (0.90-2.07)
Neonatal intensive care unit (ICU) admission	33 (6.5)	35 (6.8)	0.96 (0.58-1.57)
Apgar score at 5 min < 7	16 (3.2)	18 (3.5)	0.91 (0.45-1.80)
Cord-blood pH < 7	4 (0.8)	7 (1.4)	0.57 (0.17-1.97)
Intraventricular haemorrhage	2 (0.4)	3 (0.6)	0.67 (0.11-4.05)
Neonatal convulsions	5 (1.0)	5 (1.0)	1.02 (0.29-3.54)
Congenital abnormalities	9 (1.8)	13 (2.5)	0.70 (0.30-1.66)
Necrotising enterocolitis	3 (0.6)	6 (1.2)	0.50 (0.12-2.03)
Infant on ventilator > 24 h	10 (2.0)	11 (2.1)	0.93 (0.39-2.22)
Assisted vaginal delivery	38 (7.5)	43 (8.3)	0.95 (0.59-1.50)
Caesarean delivery	105 (20.7)	79 (15.3)	1.45 (1.05-2.01)
			Mean difference (95%CI)
Unadjusted birthweight (kg)	3.18±0.61	3.20±0.59	–0.02 (–0.10 to 0.05)
Birth weight z score	–0.36±0.99	–0.31± 1.02	–0.05 (–0.17 to 0.08)
Gestational age (wk)	39.5 ± 2.1	39.5±2.1	0.00 (–0.2 to 0.3)
Adverse events			
Total available for evaluation	*515*	*521*	
Serious adverse events			
Maternal death	0	0	
Other serious adverse events	9 (1.7)	6 (1.1)	
Maternal adverse events potentially related to treatment			
Patch use stopped permanently due to adverse event	46 (8.8)	32 (6.0)	

Outcome	NRT	placebo	Comparison NRT vs. placebo
Maternal adverse events as probable complications of pregnancy			
Blood pressure >140/90 mm Hg on at least 2 occasions	24 (4.6)	25 (4.7)	
Nausea or vomiting	16 (3.1)	19 (3.6)	
Headache	25 (4.8)	16 (3.0)	
Abdominal pain	54 (10.4)	50 (9.5)	
Vaginal bleeding or haemorrhage	35 (6.7)	38 (7.2)	
Premature rupture of membranes	6 (1.2)	10 (1.9)	
Uterine contractions during pregnancy	24 (4.6)	30 (5.7)	
Gestational diabetes	3 (0.6)	3 (0.6)	
Preeclampsia or eclampsia	3 (0.6)	5 (0.9)	
Hospital admission for other pregnancy complication	44 (8.4)	41 (7.8)	
Other, less frequent events	63 (12.1)	73 (13.8)	
Fetal adverse events as probable complications of pregnancy			
Decreased fetal movement	58 (11.1)	46 (8.7)	
Other events	5 (1.0)	5 (0.9)	
Neonatal adverse events	32 (6.1)	29 (5.5)	
Total adverse events	535	450	

38. A follow-up of SNAP trial participants assessed infant development at 2 years post-delivery (Cooper et al. 2014b). Data were gathered via questionnaire posted to either the participant or their family physician, with a response rate of 88% (from participant and/or physician) in both the NRT and placebo groups. The primary outcome was ‘infant survival without development impairment’ (no disability or problems with behaviour or development) at 2 years of age (323/445 = 73% of infants in the NRT group; 290/443 = 65% of infants in the placebo group; OR = 1.40, 95% CI 1.05 to 1.86, p = 0.023 for NRT compared with placebo). Secondary outcomes were ‘definite impairment’, ‘respiratory problems’ and ‘maternal smoking behaviour’. Definitive developmental impairment was reported for 48/445 (11%) of infants in the NRT group and 64/443 (14%) of infants in the placebo group (OR = 0.71, 95% CI 0.47 to 1.09, p = 0.12 for NRT compared with placebo). Respiratory problems were reported for 132/444 infants in the NRT group (30%) and 111/444 infants (25%) in the placebo group (OR = 1.30, 95% CI 0.97 to 1.74, p = 0.08 for NRT compared with placebo). Response rates regarding postnatal smoking were lower (around 58% at 2 years) as this question was only included in the participant, but not physician, questionnaire. Smoking abstinence rates were low, with 15/302 (3%) NRT and 9/304 (2%) placebo participants reporting smoking abstinence at 2 years post-delivery (OR = 1.71, 95% CI 0.74 to 3.94, p = 0.20 for NRT compared with placebo). On the basis of the results from the primary outcome measure, the

authors concluded that infants born to women who used NRT for smoking cessation in pregnancy were more likely to have unimpaired development than those born to women not using NRT, and suggested that this may be explained by the increased rate of smoking cessation for a short period after randomisation during pregnancy (Cooper et al. 2014b).

Denmark

39. Wisborg and colleagues analysed the effects of using NRT patches in pregnancy in women in Denmark who smoked beyond the 1st trimester, evaluating rates of smoking cessation, birth weight, and preterm delivery . A total of 250 healthy women smokers (≥ 10 cigarettes per day) who were less than 22 weeks pregnant were randomised to either nicotine (n = 124) or placebo (n = 126) patches, in a double-blind manner. Patch treatment was as follows: 15 mg nicotine patch, 16 hours per day for 8 weeks, then 10 mg nicotine patch, 16 hours per day for 3 weeks (NRT group), or equivalent treatment with patches containing no nicotine (placebo group). Salivary cotinine was evaluated at antenatal visits. Compliance with treatment was low in both groups: 17% and 11% (NRT) and 8% and 7% (placebo) used all 15 mg and 10 mg (or placebo equivalent) patches, respectively. Mean salivary cotinine levels at the 1st (gestation week 16) and 4th (4 weeks preterm) antenatal visits, respectively, were 231 and 120 ng/mL (NRT), and 226 and 153 ng/mL (placebo). Mean birth weight was 186 g higher in the NRT compared with placebo group (95% CI 35 to 336 g), while rates of preterm birth were 8% in the NRT group and 10% in the placebo group (relative risk (RR) = 0.8, 95% CI 0.4 to 1.7, for NRT compared with placebo). Overall, 26% of participants reported not smoking at the 4th antenatal visit and 14% were nonsmokers 1 year after delivery. The proportions of women described as “continuously abstinent from the start of the intervention” were 21% in the NRT group and 19% in the placebo group (RR = 1.1, 95% CI 0.7 to 1.8, for NRT compared with placebo), though the paper does not state when this was assessed. The authors concluded that “Nicotine patches had no influence on smoking cessation during pregnancy, although they might increase birth weight in comparison with placebo” (Wisborg et al. 2000).

France – Study of Nicotine Patch in Pregnancy (SNIPP)

40. A multicentre, randomised, double-blind, placebo-controlled trial, the ‘Study of Nicotine Patch in Pregnancy’ (SNIPP), was carried out in 23 maternity wards in France between October 2007 and January 2013 (Berlin et al. 2014). A total of 402 pregnant smokers (≥ 5 cigarettes per day), aged 18 years and over, were randomised between 12 to 20 weeks of gestation to receive either daily 16-hour nicotine patches (n = 203) (NRT) or equivalent placebo patches (n = 199) for the duration of the period from ‘quit date’ (start of patch treatment) to delivery. Nicotine daily dose in the patch (within the range of 10–30 mg/day) was adjusted individually throughout gestation with the aim to provide complete substitution of the baseline nicotine level, based on measured salivary cotinine levels at baseline and at 2, 4, 8, and 12 weeks post quit date. Patients were assessed at hospital visits: at inclusion,

at randomisation/quit date (2 weeks after inclusion), 2 weeks and 4 weeks after quit date, then monthly until 1 month before due date). Overall, 96 women in the NRT group and 76 women in the placebo group completed all hospital visits. Participants received behavioural smoking cessation support at every visit. Data were collected on 192 live births from each group. Median self-reported compliance with patch use, assessed at a total of 1016 hospital visits, was 85% in the 164/203 of women in the NRT group for whom these data were available, and 83% in the 143/199 women in the placebo group for whom these data were available. The primary outcome measures were complete abstinence from smoking (by self-report and exhaled CO measurement) from quit date to delivery, and birth weight. Secondary outcome measures were point prevalence of abstinence (smoking abstinence during the previous 7 days), time to lapse or relapse, and delivery and birth characteristics (head circumference, length, Apgar score, intrauterine growth restriction). Complete abstinence was achieved by 11 (5.5%) participants in the NRT group and 10 (5.1%) in the placebo group (OR = 1.08, 95% CI 0.45 to 2.60, for NRT compared with placebo). Median time to relapse was 15 days (both groups) and point prevalence abstinence at hospital visits (at weeks 2, 4, 8, 12, 16, and 20 after quit date) was 8–12.5% in the NRT group and 8–9% in the placebo group. No statistically significant differences were observed between the NRT and placebo groups. Mean birth weight was 3065 g (standard error, 44 g) in the NRT group and 3015 g (standard error, 44g) in the placebo group (Berlin et al. 2014). Overall delivery and birth characteristics, as reported in Table 3 of the publication by Berlin et al. (2014), are summarised in Table 2**.**

Table 2. Birth outcomes and adverse events according to study group in the 'SNIPP' trial of NRT for smoking cessation in pregnancy, carried out in France.
Data are summarised from Table 3 of (Berlin et al. 2014)

Outcome	NRT	Placebo	Comparison NRT vs. placebo
Birth outcomes			
n available for evaluation	*192*	*192*	
	Least square mean (standard error)	Least square mean (standard error)	Difference of least squares, mean value (95% CI), p value
Birth weight (g)	3065 (44)	3015 (44)	0 (−71.1 to 172.3), 0.41
z score	−0.40 (0.08)	−0.49 (0.08)	0.09 (−0.13 to 0.32), 0.41
Centile	40.7 (2.1)	37.6 (2.1)	3.18 (−2.57 to 8.94, 0.28
Length at birth (cm)	48.3 (0.23)	48 (0.23)	0.34 (−0.31 to 0.98), 0.31
Head circumference (cm)	33.7 (0.16)	33.9 (0.16)	−0.2 (−0.63 to 0.24), 0.37
Cord blood arterial pH	7.26 (0.008)	7.25 (0.008)	0.004 (−0.02 to 0.03), 0.75
			OR (95% CI), p value
Fetal growth restriction	0.18 (0.19)	0.24 (0.17)	0.68 (0.41 to 1.11), 0.12
Low birth weight (<2500 g)	0.14 (0.21)	0.17 (0.19)	0.79 (0.45 to 1.39), 0.42
Apgar score <10 at 5 min	0.07 (0.28)	0.06 (0.3)	1.2 (0.54 to 2.63), 0.65
Delivery outcomes			
n available for evaluation	*200*	*199*	
	n (%)	n (%)	Difference of least squares, mean value (95% CI), p value
Mean gestational age (wk)	38.3 (3.1)	38.5 (2.99)	−0.22 (−0.82 to 0.38), 0.16
			OR (95% CI), p value
Spontaneous vaginal delivery	138 (69)	147 (75)	0.76 (0.49 to 1.17), 0.21
Epidural anaesthesia	154 (78)	135 (70)	1.57 (0.99 to 2.47), 0.07
Use of oxytocin	108 (55.7)	103 (54.8)	1.04 (0.69 to 1.55), 0.92
Preterm birth	27 (13.5)	26 (13)	1.04 (0.58 to 1.85), 0.99
Caesarean section	51 (25.5)	44 (22.3)	1.21 (0.76 to 1.91), 0.48
Haemorrhagia at delivery	10 (5.1)	9 (4.7)	1.08 (0.43 to 2.72), 0.99
Transfer to neonatal ICU	14 (7.1)	14 (7.2)	0.83 (0.38 to 1.85), 0.99

41. The frequency of serious adverse events (SAE) was similar in both groups (n = 60 in the NRT group; n = 54 in the placebo group). Non-serious adverse events (non-SAE) (e.g. skin reactions to patch) were described as being more common in the NRT group. Diastolic blood pressure increased significantly over time in the NRT group but not in the placebo group (p = 0.01 for time by group interaction). At the final antenatal visit (approximately 1 month preterm) the median diastolic blood pressure was 70 mm Hg (interquartile range 60–80) in the NRT group and 62 (60–70) mm Hg in the placebo group. The authors noted that this finding had not been reported in previous studies of NRT patches in pregnant women and thus would require further evaluation (Berlin et al. 2014). A detailed breakdown of reported adverse events, as reported in Table 5 of the publication by Berlin et al. (2014), can be accessed at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3950302/table/tbl5/?report=objectonly (accessed 17/10/2018).

USA

42. A randomised, double-blind, placebo-controlled study of the use of nicotine-containing gum for reduction of cigarette smoking in pregnant women in Connecticut, USA, showed that gum use was associated with successful reduction in cigarette smoking levels and with increased birth weight and gestational age (Oncken et al. 2008). Pregnant women smokers (≥ 1 cigarette per day; average 10 cigarettes per day immediately prior to the study) with a singleton pregnancy at less than 26 weeks gestation, aged ≥ 16 years, were recruited between July 30, 2003 to April 17, 2007, and randomised to receive study gum containing either 2 mg nicotine (NRT) (n = 100) or no nicotine (placebo) (n = 94), with instructions to chew 1 piece of gum in replacement of smoking 1 cigarette (maximum 20 per day). Treatment was dispensed for 6 weeks and smoking cessation counselling was given to all patients. Gum usage did not differ significantly between the 2 groups. Urinary cotinine was measured at baseline and at regular follow-up visits. Adverse events (AEs) and SAEs during pregnancy were recorded, in addition to the following neonatal outcomes: preterm delivery (< 37 weeks); low birth weight (< 2500 g); spontaneous abortion (unintended loss at < 20 weeks); intrauterine fetal demise (fetal death *in utero* at > 20 weeks but prior to delivery); newborn death (age 0 to 28 days); maternal hospitalisation (other than labour or delivery); neonatal ICU admission. After 6 weeks of treatment, smoking cessation rates did not differ between the 2 groups, although the mean reduction in number of cigarettes smoked per day was significantly greater in the NRT group (–5.7 (standard deviation (SD) = 6.0)) than the placebo group (–3.5 (SD = 5.7)). Urinary cotinine levels were also significantly more reduced in the NRT group (–249 mg/mL (SD = 397)) than the placebo group (–112 mg/mL (SD = 333)). Mean birth weight was significantly higher in the NRT group (3287 g (SD = 566)) compared with the placebo group (2950 g (SD = 653)) (p < 0.0001). Mean gestational age was also significantly higher in the NRT group (38.9 weeks (SD = 1.7)) compared with the placebo group (38.0 weeks (SD = 3.3)). Other outcomes were not significantly different between the two groups, although the

authors noted some trends towards improved outcomes (e.g. birth outcomes, SAEs) in the NRT group compared with placebo (Oncken et al. 2008).

43. An open-label, randomised study of cognitive behaviour therapy (CBT) compared with CBT plus NRT for smoking cessation in pregnancy showed some effectiveness of NRT in achieving smoking cessation (Pollak et al. 2007). However recruitment to the trial was suspended when an *a priori* 2-fold level of SAEs was reached in the CBT+NRT compared with the CBT-only group (Pollak et al. 2007). In this study ('Baby Steps'), carried out from 2003 to 2005, smokers (≥ 5 cigarettes per day), aged ≥ 18 years, and between weeks 13 and 25 of pregnancy, were randomised to 6 weeks of treatment with CBT+NRT (n = 122) or CBT (n = 59). Women in the former group were able to choose one of four options: nicotine patches (7, 14, or 21 mg/day, depending on baseline smoking level) (n = 72), gum (2 mg to replace 1 cigarette) (n = 32), lozenge (2 mg to replace 1 cigarette) (n = 12), or no NRT (n = 6), and were able to switch between these modalities freely during the trial. Four women in the CBT arm reported using NRT. The primary endpoints were smoking cessation at 7 weeks post randomisation and at 38 weeks gestation. Women in the CBT+NRT arm were more likely to report being abstinent than the women in the CBT-only arm at 7 weeks post randomisation (risk difference (RD) = 0.19, 95% CI 0.08 to 0.30, p = 0.005) and at 38 weeks of gestation (RD = 0.16, 95% CI 0.06 to 0.26, p = 0.008). Birth outcomes and SAEs were also recorded. There were no differences between the two treatment groups in mean birthweight (3061 g (± 661) for CBT+NRT and 3132 g (± 688) for CBT, p = 0.51) or gestational age (37.9 weeks (± 3.1) for CBT+NRT and 38.6 weeks (± 2.7) for CBT, p = 0.14). A total of 44 women had SAEs, of whom 34/113 (30%) were in the NRT+CBT arm and 10/58 (17%) in the CBT arm. As this incidence reached a predefined cutoff level, recruitment to the trial was stopped by the Data and Safety Monitoring Board (DSMB). However, the DSMB noted that this effect was not considered to be related to NRT treatment, as the majority of the excess of SAEs in the NRT+CBT group were preterm births (at 35–37 weeks) and this group had a (chance) statistically higher level of previous preterm births at randomisation (Pollak et al. 2007).

44. A follow-up analysis, in which the medical records of participants in the Baby Steps trial were evaluated retrospectively, determined that SAEs that occurred during the trial were statistically significantly associated with black race, adverse pregnancy history, and use of analgesic medication during pregnancy, but not with randomisation to NRT (Swamy et al. 2009).

45. El-Mohandes and colleagues conducted a randomised controlled trial of CBT or CBT + NRT patches to aid smoking cessation in pregnant African-American women recruited from 3 antenatal clinics in Washington D.C. between July 2006 and December 2009, with follow-up to May 2010 (El-Mohandes et al. 2013). Women included were 18 years or older, less than 30 weeks pregnant, and a smoker wishing to quit. Participants were stratified according to salivary and urinary cotinine level measured at the start of the study. Women who did not quit smoking after 1 session of CBT at visit 1 (n = 52) were randomised at visit 2 (mean gestational age, 22.7

weeks) to subsequent CBT (n = 26) or CBT+NRT (n = 26)[3] with transdermal patch regimes of either 21 mg for 2 weeks, then 14 mg for 4 weeks, then 7 mg for 4 weeks (women with baseline salivary cotinine ≥ 100 ng/mL) or 14 mg for 6 weeks, then 7 mg for 4 weeks (women with baseline salivary cotinine ≥ 20 and ≤ 100 ng/mL). Data on patch use were collected at the subsequent visit. The CBT-only arm did not include placebo patches, thus participants were not blinded to study treatment. Women in the NRT arm for whom breath CO levels were repeatedly above a certain limit (indicating smoking) were discontinued from NRT treatment. Smoking behaviour was reported at visits 2 to 6 and data on pregnancy outcomes were obtained from medical records at the delivery hospital. During the study, smoking quit rates were mostly better in the CBT+NRT group (varying between 12% and 31% at evaluation points during the study) compared with the CBT-only group (varying between 0% and 12% at evaluation points during the study), although the difference overall was not statistically significant. Mean cotinine levels did not differ significantly between the groups during the study. Mean gestational age was higher in the NRT+CBT group (39.4 weeks) compared with the CBT group (38.4 weeks) (p = 0.02). Prematurity rate was 4% (n = 1) in the CBT+NRT group and 8% (n = 2) in the CBT group. Mean birth weight was higher in the CBT+NRT group (3204 g) compared with CBT group (2997 g) (p = 0.18). Rates of low birthweight were 12% (n = 3) for CBT+NRT and 16% (n = 4) for CBT, and no participant delivered an extremely low birth weight infant (El-Mohandes et al. 2013).

5.3.2 Population-based epidemiological studies

UK – The Health Improvement Network UK general practitioner database (THIN)

46. In addition to RCTs of NRT treatment, researchers at the University of Nottingham have also carried out epidemiological studies of potential adverse effects of NRT use during pregnancy. These studies used data from 'The Health Improvement Network' (THIN) database, which contains anonymised primary care records collected from 570 general practices in the UK, covering 6% of the UK population (Dhalwani et al. 2015, Dhalwani et al. 2018).

47. A cohort was created of all women in THIN aged 15–49 years who had live births of children registered in the same household between January 2001 and December 2012 (n = 192,498) (Dhalwani et al. 2015). Women were classified into 3 groups: 1] NRT (NRT prescribed during the period from 4 weeks prior to conception to the end of the 1st trimester of pregnancy) (n = 2677, 1.4%); 2] smokers (smoked during the 1st trimester of pregnancy, as ascertained at antenatal consultation, but not prescribed NRT) (n = 9980, 5.2%); 3] control (no NRT prescribed, nonsmoker between conception and the end of the 1st trimester of pregnancy) (n = 179,841, 93.4%). Socioeconomic and biomedical indices were also recorded. The proportions

[3] n = 26 per randomisation group, of whom 17 (NRT+CBT) and 23 (CBT) actually completed the protocol. All 52 were included in the final analysis. Two women were randomised and included twice; thus counted as two separate study participations in each case: their randomisations group(s) were not stated.

of women in the most socioeconomically deprived category were higher in the smoking (25.3%) and NRT (22.8%) groups compared with the control group (10.1%). Women in the smoking group were younger compared with the other two groups. Women in the NRT and smoking groups had a higher proportion of some maternal morbidities (asthma and mental illness) than controls, with percentages per group as follows: asthma (14.5% NRT, 10.4% smokers, 8.2% controls); hypertension (2.4% NRT, 1.8% smokers, 3.1% controls); diabetes (3.4% NRT, 2.2% smokers, 3.2% controls); mental illness (20.7% NRT, 15.3% smokers, 6.9% controls); epilepsy (0.4% NRT, 0.7% smokers, 0.4% controls). All major congenital anomalies (MCAs) recorded at any age during primary care registration were identified based on ICD-10[4] codes recorded in the database, and a breakdown was given by major types of anomaly. Minor congenital anomalies, fetal alcohol syndrome, and fetal valproate syndrome were excluded. Absolute risks of MCA (all MCAs combined) in each of the 3 groups were calculated as 336 per 10,000 live births (NRT), 315 per 10,000 live births (smokers), and 285 per 10,000 live births (control). Statistical analyses were carried out, comparing against the control and smoker groups, respectively, as the referent. Odds ratios were calculated with 99% CIs and p values < 0.01 were considered to be statistically significant, the reason given by the authors being to account for the large number of exposure and outcome categories. Data, and statistical analyses, as reported in Tables 3, 4, and 5 of the publication of Dhalwani et al. (2015), are summarised in Table 3.

[4] International Statistical Classification of Diseases and Related Health Problems (10th revision)

Table 3. Absolute risks, and adjusted ORs for MCAs in children according to maternal exposure to NRT and smoking during the 1st trimester of pregnancy. Data are taken from Tables 3, 4, and 5 of the publication of Dhalwani et al. (2015). Comparisons showing statistically significant difference at $p < 0.01$ are highlighted in bold.

	Number of children with anomaly (n)						Adjusted[1] OR (99% CI), p value		
	CONTROL (n_{total}=179,841)		NRT (n_{total}=2677)		SMOKERS (n_{total}=9980)		Smoking vs. control	NRT vs. control	NRT vs smoking
	n	n per 10,000	n	n per 10,000	n	n per 10,000			
All MCAs combined	5131	285	90	336	314	315	1.05 (0.89–1.23), 0.47	1.12 (0.84–1.48), 0.31	1.07 (0.78–1.47), 0.58
Heart	1652	92	26	97	104	104	1.09 (0.83–1.46), 0.39	1.01 (0.60–1.70), 0.96	0.92 (0.52–1.62), 0.72
Limb	996	55	15	56	60	60	1.04 (0.73–1.48), 0.76	0.99 (0.50–1.95), 0.98	0.95 (0.45–2.01), 0.87
Genital system	847	47	16	60	51	51	0.94 (0.63–1.38), 0.66	1.14 (0.59–2.20), 0.62	1.21 (0.58–2.54), 0.50
Urinary system	479	27	12	45	20	19	0.86 (0.47–1.57), 0.51	1.82 (0.85–3.89), 0.04	1.98 (0.76–5.13), 0.06
Chromosomal	383	21	4	15	13	13	0.71 (0.33–1.49), 0.24	0.74 (0.19–2.78), 0.56	1.05 (0.24–4.62), 0.92
Musculoskeletal	342	19	10	37	28	28	1.32 (0.76–2.29), 0.19	1.79 (0.76–4.20), 0.08	1.37 (0.53–3.54), 0.39
Orofacial cleft	252	14	3	11	20	19	1.40 (0.76–2.59), 0.16	0.75 (0.17–3.40), 0.63	0.55 (0.11–2.71), 0.33
Digestive system	261	15	6	22	18	18	1.30 (0.66–2.54), 0.32	1.52 (0.51–4.51), 0.32	1.17 (0.34–3.97), 0.74
Nervous system	273	15	4	15	18	17	1.01 (0.51–2.03), 0.96	0.83 (0.22–3.09), 0.72	0.82 (0.20–3.41), 0.72
Other malformations	257	14	5	19	18	18	1.08 (0.55–2.11), 0.76	1.16 (0.36–3.79), 0.74	1.08 (0.29–3.97), 0.88
Eye	208	12	3	11	15	14	1.19 (0.58–2.46), 0.52	0.89 (0.19–4.06), 0.85	0.74 (0.15–3.77), 0.64
Respiratory system	137	8	10	37	10	10	1.34 (0.54–3.31), 0.41	**4.65 (1.76–12.25), <0.001**	**3.49 (1.05–11.62), 0.007**
Genetic	153	9	0	-	9	9	1.02 (0.37–2.81), 0.96	-	-
Abdominal wall	29	2	0	-	3	2	0.77 (0.15–3.96), 0.68	-	-
Ear, face, and neck	35	2	0	-	3	2	1.51 (0.34–6.64), 0.48	-	-

[1]Adjusted for maternal age at conception, Townsend deprivation index score, maternal diabetes, asthma, mental illnesses, and multiple births

48. Risk for all MCAs combined was not statistically significantly different for NRT compared with control (OR = 1.12, 99% CI 0.84 to 1.48, p = 0.31), smokers compared with control (OR = 1.05, 99% CI 0.89 to 1.23, p = 0.47) or NRT compared with smokers (OR = 1.07, 99% CI 0.78 to 1.47, p = 0.58). However, analysis of system-specific anomalies indicated a higher incidence of respiratory anomalies in the NRT group compared with the control group (OR = 4.65, 99% CI 1.76 to 12.25, p < 0.001) and in the NRT group compared with smokers (OR = 3.49, 99% CI 1.05 to 11.62, p = 0.007). These results were based on analysis of a total of 157 cases with respiratory anomalies, of whom 10/2677 mothers were in the NRT group (37 per 10,000 live births), 10/9980 were in the smoker group (10 per 10,000 live births), and 137/179,841 were in the control group (8 per 10,000 live births). The authors concluded that for most system-specific MCAs there were no statistically significant increased risks associated with maternal NRT prescribed during pregnancy, with the exception of respiratory anomalies. They commented that despite the size of the study population, statistical power was limited by the relatively rare use of NRT in pregnancy. They also felt that results may have been impacted by the fact that women prescribed NRT had a higher rate of morbidities compared with women not prescribed NRT (Dhalwani et al. 2015).

49. A study reporting on rates of stillbirth in the same cohort was published by Dhalwani et al. (2018). A total of 220,630 singleton pregnancies that underwent a delivery were included in the cohort for this analysis. The NRT group (n = 5221) was classed as women who had been prescribed NRT at any time during pregnancy or the preceding 4 weeks, with the average NRT prescription duration being 2 weeks. Smokers (n = 18,407) were defined as women who smoked at any time from conception to delivery[5]. Controls (n = 197,002) neither smoked nor were prescribed NRT during pregnancy. The proportions of women from the most deprived socioeconomic categories and with mental illness were higher in the NRT (24.3% and 20.0%, respectively) and smoker (26.2% and 16.0%, respectively) groups than the control group (11.9% and 7.8%, respectively). Stillbirth was defined as a baby born with no signs of life at or after 28 weeks of gestation (WHO definition). A total of 805 deliveries were stillbirths (equivalent to 3.4 per 1000 births), of which 26 in the NRT group (0.50%), 96 in the smoker group (0.52%), and 683 in the control group (0.35%). Compared with the control group, unadjusted risks of stillbirth were: NRT, OR = 1.44, 95% CI 0.97 to 2.14, p = 0.069; smokers, OR = 1.52, 95% CI 1.23 to 1.89, p < 0.001. After adjustment for maternal age, socioeconomic status, pre-pregnancy body mass index, and diabetes, these values were: NRT, OR = 1.35, 95% CI 0.91 to 2.00, p = 0.139; smokers, OR = 1.41, 95% CI 1.13 to 1.77, p = 0.003. The risk for stillbirth in the NRT group compared with smokers was: OR = 0.95, 95% CI 0.62 - 1.48[6]. The authors concluded that from the results of this study, there was no evidence of a statistically significant association between being prescribed NRT during pregnancy and the odds of stillbirth, as compared with nonsmoking women. However, they considered that a larger study, including biochemically validated

[5] Presumed to exclude those who were prescribed NRT, although this is not stated in the report.
[6] The report does not state whether or not this comparison is adjusted for potential confounders.

smoking exposure data and close monitoring of NRT use throughout pregnancy, is required to produce more definitive results (Dhalwani et al. 2018).

Denmark – The Danish National Birth Cohort (DNBC)

50. The Danish National Birth Cohort (DNBC)[7] is a population-based cohort of pregnant women recruited from 1996–2002, derived from initial, general practitioner-based invitation to approximately 50% of the pregnant population, of whom around 60% consented to join the cohort. The original cohort included 101,042 pregnancies. Data were gathered via telephone interviews during the 2nd and 3rd trimesters of pregnancy, including exposures during pregnancy, mother's health status, medical and obstetric history, lifestyle and diet, working and living conditions, psychosocial stress, and socioeconomic status (Olsen et al. 2001).

51. A study by Morales-Suarez-Varela et al. (2006) used data from the DNBC and considered three subgroups: non-smokers and non-nicotine users, smokers and non-nicotine users, and non-smokers and nicotine users. The authors reported an increase in congenital malformation associated with use of nicotine substitutes in nonsmoking women during the first 12 weeks of pregnancy. A total of 76,768 singleton pregnancies that produced a live birth were included, of whom 20,603 (26.8%) mothers smoked ('mostly cigarettes') and 56,165 mothers did not smoke during the first 12 weeks of pregnancy. Data on congenital malformations listed from birth to 12 months of age were obtained from the Medical Birth Registry, and data on hereditary diseases and chromosome abnormalities from the National Hospital Discharge Registry. Congenital malformations were classed as minor or major according to 'EUROCAT' criteria[8], and relative prevalence ratios (RPR) were calculated for subcategories. Data on potential confounders (biomedical, behavioural, and socioeconomic indices) were also obtained. A total of 3767 (4.9%) infants had congenital malformations, with overall similar prevalence in smokers (n = 1034, 5.0%) and nonsmokers (n = 2773, 4.9%). Breakdown by type of congenital malformation showed a similar distribution in smokers and nonsmokers, except for a slightly higher prevalence of circulatory system malformations in smokers (n = 229, 22.1%) compared with nonsmokers (n = 517, 18.9%). The RPR for congenital malformations overall in smokers compared with nonsmokers was 1.1 (95% CI 1.0 to 1.2). Breakdown by type of congenital malformation showed prevalences that were significantly: increased for circulatory system (RPR = 1.2, 95% CI 1.0 to 1.4), cleft lip and palate (RPR = 1.5, 95% CI 1.0 to 1.2), and digestive system (RPR = 1.3, 95% CI 1.0 to 1.7); and decreased for eye, ear, face and neck (RPR = 0.7, 95% CI 0.5 to 1.0), urinary system (RPR = 0.7, 95% CI 0.5 to 1.0), and musculoskeletal system (RPR = 0.9, 95% CI 0.8 to 1.0). RPRs were then calculated according to 4 groups: nonsmokers who did not use nicotine substitutes (n = 55,915 births; n = 2719 malformations); smokers (≤ 10 cigarettes per day) who did not use nicotine substitutes (n = 12,365 births; n = 651 malformations); smokers (> 10 cigarettes per

[7] See: https://www.ssi.dk/English/RandD/Research%20areas/Epidemiology/DNBC/ (accessed 10/09/2018)
[8] European Surveillance of Congenital Anomalies

day) who did not use nicotine substitutes (n = 4447 births; n = 220 malformations); nonsmokers who used nicotine substitutes) (n = 250 births; n = 19 malformations). Prevalence ratios, taking nonsmokers who did not use nicotine substitute as the comparison group, were: RPR = 1.09, 95% CI 1.00 to 1.19 (≤ 10 cigarettes/day); RPR = 1.02, 95% CI 0.89 to 1.17 (> 10 cigarettes/day); RPR = 1.6, 95% CI 1.01 to 2.58 (nonsmokers who used nicotine substitute). In the latter group (nonsmokers who used nicotine substitute), of the 19 malformations observed: 11 were major and 8 minor; 14 were musculoskeletal, of which 6 major and 8 minor (7 minor malformations were dislocation of the hip). Compared with nonsmokers who did not use nicotine substitutes, prevalence ratios were: all major malformations (RPR = 1.13, 95% CI 0.62 to 2.07); musculoskeletal malformations, excluding dislocation of the hip (RPR = 2.63, 95% CI 1.53 to 4.52); major musculoskeletal malformations (RPR = 2.05, 95% CI 0.91 to 4.63). The authors concluded that there was no increase in overall prevalence of congenital malformations in smokers, although there was an increased prevalence of some types of malformations (cleft lip and palate, respiratory, circulatory), in line with other studies of smoking in pregnancy (cleft lip). They also concluded that their findings indicated that nicotine may be teratogenic when used in nicotine substitutes, although they noted that this conclusion was based on small numbers of cases (Morales-Suarez-Varela et al. 2006).

52. Another study of data from the DNBC showed no association between NRT use during pregnancy and risk of stillbirth (Strandberg-Larsen et al. 2008). Participants, women who were pregnant during 1996–2002, were interviewed at gestational weeks 12–16 regarding NRT use (user or nonuser; type of product – patches only, gum only, inhaled substances only, or a mixture of products), smoking habits (nonsmoker, ex-smoker, current smoker ≤ 10 g tobacco per day, current smoker > 10 g tobacco per day), and other potential confounders. A total of 87,032 singleton pregnancies that continued until at least 20 weeks of gestation were included in the final analysis. Stillbirth was defined as any fetus that did not breathe or show any sign of life at birth after a minimum of 20 weeks of gestation. Stillbirth was recorded for 8 of the 1927 women who used NRT (4.2 per 1000 births) and 487 of the 85,105 women who did not use NRT (5.8 per 1000 births). Smoking, but not NRT use, was associated with a statistically significant increased risk of stillbirth, and no interaction between smoking and NRT use was identified. Adjusted hazard ratios (HR) compared with non-NRT users who did not smoke were: NRT users who did not smoke (HR = 0.57, 95% CI 0.28 to 1.16); dual NRT users/smokers (HR = 0.83, 95% CI 0.34 to 2.00); smokers who did not use NRT (HR = 1.46, 95% CI 1.17 to 1.82). For smoking subgroups (compared with nonsmokers), a statistically significant increase in risk of stillbirth was observed for women who continued to smoke during pregnancy, with the highest risk in women in the higher tobacco use group (HR = 1.94, 95% CI 1.36 to 2.77[9]). The authors concluded that NRT use during pregnancy had no serious impact on the risk of stillbirth but that further studies would be needed

[9] Adjusted for maternal age, household socio-occupational status and use of NRT.

to examine other outcomes, such as preterm birth, low birthweight, and long-term neonatal and paediatric health outcomes (Strandberg-Larsen et al. 2008).

53. Lassen et al. (2010) conducted a study of NRT use and birthweight using data gathered from 72,761 women enrolled in the DNBC who had given birth to live singletons. Information on NRT use during the first 2 trimesters (up to and including 27 weeks) of pregnancy (type – gum, patch, inhaler; during which weeks of pregnancy) was extracted from responses recorded from 2nd and 3rd trimester interviews. Information was also collected on smoking habits during pregnancy (pack-weeks as a categorical variable) and other potentially confounding variables (e.g. exercise levels, alcohol intake, biomedical indices, partner smoking status). Information on gestational age and birthweight were obtained from the Danish National Patient Registry. Of the cohort of 72,761 women, a total of 1828 (2.5%) had reported NRT use (56.3% gum, 30.4% patches, 27.3% inhaler; 10% more than one type within the same week). Data on both NRT use and smoking status were available for 71,320 women. Of these women, 1753 had used NRT, 15,796 were smokers who had not used NRT, and 53,771 were nonsmokers who had not used NRT. Most (88%) of the NRT users had also smoked to some extent during the first 2 trimesters. Median NRT use was for 2 weeks, while 2.7% of women had used NRT until the end of the 2nd trimester. Median smoking level in NRT users during the study period was 9.5 pack-weeks (6.1 pack-weeks for gum, 8.9 pack-weeks for inhaler, 10.4 pack-weeks for patch, 11.1 pack weeks for multiple NRT types) and in non-NRT-users, 6.9 pack-weeks. Overall, NRT use was not associated with a difference in mean birthweight (b) compared with no NRT use: b (adjusted[10]) = 0.25 g per week of NRT use (95% CI −2.31 to 2.81). Simultaneous use of more than one NRT product was associated with a decrease in mean birthweight but the difference was not statistically significant: b (adjusted) = −10.73 g per week of NRT use (95% CI −26.51 to 5.05). No significant changes were observed by analysis for individual types of NRT product. Smoking ≥ 3 pack-weeks was associated with significantly decreased birthweight, with mean birthweight decreasing proportionately to increasing pack-weeks (at 20+ pack-weeks, b (adjusted[11]) = −266.18, 95% CI −289.02 to −243.34). A further analysis did not show a significant effect on birthweight of NRT use at any time during the first 35 weeks of gestation (b = 0.87 g per week of NRT use, 95% CI −3.41 to 5.15) or during the 3rd trimester of pregnancy (b = 0.30 g per week of NRT use, 95% CI −24.97 to 25.56. The authors concluded that the results of this study suggested that NRT use during pregnancy is not strongly associated with a change in offspring birthweight, but there may be a

[10] Adjusted for gestational age, smoking status (pack-weeks), smoking status of partner, parity, pre-pregnancy body mass index, maternal height, alcohol consumption, coffee intake, physical exercise, infant sex, socioeconomic status, weight loss, eating disorder, fertility problems, vaginal bleeding, nausea, hypertension.

[11] Adjusted for gestational age, total NRT use within the first 27 completed weeks of gestation, smoking status of partner, parity, pre-pregnancy body mass index, maternal height, alcohol consumption, coffee intake, physical exercise, infant sex, socioeconomic status, weight loss, eating disorder, fertility problems, vaginal bleeding, nausea, hypertension.

potential negative effect on birthweight associated with simultaneous use of more than one NRT product in the same week (Lassen et al. 2010).

54. Torp-Pedersen and colleagues reported that NRT use during pregnancy was not associated with increased risk of strabismus in children born to women registered in the DNBC (Torp-Pedersen et al. 2010). This study evaluated the potential risk of strabismus associated with a number of different factors, including dietary (coffee, tea, alcohol) and other (smoking, NRT) exposures, during pregnancy. Smoking and NRT use during the whole of pregnancy were ascertained from information reported by women at 3 interviews during pregnancy, and women were categorised for average daily smoking during pregnancy and per trimester, and NRT use at any point during pregnancy and during each trimester. A total of 1320 strabismus cases were included for analysis (n = 415 mothers were smokers and n = 884 mothers were nonsmokers during pregnancy; n = 61 mothers used NRT and n = 1239 mothers did not use NRT during pregnancy). Smoking compared with not smoking during pregnancy was associated with increased risk of strabismus (RR = 1.26, 95% CI 1.11 to 1.43), with an increasing dose–response for the number of cigarettes smoked per day (RR = 1.05, 95% CI 1.03 to 1.06, for each extra cigarette smoked daily during pregnancy). Smoking in the first trimester only was not associated with increased risk (RR = 1.03, 95% CI 0.84 to 1.27, compared with not smoking). However, smoking in the first 2 or all 3 trimesters were both associated with a statistically significant increased risk of strabismus: first 2 trimesters (RR = 1.43, 95% CI 1.14 to 1.81); all 3 trimesters (RR = 1.35, 95% CI 1.16 to 1.57), compared with not smoking. In children of women who used NRT during pregnancy, strabismus risk was: RR = 1.22, 95% (CI 0.92 to 1.61), compared with children of women who did not use NRT during pregnancy (RRs were adjusted for year of birth, social class, maternal age at birth, maternal smoking dose (except for the smoking analyses), and maternal coffee and tea consumption). The authors concluded that risk of strabismus in offspring increased in line with numbers of cigarettes smoked per day during pregnancy but that use of NRT during pregnancy was not associated with strabismus in the offspring (Torp-Pedersen et al. 2010).

55. Data from the DNBC indicated an association of NRT use and/or smoking during pregnancy with risk of infant colic in the offspring (Milidou et al. 2012). Based on data gathered by 2nd and 3rd trimester interview from a total of 63,128 singleton pregnancies that produced a live birth, women were categorised as NRT users (n = 207; 0.3%), smokers (n = 15,016; 23.8%), smokers using NRT (combination) (n = 1245; 2.0%), or unexposed (n = 46,660; 73.9%). Infantile colic (n = 4974; 7.9%) was determined from 6-month postpartum telephone interviews, based on crying or fussing for greater than 3 hours per day for more than 3 days per week, starting before 3 months of age, and unrelated to teeth cutting or any recognised disease. A total of 4974 women reported infants with colic, of whom 23 (11.1%) were NRT users, 1417[12] (9.4%) smokers, 137 (11.0%) smokers using NRT, and 3397 (7.3%) unexposed. Analysis showed a statistically increased risk of infantile colic in all

[12] This value has been re-calculated from data provided in the publication of Milidou et al. (2012) as there is an error in the calculated value reported in Table 2 of the publication.

nicotine-exposed groups compared with unexposed: NRT users (adjusted OR (AOR) = 1.6, 95% CI 1.0 to 2.5); smokers (AOR = 1.3, 95% CI 1.2 to 1.4); smokers using NRT (AOR = 1.5, 95% CI 1.3 to 1.8). Adjustment was made for maternal age, first parity, daily coffee consumption, weekly alcohol consumption, binge drinking episodes, couple's combined educational and occupational status. It was noted that adjustment for potential confounders had negligible impact on outcomes. The authors concluded that the findings indicated that nicotine may be the component in tobacco smoke that is responsible for increased risk of infantile colic, however the number of exclusive NRT users was limited and some estimates had large CIs. They commented that adjusted and unadjusted models (including for socioeconomic status) yielded similar results, suggesting that lifestyle factors related to smoking behaviour are not responsible for the increased risk of infantile colic, a hypothesis that has been suggested by some other investigators (Milidou et al. 2012).

56. Zhu and colleagues evaluated the input of maternal smoking and/or NRT use and paternal smoking on the risk of attention deficit hyperactivity disorder (ADHD) in children born to women participants in the DNBC (Zhu et al. 2014). Children with ADHD were identified from various Danish healthcare registers and/or by a scoring system of responses from a parent questionnaire completed at the 7-year follow-up of the DNBC (when children were aged between 8–14 years). The analysis was based on a total of 84,803 singleton pregnancies for which early-pregnancy (around 16 weeks) interview data on smoking status were available. Of these, 50,870 participated in the 7-year follow-up interview. Based on 16-week interview data, women were classed as: smokers (smoking at time of interview, with or without NRT use); NRT users (nonsmoker at the time of interview); quitters (had quit by time of interview without using NRT); or non-smokers. Fathers were classed as smokers or nonsmokers based on the reports of their pregnant partners. Thus, 8 groups were specified in total and hazard ratios were calculated, with the referent group being children of nonsmoking parents. Data are summarised in Table 4. Compared with not smoking, both maternal and paternal smoking during pregnancy were associated with statistically significant increased risk of ADHD in offspring, with the association stronger for maternal than paternal smoking. Use of NRT by women during pregnancy was also associated with statistically significant increased risk of ADHD in the child, but only in women with nonsmoking partners. Women who quit smoking during early pregnancy had increased risk of an ADHD child, but only in the case where the partner was a smoker. The authors commented that these findings suggest that exposure to prenatal tobacco smoke, possibly nicotine, may have a prenatal programming effect on the risk of ADHD in children, or that, alternatively, the results may reflect confounding by family factors that are more linked to maternal than paternal smoking, including both genetic and postpartum caring factors (Zhu et al. 2014).

Table 4. Number of children and HRs for ADHD by parental smoking and NRT use status. Children of nonsmoking parents are the referent group. Data are summarised from Tables 1 and 3 of Zhu et al. (2014).

Mother	Father	Children (total)	Children (ADHD)		HR (95% CI) [1] for ADHD
		n	n	%	
Smoker	Smoker	8771	368	4.2	1.83 (1.60 to 2.10)
NRT user	Smoker	240	7	2.9	1.28 (0.57 to 2.89)
Quitter	Smoker	3199	113	3.5	1.70 (1.38 to 2.10)
Nonsmoker	Smoker	14,004	360	2.6	1.29 (1.14 to 1.47)
Smoker	Nonsmoker	4776	164	3.4	1.63 (1.36 to 1.94)
NRT user	Nonsmoker	574	22	3.8	2.28 (1.48 to 3.51)
Quitter	Nonsmoker	4167	83	2.0	1.08 (0.85 to 1.36)
Nonsmoker	Nonsmoker	49,072	892	1.8	Referent
Total		84,803	2009	2.4	

[1] Cox regression, adjusted for maternal age, parity, alcohol intake during pregnancy, parental socioeconomic status, parental psychopathology, and child's gender.

Canada – The Quebec Pregnancy Cohort

57. A questionnaire was mailed to 8505 women randomly selected from the Quebec Pregnancy Cohort, a database of pregnancies listed in Quebec, Canada from January 1998 to December 2009. Of the respondents, 1288 women who had a live birth and described themselves as smokers before pregnancy were included. These women were classified into 3 groups: prescribed or reported purchasing patch NRT during pregnancy, with or without smoking (n = 316, 24.5%); prescribed bupropion (an antidepressant and smoking-cessation aid medication) during pregnancy, with or without smoking (n = 72, 5.6%); smoked during pregnancy without using NRT or buproprion (n = 900, 69.9%). Information on maternal smoking during pregnancy was collected retrospectively via the questionnaire. Data were not collected on use of alcohol or illicit drugs, but the authors noted that these were 'likely similar' between groups as all participants were smokers. Analyses were performed comparing against the smoking-only group as the referent. Median duration of use was 54 days for NRT and 87 days for buproprion, with initiation during the 1st trimester. Endpoints evaluated were smoking cessation, premature birth (< 37 weeks), and small for gestational age (SGA). Absolute smoking cessation was described for 79% of NRT users and 81% of buproprion users, of whom 68% and 60%, respectively, did not smoke again during pregnancy after ceasing the

quitting medication. Compared with smokers during pregnancy, adjusted[13] ORs for prematurity were: NRT (OR = 0.21, 95% CI 0.13 to 0.34); buproprion (OR = 0.12, 95% CI 0.03 to 0.50). Compared with smokers during pregnancy, adjusted ORs for small for gestational age were: NRT (OR = 0.61, 95% CI 0.41 to 0.90), buproprion (OR = 0.97, 95% CI 0.50 to 1.89) (Bérard et al. 2016).

USA – Pregnancy Risk and Assessment Monitoring System (PRAMS)

58. Gaither and colleagues reported an evaluation of data from the US 'Pregnancy Risk and Assessment Monitoring System' (PRAMS) population database, which includes self-administered questionnaire information gathered from women before, during, and after pregnancy, covering 26 US states (Gaither et al. 2009). Data were collected from a total of 5716 women, and 3 groups were specified: prescribed (spray, inhaler, or pill) or recommended to use (patch or gum) NRT during pregnancy (NRT, n = 225); women who smoked during pregnancy (smoker, n = 637); women who did not smoke and were not prescribed or recommended to use NRT during pregnancy (nonsmoker, n = 4854). Two serious adverse pregnancy outcomes were investigated using data from birth certificates: preterm birth (< 37 weeks gestation) and low birthweight (< 2500 g). Several potential confounding factors were also recorded (e.g. physical/health, behavioural, ethnicity and socioeconomic characteristics). Risks of low birthweight and preterm birth were highest for women prescribed or recommended NRT. Low birthweight was noted in 84 (13.05%) of the NRT group, 205 (9.26%) of the smoker group, and 1303 (6.99%) of nonsmokers. Unadjusted ORs for low birthweight compared with nonsmokers were: NRT (OR = 2.00, 95% CI 1.13 to 3.45); smokers (OR = 1.36, 95% CI 0.98 to 1.88), with similar outcomes after adjustment for age, marital status, education, and race/ethnicity. Preterm birth occurred in 66 (17.54%) of the NRT group, 156 (10%) of smokers, and 1165 (9.42%) of nonsmokers. Unadjusted ORs for preterm birth compared with nonsmokers were: NRT (OR = 2.05, 95% CI 1.14 to 3.63); smokers (OR = 1.09, 95% CI 0.74 to 1.61), with similar outcomes after adjustment for age, marital status, education, and race/ethnicity. The authors suggested that these findings may have been related to frequency of maternal smoking (e.g. heavier smokers might be more likely to be prescribed/recommended NRT and also less likely to stop smoking). They also noted that no data were gathered on actual use of NRT during pregnancy. Some, limited, information was available on smoking levels during pregnancy and this suggested that women prescribed/recommended NRT who were 'low-frequency' smokers (≤ 10 cigarettes per day) had a slightly increased risk of low birthweight and preterm birth compared with no NRT, whereas for 'high-frequency' smokers (≥ 10 cigarettes per day), NRT prescription/recommendation may be associated with up to 3-fold increased risk. However, the authors noted that the dataset was inadequate for useful statistical analysis of these aspects. They also noted that the adverse findings may have been

[13] Adjusted for maternal age, work status, place of living, education level, annual family income, welfare status, maternal hypertension, diabetes, asthma, depression, and health services utilization before pregnancy.

related to adverse effects of nicotine and/or other constituents of NRT products on the developing fetus (Gaither et al. 2009).

5.3.3 Systematic reviews, pooled and meta-analyses

59. The group of Coleman and colleagues from the University of Nottingham have published a series of systematic reviews on the efficacy and potential adverse effects indicated from RCTs of NRT for smoking cessation in pregnancy (Agboola et al. 2010, Coleman et al. 2011, Coleman et al. 2012a, Coleman et al. 2015). An overview of the most recent summary, a Cochrane Database Systematic Review updated to July 2015 (Coleman et al. 2015), is given in the following paragraphs.

60. A search of the Pregnancy and Childbirth Group's Trial Register was carried out on 11/07/2015 with the selection criteria 'Randomised controlled trials conducted in pregnant women with designs that permit the independent effects of any type of pharmacotherapy of electronic nicotine delivery systems (ENDS) on smoking cessation to be ascertained'. A total of 8 trials that had evaluated NRT were identified, while no trials had evaluated ENDS. The 8 studies of NRT included were: Wisborg et al. (2000), Kapur (2001), Oncken et al. (2008), Coleman et al. (2012b) / Cooper et al. (2014b), and Berlin et al. (2014) (placebo-controlled trials); Hotham et al. (2006), Pollak et al. (2007), and El-Mohandes et al. (2013) (non-placebo-controlled trials). A pooled analysis of data from these 8 trials (n = 2199 participants in total) for the primary outcome, efficacy of intervention for smoking cessation, produced a risk ratio (RR) of: RR = 1.41, 95% CI 1.03 to 1.93, $Tau^2 = 0.03$, $I^2 = 18\%$. Subgroup analysis for the 5 studies that were placebo controlled (n = 1926 women) indicated: RR = 1.28, 95% CI 0.99 to 1.66, $Tau^2 = 0.00$, $I^2 = 0\%$. Subgroup analysis for the 3 studies that were not placebo controlled (n = 273 women) indicated: RR = 8.51, 95% CI 2.05 to 35.28, $Tau^2 = 0.00$, $I^2 = 0\%$. Birth outcomes were analysed as secondary endpoints. Pooled analyses indicated no statistically significant difference between NRT and control interventions in stillbirth rate (RR = 1.24, 95% CI 0.54 to 2.84, $Tau^2 = 0.00$, $I^2 = 0\%$, 4 studies, n = 1777 women), birthweight (mean difference 100.54 g, 95% CI −20.84 to 222.91, $Tau^2 = 15624.49$, $I^2 = 75\%$, 6 studies, n = 2068 women), preterm birth (RR = 0.87, 95% CI 0.67 to 1.14, $Tau^2 = 0.00$, $I^2 = 0\%$, 6 studies, n = 2048 women), neonatal ICU admissions (RR = 0.90, 95% CI 0.64 to 1.27, $Tau^2 = 0.00$, $I^2 = 0\%$, 4 studies, n = 1756 women), neonatal deaths (RR = 0.66, 95% CI 0.17 to 2.62, $Tau^2 = 0.00$, $I^2 = 0\%$, 4 studies, n = 1746 women), congenital abnormalities (RR = 0.73, 95% CI 0.36 to 1.48, $Tau^2 = 0.00$, $I^2 = 0\%$, 2 studies, n = 1401 women), or caesarean birth rate (RR = 1.18, 95% CI 0.83 to 1.69, $Tau^2 = 0.03$, $I^2 = 46\%$, 2 studies, n = 1401 women) (Coleman et al. 2015).

61. The narrative of the systematic review noted that 2 studies had reported on maternal blood pressure: Coleman et al. (2012b) had reported hypertension in 4.6% of the NRT group and 4.7% of the control group, while Berlin et al. (2014) had reported significantly higher median diastolic blood pressure in the NRT compared with control group (NRT: median = 70 mm Hg, IQR 60–80 mm Hg; control: median = 62 mm Hg, IQR 60–80 mm Hg; p = 0.02). The trials of Coleman et al. (2012b) and

Berlin et al. (2014) were also noted to have reported the distribution of other birth outcomes between NRT and placebo groups, such as Apgar score at 5 min after birth, cord arterial blood pH, intraventricular haemorrhage, neonatal convulsions, necrotising enterocolitis, mechanical ventilation of infant, assisted vaginal delivery, and maternal death. The systematic review by Coleman et al. (2015) commented that no statistically significant differences were noted.

62. The systematic review of Coleman et al. (2015) noted that the study by Coleman et al. (2012b) had been the only one to report on postnatal outcomes. This study had shown significantly better developmental outcomes (parent-reported 'survival without impairment' at 2 years old) for infants born to NRT-group mothers compared with placebo-group mothers (OR = 1.40, 95% CI 1.05 to 1.86), although respiratory outcomes (parent-reported respiratory symptoms) were not significantly different between the groups (OR = 1.32, 95% CI 0.97 to 1.74, for NRT compared with placebo) (Coleman et al. 2015).

63. A narrative evaluation of adherence and side effects of NRT was given, noting that adherence to treatment in the studies assessed was generally low; only the study of Berlin et al. (2014) had reported high rates of adherence, but these were difficult to reconcile with other outcomes in this study (e.g. rates of discontinuation of the intervention). Five trials had reported on non-serious side effects, including 'unpleasant effects of patches' (Hotham et al. 2006), headache, dizziness, fatigue, heartburn, nausea or vomiting (discontinuation by 15% of NRT, 12% of control) (Oncken et al. 2008), discontinuation by 11 participants due to skin irritation and headache, plus 5 participants reported palpitations and 2 nausea in the trial of Wisborg et al. (2000). In the study of Coleman et al. (2012b), 535 and 450 non-SAE were reported, respectively, by NRT (n = 521) and placebo (n = 529) group participants, while the report of Berlin et al. (2014) listed a range of non-serious adverse events, with skin reactions more common in the NRT (11%) compared with placebo (4%) group (Coleman et al. 2015).

64. Overall, Coleman et al. (2015) concluded that evaluation of all 8 studies included in this Cochrane Database Review of RCTs for NRT in pregnancy indicated that NRT use in pregnancy for smoking cessation increases smoking cessation rates in late pregnancy by approximately 40%. However, exclusion of 3 non-placebo-controlled trials indicated that cessation rates with NRT and placebo are similar. The authors highlighted the apparent lack of efficacy of NRT for smoking cessation in pregnancy in comparison with established efficacy in non-pregnant women, noting that in the trials conducted, pregnant women have generally shown low adherence to NRT and also that increased nicotine metabolism in pregnancy may be implicated (i.e. reduced adherence may be related to a lack of efficacy of NRT to maintain a suitable blood nicotine level). They considered that there was no evidence that use of NRT for smoking cessation in pregnancy had a positive or negative effect on birth outcomes, and that the trial of Coleman et al. (2012b), had suggested that NRT promotes healthy developmental outcomes in infants. Further placebo-controlled trials would be required with higher adherence rates and monitoring of outcomes in

infancy and childhood. The authors suggested that data indicate that it would be ethical to conduct such RCTs using higher doses of NRT than those tested in the 8 studies that had been included in this evaluation (Coleman et al. 2015).

65. A group of investigators from the University of Oxford reported a meta-analysis of data from RCTs listed in the Cochrane Tobacco Addiction Group trials register as of July 2017, to evaluate the efficacy of NRT for smoking cessation (Hartmann-Boyce et al. 2018). This analysis was not limited to pregnancy, but did include specific analysis of a subset of 6 trials conducted in pregnant women (Wisborg et al. 2000, Pollak et al. 2007, Oncken et al. 2008, Coleman et al. 2012b, El-Mohandes et al. 2013, Berlin et al. 2014). The authors noted that these 6 trials did not detect significant increases in SAE among the treatment groups; recruitment for Pollak et al. (2007) had been suspended early when interim analysis found a higher rate of adverse birth outcomes (mostly preterm birth) in the NRT arm (primarily preterm birth), but there was no significant finding in the final analysis adjusted for previous birth outcomes. Analysis of 2-year follow-up data from the study by Coleman et al. (2012b) had shown that 2-year-olds born to women who used NRT were more likely to have survived without developmental impairment compared with 2-year-olds born to women who used placebo (OR = 1.40, 95% CI 1.05 to 1.86). In conclusion to their analysis of pregnancy-based studies, noted that “We found no significant benefit of treatment at longest follow-up/post-partum follow-up. None of the studies found evidence of a significant increase in serious adverse events in the NRT arms.” (Hartmann-Boyce et al. 2018).

66. Lee and Fariss (2017) conducted a systematic review and meta-analysis, sponsored by Altria Client Services[14], of serious adverse health effects (SAHEs) associated with use of pharmaceutical[15] NRT products, including data from both RCTs and population-based epidemiological studies. The main aim of the meta-analysis with regard to reproduction/development was, where possible, to use published study data to evaluate outcomes for NRT use compared with no NRT use, in women who were smokers. The definition of SAHEs was “serious adverse events leading to substantial disruption of the ability to conduct normal life functions, including those that lead to hospitalization, significant disability or birth defects, are life-threatening or result in death or require medical attention to prevent one of the above outcomes”. Acute side effects were not included. Searches were carried out of PubMed (to 30 Nov, 2015) and Cochrane Library clinical trial reviews (search date not specified). Study reports or meta-analyses identified were each evaluated using a critical assessment form (CAF) to rate quality, and CAFs were then categorised and findings summarised by endpoint: cancer (CAF 1), reproduction/development (CAF 2 – CAF 20), cardiovascular disease, stroke, and other SAHEs (CAFs 21 onwards for the latter 3 categories). Only the findings relating to

[14] Altria Client Services Inc. operates as a subsidiary of Altria Group Inc, which is the parent company of Philip Morris USA, U.S. Smokeless Tobacco Company, John Middleton, Nat Sherman, Nu Mark, Ste. Michelle Wine Estates and Philip Morris Capital Corporation.

[15] Thus excluding smokeless tobacco products and ENDS, which are not currently approved and licensed as drugs or medicines to aid smoking cessation (Royal College of Physicians, 2016; US Food and Drug Administration, 2016, *cited by* Lee and Fariss, 2017)

reproduction/development are described here. A total of 19 citations related to reproduction/development, reporting on 16 studies (8 epidemiological studies, all but 2 of which were based on the DNBC, and 8 RCTs). Study follow-up times were noted to be generally very short, except for two reports from the DNBC (Torp-Pedersen et al. 2010, Zhu et al. 2014). A summary of the reports/studies included is shown in Table 5.

Table 5. Supplementary file 3 from the publication of Lee and Fariss (2017). Studies on NRT and reproduction/development.

CAF No.	Reference	Brief study description
Epidemiological studies		
2	Morales-Suarez-Varela et al. (2006)	Prospective study based on Danish National Birth Cohort, involving 76,768 pregnant women of which 250 used NRT and did not smoke. Compared rates of congenital malformations in offspring by maternal smoking and NRT use in pregnancy. STUDY QUALITY = GOOD
3	Strandberg-Larsen et al. (2008)	Prospective study based on Danish National Birth Cohort, involving 87,032 pregnant women of which 3,118 used NRT. Compared rates of stillbirth by maternal smoking and NRT use in pregnancy. STUDY QUALITY = GOOD
4	Lassen et al. (2010)	Prospective study based on Danish National Birth Cohort, involving 68,156 women of which 1,825 used NRT. Related birthweight to maternal use of NRT in pregnancy. STUDY QUALITY = GOOD
5	Torp-Pedersen et al. (2010)	Prospective study based on Danish National Birth Cohort, involving 96,842 children, with an estimated 2,800 women using NRT. Compared rates of strabismus by maternal NRT use in pregnancy. STUDY QUALITY = GOOD
6	Milidou et al. (2012)	Prospective study based on Danish National Birth Cohort, involving 63,128 children, with 1,452 mothers using NRT. Related odds of infantile colic in the first six months to maternal smoking and NRT use in pregnancy. STUDY QUALITY = GOOD
7	Zhu et al. (2014)	Prospective study based on Danish National Birth Cohort, involving 84,803 children, with 814 mothers using NRT and not smoking. Compared rates of attention-deficit/hyperactivity disorder, parent-rated hyperactivity/inattention score and birthweight by maternal smoking and NRT use in pregnancy. STUDY QUALITY = GOOD
8	Gaither et al. (2009)	Cross-sectional study in four US states in 5,716 women assessed postnatally, of which 225 had been recommended or prescribed NRT in pregnancy. Compared rates of low birthweight and preterm birth by smoking and NRT use in pregnancy. STUDY QUALITY = FAIR
9	Dhalwani et al. (2015)	Prospective study using another mother-child primary care records for 192,498 children born in the UK. Compared rates of major congenital abnormalities in 2,677 smokers prescribed NRT, 9,980 smokers not prescribed NRT and 179,841 nonsmokers. STUDY QUALITY = FAIR

CAF No.	Reference	Brief study description
Clinical trials		
10	Wisborg et al. (2000)	Double-blind placebo-controlled RCT in Aarhus, Denmark. Compared birthweight, low birthweight and preterm delivery in 124 pregnancy smokers allocated to nicotine patches and 126 allocated to placebo patches. RISK OF BIAS = LOW
11	Kapur (2001)	Double-blind placebo-controlled RCT in Ontario, Canada. 17 pregnant smokers were allocated to nicotine patches and 13 to placebo patches, but study terminated prematurely due to an adverse event. RISK OF BIAS = LOW
12	Schroeder et al. (2002)	One-sample clinical trial in Minnesota, USA. 21 pregnant smokers initiated nicotine patches, with cases of severe infant morbidity noted. RISK OF BIAS = HIGH
13	Pollak et al. (2007)	Open-label multicentre RCT in North Carolina, USA. A range of endpoints were compared in 59 pregnant smokers allocated to cognitive behavioural therapy only, and 122 allocated to cognitive behavioural therapy in conjunction with NRT. RISK OF BIAS = UNCLEAR
14	Oncken et al. (2008)	Placebo-controlled RCT in Connecticut and Maine, USA. A range of endpoints were compared in 100 pregnant smokers allocated to nicotine gum and 94 allocated to placebo gum. RISK OF BIAS = LOW
15	Swamy et al. (2009)	Same study as considered in CAF 13. The analyses relate to serious adverse events following a review of medical records of 52 of the pregnant smokers allocated to cognitive behavioural therapy only, and 105 allocated to cognitive behavioural therapy in conjunction with NRT.
16	Coleman et al. (2012b)	Double-blind placebo-controlled multicentre RCT in the Midlands and North-West England. A range of endpoints recorded at or before birth were compared in 521 pregnant smokers allocated to nicotine patches and 529 allocated to placebo patches. RISK OF BIAS = LOW
17	El-Mohandes et al. (2013)	Placebo-controlled RCT in Washington D.C., USA. Gestational age and birthweight were compared in 26 pregnant women allocated to cognitive behavioural therapy only and 26 allocated to cognitive behavioural therapy in conjunction with nicotine patches. RISK OF BIAS = LOW
18	Berlin et al. (2014)	Double-blind placebo-controlled multicentre RCT in France. A range of endpoints were compared in 203 pregnant women allocated nicotine patches and 199 allocated placebo patches. RISK OF BIAS = LOW
19	Cooper et al. (2014a)	Same study as considered in CAFs 16 and 20. Provides fuller details of the study, but no additional relevant results.
20	Cooper et al. (2014b)	Same study as considered in CAFs 16 AND 19. Provides results relating to infant and maternal outcomes at 2 years.

67. Analyses were then reported based on breakdown by the endpoints listed in the following paragraphs. Depending on the availability of study data from each original publication, estimates for HR, RR or OR values were calculated for the comparison ‘NRT/non-NRT’, with mean differences as NRT minus non-NRT. Analyses were restricted to smokers only, except in a cases where the original,

published study data were not suitable to make this comparison (Lee and Fariss 2017).

Fetal loss and spontaneous abortion

68. Effect estimates were based on small numbers of cases. No statistically significant association was identified. One study, Strandberg-Larsen et al. (2008), had noted that stillbirth risk was not affected by NRT type. The meta-analysis estimate of stillbirth/fetal loss for NRT compared with no NRT was 0.78, 95% CI 0.45 to 1.33, with no heterogeneity (Lee and Fariss 2017).

Birthweight

69. A random-effect meta-analysis from 8 studies showed no overall statistically significant effect of NRT compared with no NRT on birthweight (RR = 0.81, 95%CI 0.48 to 1.39). Two placebo-controlled RCTs (Wisborg et al. 2000, Oncken et al. 2008) had shown significantly higher birthweight in the NRT arm, while some other studies had shown nonsignificant changes. The inverse-variance weighted mean difference of birthweight associated with NRT use was estimated as 142 g (95% CI −53 to 336) (Lee and Fariss 2017).

Gestational age

70. Two out of 5 trials had reported significant association of NRT use with gestational age (Oncken et al. 2008, El-Mohandes et al. 2013). The combined weighted estimate of difference in gestational age for NRT compared with no NRT use was 0.1 weeks (95% CI −0.5 to 0.6) (Lee and Fariss 2017).

Head circumference and infant length at birth

71. No significant differences were found in the 2 trials reporting results for these endpoints (Lee and Fariss 2017).

Preterm birth

72. One RCT had shown a significantly reduced OR associated with NRT (Oncken et al. 2008) while results from 6 other studies were not statistically significant. A meta-analysis of data from the 7 studies showed no association of NRT with preterm birth, compared with no NRT, with a random-effect estimate of 0.98 (95% CI 0.70 to 1.37) (Lee and Fariss 2017).

Neonatal intensive care admissions

73. Four RCTs had shown no significant differences between NRT and control groups. The overall random-effect estimate for NRT compared with no NRT was 0.95 (95% CI 0.61 to 1.47) (Lee and Fariss 2017).

Neonatal death

74. Data available from 2 trials, described as 'extremely limited', had not indicated any major effect of NRT on neonatal death (Lee and Fariss 2017).

Congenital abnormalities

75. For 'any congenital abnormality', two RCTs (Coleman et al. 2012b, Berlin et al. 2014)) had reported a non-statistically significant reduced risk, while one epidemiological study from the DNBC (Morales-Suarez-Varela et al. 2006) had indicated an increased risk with NRT use in nonsmokers compared with no NRT use in nonsmokers (RR = 1.61, 95% CI 1.01 to 2.58). The random effect estimate for any congenital abnormality calculated from all available study data was 1.10 (95% CI 0.86 to 1.41), and the estimate after removal of data for nonsmokers in the Danish cohort of (Morales-Suarez-Varela et al. 2006) was 1.02 (95% CI 0.81 to 1.28). Breakdown by type of abnormality had been reported in 2 studies. The large UK study of Dhalwani et al. (2015) had reported a significant increase in respiratory system abnormalities for women prescribed NRT (HR = 3.49, 95% CI 1.40 to 8.71, from the calculation by Lee and Fariss (2017), for NRT compared with no NRT in smokers). The analysis of DNBC data by Morales-Suarez-Varela et al. (2006) had indicated no significant major musculoskeletal system abnormalities associated with NRT (Lee and Fariss 2017).

Apgar score

76. Of 3 RCTs that evaluated Apgar score, none had shown a significant effect of NRT use (Lee and Fariss 2017).

Other endpoints for NRT and reproduction/development

77. No significant relationships were evident from the 10 publications. Lee and Fariss (2017) commented that authors' conclusions varied as to whether NRT was beneficial, harmful, or neither, but that opinions were generally not expressed with confidence. Four RCTs had concluded possible beneficial effects (Wisborg et al. 2000, Oncken et al. 2008, El-Mohandes et al. 2013, Cooper et al. 2014b), while possible adverse effects had been noted by Kapur (2001), Morales-Suarez-Varela et al. (2006), Pollak et al. (2007), Gaither et al. (2009), Milidou et al. (2012), and Zhu et al. (2014). Lee and Fariss (2017) noted that some of these possible adverse effects were based on comparisons with nonsmokers and were no longer evident if the comparison group was switched to smokers.

78. In the summary of their systematic evaluation/meta-analysis of studies on reproductive and developmental effects of NRT, Lee and Fariss (2017) commented that the evidence base has various limitations, including the few NRT exposed cases for some endpoints, the lack of dose–response data on dose or duration of use, the minimal data by type of NRT used, and failure to adjust for extent and duration of smoking before NRT prescription, or the extent of change in smoking afterwards (Lee and Fariss 2017).

6 Studies in animal models

79. Experimental studies in animal models have shown adverse effects of maternal and/or early postnatal exposure to nicotine on the development of several organ systems in the fetus and offspring, with major targets reported as the nervous and pulmonary systems. These studies have mostly been carried out in rodents or non-human primates with continuous nicotine exposure applied via subcutaneous osmotic minipump, although some studies have used other routes of exposure such as bolus injection or via drinking water. Doses tested have mostly been in the range of approximately 1–6 mg/kg bw/day (with the aim to achieve plasma nicotine levels in the range of those of average human CC smokers[16]), although some studies have used doses outside this range (lower and higher). Reports do not always state clearly whether the dose described refers to nicotine (free base) or total dose of the nicotine compound (e.g. nicotine bitartrate) used. A very limited data set was identified relating to effects of paternal exposure to nicotine prior to mating.

80. A small number of studies have been reported that have investigated effects of maternal exposure to nicotine via inhalation of E(N)NDS aerosols in rodent models. These studies are described in the accompanying discussion paper, TOX/2018/46.

6.1 Neurodevelopment

6.1.1 Neurobiology (fetal brain development)

81. Studies in animal models have shown that *in utero* exposure to nicotine can adversely affect all stages of fetal brain development, occurring at exposure levels that do not affect somatic growth (*reviewed by* Slotkin 2004, Slotkin 2008). nAChRs are present and functional in fetal brain from early stages of development, involved in the regulation of brain development normally via binding of acetylcholine (*reviewed by* Dwyer et al. 2008), with the effects of nicotine on neurodevelopment considered to be modulated via binding to nAChRs (Slotkin 2004, Slotkin 2008, England et al. 2015, England et al. 2017).

82. Much of the work on animal models of nicotine neurodevelopmental toxicity has been carried out by Slotkin and coworkers, using rat models of exposure during gestation. In a review of the field in 2004, Slotkin noted that the design of animal models of nicotine exposure in gestation is complicated by the fact that treatment via daily nicotine injections leads to acute high plasma nicotine levels, associated uteroplacental vasoconstriction, and fetal hypoxia. Thus, a model was developed

[16] Matta et al. (2007) published a detailed review of guidelines for nicotine dose selection in *in vivo* research. The following data were cited in the review: in non-human primates, 1.0-2.0 mg/kg bw/day nicotine dose by subcutaneous osmotic minipump produces nicotine levels of around 27 ng/mL (plasma, non-pregnant baboons) and 14 ng/mL (amniotic fluid in pregnant monkeys); in rats, a nicotine dose of 1.0 mg/kg bw/day by subcutaneous osmotic minipump produces a plasma nicotine level of approximately 25 ng/mL. The full text of this review article can be viewed at: http://www2.mrc-lmb.cam.ac.uk/groups/wschafer/Matta2006.pdf (accessed 20/11/2018).

using a subcutaneous osmotic minipump to achieve delivery of continuous, low-level infusions, leading to steady-state plasma nicotine levels considered to mimic those of smokers or NRT patch users (Slotkin (2004) *and references therein*). Given the differences in nicotine pharmacokinetics, higher doses are required to achieve equivalent plasma levels in rodents than in humans, thus doses of 2 to 6 mg/kg bw/day have been used in rats to reproduce plasma nicotine levels observed in moderate (10–20 CC/day) and heavy (20–40 CC/day) smokers. The dose of 6 mg/kg bw/day was noted to induce a similar level of nAChR upregulation (30%) as maternal cigarette smoking (20–50% across different brain regions) (Slotkin (2004), Slotkin (2008), *and references therein*). The findings of some key studies in the development of the field are described in the following paragraphs, while more information may be found in detailed summaries and reviews by Slotkin and colleagues (*see, for example,* Slotkin (2004), Slotkin (2008)) and general overviews of the developmental toxicity of nicotine by other authors (*including* Wickstrom (2007), Bruin, Gerstein and Holloway (2010), England et al. (2015), England et al. (2017)). This is a field in which a large amount of research has been published and is still actively in progress, thus the data presented here are intended to give an overview of the potential for nicotine to disrupt neurodevelopment, but not to represent a complete description of knowledge and studies reported in the field.

83. In a rat embryo culture system, first trimester exposure to nicotine was shown to specifically disrupt neural development at concentrations that did not cause dysmorphogenesis, including disrupted cell development in the brain primordium, with damage to the neuroepithelium including cytoplasmic vacuolation, enlargement of intercellular spaces, and increased cell apoptosis (Roy 1998). These effects occurred early, during the neural tube stage of development, and the potential role of nAChRs was supported by subsequent findings that functional nAChRs are indeed present by the early neural tube stage, before differentiation into cholinergic neurons (Atluri et al. 2001, Schneider et al. 2002).

84. Studies *in vivo* using the osmotic minipump system initially evaluated the effects of prenatal exposure to 6 mg/kg bw/day nicotine (free base). Pregnant Sprague-Dawley rats were exposed via continuous subcutaneous osmotic minipump from gestation day (GD) 4 to GD20, while control animals received equivalent minipump treatment without nicotine (Slotkin, Cho and Whitmore 1987a, Slotkin, Orband-Miller and Queen 1987b, Navarro et al. 1989a). Maternal weight gain was slightly reduced and nearly half of dams failed to give birth, but there was no maternal toxicity. At GD18, fetal brain showed reduced cell number (DNA, RNA, and protein content) and decreased weight, while the fetus as a whole was generally not affected. Postnatally, brain growth was spared, but there was disruption of the timing of macromolecule synthesis in all brain regions (indicated by ornithine decarboxylase activity), and this was particularly evident in the cerebellum (Slotkin et al. 1987a). Prenatal nicotine exposure was associated with abnormalities in [3H] nicotine binding sites in the brain. In the absence of nicotine, [3H]nicotine binding in whole brain from GD18 fetuses was low, with a substantial increase after birth, particularly during the period of cholinergic synapse formation. Three weeks after birth, the adult

pattern of binding was evident: midbrain > brainstem > cerebral cortex >> cerebellum. However, in the presence of fetal nicotine exposure, [3H] nicotine uptake to synaptosomes was significantly elevated in GD18 fetus, and subsequently disrupted postnatally (in the absence of postnatal nicotine exposure) (Slotkin et al. 1987b). Brain choline acetyltransferase (ChAT) activity in GD18 fetuses was significantly elevated by the prenatal nicotine exposure, but reduced during postnatal development, whereas in offspring not prenatally exposed to nicotine, a rise in CNS ChAT was observed during postnatal weeks 2 and 3. Postnatal [3H] choline uptake was also affected by prenatal nicotine exposure, in a brain-region-specific manner. The authors of this report concluded that nicotine may prematurely stimulate a surge in cholinergic activity, associated with a premature switch from neurogenesis to differentiation, and disruption of cortical development (Navarro et al. 1989a).

85. Subsequent studies were carried out at lower nicotine doses that did not affect maternal weight gain or fetal resorption rate (Navarro et al. 1989b). Pregnant Sprague-Dawley rats were exposed via the subcutaneous continuous osmotic minipump system to 2 mg/kg bw/day nicotine (free base) from GD4 to GD20. Nicotine exposure did not affect fetus body or brain weight at GD18 but was associated with slightly increased weight gain of offspring postnatally. At GD18, [3H] nicotine binding in the brain was increased and ornithine decarboxylase activity decreased, and, postnatally, similar effects were seen to those reported from earlier experiments using 6 mg/kg bw/day nicotine (increased [3H] nicotine binding in the brain, transiently increased ornithine decarboxylase activity, interference of cell acquisition in the cerebellum). The authors commented that for nicotine, standard growth-related markers are poor predictors of perturbed development, hypothesising that this may be due to the action of nicotine on a specific neurotransmitter receptor population, rather than generalised toxic insults on cell replication and/or growth (Navarro et al. 1989b). Subsequent commentaries in the field have reiterated this point: effects are seen at doses that are not associated with growth retardation or other secondary effects, emphasizing that growth retardation *per se* is not an adequate indicator of developmental neurotoxicity of nicotine (Slotkin 2008, England et al. 2017).

86. Subsequent studies (using either 6 mg/kg bw/day or 2 mg/kg bw/day exposures) showed that the effects on neuronal damage and cell loss involve the constitutive activation of apoptosis-associated genes, and these effects intensify postnatally, in the absence of continued nicotine exposure (Slotkin, McCook and Seidler 1997, Trauth et al. 1999).

87. By early adulthood, morphologically, gross structure may be normal but with fine abnormalities (layer thinning, loss of neuropil, glial ‘scarring’), notably in the hippocampus and somatosensory cortex, which are areas that are critical for attention and cognitive function (Roy, Seidler and Slotkin 2002)[17]. Nicotine has therefore been described as a ‘subtle neuroteratogen’ (morphologically) rather than a

[17] 2 mg/kg bw/day during gestation [subcutaneous osmotic minipump]; offspring evaluated at PND21 and PND30.

'classic neurotoxin', and with functional consequences exceeding the visible evidence of disrupted development (Slotkin 2008, England et al. 2017).

88. Some effects that are seen in infants, but show subsequent recovery, reappear in adolescence, for example patterns of synaptic hypoactivity may occur early after birth, recover during subsequent development, but then reoccur in adolescence and remain through adult life (England et al. (2017) *and references therein*). Thus the mechanisms via which the effects of prenatal nicotine exposure are mediated differ, from early effects of direct exposure (modulation via direct interaction with nAChRs) to later effects (compromised synaptic function, even in regions that have relatively few nAChRs, and which can impact on a wide range of neurotransmitter systems) (*discussed by* Slotkin 2008, England et al. 2017). Studies have indicated that the type and persistence of effects in adulthood may show variation between males and females, with a greater magnitude in males (Slotkin 2008).

89. Studies have also been carried out in rats exposed to nicotine during the early postnatal period, which is functionally equivalent to the 3rd trimester of gestation in humans. Injection of rat pups twice daily with 1 or 2 mg/kg bw nicotine hydrogen tartrate (0.35 mg/kg bw or 0.7 mg/kg bw nicotine base, respectively) during postnatal week 2, but not weeks 1 or 4, disrupted glutamate synapses in the auditory cortex (Aramakis et al. 2000). In a subsequent study by the same group, injection twice daily with 2 mg/kg bw nicotine hydrogen tartrate from PND8 to PND12 altered auditory–cognitive responses in adulthood, with the authors noting that this effect, which is not observed on treatment of adult rats with chronic nicotine exposure, may be due to transient disruption of cortical nAChRs (Liang et al. 2006). Postnatal nicotine exposure (Sprague-Dawley rat pups treated with 6 mg/kg bw/day nicotine, from PND4 to PND9, or PND1 to PND7) also disrupted morphological assembly of the hippocampus and cerebellum, which are late-maturing structures and which exhibit unique regulation by nAChRs (Chen, Parnell and West 1998, Huang et al. 2007).

90. Slotkin et al. (2015a) evaluated the potential proportion of disruption of neurodevelopment associated with maternal CC smoking that is estimated to be due to the nicotine exposure. Groups of female Sprague-Dawley rats were exposed, via subcutaneous osmotic minipump, prior to mating, throughout gestation, and into the first 2 postnatal weeks, to either vehicle control, 0.2 or 2.0 mg/kg bw/day nicotine free base, or tobacco smoke extract[18] (TSE) with an equivalent nicotine (free base) concentration of 0.18 mg/kg bw/day. The high (2.0 mg/kg bw/day) and low (0.2 mg/kg bw/day and 0.18 mg/kg bw/day) nicotine dose levels were intended to represent general exposure levels experienced via moderate-level (direct) CC smoking or from exposure to secondhand smoke, respectively. Bioequivalence of TSE/0.2 mg/kg bw/day nicotine was demonstrated by a similar upregulation of cerebral cortex nAChRs in adult rats, and with higher upregulation by 2 mg/kg

[18] Smoke condensate collected on filter pad, then resuspended in dimethylsulphoxide to 20 mg condensate per mL.

bw/day nicotine. Treatments did not affect maternal weight gain or cause maternal toxicity. TSE treatment was associated with a small decrease in offspring postnatal weight gain compared with the other 3 groups, but brain weights were not significantly different. Offspring were evaluated during a timeline from birth to adulthood (PND30, PND60, PND100, PND150) for markers of cholinergic (ACh) and serotonergic (5-HT) signalling systems in different brain regions. Markers for ACh were: ChAT activity – an index of the development of cholinergic innervation; concentration of high-affinity choline transporters ([HC3] binding) – an indicator of presynaptic impulse activity relative to number of nerve terminals; nAChR binding). Markers for 5-HT systems were 5-HT_{1A} and 5-HT_2 receptors – known major targets for developmental toxicity of nicotine (Slotkin et al. 2015a).

91. Multivariate analysis incorporating all 4 treatment groups, sex, age, brain region, and the 3 ACh markers showed that all treatment groups differed significantly from control, and TSE group differed significantly from each nicotine group. For ChAT activity, ANOVA indicated a main effect of treatment that was dependent on sex (treatment x sex, $p < 0.04$). TSE increased ChAT activity in males, but reduced it in females. 0.2 mg/kg bw/day nicotine increased activity with no sex distinction, while 2.0 mg/kg bw/day nicotine increased activity to a greater extent in males than in females. The effects of either dose of nicotine were of greater magnitude than the effects of TSE. For HC3 binding, ANOVA showed a main effect of treatment that was dependent on age (treatment x age, $p < 0.0003$) but not sex or brain region. Both TSE and 2.0 mg/kg bw/day nicotine decreased HC3 binding, with TSE showing an effect of greater magnitude. For 2.0 mg/kg bw/day nicotine, the effects were more prominent in adolescence than in adulthood. HC3 binding was increased by 0.2 mg/kg bw/day nicotine. HC3/ChAT ratio showed effects across treatment groups that interacted with sex (treatment x sex, $p < 0.05$) and age (treatment x age, $p < 0.0002$). The ratio was decreased by TSE, 0.2 mg/kg bw/day nicotine, and 2.0 mg/kg bw/day nicotine, with greater effect in males than females for TSE and 0.2 mg/kg bw/day nicotine. Effects were greater in adolescence than adulthood for 2.0 mg/kg bw/day nicotine. The magnitude of ratio decrease was greater with TSE and 2.0 mg/kg bw/day nicotine than with 0.2 mg/kg bw/day nicotine. For nAChR binding, effects interacted with all other factors (treatment x age, $p < 0.0001$; treatment x sex x age, $p < 0.05$; treatment x age x brain region, $p < 0.03$). TSE reduced nAChR binding, while 0.2 mg/kg bw/day nicotine and 2.0 mg/kg bw/day nicotine increased nAChR binding, in all cases with effects greater in females than males (Slotkin et al. 2015a).

92. Multivariate ANOVA incorporating all 4 treatment groups, sex, brain region, and the two 5-HT receptor measurements showed differences for treated groups versus control and for TSE versus both nicotine groups. For 5-HT_{1A} receptors, main treatment effects were highly dependent on sex (treatment x sex, $p < 0.0001$), with TSE-induced decrease in females, and increase with 0.2 mg/kg bw/day nicotine in females. For 5-HT_2 receptors, some sex-dependent treatment effects were seen (treatment x sex, $p < 0.0001$). Binding was increased with TSE, 0.2 mg/kg bw/day

and 2.0 mg/kg bw/day nicotine in males, and with 0.2 mg/kg bw/day and 2.0 mg/kg bw/day nicotine in females, but decreased with TSE in females (Slotkin et al. 2015a).

93. Overall, the authors calculated that for males, the effects of low-dose nicotine were significantly correlated with those of TSE and accounted for 36% of the TSE effects, while the high dose of nicotine accounted for 46%. For females, nicotine accounted for 13% of the TSE effect at low dose and 7% at the higher dose. The authors concluded that sex differences in the impact of TSE on brain development reflect differing contributions of nicotine in males and females, with nicotine responsible for a greater proportion of the adverse effects in males, and with the TSE components exacerbating the impact of nicotine in males but not females. The authors cautioned, however, that these findings should not be taken as reassurance of harm reduction for nicotine alternatives, given that nicotine could account for as much as 35-45% of overall effect on serotonergic and cholinergic systems, and also that significant adverse effects of nicotine alone were observed at levels commensurate with secondhand smoke exposure, 10-fold below those of active smokers (Slotkin et al. 2015a).

6.1.2 Neurodevelopmental outcomes in offspring

94. A large body of research is available on the effects of prenatal nicotine exposure on subsequent neurodevelopmental outcomes in offspring. This topic has been discussed in a number of review articles, including U.S. Department of Health and Human Services (2016) and England et al. (2017). Due to the extensive amount of data, detailed descriptions of individual studies are mostly not given here, but the following paragraphs contain an overview of the field, and a summary of cited studies given in Table 6.

Table 6. Studies of the effects of prenatal or early postnatal nicotine exposure on cognitive and behavioural development in rodent offspring.

Study	**Nicotine dose reported** (mg/kg bw/day); maternal dose unless otherwise stated)	**Route of administration**	**Duration of administration**	**Outcome** (offspring unless otherwise stated)
Nakauchi et al. (2015) [mouse]	21 (to nursing dams)	Subcutaneous osmotic minipump	PND1 - PND15	Deficits in long-term memory for object location and increased anxiety (male offspring tested at adolescence)

Study	**Nicotine dose reported** (mg/kg bw/day); maternal dose unless otherwise stated)	**Route of administration**	**Duration of administration**	**Outcome** (offspring unless otherwise stated)
Vaglenova et al. (2004) [rat]	6	Subcutaneous osmotic minipump	GD3 - delivery	Reduced pup body weight; Alterations in locomotion, adaptation, anxiety, and cognitive behaviours, from birth into adulthood; effects more severe in females than males
Sorenson, Raskin and Suh (1991) [rat]	6.0 (approximately)	Drinking water	15 days prior to mating and throughout gestation	Reduced pup birth weight; Impaired ability to learn an 8-arm radial maze (both sexes, tested at PND45-65)
Sobrian et al. (1995) [rat]	5.0 (+ daily subcutaneous injections of saline)	Subcutaneous osmotic minipump	GD8 - GD21	Transient increase in locomotor activity that occurred within a 30-min delay following acute challenge with apomorphine on PND20-PND22
Sobrian, Marr and Ressman (2003) [rat]	5.0, 2.5 (+ daily subcutaneous injections of saline)	Subcutaneous osmotic minipump	GD8 - GD21	Increased risk taking behaviour in offspring at 12-14 months age at 5.0 mg/kg bw/day
Schneider et al. (2011) [rat]	4.61 ± 0.54	Drinking water	3 weeks prior to mating – delivery	Reduced pup birth weight and delayed sensorineural development; Deficits in tests of attention and impulsivity and altered learning ability in adulthood
Cutler et al. (1996) [rat]	4	Subcutaneous osmotic minipump	GD4 - GD21	Reduced maternal weight gain and pup birth weight; Altered cognitive function in response to adrenergic challenge in radial arm maze in adulthood

Study	**Nicotine dose reported** (mg/kg bw/day); maternal dose unless otherwise stated)	**Route of administration**	**Duration of administration**	**Outcome** (offspring unless otherwise stated)
Paz et al. (2007) [mouse]	3.5 ± 0.4 (average)	Drinking water	2 weeks prior to mating, during gestation, with gradual step-down postnatally	Alterations in spontaneous locomotion, preference for cocaine-associated place, learned helplessness, increased learning of trace-conditioned, fear-associated cues in adult offspring
Yanai et al. (1992) [mouse]	3.0 (1.5, 2x per day) (pregnant dams) 1.5 (pups)	Subcutaneous injection	GD9 - GD18 (dams) or PND2 - PND21 (pups)	Reduced birthweight/body weight associated with nicotine treatments; weights had normalised before behavioural testing was carried out. Deficit in hippocampus-related behaviours in pups tested at PND50 in association with both maternal or postnatal exposure
Franke et al. (2008) [rat]	3	Subcutaneous osmotic minipump	GD4 - GD18	Alterations in natural and drug-induced reinforcement in adolescent male offspring
Levin et al. (1993) [rat]	2	Subcutaneous osmotic minipump	GD4 - GD20	'Subtle alterations' in various tests of cognitive performance that were magnified by challenges on nicotinic and adrenergic systems in adult offspring
Levin et al. (1996) [rat]	2	Subcutaneous osmotic minipump	GD4 - GD20	'Subtle changes' in cognitive function; choice accuracy, response to behavioural challenge and response to effect of drug challenge in adult offspring; sex dependent

Study	**Nicotine dose reported** (mg/kg bw/day); maternal dose unless otherwise stated)	**Route of administration**	**Duration of administration**	**Outcome** (offspring unless otherwise stated)
Liang et al. (2006) [rat]	1.4 (to pups) (0.7, 2x per day)	Subcutaneous injection	PND8 - PND12	Impaired nicotinic enhancement of central auditory processing and auditory learning in adulthood
Eppolito et al. (2010)	0.96, 2.0	Subcutaneous osmotic minipump	GD4 - PND10	Decreased preweaning pup weight gain, with recovery after weaning; Subtle cognitive deficits and increased anxiogenic behaviours in offspring in adulthood but not adolescence; some sex differences noted
Eppolito and Smith (2006) [rat]	0.96	Subcutaneous osmotic minipump	GD4 - PND10	Significant reduction in weight gain of female offspring starting at puberty; Mild deficits in spatial learning and memory in females tested at PND60; Slower swim speed in male offspring
Hall et al. (2016) [rat]	0.2, 2.0, or tobacco-smoke extract (TSE) corresponding to 0.18 mg/kg bw/day nicotine)	Subcutaneous osmotic minipump	3 days prior to mating - PND12	Early mild reduction in pup body weight, with recovery at later ages; Effects of both nicotine doses and TSE on behavioural test outcomes in adolescence; magnitude of effects of TSE considered to correlate with those of the 2.0 mg/kg bw/day nicotine treatment
Lacy, Mactutus and Harrod (2011) [rat]	0.15 (0.05, 3x per day)	Intravenous injection	GD8 - GD21	Long-lasting alterations in sensorimotor gating, with sex-specific differences in adulthood

Cognitive and behavioural development

95. Studies in rodents have consistently indicated that nicotine exposure during gestation and/or lactation is associated with global impairments of learning and memory. Eight studies relating to exposure during gestation and/or lactation were listed by the review of England et al. (2017) (Sorenson et al. 1991, Yanai et al. 1992, Levin et al. 1993, Cutler et al. 1996, Levin et al. 1996, Vaglenova et al. 2004, Eppolito and Smith 2006, Nakauchi et al. 2015). All except Sorenson et al. (1991) (drinking water) and (Yanai et al. 1992) (twice-daily subcutaneous injection) evaluated nicotine exposures as continuous infusion of dams via subcutaneous osmotic minipump, with adverse effects on offspring noted at gestational doses in the range of 0.96–6.0 mg/kg bw/day, and 1 study of postnatal-only exposure in which nursing dams were treated with 21 mg/kg bw/day nicotine. England et al. (2017) noted that effects appear to be related to dose, timing of exposure, and sex of offspring. It is thought that effects are mediated through nAChR modulation of long-term potentiation in the hippocampus (Nakauchi et al. 2015).

96. England et al. (2017) also reported that animal studies have shown adverse outcomes of nicotine exposure during gestation or gestation/early postnatal period on affective behaviour in offspring at various stages of development through to adulthood. Two studies were cited that reported effects on learned helplessness, fear trace conditioning, and anhedonia. In one of these studies, female mice were exposed to 3.5 mg/kg bw/day nicotine in drinking water during gestation (with dosing tailed off during lactation) (Paz et al. 2007) and in the other, rats were exposed to 3 mg/kg bw/day by continuous infusion from GD4 to GD18 (Franke et al. 2008). Three studies were cited relating to increased anxiety levels, poor adaptation to a new environment and decreased novelty seeking. In these studies, rats were exposed to nicotine doses between 0.96 mg/kg bw/day (via drinking water) and 6 mg/kg bw/day (osmotic minipump) (Sobrian et al. 2003, Vaglenova et al. 2004, Eppolito et al. 2010).

97. In a follow-up to the study reported by Slotkin et al. (2015a) (described in section 6.1.1, paragraphs 89–92), Slotkin and colleagues compared effects of TSE or nicotine on cognitive and behavioural function of offspring in adolescence and adulthood (postnatal weeks 4–40). Female rats were exposed to TSE (containing 0.18 mg/kg bw/day nicotine), 0.2 or 2.0 mg/kg bw/day nicotine base, via continuous subcutaneous osmotic minipump prior to and during gestation and into the first 2 weeks postnatally. All exposures led to disruptions in cognitive and behavioural function, producing hyperactivity, working memory deficits, and impairments in emotional processing. Effects were greater with TSE than those seen at the equivalent nicotine dose (0.2 mg/kg bw/day), and were more similar in magnitude to those resulting from the 2.0 mg/kg bw/day nicotine dose. The authors concluded that exposure to secondhand smoke causes neurodevelopmental deficits, which originate in disruption of neurodifferentiation, leading to miswiring of circuits, and culminating in behavioural dysfunction. Overall, the authors concluded that the fact that TSE effects were much greater than a comparable nicotine exposure indicate that

substitution of nicotine instead of tobacco is likely to provide harm reduction in terms of neurodevelopmental outcomes, but given that significant effects were also apparent with low-dose nicotine alone, the concept is of 'harm reduction' but not 'harm elimination'. They commented that there would still be a need to avoid second hand smoke during pregnancy, and to highlight the potential adverse effects of electronic nicotine delivery devices and shisha, which would expose users and bystanders to significant amounts of nicotine (Hall et al. 2016).

98. The review by U.S. Department of Health and Human Services (2016) also noted that animal studies have linked prenatal exposure to nicotine with subsequent appetitive and consummatory behaviours in offspring, including behavioural responses for drug use and dependence in adolescent and adult experimental animals, with effects likely to be modulated via alteration of dopamine signalling pathways.

99. Three publications were identified that reported behavioural alterations in offspring associated with paternal exposure to nicotine prior to mating (Dai et al. 2017, Zhang et al. 2018, McCarthy et al. 2018).

100. In a study reported by McCarthy and colleagues, groups of male C57BL/6 mice were given drinking water containing 200 µg/mL nicotine, or plain drinking water (control), for 12 weeks prior to and continuing throughout mating with non-nicotine-exposed females. Serum cotinine level was 77.18 ± 3.06 ng/mL in nicotine-treated males, and undetectable in males given plain drinking water. Daily drinking water consumption was approximately 5.5 mL in both groups[19]. There were no significant differences between groups in terms of water or food consumption, body weight gain, sperm count in the fathers, or in litter size, weight, weight gain, or developmental milestones in the offspring. Tests of F1 pups showed significantly increased locomotor activity at PND60 (male and female offspring), attention deficit (male offspring only), and reversal learning deficits (male and female offspring) in the paternal nicotine-exposed compared with control group. Male F1 pups also had significantly increased brain monoamine content and dopamine receptor mRNA expression. In the F2 generation, male offspring from female F1 mice with paternal nicotine exposure showed reversal learning deficits. This effect was not observed in male F2 offspring from male F1 mice with paternal nicotine exposure, or in any female F2 offspring. Epigenetic changes were observed in spermatozoal DNA from F0 males exposed to nicotine, with increased global DNA methylation and reduced methylation at the D2 dopamine receptor promotor region. The authors concluded that nicotine exposure of male mice produces behavioural changes in multiple generations of descendants, and that changes in spermatozoal DNA methylation are a plausible mechanism for the transmission of the phenotypes across generations (McCarthy et al. 2018).

[19] Assuming an average adult male mouse weight of 25 g, the calculated nicotine dose would be 44 mg/kg bw/day. However, calculation of daily dose per kg/bw was not provided in the report.

101. Dai and colleagues evaluated effects of pre-mating exposure of male mice to nicotine or tobacco smoke on behavioural changes in offspring, and the possible underlying molecular basis in the case of nicotine exposure. Six-week-old male C57BL/6 mice were treated with either 2 mg/kg bw/day nicotine (4x 0.5 mg/kg bw intraperitoneal injections), considered to represent smoking ≥ 20 CC per day, or saline (control). After 5 weeks of treatment, males were bred with non-nicotine-treated females, with continued nicotine or saline treatment of males for 5 days during mating. For tobacco smoke exposure, mice were exposed in a chamber to smoke from 1 CC for approximately 1 hour, twice per day, or an equivalent procedure without the presence of a burning cigarette (control), and mated with unexposed females after 5 weeks of treatment. In behavioural tests performed on F0 males after 5 weeks of treatment, tobacco smoke exposure led to depression and hypoactivity, and nicotine exposure was associated with depression-like behaviour. Tests in F1 offspring at sexual maturity showed hyperactivity and activated social behaviour in offspring of tobacco smoke-exposed males, and hyperactivity in offspring of nicotine-exposed males. mRNA analyses showed nicotine-associated increased expression of *Wnt4* in F0 spermatozoa and F1 brain, and of *Dvl2* in F1 brain. Protein analyses showed nicotine-associated upregulation of Wnt4 and DVL2 in F0 testis and F1 brain, but not F0 brain. Wnt4 expression was mostly seen in the thalamus, and DVL2 in the hippocampal formation, particularly in the CA3 region. Expression of the miRNA, mmu-miR-15b, was downregulated due to CpG hypermethylation in F0 sperm and F1 brain. An F2 generation was bred on from the F0 nicotine or control groups, using a cross-fostering method. No differences were seen between groups in behavioural tests of F2 offspring, nor were there any differences in levels of Wnt4 and DVL2 proteins or mmu-miR-15b in brain tissue of F2 offspring or of mmu-miR-15b in sperm of F1 offspring (Dai et al. 2017).

102. A follow-on study from that of Dai et al. (2017) described in the preceding paragraph compared effects on offspring behavioural parameters of maternal or biparental nicotine exposure before pregnancy (Zhang et al. 2018). Nicotine and control exposure regimes were given as described in paragraph 101 above (2 mg/kg bw/day nicotine, as 4x 0.5 mg/kg bw/day intraperitoneal injections, or control saline injections), but in this study both males and females were treated for 5 weeks prior to mating. Mice were mated in combinations of control-male/control-female (control parents), nicotine-male/nicotine-female (biparental nicotine), nicotine-male/control-female (paternal nicotine), and control-male/nicotine-female (maternal nicotine), and F1 mice were cross-fostered. There were no differences between groups in birth rates. Male offspring underwent behavioural testing at 10-12 weeks postnatally. In the open field test, in keeping with the findings reported by Dai et al. (2017), paternal nicotine exposure was associated with increased hyperactivity in offspring. Conversely, maternal nicotine exposure was associated with reduced activity compared with offspring of control parents. The phenotype for offspring from biparental nicotine exposure was split, with 70% of offspring showing reduced activity (as for the maternal nicotine exposure group), 20% showing hyperactivity (as for the paternal nicotine exposure group), and 10% no difference compared with offspring of control parents. Offspring with maternal nicotine or biparental nicotine

exposure showed characteristics of depression-like behaviours in other tests, including reduced sucrose preference and reduced time spent in a social chamber. The authors concluded that maternal pre-pregnancy nicotine exposure has a greater impact on offspring behaviour than that of paternal nicotine exposure (Zhang et al. 2018).

Auditory processing deficits

103. Studies have shown that exposure of rats to nicotine (0.35 or 0.7 mg/kg bw injected twice per day) during the second postnatal week (equivalent to the 3rd trimester in humans) disrupts development of glutamate synapses in the auditory cortex (Aramakis et al. 2000), and subsequent development of normal auditory learning (Liang et al. 2006) (described in section 6.1.1, paragraph 89). These aspects are discussed in more detail in U.S. Department of Health and Human Services (2016) (page 110) and England et al. (2017).

Reflex maturation

104. The review of England et al. (2017) noted that rodent studies have indicated that developmental nicotine exposure is associated with delayed maturation of reflexes, including negative geotaxis and surface righting (limb coordination and motor development), long term filtering of auditory information, and activity levels. However it was noted that study findings are inconsistent and this may reflect inconsistencies in study design, for example different exposure methods and/or developmental periods. Three studies were listed, in which rats were exposed during gestation to doses of nicotine from 0.15 to 5.0 mg/kg bw/day, by various routes including intravenous injection and in the drinking water (Sobrian et al. 1995, Lacy et al. 2011, Schneider et al. 2011).

6.2 Stillbirth, perinatal mortality, and SIDS

105. Data from animal studies have provided evidence for a link between prenatal nicotine exposure and stillbirth and perinatal mortality, implicating altered responses of the fetus and newborn to hypoxic stress and subsequent inadequate respiratory response.

106. The U.S. Surgeon General report on the health consequences of smoking noted that there has been an extensive amount of animal research carried out that investigated the role of nicotine in the risk of perinatal mortality, implicating loss of a critical protective response to hypoxia. Release of catecholamines from the fetal adrenal medulla during parturition protects the fetus from hypoxia and maintains blood flow to the brain and heart. However, prenatal exposure of rats to nicotine results in immature chromaffin cells in the adrenal gland differentiating prematurely, with loss of the normal direct stimulation of the adrenal gland by hypoxia, absence of catecholamine release, and impaired cardiac response (HHS (2014) *and references therein*).

107. A risk model for SIDS proposes the presence of 3 overlapping risk factors: a vulnerable infant, a critical developmental period in homeostatic control, and an external stressor. Prenatal nicotine exposure has been implicated in infant vulnerability and studies in animal models have supported this hypothesis, with evidence suggesting that prenatal nicotine exposure leads to changes affecting hypoxia sensing, cardiovascular control and arousal in neonates and infants (*reviewed by* Hafstom et al. 2005, HHS 2014, England et al. 2017, Duncan 2018).

108. Newborn rats of dams exposed to 6 mg/kg bw/day nicotine base from GD5 to birth had increased mortality during a hypoxic challenge[20] (Slotkin et al. 1995), while administration of 1.5 mg/kg bw/day[21] nicotine base to pregnant ewes via subcutaneous osmotic minipump during the last trimester of gestation affected lung mechanical response to hypoxia in newborn lambs (Sandberg et al. 2007). Ventilatory response to acute hypoxia was found in prenatally exposed 5-day-old lambs (maternal dose, 0.5 mg/kg bw/day nicotine base via subcutaneous osmotic minipump) (Hafstrom, Milerad and Sundell 2002). However, the response was not further compromised by subsequent postnatal nicotine exposure (maternal dose, 0.5 mg/kg bw/day, followed by 1.6–2.0 mg/kg bw/day postnatal dose via subcutaneous osmotic minipump from birth to PND21) indicating effects specific to prenatal exposure (Hafstrom, Milerad and Sundell 2004). In a review of the field, Wickstrom (2007) noted that the effects associated with prenatal exposure were observed at a maternal dose as low as 0.5 mg/kg bw/day nicotine free base, associated with an average maternal nicotine concentration of 7 ng/mL (Hafstrom et al. 2002, 2004, *cited in* Wickstrom 2007).

109. In a recent overview of SIDS, Duncan (2018) noted that there is now a large body of literature suggesting that dysfunction of the nervous system, particularly certain brainstem regions, is involved in SIDS. Abnormal development or maturational delay may underlie these changes, and they are hypothesised to play a key role in SIDS due to their direct impact on homeostatic processes, including cardiorespiratory control, sleep regulation, and arousal.

110. Given the extensive literature and complexity of this topic, it is not covered in more depth here, but more detailed discussions can be found in review articles and book chapters, for example Hafstrom et al. (2005), HHS (2014), U.S. Department of Health and Human Services (2016), England et al. (2017), Duncan (2018).

6.3 Pulmonary development

111. Investigations carried out in a range of animal species have shown that nicotine exposure *in utero* is associated with adverse effects on development of the respiratory system, leading to deficits in pulmonary function that are related to alterations in the structure of the respiratory system (*discussed by* McEvoy and

[20] Although effects were not seen at 2 mg/kg bw/day maternal dose
[21] But not 0.5 mg/kg bw/day

Spindel 2017). Studies demonstrating key findings in this area are summarised in the following section.

6.3.1 Non-human primates

112. The group of Spindel and colleagues reported a series of studies to examine the effects of *in utero* nicotine exposure on pulmonary development in rhesus monkeys (Sekhon et al. 1999, Sekhon et al. 2001, Sekhon et al. 2002, Sekhon et al. 2004).

113. One of these studies evaluated the effects of *in utero* exposure to nicotine on pulmonary function in newborn monkeys (Sekhon et al. 2001). Timed pregnant rhesus monkeys were treated with 1.5 mg/kg bw/day nicotine (n = 7) or saline (n = 7), as nicotine bitartrate, by subcutaneous osmotic minipump from GD26 to GD160 (standard gestation, 165 days). Caesarean section was carried out on GD160, pulmonary function tests (whole body plethysmography) were carried out on offspring 1 day later, following which the offspring were euthanized and lungs were weighed and fixed. Average nicotine (13.2 ± 3 ng/mL) and cotinine (66.9 ± 6 ng/mL) levels measured in amniotic fluid at intervals during gestation (GD119, GD140, GD160) were reported to be similar to those in pregnant human smokers (range cited by the authors as 3.3 to 28 ng/mL for nicotine and 93 to 109 ng/mL for cotinine). Nicotine and cotinine were not detected in control group amniotic fluid. Nicotine treatment led to a slight, but non-significant, decrease in fetal weight. Offspring of nicotine-exposed monkeys had significantly lower lung weight (16%) and volume (14%) than controls. Nicotine treatment was associated with changes in lung function test outcomes, including significantly decreased forced expiratory volume during first 0.2 seconds ($FEV_{0.2}$) (23%), peak tidal expiratory flow (PTEF) (17%), mean mid-expiratory flow ($FEF_{25-75\%}$)[22] (29%), forced expiratory volume to achieve peak expiratory flow (FEV_{PEF}) (32%), and FEV_{PEF}/forced vital capacity (FVC) (27%). The nicotine-treated group had significantly higher values of absolute and specific (corrected for lung volume) pulmonary resistance (132% and 100%, respectively), while although measurements of lung compliance were lower in nicotine-treated animals, the differences were not statistically significant. The authors commented that these changes in pulmonary function were strikingly similar to the changes observed in offspring of human smokers, suggesting that the interaction of nicotine with nAChR in developing lung is responsible for the altered pulmonary mechanics observed in human infants whose mothers smoked during pregnancy (Sekhon et al. 2001).

114. The series of studies by Spindel and colleagues in rhesus monkeys also evaluated effects on airways structure.

[22] mid-expiratory flow over middle 50% of FVC

115. Timed pregnant rhesus monkeys were treated with 1 mg/kg bw/day nicotine[23], as nicotine tartrate in saline, via subcutaneous osmotic minipump (n = 3) from GD26 to GD134, or no pump/no nicotine (n = 3 controls). The nicotine dose was described by the authors as equivalent to exposure in heavy smokers, and average nicotine level in amniotic fluid of nicotine-treated monkeys measured at day 134 was 15.5 ± 3.8 ng/mL, which was noted to be consistent with average levels in amniotic fluid of pregnant human smokers. The authors further commented that light smokers would generally receive around one-half of this dose, users of transdermal nicotine patches, one-quarter, and users of nicotine gum, one-eighth. Caesarean sections were performed on GD134 (equivalent to 32 weeks of human pregnancy, when the lungs are in the late saccular/early alveolar phase). Fetal body weight was reduced by 8% in nicotine-treated compared with control offspring, and individual organ weights were generally also decreased, including a 13% decrease in lung weight. However weight differences between nicotine and control groups did not show statistical significance. Morphometric analysis of lung blocks indicated that nicotine exposure was associated with increased size and volume density of primitive alveoli, and decreased surface area. These observations were noted to correlate with those previously reported for rats exposed to CC smoke (Collins et al. 1985) and rats prenatally or postnatally exposed to nicotine (Maritz, Woolward and du Toit 1993, Maritz and Thomas 1994). Immunohistochemical analysis indicated the presence of α7nAChRs in airway epithelial cells, cells surrounding large airways and blood vessels, alveolar type II cells, free alveolar macrophages, and pulmonary neuroendocrine cells. Nicotine treatment was associated with substantial upregulation of the α7nAChR in airway epithelial cells and airway and vessel walls, correlating with increased collagen expression. This was noted by the authors to be consistent with reports of increased airway wall thickness in the SIDS-afflicted infants of mothers who smoked during pregnancy (Elliot, Vullermin and Robinson 1998). α7nAChR was also observed in submucous glands, airway-associated nerve fibres, and free pulmonary macrophages. The authors hypothesised that nicotine interaction with fibroblasts stimulates increased collagen deposition, leading to increased airway wall thickness. This would also be consistent with decreased respiratory function in infants whose mothers smoked during pregnancy, with increased collagen deposition around pulmonary vessels underlying the increased incidence of pulmonary hypertension observed (Sekhon et al. 1999).

116. A subsequent report described evaluations on whether nicotine exposure alters regulation of collagen and elastin expression in fetal lungs and/or alters morphometric wall dimensions of the developing lung, and whether α7nAChRs are expressed in pulmonary fibroblasts (Sekhon et al. 2002). These evaluations were carried out on the monkeys that had undergone the nicotine or control treatments described in previous publications. Briefly, one set of pregnant rhesus monkeys (n = 3 per group) were treated with 1 mg/kg bw/day nicotine[24] via subcutaneous

[23] It is not clear from the report whether the dose of 1 mg/kg bw/day refers to nicotine or nicotine tartrate.
[24] It is not clear from the report whether the dose of 1 mg/kg bw/day refers to nicotine or nicotine tartrate.

minipump or no nicotine/pump from GD26 to GD134, and delivered at GD160 by caesarean section, as described in Sekhon et al. (1999) (paragraph 115). A second set of monkeys (n = 7 per group) were treated with 1.5 mg/kg bw/day nicotine or saline via subcutaneous pump and delivered at GD160 by caesarean section, as described in Sekhon et al. (2001) (paragraph 113). The majority of the results presented relate to studies on the set of monkeys treated with nicotine to GD134. Lung tissue sections were stained with antibodies for α7nAChR, collagen types I and III, and α-elastin, and RT-PCR was performed for collagen I and III and elastin subunit expression. Morphometric analysis was performed to assess collagen type I and III expression in cartilaginous and noncartilaginous airways, and elastin expression in parenchyma[25]. *In utero* nicotine exposure significantly increased airway wall thickness in at all levels of airways (cartilaginous, membranous, and terminal airways). Collagen type I and III mRNA expression and immunostaining were significantly increased in the airway and alveolar walls of the nicotine-treated group. The distribution of α7nAChR was similar to that of collagen, and co-localization of α7nAChR and collagen was confirmed in primary culture of pulmonary fibroblasts isolated from a GD100 fetal monkey lung. Elastin expression was low and not affected by nicotine exposure. Elastin mRNA expression increased but protein expression was unchanged by nicotine treatment in the lung parenchyma. In the set of monkeys treated with 1.5 mg/kg bw/day nicotine or saline control through to GD160, for newborns in whom pulmonary function tests were altered by nicotine treatment (paragraph 113), collagen mRNA expression was also significantly upregulated compared with controls. The authors commented that these findings suggest that nicotine may directly interact with α7nAChR to increase collagen accumulation in airway and alveolar walls following *in utero* nicotine exposure, and that these alterations may in part contribute to the observed lung function abnormalities in infants exposed to tobacco smoke *in utero* (Sekhon et al. 2002).

117. An additional publication from the study of Sekhon et al. (1999) in which monkeys were treated with 1 mg/kg bw/day nicotine[26] from GD26 to GD134 (paragraph 115) addressed potential effects on pulmonary vasculature (Sekhon et al. 2004). In airways-associated pulmonary vessels, nicotine exposure was associated with increased total vessel wall dimensions, increased thickness of tunica adventitia but not tunica media, and with increased endothelial cell proliferation index. Collagen I and III mRNA and protein expression were significantly increased in tunica adventitia but not in tunica media. Elastin, primarily expressed in the tunica media, showed upregulated mRNA, but significantly downregulated protein levels in the tunica media associated with nicotine exposure, which the authors noted to be consistent with the lack of effect of nicotine exposure on tunica media thickness. Concurrent expression of collagen and α7nAChR was demonstrated on vessel fibroblasts in the tunica adventitia, and expression of ChAT, which mediates the production of the α7nAChR ligand, ACh, was also noted in the tunica adventitia. No effects were observed on TGF-β1 or bFGF levels. The authors proposed that in the

[25] Elastin expression was low in airway cells

[26] It is not clear from the report whether the dose of 1 mg/kg bw/day refers to nicotine or nicotine tartrate.

developing lung, locally synthesized ACh acts in a paracrine manner and that the effects of nicotine in lung vessels may be to modulate this local paracrine cholinergic signalling pathway. They concluded that their findings suggested that with smoking during pregnancy, nicotine is transported across the placenta and directly interacts with nAChRs in pulmonary vessels to alter connective tissue expression and therefore produce vascular structural alterations (Sekhon et al. 2004).

6.3.2 Rodents

118. Following from the above mentioned findings in rhesus monkeys, Wongtrakool and colleagues investigated potential mechanisms by which nicotine may affect lung development *in utero*, by studying the effects of nicotine on lung branching morphogenesis, and potential implication of the α7nAChR, using embryonic murine lung explants (Wongtrakool et al. 2007). Initial evaluations of embryo lung tissues obtained from pregnant CD57BL/6J mice at intervals from GD11 to PND1 using semi-quantitative RT-PCR, quantitative real-time PCR, and immunohistochemistry indicated expression of α7nAChR mRNA at all stages of lung development, with highest expression occurring in lungs during the pseudoglandular stage compared with lungs at later stages. α7nAChR protein was expressed in epithelial and mesenchymal cells. For *ex vivo* studies, mouse embryo lungs were explanted on day GD11, for 5 days, into culture medium containing various combinations of 1 μM or 5 μM nicotine, α7nAChR antagonist (5 nM α-bungarotoxin), or α7nAChR agonist (3 μM GTS-21). The number of lung branching clefts was counted daily for 5 days. Compared with control treatment (medium without nicotine), nicotine stimulated increased lung branching, with the effect reported to be dose dependent (although results were only reported for the 1 μM nicotine dose). This stimulatory effect on lung branching was completely inhibited by co-addition of α-bungarotoxin. In the absence of nicotine, α-bungarotoxin alone had no effect. Explants cultured with GTS-21 (alone) also showed increased lung branching compared with no treatment. Nicotine also stimulated airway growth, an effect that was partially, but not completely, blocked by co-addition of α-bungarotoxin. Nicotine-exposed lung explants also expressed increased mRNA levels of fibronectin (involved in morphogenesis). Further studies performed using lung explants from α7nAChR-deficient mouse embryos revealed that, in this case, nicotine did not stimulate increased lung branching. The authors concluded that nicotine stimulates lung branching morphogenesis during the pseudoglandular stage through nAChRs and may contribute to dysanaptic lung growth (disproportionate growth between conducting airway and alveolar parenchyma, which may underlie variability in expiratory flow volume curves). The possibility that this may be partially mediated through increased fibronectin expression would require further investigation. Authors stated that these effects may lead to abnormal pulmonary function in infants and children (Wongtrakool et al. 2007).

119. Subsequent studies by the same group of researchers investigated the effects of nicotine exposure during different periods of gestation and early postnatal development on pulmonary development and subsequent pulmonary function *in vivo*.

C57BL/6 (wild-type) and α7nAChR-knockout mice were exposed to nicotine (2 mg/kg bw/day by subcutaneous osmotic minipump) from GD7 to GD21, GD14 to PND7, or PND3 to PND15. Details of control group mice were not described, but it is assumed that they did not receive nicotine exposure. As compared with controls, pulmonary function (FEF in response to methacholine challenge in anaesthetized animals), measured at PND15, was significantly reduced in offspring of the GD14–PN7 group, but not in the other exposure groups, indicating a critical period for the effects of nicotine. This developmental period was noted as representing the last half of lung branching morphogenesis and all the cannicular and saccular periods of lung development, before extensive alveolarization, suggesting that nicotine affects development of the conducting airways rather than alveolarization. There were no differences in FEF values between control and nicotine-treated groups for α7nAChR-knockout infants (Wongtrakool et al. 2012).

120. In a separate study reported in the same publication, airway anatomy was evaluated in 8-week-old adult offspring of wild-type and α7nAChR-knockout C57BL/6 mouse dams administered 100 µg/mL nicotine in drinking water for 6 weeks prior to and during gestation. The offspring were also administered 100 µg/mL nicotine in drinking water from weaning. In wild-type mice, total airway length was increased in nicotine-exposed offspring compared with 'untreated' controls, with a significantly greater total number of both bronchi and bronchioles. Average bronchial diameter and bronchiolar diameter did not differ between control and nicotine groups, although lungs from offspring in the nicotine group had a higher number of small bronchioles compared with controls. There were no effects of nicotine in α7nAChR-knockout mice. Nicotine exposure did not alter lung volume in any group. Increased collagen around airways and vessels was noted in wild-type offspring exposed to prenatal nicotine, but this was not seen in α7nAChR-knockout offspring[27]. The authors concluded that α7nAChRs mediate effects of nicotine on airway growth, stimulating epithelial cell growth and lung branching, leading to increased numbers of airways with small diameter (Wongtrakool et al. 2012).

121. Studies have also shown effects of prenatal nicotine exposure on pulmonary development in rats, with exposures generally given by subcutaneous injection.

122. Early studies by Maritz and coworkers showed that nicotine exposure (1 mg/kg bw/day by subcutaneous injection, during gestation and lactation) caused similar changes in rat pup lungs to those observed in rats exposed *in utero* to CC smoke, with impaired lung growth and alveolar development (Maritz and van Wyk 1997, Maritz 2002).

123. Petre and colleagues showed that exposure to nicotine during fetal and early postnatal life affects vascularization of the lung in a rat model (Petre et al. 2011). Female Wistar rats were treated with 1 mg/kg bw/day nicotine bitartrate or saline, once per day by subcutaneous injection from 2 weeks prior to mating through to pup weaning at PND21. Pups were evaluated at various time points postnatally. At

[27] It is not clear from the report which nicotine exposure regime these animals had received.

3 weeks postnatally, relative lung weight was reduced in pups exposed to nicotine compared with saline, but values had normalised by 12 weeks. Airspace size was reduced and secondary septal buds were shortened in nicotine-exposed pups at PND1 (saccular stage of development) and 3 weeks, but no differences were observed in airspace size compared with controls at 12 weeks of age. There were no differences between nicotine and control pups in lung compliance, airway resistance, or response to methacholine tests at 3 weeks or 12 weeks postnatally. However, lung blood vessel density was significantly reduced by nicotine exposure at PND1, 3 weeks, and 12 weeks of age. This was associated with decreased vascular endothelial growth factor receptor 2 (VEGF-R2) expression at 3 weeks of age, but not at PND1 or 12 weeks (Petre et al., 2011).

124. In keeping with reports of potential epigenetic effects on lung function of CC smoking during pregnancy (see section 5.1.3, paragraphs 29–30), rat studies of *in utero* nicotine exposure also support a role for epigenetic effects, which may be multigenerational.

125. Rehan and colleagues reported a series of studies in rats that indicated that methylation changes may persist for more than one generation, associated with increased risk of asthma (Liu et al. 2011, Rehan et al. 2012, Liu et al. 2013, Rehan et al. 2013, Liu, Sakurai and Rehan 2015, Sakurai et al. 2016). In these studies, pregnant Sprague-Dawley rats were treated once daily from GD6 to PND21 with 100 µL subcutaneous 1 mg/kg bw nicotine[28] or saline control, with or without rosiglitazone, a selective PPARɣ agonist. The dose of 1 mg/kg bw nicotine was reported as consistent with the dose of nicotine to which habitual smokers are exposed. F1 pups were allowed to suckle to PND21, when they either underwent pulmonary function tests, were euthanized for collection of tissue specimens, or were separated and bred on to F2 and F3 generations, without subsequent nicotine exposure. F2 and F3 pups also underwent lung function tests at PND21.

126. Nicotine exposure of F0 dams led to a significantly increased respiratory resistance and decreased compliance in F1 offspring at PND21, associated with increased markers of airway contractility. These effects were more pronounced in male than female offspring, were blocked by concomitant exposure to rosiglitazone with nicotine (Liu et al. 2011, Liu et al. 2013), and were subsequently shown to be reversed by rosiglitazone treatment from PND1 to PND21, after gestational exposure to nicotine (Liu et al. 2015).

127. Pulmonary function tests at PND21 also showed significantly increased respiratory resistance and decreased compliance in F2 offspring, with greater effects being observed in male compared with female offspring. These effects were blocked if F0 dams had been co-treated with rosiglitazone and nicotine. Nicotine also

[28] The reports of Liu, Rehan and colleagues describe the dose as ‘1 mg/kg bw nicotine’. Two of these reports (Rehan et al. 2012, 2013) describe the form used as nicotine tartrate, while the other reports do not describe the chemical form of nicotine used. Thus, it is not entirely certain to what the dose of 1 mg/kg bw actually refers.

increased contractile protein content of whole lung and isolated lung fibroblasts, with decreased PPARɣ expression. Evaluation of F1 offspring gonads showed that in nicotine-exposed offspring, global DNA methylation was increased in the testes, decreased in the ovaries, and did not change in the lungs. H3 and H4 acetylation were variously changed in lungs, testes, and ovaries. Effects on H3 acetylation, but not H4 acetylation or global DNA methylation, were blocked by rosiglitazone. The authors concluded that the pulmonary effects of nicotine exposure during pregnancy are not restricted to the offspring of the exposed pregnancy, but are also transmitted to the subsequent generation, possibly through germline epigenetic alterations (Rehan et al. 2012).

128. In F3 offspring, pulmonary function tests at PND21 also showed significantly increased respiratory resistance and decreased compliance, with changes being greater in males than females. Fibronectin was increased and PPARɣ decreased in isolated airway fibroblasts, confirmed by immunofluorescent staining of lung sections (Rehan et al. 2013).

129. The potential mechanism by which nicotine exposure alters offspring lung structure and function was investigated by immunohistochemical and molecular analyses of PND21 tissue specimens from the F1 and F3 generations. An enhanced myogenic profile was observed, with no evidence of epithelial-mesenchymal transition (EMT), indicating that nicotine-associated effects are mediated via myogenesis, but not EMT (Sakurai et al. 2016). However, the authors noted that conversely, a study by Chen, Chou and Huang (2015b), in which Sprague-Dawley rats had been exposed to 6 mg/kg bw/day nicotine via osmotic minipump from GD7 to either GD14 or GD21, had indicated effects of EMT in offspring lung tissues.

130. In a separate study, Suter et al. (2015) reported that exposure of Sprague-Dawley rats to 2 mg/kg bw/day nicotine[29], by daily intraperitoneal injection, from GD6 to GD22, was associated with alterations in histone H3 methylation and acetylation, and decreased histone acetylase activity in brain and lung of pups on PND1, and with reduced expression of splice variant 1.7 of the glucocorticoid receptor.

6.3.3 Sheep

131. Exposure of ewes during the last trimester of gestation to 0.5 or 1.5 mg/kg bw/day nicotine bitartrate[30] via subcutaneous osmotic minipump was associated with reduced proximal airway conductance in lambs, compared with saline-exposed controls, with effects persisting during the evaluation period of PND12 to PND51 (Sandberg et al. 2004). A subsequent study described association of 3rd trimester exposure to 0.5 or 1.5 mg/kg bw/day nicotine (base) with hyper-reactive proximal

[29] The dose is described as '2mg/kg of nicotine' but further details are not given.
[30] The Methods section of the report states that pumps delivered nicotine bitartrate of approximately 0.5 or 1.5 mg/kg/d. However, other parts of the report refer to doses of 0.5 or 1.5 mg/kg nicotine, so it is unclear whether the doses referred to are nicotine or nicotine bitartrate.

airways, and changes in proximal airway wall composition with associated airflow limitation in lambs evaluated between PND12 to PND52 (Sandberg et al. 2011).

6.4 Other systems

132. Studies in animal models have shown that nicotine exposure may be associated with adverse effects on the development of several other body systems. These studies have used various routes of exposure to nicotine, including injection, drinking water, and continuous subcutaneous osmotic minipump. An overview of some main findings is given below.

6.4.1 Adipose, endocrine, obesity

133. Studies in rats have indicated that maternal nicotine exposure can be associated with increased postnatal body weight and body fat in offspring into adulthood, and endocrine and metabolic changes in adult offspring consistent with type II diabetes, including adverse effects on pancreatic development and function, and impaired insulin sensitivity in peripheral tissues. Exposure regimes have included continuous exposure via continuous subcutaneous osmotic minipump to 3 mg/kg bw/day (group of Somm and colleagues) or 6 mg/kg bw/day (group of Oliveira and colleagues) nicotine, and daily subcutaneous injection with 1 mg/kg bw/day nicotine bitartrate (group of Holloway and colleagues) (*see* Bruin et al. (2010) *and references therein* for an overview and individual study citations).

6.4.2 Xenobiotic metabolism

134. A study in mice indicated that high-dose paternal nicotine exposure led to altered hepatic xenobiotic metabolism in offspring. Male C57BL/6J mice were given drinking water containing nicotine hydrogen tartrate (200 µg/mL nicotine free base) and saccharin, or tartaric acid and saccharin (control) from 3 to 8 weeks of age, withdrawn from treatment for 1 week, and then mated with untreated females. There were no effects on litter size, sex ratio or body weight of offspring. Offspring were tested at 8 weeks of age. There were no differences between groups in offspring behavioural response following an acute nicotine exposure (injection), or of nicotine self-administration reward behaviour in male offspring. However, compared with controls, male offspring with paternal nicotine exposure showed increased resistance to lethality associated with an acute, high-dose nicotine injection following a 1-week period of chronic, low-level nicotine exposure. This effect of tolerance was not seen in the absence of the 1-week low-level nicotine acclimatisation. Further investigations indicated that this effect was likely not modulated via specific brain nAChR pathways, but rather via increased hepatic detoxification of nicotine, and gene-expression analysis showed upregulation of genes involved in drug metabolism in the liver. Finally, studies were carried out to determine whether these effects were nicotine specific, with the finding that similar effects could be induced by several different paternal drug exposures, suggesting a generic effect of enhanced tolerance to xenobiotics. Effects were restricted to male offspring and were not observed in females (Vallaster et al. 2017).

6.4.3 Reproductive/fertility outcomes

135. Studies in animal models have identified that prenatal exposure to nicotine can result in adverse effects on reproductive function in male and female offspring (*reviewed by* Wong et al. 2015)).

136. In a study of male offspring, exposure of pregnant Wistar rats to 2 mg/kg bw/day nicotine via continuous subcutaneous osmotic minipump from GD1 to the end of weaning induced morphofunctional alterations of fetal and mature Leydig cells and affected cholesterol and testosterone, evaluated over a period from PND1 to PND90 (Paccola et al. 2014). Follow-up using the same dosing regime also indicated adverse effects on spermatogenesis in male offspring followed up to PND196 (Miranda-Spooner et al. 2016).

137. Nicotine exposure (1 mg/kg bw/day nicotine bitartrate, by subcutaneous injection) of pregnant Wistar rats from 14 days prior to mating, through gestation and lactation, led to germ cell depletion and altered steroidogenesis in male offspring (Lagunov et al. 2011) and increased ovarian cell apoptosis, increased serum progesterone levels and lower oestrogen/progesterone ratios, and impaired fertility in female offspring (Holloway, Kellenberger and Petrik 2006, Petrik et al. 2009).

6.4.4 Immune and haematopoietic effects

138. Basta et al. (2000) (abstract only) reported that exposure of rats to 6 mg/kg bw/day nicotine by continuous subcutaneous osmotic minipump from GD4 to GD20 suppressed proliferative response of immune cells in offspring, with or without ethanol in drinking water, with more severe effects in adult offspring in the combined exposure group.

139. Mohsenzadeh et al. (2014) reported that prenatal exposure of rats to 2, 4, or 6 mg/kg bw/day nicotine[31] via continuous subcutaneous osmotic minipump, throughout gestation, led to significant dose-dependent increases in newborn serum levels of acute phase reactant hs-CRP at all doses, and of pro-inflammatory cytokines IL-6 and TNFα in the 2 highest dose groups.

140. Studies in mice have indicated that intravenous administration of nicotine during gestation can alter fetal development of the hematopoietic system, with delayed release of primitive precursors from fetal liver, impaired colonization of fetal bone marrow and imbalance in the production of mature blood cells, including immune system cells, in newborns (Demina et al. 2005, Serobyan et al. 2005) (abstract only).

6.4.5 Renal effects

141. The offspring of Sprague-Dawley rats exposed via continuous subcutaneous osmotic minipump to 6 mg/kg bw/day nicotine from GD7 to birth or GD7 to PN14 had

[31] It is not entirely clear from the report whether the doses refer to nicotine or nicotine bitartrate.

higher levels of kidney fibrosis at PND7 and PND21 compared with offspring of saline-treated rats. Nicotine treatment was associated with smaller mean glomerular size (Chen, Chou and Huang 2015a).

142. Shariati Kohbanani et al. (2016) (abstract only) reported that offspring of mice treated subcutaneously with 2 mg/kg bw/day nicotine from GD7 to the end of gestation had significantly increased kidney laminin α5 in newborns compared with offspring of saline-injected controls.

143. Exposure of rats to 20 µg/mL nicotine in drinking water during gestation, in combination with reduced uterine perfusion pressure from GD14 to GD21 was associated with adverse effects on the kidneys of male fetuses evaluated at GD21, and accumulation of nicotine in the plasma and kidney of the exposed fetuses (Ojeda et al. 2016) (abstract only).

6.4.6 Skeletal effects

144. A set of studies from a research group in China, in which rats were treated by daily injection with 2 mg/kg bw/day nicotine starting on GD9 or GD11 through to GD20, has shown adverse effects on bone and cartilage development, including poor cartilage quality in female offspring of rats co-fed a high-fat diet, delayed chondrogenesis, and retarded osteoclastogenesis and endochondral ossification in fetal long bones (Deng et al. 2013, Tie et al. 2016, Hu et al. 2018) (abstract only).

7 Summary

7.1 Scope of the review

145. This discussion paper aims to summarise data of relevance to assessing the potential for developmental toxicity in human offspring following parental exposure to nicotine. The vast majority of data reviewed are of relevance to maternal nicotine exposure, with a very limited dataset relating to paternal exposure. Data from studies in humans include an overview of developmental effects in offspring following maternal or paternal exposure to tobacco products, and a detailed review of studies that have investigated effects of NRT use in pregnancy. Experimental studies in animal models are also reviewed, including those that have evaluated the effects of nicotine exposure *in utero*, via breast milk, directly to offspring during the early postnatal period in rodents, which corresponds to the 3rd trimester of pregnancy in humans, and a very small number of studies that evaluated paternal exposures. Data relating to effects of exposure to E(N)NDS aerosols, with and without nicotine, on developmental outcomes are provided in the accompanying paper, TOX/2018/46.

7.2 Data in humans

7.2.1 Maternal exposure to tobacco products

146. In humans, maternal CC smoking in pregnancy has been causally associated with adverse pregnancy outcomes including ectopic pregnancy, fetal growth

restriction, increased risk of preterm delivery, placental abruption, perinatal mortality (stillbirth and neonatal death), SIDS, and orofacial clefts. Use of smokeless tobacco has been associated with increased risk of some (preterm delivery, stillbirth, orofacial clefts) but not other (fetal growth restriction) of these effects (HHS 2014). Maternal CC smoking in pregnancy is also associated with adverse effects on brain structure, altered behaviour and stress response in newborns, and with longer term alterations in behavioural outcomes during childhood and adolescence, particularly externalising and disruptive behaviours such as ADHD (HHS 2014). Exposure to CC smoke *in utero* via maternal smoking during pregnancy has also been associated with adverse effects on pulmonary development and infant/childhood respiratory health, including altered pulmonary function tests in infants, persisting into childhood and early adulthood, and increased rates of bronchitis, lower respiratory tract infections, wheezing and asthma in infants. Some studies have indicated that some of these effects may be transmitted through to grandchildren (McEvoy and Spindel 2017, England et al. 2017). A limited amount of literature was identified suggesting possible association of CC smoking in fathers with birth defects and childhood cancers (Esakky and Moley 2016)

7.2.2 Maternal exposure to NRT products

147. Some data on the developmental effects in humans following maternal exposure to nicotine from non-tobacco sources are available from published studies on NRT use during pregnancy, including clinical trials (RCTs) and population-based epidemiological studies. Overall, outcomes reported from studies of nicotine exposure via NRT use during pregnancy have been variable. Limiting factors include generally low rates of adherence to NRT use and of smoking cessation in RCTs, a lack of data on the extent of CC smoking in association with NRT use, and the small proportion of women in the NRT groups, leading to limited power of epidemiological studies. Studies have mostly evaluated immediate endpoints such as birth outcomes, thus with limited capability to identify adverse outcomes such as neurobehavioral effects which may only become apparent later in life.

148. RCTs of NRT use in pregnancy reviewed include studies carried out in Europe (UK, Denmark, France), and the USA. The largest of these studies was the ‘SNAP’ trial for smoking cessation in pregnancy, carried out by Coleman and colleagues at the University of Nottingham, UK. This study included 1050 pregnant smokers randomised to NRT (15 mg/day over 16 hours) or placebo transdermal patches for periods of up to 8 weeks, starting between 12–24 weeks of pregnancy. No differences were noted in birth outcomes between NRT and placebo groups, other than a significantly increased rate of birth by caesarean section in the NRT group (OR = 1.45, 95% CI 1.05 to 2.01 for NRT compared with placebo). However, overall adherence to patch use and smoking cessation rates was poor (Coleman et al. 2012b). A 2-year follow up of SNAP data suggested that ‘survival without impairment’ was higher in infants from the NRT compared with placebo group (OR = 1.40, 95% CI 1.05 to 1.86, p = 0.023) (Cooper et al. 2014b).

149. Several epidemiological studies have used data from national databases to assess parameters of potential NRT use/exposure during pregnancy and their correlation with various pregnancy and infant/childhood developmental outcomes. Researchers at the University of Nottingham used data from 192,498 births registered between 2001–2012 in the UK 'THIN' general practitioner database. Women who had been prescribed NRT during a period from 4 weeks before conception to the end of the 1st trimester of pregnancy were identified and their smoking status was ascertained. Analyses of birth outcomes were carried out based on these data. Overall, approximately 1.4% of women had been prescribed NRT, 5.2% were smokers not prescribed NRT, and 93.4 % were nonsmokers who were not prescribed NRT. Evaluation of system-specific MCAs in infants born to these women, indexed by ICD-10 codes, showed no statistically significant difference between groups except for a significant increase in respiratory system anomalies in infants born to women prescribed NRT in comparison with both non-NRT/nonsmokers (OR = 4.65, 99% CI 1.76 to 12.25, $p < 0.001$) and non-NRT/smokers (OR = 3.49, 99% CI 1.05 to 11.62, $p = 0.007$). The authors commented that the overall study findings had been limited by the low rate of NRT prescription during pregnancy and by the fact that certain morbidity rates were different between comparison groups. Notably the rate of asthma was higher in the NRT group (14.5%) compared with the no-NRT/smoker (10.4%) and no-NRT/nonsmoker (8.2%) groups (Dhalwani et al. 2015). A follow-up study of stillbirth in the same cohort found no effect of NRT prescription during the whole of pregnancy or the preceding 4 weeks on rate of stillbirth (Dhalwani et al. 2018).

150. Several studies have been carried out using data from the Danish National Birth Cohort (DNBC), which contains data from around 100,000 pregnancies in Denmark during 1996–2002. Studies of this cohort have reported that NRT use during pregnancy was associated with increased rates of congenital malformations (RPR = 1.6, 95% CI 1.01 to 2.58 for nonsmokers who used NRT compared with nonsmokers who did not use NRT) (Morales-Suarez-Varela et al. 2006) and infantile colic (AOR = 1.6, 95% CI 1.0 to 2.5 for nonsmoker NRT users compared with nonsmokers who did not use NRT, and AOR = 1.5, 95% CI 1.3 to 1.8 for smokers using NRT compared with nonsmokers who did not use NRT) (Milidou et al. 2012). Use of NRT by women during pregnancy was also associated with an increased risk of ADHD in the child, but the findings were only statistically significant for women with nonsmoking partners (HR = 2.28, 95% CI 1.48 to 3.51 for NRT use/partner a nonsmoker; HR = 1.28, 95% CI 0.57 to 2.89 for NRT use/partner a smoker compared with children whose parents were nonsmokers and the mother did not use NRT) (Zhu et al. 2014). No significant effects of NRT use during pregnancy were found for stillbirth, birthweight, or strabismus. Some smaller epidemiological studies carried out on populations in North America have suggested either beneficial or adverse effects of NRT use during pregnancy on birth outcomes, but with no particularly striking findings.

7.3 Studies in animals

151. Animal studies of nicotine exposure have shown adverse effects on the development of several organ systems, with a large part of the literature focussed on effects on development of the neurological and respiratory systems. The majority of studies administered nicotine during gestation via continuous subcutaneous osmotic minipump in order to avoid the acute high plasma nicotine levels associated with bolus injections, which may cause uteroplacental vasoconstriction and fetal hypoxia (Slotkin 2004). Nicotine (free base) doses in the range of approximately 1–6 mg/kg bw/day have been used in rodents, with 2 mg/kg bw/day and 6 mg/kg bw/day, respectively, reported to reproduce plasma nicotine levels observed with moderate (10–20 CC/day) and high (20–40 CC/day) levels of smoking (Slotkin 2004). The higher dose of 6 mg/kg bw/day has been associated with reduced maternal weight gain and fetal resorption, but these effects are generally not observed at 2 mg/kg bw/day.

152. Studies in rodents, mostly rats, have shown that nicotine exposure *in utero* adversely affects structural brain development via interaction with nAChRs, leading to subtle structural alterations in the CNS that persist after birth. These alterations correspond with adverse effects on cognitive and behavioural development in offspring, with effects persisting through infancy, to adolescence and adulthood. It has been emphasized that effects are subtle and seen at exposures that do not lead to other secondary effects, indicating that growth retardation *per se* is not an adequate measure for developmental neurotoxicity of nicotine (Slotkin 2008, England et al. 2017). Further studies in this area have indicated that neurodevelopmental effects of nicotine vary between male and female offspring. One study modelled the adverse neurodevelopmental effects of nicotine at an exposure concentration equivalent to that in secondhand CC smoke (0.2 mg/kg bw/day) compared with tobacco smoke extract (TSE) containing an approximately equivalent nicotine concentration, and with a 10-fold higher nicotine exposure (2 mg/kg bw/day, representing exposure to ‘moderate’ direct CC smoking). There were no effects on maternal weight gain or toxicity, and only a small decrease in offspring postnatal weight gain in the TSE group. Various adverse effects were identified on cholinergic and serotonergic signalling systems in different brain regions of offspring in all exposure groups, with some sex-specific effects. Overall, the authors calculated that for male offspring, the effects of 0.2 mg/kg bw/day nicotine were significantly correlated with those of TSE and accounted for 36% of the TSE effects, while 2.0 mg/kg bw/day nicotine accounted for 46%. For female offspring, nicotine accounted for 13% of the TSE effect at 0.2 mg/kg bw/day and 7% at 2.0 mg/kg bw/day (Slotkin et al. 2015a). A follow-up study with the same exposure regimes found that all exposures led to disruptions of cognitive and behavioural function, with hyperactivity, working memory deficits, and impairments in emotional processing. Effects were greater with TSE exposure than with either dose of nicotine alone, with the magnitude of effects in the TSE-exposed group more in line with 2.0 mg/kg bw/day nicotine, and a lower level of effects seen at 0.2 mg/kg bw/day. This indicated that exposure to an approximately equivalent level of nicotine incurs a greater magnitude

of adverse effects on cognitive and behavioural development in combination with other components of TSE than alone (Hall et al. 2016). In addition to studies of maternal exposure, a small number of recent reports have suggested that paternal exposure of mice to nicotine prior to and during mating may have an effect on behavioural outcomes in offspring (Dai et al. 2017, McCarthy et al. 2018, Zhang et al. 2018).

153. Adverse effects of nicotine exposure on development of the respiratory system have been demonstrated in a range of animal species, including deficits in pulmonary function that are related to alterations in the structure of the respiratory system (McEvoy and Spindel 2017). Maternal exposure in rhesus monkeys to 1.5 mg/kg bw/day nicotine base via the continuous subcutaneous osmotic minipump system throughout gestation led to alterations in pulmonary function tests in newborn offspring (Sekhon et al. 2001). Moreover, exposure to 1.0 mg/kg bw/day nicotine was associated with altered connective tissue in airways and surrounding vasculature (Sekhon et al. 1999, Sekhon et al. 2002, Sekhon et al. 2004). Follow-up studies in mice were carried out to elaborate the molecular/structural alterations involved and developmental timing of the effects. Authors concluded that α7nAChRs mediate effects of nicotine on airway growth, stimulating epithelial cell growth and lung branching, leading to increased numbers of airways with small diameter (Wongtrakool et al. 2007, Wongtrakool et al. 2012). Similar effects of nicotine exposure on respiratory system development have also been reported in other species, including rats and sheep. Adverse effects in offspring were observed after maternal exposures of 0.5 mg/kg bw/day nicotine during the 3rd trimester of gestation in sheep (Sandberg et al. 2004, Sandberg et al. 2011).

7.4 Main conclusions

154. In humans, maternal CC smoking during pregnancy is causally associated with adverse pregnancy outcomes including ectopic pregnancy, fetal growth restriction, increased risk of preterm delivery, placental abruption, perinatal mortality (stillbirth and neonatal death), SIDS, and orofacial clefts, and has also been associated with adverse effects on brain structure, altered behaviour and stress response in newborns, and with longer term alterations in behavioural outcomes during childhood and adolescence, particularly externalising and disruptive behaviours such as ADHD. Exposure to CC smoke *in utero* via maternal smoking during pregnancy has also been associated with adverse effects on pulmonary development and infant/childhood respiratory health, including altered pulmonary function tests in infants, persisting into childhood and early adulthood, and increased rates of bronchitis, lower respiratory tract infections, wheezing and asthma in infants. Use of smokeless tobacco is associated with increased risk of preterm delivery, stillbirth, and orofacial clefts, but with little effect on fetal growth restriction. A large epidemiological study in the UK showed an association of being prescribed NRT during early pregnancy with a higher rate of respiratory system anomalies in offspring, although this finding may have been related to the higher prevalence of asthma in the women prescribed NRT. Studies from the Danish National Birth Cohort

indicated a possible increased risk of congenital anomalies, infantile colic, and ADHD in children whose mothers used NRT during pregnancy.

155. Animal studies have demonstrated developmental toxicity following prenatal and/or early postnatal nicotine exposure on several organ systems, notably the neurological and respiratory systems. Effects can be subtle, may be expressed during specific life periods, and persist into adulthood. Exposure levels have been evaluated to model systemic nicotine exposures that would be achieved via direct CC smoking or from secondhand smoke exposure, and thus may also represent nicotine levels achieved by some ENDS users. These studies have used exposure regimes that include continuous subcutaneous infusion, drinking water, or bolus injection, but not inhalation.

8 Questions for the Committee

156. Members are invited to comment on the information provided in this paper and to consider the following questions:

i. Is the Committee able to draw any conclusions from the data presented on the risks to development in the offspring of parental exposure to nicotine?

ii. Are there any particular aspects of this paper that should be captured when a COT statement on E(N)NDS is prepared?

NCET at WRc/IEH-C under contract supporting the PHE COT Secretariat
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Abbreviations

5-HT	5-hydroxytryptamine
ACh	Acetylcholine
ADHD	Attention deficit hyperactivity disorder
AE	Adverse event
AOR	Adjusted odds ratio
b	Birthweight
bFGF	Basic fibroblast growth factor
CAF	Critical assessment form
CBT	Cognitive behaviour therapy
CC	Conventional cigarette
ChAT	Choline acetyltransferase
CI	Confidence interval
CNS	Central nervous system
CO	Carbon monoxide
DNBC	Danish National Birth Cohort
DSMB	Data and safety monitoring board
EMT	Epithelial-mesenchymal transition
E(N)NDS	Electronic nicotine (or non-nicotine) delivery system
ENDS	Electronic nicotine delivery system
ENNDS	Electronic non-nicotine delivery system
EUROCAT	European Surveillance of Congenital Anomalies
FEF	Forced expiratory flow
$FEF_{25-75\%}$	Mean mid-expiratory flow
$FEV_{0.2}$	Forced expiratory volume during first 0.2 seconds
FEV_{PEF}	Forced expiratory volume to achieve peak expiratory flow
FVC	Forced vital capacity
GD	Gestation day
HR	Hazard ratio
ICD-10	International Statistical Classification of Diseases and Related Health Problems (10^{th} revision)
ICU	Intensive care unit
IQR	Interquartile range
MCA	Major congenital anomaly
MRI	Magnetic resonance imaging
nAChR	Nicotinic acetylcholine receptor
NAS	U.S. National Academy of Sciences
NRT	Nicotine replacement therapy
OR	Odds ratio
PAH	Polycyclic aromatic hydrocarbon
PG	Propylene glycol
PND	Postnatal day
PPARɣ	Peroxisome proliferator-activated receptor gamma
PRAMS	Pregnancy Risk and Assessment Monitoring System (US)
PTEF	Peak tidal expiratory flow
RCT	Randomised clinical trial

RD	Risk difference
RPR	Relative prevalence ratio
RR	Relative risk or Risk ratio
SAE	Serious adverse event
SAHE	Serious adverse health effect
SD	Standard deviation
SGA	Small for gestational age
SIDS	Sudden infant death syndrome
SNAP	Smoking, Nicotine, and Pregnancy (clinical trial)
SNIPP	Study of Nicotine Patch in Pregnancy (clinical trial)
TGF-β1	Transforming growth factor beta 1
THIN	The Health Improvement Network (UK general practice database)
TSE	Tobacco smoke extract
TSNA	Tobacco-specific nitrosamine
VEGF-R2	Vascular endothelial growth factor receptor 2
VG	Vegetable glycerine (glycerol)
VOC	Volatile organic compound
WHO	World Health Organization

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COMMITTEE ON TOXICITY OF CHEMICALS IN FOOD, CONSUMER PRODUCTS AND THE ENVIRONMENT (COT)

Potential toxicological risks from electronic nicotine (and non-nicotine) delivery systems (E(N)NDS – e-cigarettes). Paper 6a: A review of data relating to developmental toxicity in offspring following parental exposure to nicotine.

Details of Literature search carried out by NCET at WRc/IEH-C

Searches were carried out to identify literature relating to developmental toxicity of nicotine as a result of maternal exposure, as follows.

Search 1 (n = 805 citations total)

Literature searches were performed by NCET at WRc/IEH-C under contract to PHE on 16/07/18 in Scopus and PubMed, with no limit of publication date.

Scopus

(TITLE-ABS-KEY (nicotine OR "nicotine replacement therap*") AND TITLE-ABS-KEY ("development* toxicity" OR "fetal development*" OR "foetus development*" OR embryo* OR teratogen* OR "birth defect*" OR "congenital abnormalit*" OR "congenital anomal*" OR "prenatal development*")) AND (LIMIT-TO (DOCTYPE , "ar ") OR LIMIT-TO (DOCTYPE , "re ") OR LIMIT-TO (DOCTYPE , "sh ") OR LIMIT-TO (DOCTYPE , "ip ")) AND (LIMIT-TO (SUBJAREA , "PHAR ") OR LIMIT-TO (SUBJAREA , "BIOC ") OR LIMIT-TO (SUBJAREA , "NEUR ") OR LIMIT-TO (SUBJAREA , "PSYC ") OR EXCLUDE (SUBJAREA , "MEDI ") OR EXCLUDE (SUBJAREA , "ENVI ") OR EXCLUDE (SUBJAREA , "AGRI ") OR EXCLUDE (SUBJAREA , "CHEM ") OR EXCLUDE (SUBJAREA , "SOCI ") OR EXCLUDE (SUBJAREA , "ARTS ") OR EXCLUDE (SUBJAREA , "HEAL ") OR EXCLUDE (SUBJAREA , "NURS ") OR EXCLUDE (SUBJAREA , "COMP ") OR EXCLUDE (SUBJAREA , "ENGI ") OR EXCLUDE (SUBJAREA , "BUSI ") OR EXCLUDE (SUBJAREA , "CENG ") OR EXCLUDE (SUBJAREA , "ECON ")) AND (LIMIT-TO (LANGUAGE , "English ")): 576 citations

PubMed

(english[Language]) AND (((nicotine[Title/Abstract] OR "nicotine replacement therapy"[Title/Abstract])) AND ("developmental toxicity"[Title/Abstract] OR "fetal development"[Title/Abstract] OR teratogen*[Title/Abstract] OR embryo*[Title/Abstract] OR "birth deffect*"[Title/Abstract] OR "foetus development"[Title/Abstract] OR "congenital abnormalit*"[Title/Abstract] OR "congenital anomal*"[Title/Abstract] OR "prenatal development*"[Title/Abstract]): 417 citations

Search 2

Additional searches of PubMed were carried out, as follows:

(Nicotine replacement therapy) AND pregnancy (10/07/18); 229 citations

Nicotine AND development* NOT addicti* NOT dependence (01/01/2017 - 10/07/18); 373 citations

'Nicotine developmental toxicity' (20/09/18); 174 citations

Nicotine AND paternal (15/11/18); 68 citations

Search 3

The reference lists of selected papers identified from Searches 1 and 2 were examined for further relevant publications. Additional *ad hoc* searches were carried out as considered appropriate.

NCET at WRc/IEH-C under contract supporting the PHE COT Secretariat November 2018