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Risks and Benefits of Nicotine to Aid Smoking Cessation in Pregnancy

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Abstract

Cigarette smoking during pregnancy is the single largest modifiable risk for pregnancy-related morbidity and mortality in the US. Addiction to nicotine prevents many pregnant women who wish to quit smoking from doing so. The safety and efficacy of nicotine replacement therapy (NRT) for smoking cessation during pregnancy have not been well studied.

Nicotine is classified by the US Food and Drug Administration as a Pregnancy Category D drug. Animal studies indicate that nicotine adversely affects the developing fetal CNS, and nicotine effects on the brain may be involved in the pathophysiology of sudden infant death syndrome (SIDS). It has been assumed that the cardiovascular effects of nicotine resulting in reduced blood flow to the placenta (uteroplacental insufficiency) is the predominant mechanism of the reproductive toxicity of cigarette smoking during pregnancy. Short term high doses of nicotine in pregnant animals do adversely affect the maternal and fetal cardiovascular systems. However, studies of the acute effects of NRT in pregnant humans indicate that nicotine alone has minimal effects upon the maternal and fetal cardiovascular systems.

Cigarette smoking delivers thousands of chemicals, some of which are well documented reproductive toxins (e.g. carbon monoxide and lead). A myriad of cellular and molecular biological abnormalities have been documented in placentas, fetuses, and newborns of pregnant women who smoke. The cumulative abnormalities produced by the various toxins in cigarette smoke are probably responsible for the numerous adverse reproductive outcomes associated with smoking. It is doubtful that the reproductive toxicity of cigarette smoking is primarily related to nicotine.

We recommend the following. Efficacy trials of NRT as adjunctive therapy for smoking cessation during pregnancy should be conducted. The initial dose of nicotine in NRT should be similar to the dose of nicotine that the pregnant woman received from smoking. Intermittent-use formulations of NRT (gum, spray, in-

haler) are preferred because the total dose of nicotine delivered to the fetus will be less than with continuous-use formulations (transdermal patch). A national registry for NRT use during pregnancy should be created to prospectively collect obstetrical outcome data from NRT efficacy trials and from individual use. The goal of this registry would be to determine the safety of NRT use during pregnancy, especially with respect to uncommon outcomes such as placental abruption. Finally, our review of the data indicate that minimal amounts of nicotine are excreted into breast milk and that NRT can be safely used by breast-feeding mothers.

1. Cigarette Smoking During Pregnancy: Overview of the Problem

It is estimated that 19 to 27% of pregnant women in the US smoke cigarettes.[1] The adverse health effects associated with maternal cigarette smoking are listed in table I. There are approximately 4 million births per year in the US, and the low estimate that 19% of pregnant women smoke cigarettes would result in nearly 800 000 babies per year who are exposed *in utero* to the products of cigarette smoke. Cigarette smoking during pregnancy is the single largest modifiable risk for pregnancy-related morbidity and mortality in the US. Cigarette smoke contains thousands of chemicals, some of which are well documented fetal toxins [e.g. carbon monoxide (CO), lead, nicotine (table II)].

Nicotine is the substance in cigarettes that is responsible for addiction to smoking. Addiction to nicotine prevents many pregnant women who wish to quit from doing so.[3] Nicotine replacement therapy (NRT) in nonpregnant adults increases quit rates when used as adjunctive therapy in a smoking cessation programme. Nicotine is classified by the US Food and Drug Administration (FDA) as a Pregnancy Category D drug. Category D implies some risk during pregnancy and requires that the risks associated with the use of the drug are outweighed by the risks of the medical problem if left untreated. Uteroplacental insufficiency has been the main accepted mechanism of smoking-related reproductive adverse outcomes. According to this mechanism, nicotine is responsible for many adverse pregnancy outcomes. It is suggested that reduced blood flow through the placenta due to the

Table I. Cigarette smoking-related outcomes of pregnancy

Pregnancy loss
spontaneous abortion
fetal demise
stillbirths
Premature rupture of membranes
preterm
term
Premature labour and delivery
Placental abruption
Placental previa
Hypertension
Pre-eclampsia
Fetal toxicity
Growth retardation
Neurotoxicity
Cleft palates and cleft lips
Pulmonary effects
Postnatal outcomes
Sudden infant death syndrome
Premature infants, especially very low birth weight infants
Hyperviscosity in the newborn
Elevated blood pressure during infancy and childhood
Behavioural, psychiatric, and cognitive outcomes of childhood
Mental retardation
Childhood cancers
Medical conditions associated with passive smoking in childhood[a]
sudden infant death syndrome
deaths due to respiratory illnesses
asthma
pneumonia and other respiratory illnesses
otitis media
burns and fire deaths

a Increased prevalence of childhood passive smoking among children born to mothers who smoke.

Table II. Selected potentially fetotoxic chemicals in cigarettes[2]

Chemical	Dose per cigarette
Carbon monoxide	10 to 23mg
Nicotine	1 to 3mg
Hydrogen cyanide	400 to 500μg
Aniline	360 to 655μg
Catechol	200 to 400μg
Nitrogen oxide	100 to 600μg
Methanol	100 to 250μg
Phenol	80 to 160μg
Acrolein	60 to 140μg
Pyridine	16 to 40μg
Ammonia	10 to 130μg
Hydrogen sulfide	10 to 90μg
Arsenic	40 to 120μg
Hexavalent chromium	4 to 70ng
Cadmium	4 to 70ng
Nickel	0 to 600ng
Lead	34 to 85ng
Carcinogens	
polynuclear aromatic hydrocarbons	60 to 190ng
heterocyclic compounds	3 to 14ng
N-Nitrosamines	200 to 4900ng
aromatic amines	30 to 670ng
N-Heterocyclic amines	40 to 300ng
aldehydes	570 to 1500ng
volatile hydrocarbons	500 to 1150ng

cardiovascular effects of nicotine is responsible for the adverse outcomes associated with smoking. Based on the review of the literature presented here, we conclude that it is doubtful that uteroplacental insufficiency is a major mechanism of the toxicity of cigarette smoking or even of nicotine. Rather, a myriad of cellular and molecular biological abnormalities produced by a number of tobacco smoke toxins, acting alone or in concert, produce a wide range of adverse pregnancy outcomes.

The category D status of NRT has discouraged the investigation of NRT use during pregnancy. Granting agencies have been hesitant to fund efficacy studies of NRT for smoking cessation during pregnancy because of its status as a category D drug. Drug manufacturers have not funded efficacy studies of pharmacotherapy to aid cessation in pregnant women. Individual providers of prenatal care may also be reluctant to prescribe a category D drug because of legal liability fears.

Nicotine use most likely does carry some risk. Animal data indicate that nicotine is toxic to the developing CNS, and may be involved in the aetiology of sudden infant death syndrome (SIDS).[4] To place the risks of nicotine in context, one must consider the risks associated with smoking tobacco in relation to the risks associated with NRT use. There are at present inadequate data with which to compare the clinical effects of cigarette smoking and the effects of nicotine during pregnancy. Our paper describes possible mechanisms of toxicity of cigarette smoking during pregnancy and tries to identify nicotine-specific toxicity versus toxicity related to other chemicals in cigarette smoke. We then summarise the risks and benefits of NRT use during pregnancy, and make recommendations regarding efficacy studies and safety studies of NRT use during pregnancy.

2. General Pharmacology of Nicotine as Related to Reproductive Toxicity

2.1 Pharmacological Actions of Nicotine

Nicotine binds to nicotinic cholinergic receptors that are located in the brain, in autonomic ganglia, the adrenal medulla, and in neuromuscular junctions. The receptors are believed to be receptors for endogenously released acetylcholine. There are different subtypes of nicotinic cholinergic receptors, composed of different subunits, different ligand binding characteristics and different functional characteristics. It is not clear which receptor subtypes mediate which pharmacological actions of nicotine. Nicotine receptor activation facilitates the release of a variety of neurotransmitters, including dopamine, noradrenaline (norepinephrine), adrenaline (epinephrine), acetylcholine, serotonin (5-hydroxytryptamine), γ-aminobutyric acid (GABA), glutamate, and β-endorphin. These effects presumably mediate the psychoactive actions of nicotine. The main effects of nicotine on the systemic circulation is that of a weak sympathomimetic drug.

2.1.1 Pharmacodynamics

The cardiovascular effects of nicotine in non-pregnant adults have been extensively studied.[3,5] Nicotine increases heart rate and myocardial contractility, constricts some blood vessels, and transiently increases blood pressure. For additional details and references, the reader is referred to another recent review.[3] With prolonged or repeated exposures to nicotine, many nicotine receptors become desensitised, and tolerance develops to the effects of nicotine via these receptors. Tolerance to the CNS effects of nicotine is associated with an increased number of nicotinic cholinergic receptors in the brain. Tolerance to the cardiovascular effects of nicotine is incomplete. For example, heart rate acceleration persists, although at less than maximal levels, despite prolonged continuous exposure to nicotine.

The dose-response to nicotine is important in understanding the risks of nicotine-mediated cardiovascular toxicity. Within the range of nicotine concentrations seen in smokers, the dose-response to heart rate acceleration and blood pressure elevation is flat, with maximal increases seen at plasma nicotine concentrations consistent with low level cigarette smoking. This flat dose-response curve has been demonstrated in studies comparing smoking cigarettes with high and low nicotine-containing tobaccos, studies of the effects of cigarette smoking with and without concomitant intravenous nicotine infusion, and studies of cigarette smoking with and without concomitant transdermal nicotine in doses of 21 to 63 mg/day. The flat dose-response curve may have implications for the risks of NRT in persons who continue to smoke cigarettes – namely, that there is little increased risk attributable to nicotine therapy. With extremely high exposures to nicotine, such as with acute nicotine poisoning, bradycardia and hypotension associated with nausea and vomiting occur, which is consistent with a vagotonic syndrome.

The pharmacological effects of nicotine depend on the rate of rise of plasma concentrations in the brain and in other target organs. Rapid administration of nicotine, such as after cigarette smoking, results in transiently high arterial nicotine concentrations and rapid delivery of nicotine to the brain. The latter means that nicotine can have its effects before there is time for the development of tolerance. Conversely, slow delivery of nicotine, such as with transdermal nicotine systems, produces less intense effects. The difference in magnitude of nicotine effects as a function of the rate of administration is most clearly seen in psychoactive effects, which are quite pronounced with cigarette smoking or rapid intravenous infusion, but which are virtually nil in individuals receiving transdermal nicotine. On the other hand, while heart rate acceleration is greater with rapid administration, there is still heart rate acceleration even with transdermal nicotine.

One pharmacological response which may have implications for cardiovascular toxicity is the nicotine effect on platelets and coagulation. *In vitro* studies do not consistently show that nicotine activates platelets. However, some *in vivo* studies indicate that after rapid intravenous administration of nicotine there is platelet activation. In studies in dogs where platelet activation was documented, the effect was blocked by α-adrenergic blockers, suggesting that the effect is mediated by nicotine-related catecholamine release. One human study has compared cigarette smoking and transdermal nicotine and showed no effect of transdermal nicotine upon platelet activation, whereas cigarette smoking activated platelets. Whether a rapidly delivered nicotine bolus without other components of cigarette smoke activates platelets remains to be determined.

2.1.2 Pharmacokinetic Considerations

Nicotine is absorbed rapidly from cigarette smoke, resulting in peak arterial nicotine concentrations of 50 to 100 μg/L immediately after the last inhalation of cigarette smoke. Venous nicotine concentrations are several-fold lower, because nicotine in the arterial circulation has been taken up by body tissues before it reaches the venous circulation. The faster the rate of administration, the larger the arterial-venous difference in blood nicotine concentration. Thus, rapid delivery systems

such as nicotine nasal spray produce greater arterial-venous nicotine differences than slower delivery systems such as transdermal nicotine. The issue of the rate of drug delivery is important to keep in mind in interpreting venous plasma concentration data that are reported in various experimental studies of nicotine pharmacology and toxicology.

Once nicotine is absorbed, it moves to virtually all body organs with particularly high affinity for brain, the heart, and the lungs. The major storage organ for nicotine in the body is skeletal muscle, primarily because of its large mass. Nicotine concentrations in adipose tissue are low because nicotine is a relatively polar compound. Nicotine is metabolised primarily to cotinine, but also by glucuronidation and by *N*-oxidation. There is nicotine metabolism in the fetus, but it proceeds at a much lower rate than in the mother (unpublished observation). Cotinine is important primarily because it has been used as a marker of nicotine exposure. Cotinine has a much longer elimination half-life than nicotine (16 *vs* 2 hours in nonpregnant adults), and concentrations in the blood are much higher for cotinine than nicotine. Thus, cotinine is more stable and easier to measure than nicotine.

2.2 Nicotine Pharmacokinetics During Pregnancy

2.2.1 Disposition of Nicotine and Cotinine

To the best of our knowledge, no systematic studies of the disposition of nicotine during pregnancy have been published. We have unpublished data on 10 women in whom the pharmacokinetics of nicotine and cotinine were studied during pregnancy and then again 3 months postpartum.[6,7] Several women were studied twice during pregnancy. The gestational ages varied from 17 to 40 weeks and gestational age was not found to affect any of the pharmacokinetic parameters studied.

Nicotine

The metabolic clearance of nicotine increased during pregnancy by a factor of 1.6.[7] During pregnancy, the mean clearance of nicotine was 1.56 L/h/kg, while 3 months after pregnancy it was 0.96 L/h/kg. This change in clearance resulted in a modest decrease in the elimination half-life of nicotine during pregnancy. The mean elimination half-life of nicotine during pregnancy was 1.6 hours, while after pregnancy it increased to 1.8 hours. The fractional conversion or the percentage of the dose of nicotine metabolised to cotinine was unchanged by pregnancy (76 *vs* 78%).

Cotinine

Pregnancy had a greater effect upon the pharmacokinetics of cotinine.[7] The nonrenal or metabolic clearance was increased by a factor of 3. During pregnancy, the nonrenal clearance of cotinine averaged 0.09 L/h/kg, while 3 months postpartum it averaged 0.03 L/h/kg. This change in clearance resulted in a substantial decrease in the elimination half-life of cotinine during pregnancy. The mean elimination half-life of cotinine during pregnancy was 9 hours, while postpartum the mean was 17 hours. Plasma cotinine concentrations appear to be significantly lower during pregnancy, which alters the correlation between cotinine concentrations and the number of cigarettes smoked per day.[8,9] The increased clearance of cotinine accounts at least in part for the decreased saliva and plasma cotinine concentrations reported during pregnancy.[8,9]

2.2.2 Plasma Concentrations Associated with Nicotine Replacement Therapy During Pregnancy

Plasma nicotine and cotinine concentrations have been determined in a few investigations of the short term effects of nicotine gum and transdermal nicotine patches in pregnant women. Two studies have reported nicotine concentrations associated with use of transdermal patches, and 4 studies have reported concentrations associated with chewing gum. All the data reported here are means.

Plasma Nicotine Concentrations Associated with Use of Transdermal Patches

Oncken et al.[10] obtained plasma nicotine samples from 15 pregnant smokers while they wore a 21mg transdermal patch for 8 hours. Blood samples were collected before and 2, 3, 4, 6, and 8 hours after patch placement. During a separate study visit, women were allowed to smoke *ad libitum*, and samples were collected hourly. Based on graphical data, plasma nicotine concentrations had

reached a mean steady state level of approximately 14 μg/L by the time of the first sample collection. There was a slight decline in nicotine concentrations over the final 4 hours of wearing the patch, and the mean concentration was approximately 10 μg/L when the patch was removed. Plasma nicotine concentrations while on the patch were very similar to nicotine concentrations associated with *ad libitum* smoking. There is a report of nicotine concentrations in saliva associated with patch use during pregnancy,[11] but the results are hard to interpret. In nonpregnant adults, nicotine concentrations in saliva are 8 times greater than those in plasma.[12] Yet in this report, the salivary nicotine concentrations were similar to plasma concentrations reported by Oncken et al.[10] Also, there was huge variability and fluctuations in the salivary nicotine concentrations. For these reasons, these data will not be reviewed here.

Ogburn et al.[8] studied 21 pregnant smokers who wore a 22mg transdermal nicotine patch for 24 hours per day for 4 days while abstaining from smoking on a hospital research ward. There was no significant difference in the afternoon nicotine concentrations seen in the women when they wore the patch as compared to when they were smoking *ad libitum*. On the first 2 days of wearing the patch, the morning nicotine concentrations were higher than those achieved while smoking, but on the third and fourth hospital days there was no difference in concentrations associated with the patch or with smoking. The plasma data indicate that nicotine patches will produce plasma concentrations in pregnant women that are similar to those seen in nonpregnant adults.

Plasma Nicotine Concentrations Associated with Gum Use

Gennser et al.[13] determined nicotine plasma concentrations in 6 pregnant smokers who chewed 2 or 4mg pieces of gum for 30 minutes. Blood samples were obtained before, and 30 and 60 minutes after the onset of chewing nicotine gum. Thirty minutes after chewing the 2mg gum, the mean plasma nicotine concentration rose by 3 μg/L and at 60 minutes it was 2.5 μg/L above the mean basal level. Chewing the 4mg gum resulted in a more pronounced rise in nicotine concentrations. After 30 minutes, the mean nicotine concentration had risen by 9.2 μg/L and after 60 minutes the mean concentration was 6.4 μg/L above basal levels. The amount of nicotine in the gum, before and after chewing, was also determined. The mean amount of nicotine released from the 2mg gum was 1.35mg, while the mean amount released from the 4mg gum was 2.88mg. Although the 4mg gum appeared to deliver twice the dose of the 2mg gum, the change in plasma nicotine concentrations for the 4mg gum was 3 times that of the 2mg gum. The reason for this is unknown.

Manning and Feyerabend[14] determined plasma nicotine concentrations in 7 pregnant smokers after chewing one 4mg piece of nicotine gum and in 5 women after concurrently chewing 2 pieces of 4mg nicotine gum. The gum was chewed for 20 minutes and the amount of nicotine released from the gum was not determined. Blood samples were obtained before and 30 and 60 minutes after the onset of chewing nicotine gum. 30 minutes after chewing the 4mg piece of nicotine gum, the mean plasma nicotine concentration rose by 4.4 μg/L and after 60 minutes it was 3.6 μg/L above the basal concentration. For the 8mg gum, the 30 minute mean nicotine plasma concentration rose by 14.9 μg/L and after 60 minutes the mean was 9 μg/L above the basal concentration. A doubling of the nicotine gum dose from 4mg to 8mg resulted in a 3.4-fold rise in the nicotine concentration.

Lindblad et al.[15] determined plasma nicotine concentrations in 12 pregnant smokers before and 25 and 45 minutes after chewing 1 or 2 pieces of 4mg nicotine gum. The 2 pieces of gum were chewed 1 after another, not concurrently. Each piece of gum was chewed for 25 minutes. Based on graphed data, 25 minutes after chewing one 4mg piece of gum, the nicotine plasma concentration had risen by 5 μg/L and was 3 μg/L above the basal concentration after 45 minutes. 25 minutes after chewing 2 pieces of gum, the concentration was approximately 6.5 μg/L above the basal concentration and

after 45 minutes it was approximately 12 μg/L above the basal concentration.

Oncken et al.[16] obtained blood samples from 19 pregnant smokers who chewed nicotine gum (2mg) *ad libitum* (maximum 30 pieces per day) for 5 days while refraining from smoking. Women were considered to be abstinent if exhaled CO concentrations were below 8 ppm. On average, the women chewed between 6 and 9 pieces of gum per day. Two of the 19 women could not maintain abstinence. Blood samples were collected on the afternoon of the fifth day. Mean peak and trough nicotine concentrations were 5.7 μg/L and 3.3 μg/ L, respectively.

These data indicate that the gum can effectively raise plasma nicotine concentrations during pregnancy, but the data are variable in the magnitude of the rise in nicotine concentrations. Also, with dose escalation, the mean plasma nicotine concentration appeared to increase by a factor that was greater than the increase in the dose.

2.2.3 Summary

Pregnancy affects the disposition of nicotine by increasing the metabolic clearance of both nicotine and cotinine. But, broadly speaking, the plasma nicotine concentrations associated with standard doses of NRT during pregnancy appear to be in the therapeutic range. For any level of intake of nicotine, cotinine concentrations are significantly lower during pregnancy, which alters the correlation between cotinine concentrations and the number of cigarettes smoked per day.[8,9]

2.3 Nicotine Disposition in the Maternal-Fetal Unit

The disposition of a drug between mother and fetus are an important consideration in understanding the potential fetal toxicity of a drug taken during pregnancy. The important anatomical elements in considering maternal-fetal drug transfer are as follows. On the maternal side (maternal compartment), blood flows through the uterine artery to the uterus and then through the subsidiary vessels into the placenta. The syncytiotrophoblast or syncytium is the very thin layer of tissue in the placenta that separates the maternal blood from the fetal blood. Nutrients, drugs, and waste products cross the syncytium between the mother and fetus. On the fetal side, the 2 umbilical arteries carry fetal blood into the placenta and the single umbilical vein carries blood away from the placenta back to the fetus. The fetus is immersed in amniotic fluid. The amniochorionic membrane, which makes and surrounds the amniotic fluid, is adhered to the inside of the uterus not covered by the placenta.

Typically, a drug diffuses from the maternal blood across the syncytium into the fetal blood. Maternal drug metabolites, depending upon their charge and concentration, may also diffuse across the syncytium. In general, the diffusion of a drug is dependent upon the blood concentration in the mother and in the fetus. When the concentration is higher in the mother, the diffusion is in the direction of the fetus. When the fetal concentration of the drug is higher, then the diffusion direction is towards the mother. How much drug actually diffuses from the mother into the fetus is dependent upon the lipid solubility of the drug, the molecular weight of the drug, and other factors. Some endogenous substances also diffuse across the amniochorionic membrane into and out of the amniotic fluid. Transfer across the amniochorionic membrane is also a potential route of transfer for drugs.

Nicotine moves readily from the maternal circulation into the fetus. The major route of fetal elimination is via transfer back into the maternal circulation. Data on maternal-fetal disposition of nicotine are available from studies in sheep, (unpublished observation) monkeys[17] and directly from humans.[18,19] Animal studies show rapid transfer of nicotine across the placenta, with the peak concentrations in the fetal circulation seen within 15 to 30 minutes of maternal administration.[17] In monkeys, nicotine concentrations in mother and fetus are similar but those in the fetus are higher and persist for a longer period of time than in the mother.[17] In sheep, nicotine concentrations in mother and fetus are similar. In the sheep fetus, the clearance of nicotine is similar to umbilical blood flow, indicating that transfer of drug to

the mother is the major route of elimination. Fetal renal clearance and metabolism account for less than 2% of total clearance in the fetal sheep (unpublished observation).

The data in humans derive from sampling maternal blood, umbilical venous blood (fetal origin) and amniotic fluid at various times during gestation. Luck and Nau.[18,19] showed a high correlation between maternal blood and amniotic fluid concentrations of nicotine and cotinine sampled at 16 to 24 weeks gestation. The average ratio of amniotic fluid to maternal blood concentrations was 1.54 for nicotine (n = 23) and 0.72 for cotinine (n = 29). The ratio of mean umbilical blood to maternal blood nicotine and cotinine concentrations were 1.12 and 1.05 in the study by Luck and Nau[18,19] A study by Donnenfeld et al.[20] of maternal and percutaneous umbilical blood samples obtained from pregnant women at 21 to 36 weeks gestation found a fetal to maternal ratio for cotinine of 0.9 (n = 11). In isolated perfused human placental cotyledons, the maternal to fetal concentration ratio of the perfusates reached 1.0 after about 60 to 80 minutes.[21]

3. Reproductive Toxicity of Nicotine

3.1 Uteroplacental Insufficiency

Uteroplacental insufficiency has been commonly cited as the mechanism responsible for fetal growth retardation and placental abruption associated with cigarette smoking. This hypothesis proposes that nicotine causes vasoconstriction of uteroplacental blood vessels which reduces blood flow to the placenta and decreases delivery of oxygen and nutrients to the fetus. The daily episodes of transient reductions in blood flow and delivery of oxygen and nutrients to the fetus have been postulated to be the pathophysiological mechanism of fetal growth retardation. This hypothesis gained widespread acceptance without solid data to support it.[22] However, the role of uteroplacental insufficiency as the mechanism of fetal growth retardation has been questioned recently because studies of surrogate measures of blood flow in humans have failed to demonstrate an association with fetal growth retardation except when the changes are profound (see section 4.5).[22-25]

3.2 Acute Effects of Nicotine During Pregnancy in Humans

The results of 42 human studies investigating the acute cardiovascular effects of nicotine and cigarette smoking during pregnancy are presented in appendix I. Studies which investigated nicotine are discussed here and those which investigated cigarette smoking are presented in section 5.1. A study in 21 pregnant smokers who wore a 22mg transdermal nicotine patch (24 hours/day) for 4 days and received extensive monitoring over 4 days, found no change in the maternal blood pressure or any fetal cardiovascular effects except for a small decrease in the fetal heart rate in the morning.[8] This decrease was statistically but not clinically significant.[8] No change in fetal heart rate reactivity was found.[8] There are 2 other studies in which a nicotine patch was worn for 6 to 8 hours.[10,11] Both studies found a gradual increase in maternal heart rate. One study found no change in any fetal parameters,[11] while the other found an increase in loss of fetal heart rate reactivity compared with cigarette smoking.[10] It is difficult to interpret the fetal heart rate reactivity data in this study, since the mean gestational age of the pregnancies was 28 weeks, and fetal heart rate reactivity is not a reliable measure for fetal well-being until 32 weeks gestation.

A number of investigations have studied the effects of nicotine gum during pregnancy.[13-16,34,42] These studies have found dose-related effects of nicotine gum on maternal heart rate and blood pressure, and attenuated effects on fetal heart rate and fetal breathing. These effects are less pronounced than the effects of cigarette smoking. Overall, nicotine patches or gum do have some measurable cardiovascular effects during pregnancy, but the effects are small and do not appear to compromise the mother or fetus. These effects should not limit the use of NRT during pregnancy in healthy smokers who wish to quit.

3.3 Acute Effects of Nicotine in Monkeys and Sheep

The acute effects of nicotine in pregnant monkeys and sheep are summarised in appendix II (includes doses, routes of administration and outcome). Prolonged enforced smoking in monkeys resulted in a persistent drop in the maternal and fetal arterial oxygen tension (PaO_2) independent of the presence of nicotine in the smoke.[57] Large bolus doses of nicotine in pregnant monkeys resulted in significant cardiovascular effects, while infusions at lower doses had much smaller cardiovascular effects.[58] Nicotine infusions in the monkey used doses that are 100 times the doses used in humans (see appendix II).[7,58] These high doses in monkeys resulted in a 39% decrease in uterine blood flow, and acidosis and hypoxia in the fetus.[59]

In pregnant sheep, large bolus doses of nicotine had significant cardiovascular effects on the ewe, and lesser effects on the fetus.[61-64,66] At lower bolus doses of nicotine or infusions given over 10 or more minutes, the effects were attenuated or did not occur (see appendix II).[62,63,65,66] Although some of these studies appear to support the uteroplacental insufficiency model, overall they do not, because very high doses were required to demonstrate these effects, the animals were stressed from instrumentation and the animals were often sedated.[23] Additionally, often no effects were found at the lowest doses used in animal studies (see appendix II), which were still far above the doses of nicotine delivered by smoking.

3.4 Rodent Studies of Long Term Nicotine Exposure and Toxicity

Many rodent studies have demonstrated deleterious reproductive effects of nicotine that are separate from the cardiovascular effects of the drug. Animal research also allows separation of the toxicity of nicotine from the toxicity of the other components of cigarette smoke. The results of studies which examined the effects of tobacco smoke and nicotine on pregnant animals are presented in appendix III (includes species, doses, routes of administration and outcomes). The outcomes include effects upon growth, behaviour, the CNS, the peripheral nervous system, neuroendocrine system, blood flow, and others. The majority of the studies used bolus or continuous parenteral nicotine administration. In most early studies, nicotine was delivered as multiple bolus doses per day by the subcutaneous or intraperitoneal route. These large doses affect the cardiovascular systems of the mother and fetus and cause ischaemic hypoxic/anoxic damage to the fetal CNS. Later, implantable osmotic minipumps were developed to give a continuous subcutaneous infusion that delivered a dose more comparable to smoking or administration of transdermal nicotine.[194] This mode of administration eliminated the acute cardiovascular effects of nicotine and enabled researchers to study the effects of nicotine on the developing fetus in the absence of excessive haemodynamic stress.

Few studies have reported plasma nicotine concentrations. Most investigators have relied upon the work of Murrin et al.[194] to estimate the plasma nicotine concentrations from the dose delivered by osmotic pump. These authors implanted minipumps which provided continuous nicotine infusions of 0, 1.5, 4.5 or 13.5 mg/kg/day to pregnant rats. Based on the Murrin data, for every 1 mg/kg/day of nicotine infused, the steady state plasma nicotine level will rise by approximately 13 to 15 μg/L. A maternal nicotine dose of 6 mg/kg/day, expected to give a plasma nicotine concentration of 80 to 90 μg/L, has consistently produced abnormalities in the offspring of pregnant rats.

A cigarette typically delivers 1mg of nicotine to the smoker. A woman who smokes 20 cigarettes per day and weighs 60kg would receive a daily nicotine dose of 0.33 mg/kg/day and would have a peak venous plasma nicotine concentration between 15 and 30 μg/L after smoking a cigarette. This concentration falls during the time interval between cigarettes. Prior to the first cigarette in the morning, the plasma nicotine concentration is usually at or below 5 μg/L. In a study in which plasma nicotine concentrations were determined in pregnant smokers after smoking and while wearing a 22mg trans-

dermal patch applied for 24 hours per day, mean early morning venous plasma nicotine concentrations were 3.7 μg/L when smoking and 5 μg/L when using the patch; mean afternoon plasma nicotine concentrations were 20 μg/L when smoking and 11.2 μg/L when using the patch.[8]

Plasma concentrations associated with a continuous nicotine infusion of 6 mg/kg/day in rats are 3 to 4 times higher than the peak concentrations seen in humans and the doses required to achieve these concentrations are substantially higher in rats than in humans. Although plasma concentrations achieved in the rats are higher than those in humans, it is reasonable to extrapolate these results to humans.

3.4.1 Growth Effects

Inhalation of cigarette smoke for even several hours a day is the most toxic route of exposure, as evidenced by decreased birthweight, decreased litter size, and decreased survival of offspring.[75-77,171] Birthweight was adversely affected by tobacco smoke even though maternal weight was unaffected. This is in contrast to nicotine administration which results in reduced maternal weights at doses that do not reduce fetal weight.[72,137,177] Bolus doses of nicotine by gavage or subcutaneous or intraperitoneal administration usually resulted in birthweight reduction, decreased litter size, and decreased survival.[4] The minipump supplies a continuous dose of nicotine at daily doses that are similar to daily bolus administration and in many studies the daily dose was the same as that delivered by bolus doses in other studies. Administration with the minipump avoids the vasoconstrictive effects of bolus dosing.[4] The vasoconstrictive effects of nicotine are thought to be responsible for growth retardation and decreased litter size associated with bolus administration. Interestingly, 1 study that compared the effect of nicotine and epinephrine upon uterine blood flow in late pregnancy found that both drugs decreased uterine blood flow and reduced maternal weight gain, but neither affected fetal weight.[72] These data suggest that growth retardation may not be secondary to the cardiovascular properties of bolus dosing of nicotine. Minipump administration allows for the study of the cellular toxicity of nicotine separate from the vasoconstrictive effects of the drug. In general, continuous delivery of nicotine by minipump usually does not affect birthweight at lower doses and has only a minimal effect at 6 mg/kg/day.[4] The smoking and nicotine data indicate that nicotine is toxic to developing rodents at relatively high doses, but that the fetal growth retardation effects of smoking are probably due to other components of cigarette smoke.

3.4.2 Behavioural Effects

Prenatal and perinatal nicotine exposure by any route has been associated with dose-dependent alterations in selected behaviours and responses to cognition tests in young and adult rodents. There appear to be sex differences, with males more affected than females.[85,87,93,98,101,102,106] Nicotine-exposed animals have exhibited alterations in spontaneous locomotion or motor activity.[84,89,98,103,105] Other alterations were found in the righting reflex, male sexual behaviour, some maze tests, and swimming behaviours.[86,87,99,104]

3.4.3 CNS Effects

The CNS is the primary target organ for the toxic effects of cigarette smoking and nicotine upon development. The development of the CNS is very complex. There is a programme of sequential development for neurogenesis and synaptogenesis.[4] Neurogenesis begins with the proliferation of cells, then there is termination of replication and a switch to cellular differentiation. Further development involves migration of neurons and the development of complex cytoarchitectural configurations of various neurons. Finally, there is programmed cell death (apoptosis) of some neurons. Synaptogenesis refers to the events required for the development of synaptic competence. This involves the development of various types of receptors, receptor densities, synthesis of neurotransmitters, regulation of neurotransmitter synthesis, mechanisms for transmitter release, mechanisms for modulation and termination of receptor stimulation, and postreceptor signalling events. The set-

point of neural activity and synaptic competence appears to be determined during gestation.

During development, neurotransmitters have unique trophic effects. They communicate with genes, and neurotransmitter stimulation at specific developmental points may initiate or terminate cellular proliferation, differentiation, migration, or apoptosis, and may determine the set-point for neuronal reactivity which may persist into adulthood.[4] Nicotine simulates the function of a neurotransmitter at nicotinic cholinergic receptors (acetylcholine is the endogenous ligand at these receptors). The existence of CNS nicotinic receptors is well documented. Maternal smoking exposes the embryo/fetus to nicotine which may stimulate nicotinic cholinergic receptors at inappropriate times during development (that is, when acetylcholine is not normally present in significant levels), and thus affect the development of the cholinergic nervous system. The cholinergic system interacts with other neural projections. The adrenergic and cholinergic systems are highly interactive; therefore, during development, stimulation of cholinergic neurons may be expected to affect the developing adrenergic system, and possibly other systems as well.[4]

Nicotine is thought to exerts its toxicity by interacting with nicotine receptors at inappropriate times during gestation. There may be multiple critical times throughout human gestation when nicotine may exert a toxic effect upon nervous system development. Rodent studies (appendix III) have unequivocally demonstrated that exposure to nicotine during gestation adversely affects neurogenesis and synaptogenesis in the developing CNS.[4] Nicotine exposure during regional nicotinic receptor development in the rat results in the most severe local adverse effects. The most severe damage occurs in areas with the highest nicotinic cholinergic receptor density. Initially, the midbrain and brainstem are affected, then the forebrain (cerebral cortex), and then the cerebellum.[117] Inappropriate cholinergic stimulation appears to prematurely end cell replication and initiate cellular differentiation in the fetal CNS, resulting in a reduced number of CNS neurons in selected areas of the brain.[118] These events occur at nicotine doses that do not cause growth retardation, and often full recovery does not occur after nicotine is stopped.

Gestational nicotine exposure appears to have wide ranging effects upon synaptic competence (appendix III).[4] Various studies have demonstrated effects of nicotine upon transmitter synthesis, release, and turnover in the fetal or neonatal brain. The affected transmitters include acetylcholine, noradrenaline, dopamine, and serotonin.[134,145,146] A variety of alterations in the receptors for these neurotransmitters after exposure to nicotine have also been found and include alterations in receptor type, receptor density, receptor binding capacity, and G protein-mediated receptor density.[115,120,124,130,136,138,141]

3.4.4 Rodent Models of Sudden Infant Death Syndrome (SIDS)

The mechanisms responsible for SIDS are unknown and are probably multifactorial. Maternal smoking during gestation increases the risk of SIDS[195] and, independent of prenatal exposure, postnatal passive smoking by the infant also increases the risk of SIDS.[195,196] Epidemiologically, it is difficult to separate prenatal and postnatal exposure. One hypothesis predicates that SIDS results from an abnormality in the cardiovascular and respiratory response to hypoxia (assumed to be secondary to transient sleep apnoea or transient airway obstruction).[159] In the normal rat, the adrenal medulla of the fetus and neonate is not innervated. In response to hypoxia, there is massive catecholamine release by the adrenal medulla, which redistributes blood flow to the brain and heart and maintains the heart rate during an episode of hypoxia. The neonatal rat is dependent upon a response to hypoxia by the adrenal medulla that is independent of central reflexes. Eventually the adrenal medulla is innervated and loses its autonomous response to hypoxia.

Some neonatal rats that have had long term exposure to nicotine *in utero* lose their autonomous adrenal response to hypoxia before innervation and the development of central reflexes.[151,159] Nicotine appears to prematurely switch off the autono-

mous response to hypoxia. This provides a window of vulnerability to hypoxia. A proportion of these animals, in response to hypoxia, do not release catecholamines. During hypoxic stress, they develop bradycardia and die. The rat studies were somewhat extreme in comparison to the human situation. The gestational dose of nicotine was 6 mg/kg/day and the hypoxic insult was 60 or 75 minutes of 5% oxygen.[4] These data may still be relevant to humans for the following reasons. Fetal and young rats are much more tolerant to hypoxia than humans. SIDS has a low incidence and animal studies can only model infrequent human events by employing high doses of potentially causative agents and extreme measures. Whether adrenal catecholamine release in response to hypoxia is a valid model for the human pathophysiology of SIDS remains to be determined.

3.5 Summary and Caveat Regarding Nicotine CNS Toxicity and SIDS Data Derived From Animals

Clearly, nicotine is a neuroteratogen. The animal data demonstrate that nicotine is toxic to the developing CNS. The question is how to apply these animal data to humans; specifically, how should these data affect recommendations for NRT during pregnancy? In an individual woman, should the dose of nicotine in NRT be limited to no greater than that which she receives from smoking? Although nicotine is a CNS fetal toxin, cigarette smoke also delivers other CNS fetal toxins. CO is a well documented fetal neurotoxin (section 5.2).[197] We know little about the fetal toxicity from the thousands of other compounds in tobacco smoke. The potential human toxicity of nicotine must be balanced against the combined toxicity of nicotine, CO, and the other compounds in cigarette smoke. Animal studies that compare the nervous system toxicity of nicotine with that of tobacco smoke are generally lacking. The growth data indicate that inhaling cigarette smoke for a few hours a day is more toxic than relatively large doses of nicotine. It is doubtful that the smoking regimens in the animal studies delivered a nicotine dose comparable to those delivered by bolus injection or continuous minipump infusion, yet 1 rat study, involving 4 hours per day of tobacco smoke exposure, found reduction in cell number in the hindbrain.[112] It cannot be assumed from these animal studies that the fetal toxicity found in epidemiological studies of pregnant smokers is attributable to nicotine because there are so many fetal toxins in cigarette smoke and the nicotine doses used in these animal studies were high relative to the doses delivered by cigarettes or NRT.

4. Toxicity of Cigarette Smoke Upon the Pregnant Human and Fetus

Studies of the acute effects of smoking 1 to 2 cigarettes after overnight abstinence are the predominant data used to support uteroplacental insufficiency as the mechanism responsible for many adverse reproductive outcomes such as fetal growth retardation and placental abruption. It is important to review these studies because they are the basis for concerns regarding the acute toxicity of nicotine upon reproduction (even though there are actually studies of smoking cigarettes).

40 studies in humans have investigated the acute effects of cigarette smoking or nicotine upon the maternal cardiovascular system or upon the fetus (appendix I).

4.1 Maternal Heart Rate and Blood Pressure

In 1 study of 292 nonsmokers and 203 smokers, no differences in basal maternal heart rates were found.[27] An acute elevation in maternal heart rate and in systolic and diastolic blood pressure was commonly found with smoking a cigarette (appendix I). The increases were dose-dependent. Heart rate elevations were not associated with placebo gum or with smoking 1 herbal (non-nicotine) cigarette, but were associated with smoking 2 herbal cigarettes.[15] The effects of smoking upon maternal heart rate and blood pressure were modest and transient.

4.2 Fetal Heart Rate

25 studies have investigated the effects on cigarette smoking or nicotine upon the fetal heart rate. Newnham et al.[27] performed serial fetal sonograms at 18, 24, 28, and 34 weeks of gestation in 263 nonsmokers, 29 quitters, and 203 smokers. They found no significant association between fetal heart rates and maternal smoking, but did find the usual dose-dependent decrease in birthweight. A small study[28] of 19 nonsmokers and 5 smokers, which also involved serial fetal sonograms, did find that after 35 weeks gestation there was a statistically significant but not clinically significant elevation in the fetal heart rate in smokers compared to nonsmokers (144 *vs* 132 beats/min). Among the remaining 23 studies, smoking 1 to 2 cigarettes generally resulted in a transient elevation in the fetal heart rate, while chewing nicotine gum, wearing a nicotine patch, or smoking herbal cigarettes did not result in an elevation in the fetal heart rate. One study comparing *ad libitum* smoking with *ad libitum* nicotine gum did find an elevation in the fetal heart rate after chewing gum; but the effect of the gum upon the fetal heart rate was less than that of smoking (appendix I).

4.3 Fetal Heart Rate Reactivity

Various measures of fetal activity have been evaluated for their ability to predict fetal well-being. The modified Biophysical Profile (BPP) is the most widely used and accepted measure to assess the well-being of the fetus.[198] The validity of its use for surveillance for fetal well-being has been confirmed.[198,199]

The modified BPP consists of a fetal nonstress test (NST) and an amniotic fluid index (AFI). The NST consists of an unprovoked, continuous measurement of the fetal heart rate using ultrasound. A reactive NST is an immediate indicator of fetal well-being. After 32 weeks of pregnancy, the vast majority (90 to 99%) of NSTs in normal fetuses should be reactive.[200,201] In clinical practice, the majority of NSTs are performed after 32 weeks. The NST is less useful before 32 weeks gestation, because of the high rate of false positives.[200,201] The AFI is a measure of the amniotic fluid volume by ultrasound and reflects the long term adequacy of placental function. A reactive NST and an AFI between 5 and 25cm constitutes a normal (or negative) modified BPP. A BPP is usually done weekly in high risk pregnancies.

10 studies have investigated fetal heart rate reactivity. There are different ways that fetal heart rate reactivity is reported in the literature. More recently, NST results have been reported as reactive or nonreactive. Earlier studies reported increased or deceased short term reactivity (also known as the differential index, DI) and long term reactivity (also known as the interval index, II). A decrease in short and long term reactivity is similar to a nonreactive NST. Phelan[26] followed 478 high risk pregnancies, of whom 128 mothers were smokers. The smokers and nonsmokers had similar high risk diagnoses, except that intrauterine growth retardation (IUGR) was more common and preeclampsia significantly less common among smokers. 13% of the nonsmokers and 21% of the smokers had nonreactive NSTs ($p < 0.005$). Among the nonsmokers with nonreactive NSTs, the NSTs remained nonreactive at subsequent NSTs which is associated with a poor prognosis; among the smokers, the subsequent NSTs were often reactive indicating a transient effect of smoking upon the NST, consistent with frequent false positive NSTs among smokers. A transient decrease in fetal heart rate reactivity has been consistently found in studies where the fetal heart rate reactivity was monitored before and after smoking 1 or 2 cigarettes (appendix I).

4.4 Blood Flow Studies in the Fetus and Mother

Four arteries, 2 uterine and 2 ovarian, supply the maternal placental circulation. These arteries extensively arborise before they reach the placenta. By the time they reach the placenta, they have become funnel shaped, low resistance, dilated uteroplacental arteries which lack a muscular layer. Across this branching arterial system there is a large drop in blood pressure. The anatomic changes

that occur and the great drop in pressure across the arterial system of the pregnant uterus allow for increased blood flow to the placenta as pregnancy advances. Blood flow in this complex system cannot be studied directly in humans because it requires radioactive isotopes. Blood velocity or the systolic : diastolic ratio are surrogate measures of flow blood and can be determined by Doppler studies. Doppler studies of these surrogate measures in the placenta and fetus are not sensitive predictors of outcome except when the changes are profound.[24,25]

19 studies examine various parameters of fetal blood flow, such as cardiac output, blood vessel diameter, velocity indices, etc. In general, there are transient effects in response to cigarette smoking, chewing nicotine gum, or wearing a nicotine patch. A large prospective study of basal umbilical artery systolic : diastolic ratios found no significant difference among 535 nonsmokers, quitters, light smokers, and heavy smokers.[27] A much smaller study of 19 nonsmokers and 5 smokers found that among smokers there was a significant increase in basal aortic blood flow, and in mean and peak aortic blood flow velocities.[28]

It is difficult to summarise the fetal blood flow data because they are inconsistent, and often different parameters are reported (pulsatile indices, resistance indices, velocity, velocity indices, systolic : diastolic ratios, blood flow) which are not easily compared. Studies, even by the same authors, have reported data suggestive of no change in fetal aortic blood flow,[29,33,34,55] increased fetal aortic blood flow,[15,29,30,202] and decreased fetal aortic blood flow.[15] Similarly, contradictory results have been reported for umbilical artery, umbilical vein, and uterine artery blood flow parameters. Even the few studies of intervillous blood flow in the placenta have been contradictory. Lehtovirta and Forss[39] reported that overall there was a decrease in the mean intervillous blood flow after smoking a cigarette which returned to baseline within 15 minutes. But an examination of the data revealed that 5 of 12 women in the study had increased intervillous blood flow.[39] Other studies[40,41,46] have also reported inconsistent findings within the same type of study. Studies of smoking or nicotine gum or patches in healthy pregnancies mainly confirm that nicotine has modest, transient pharmacological effects upon the cardiovascular system in the mother and fetus.

4.5 The Inadequacy of the Uteroplacental Insufficiency Model

There are a number of problems with uteroplacental insufficiency as an explanation for the deleterious effects of smoking upon the fetal-maternal unit. These studies were conducted in the morning after overnight abstinence and required that the mother vigorously smoke the cigarette. The acute response to smoking is maximal with the first cigarette, and some studies involved smoking 2 cigarettes in succession. With repeated administration, tolerance develops to the effects of nicotine. In nonpregnant adults the effects occurring in the morning are greater than the effects occurring through the day. Many human studies, as summarised in appendix I, found statistically significant differences in uteroplacental blood flow before and after smoking 1 to 2 cigarettes. But the clinical significance is not obvious since the changes are often within normal limits.

Fetal growth retardation associated with cigarette smoking begins in the first trimester. During the first 2 trimesters, the fetus is so small that the placenta can easily deliver more than adequate nutrition to the fetus, and uteroplacental insufficiency cannot be a mechanism for fetal growth retardation. Possibly, during the third trimester, uteroplacental insufficiency may contribute to fetal growth retardation because of rapid fetal growth. In general, Doppler studies during pregnancy have been unable to correlate placental blood flow or systolic : diastolic ratios with pregnancy outcome, except among severely growth-retarded fetuses where there is reverse diastolic blood flow.[24,25] The physiology of maternal blood flow in the placenta is that of a low pressure system designed to maintain fetal oxygenation and nutrition during labour when very strong contractions lasting 60 seconds

are reducing blood flow to the placenta. The maternal and fetal blood vessels on either side of the placenta lack musculature and are unresponsive to vasoactive agents. Physiologically, it is difficult to envision a system that can accommodate labour, but cannot accommodate the minor increases in vascular resistance associated with smoking.

Animal studies have found that nicotine does reduce blood flow to the fetal placental unit (see appendix II and section 3.3). But Lambers and Clark,[23] who conducted some of these animal studies and have written an extensive review, found them inadequate as an explanation for the deleterious effects of smoking upon pregnancy. Animal studies usually involve very high doses of nicotine. The animals are instrumented, which is stressful to the mother and fetus. Sedation, which is required to maintain instrumentation, may alter the haemodynamics of nicotine and could accentuate their cardiovascular effects.

The vascular effects of nicotine were the first effects to be demonstrated, but may not be the most important mechanism of the deleterious effects of smoking. The myriad of cellular effects presented in the next section and in appendix IV indicate that the pathophysiology of smoking-induced adverse reproductive effects are complex and many occur at the cellular level. The haemodynamic effect of nicotine is one of the many effects of cigarette smoking and uteroplacental vasoconstriction alone is insufficient to explain placental abruption or fetal growth retardation. The lack of data to support the uteroplacental insufficiency model is important when considering the use of NRT during pregnancy. The greatest concern about adverse effects of NRT on the mother during pregnancy has been that nicotine will acutely affect placental blood flow. However, the data reviewed above (section 4.4) suggest that NRT does not significantly decrease placental blood flow.

5. Toxicity of Cigarette Smoke During Pregnancy

Cigarette smoking exposes the fetus to more than 3000 chemicals in smoke.[2] The major toxic components in cigarette smoke are nicotine, CO, ammonia, nitrogen oxide, hydrogen cyanide, hydrogen sulfide, acrolein, methanol, pyridine, phenol, aniline, many carcinogens, lead, and other metals (table II).[2] Different toxins in cigarettes might cause similar adverse outcome. Nicotine, CO, and lead are all fetal neurotoxins.

5.1 Cellular Mechanisms of Nicotine- and Cigarette Smoking-Related Complications of Pregnancy

In the past 2 decades, many cellular effects of cigarette smoking upon the uterus, placenta, and fetus have been discovered and these actions may be more important than the vascular effects of nicotine. In this section (and in appendix IV) we review these cellular effects and discuss selected mechanisms of toxicity. It is important to note that, in general, the mechanisms involved in the adverse outcome of pregnancy are incompletely understood. The mechanisms underlying premature rupture of membranes, fetal growth retardation, placental abruption, placenta previa, etc., are believed to involve multifaceted pathogenic pathways, but their actual pathophysiology is still obscure.[269] For a particular adverse outcome, changes (hormone levels, enzyme expression or activity, cellular morphology, etc.) have been observed, but how the various changes are integrated into a whole and which are the most important is often unknown. The changes associated with maternal smoking must be viewed in the context of limited understanding of the underlying pathophysiological processes. Usually the smoking data are too limited to ascertain if the observed effect of smoking has a clinically significant involvement in an outcome.

5.1.1 Estrogen Production

Plasma levels of estrogen in pregnant smokers are approximately 90% of those found in nonsmokers.[232,241] During pregnancy, most estrogen is formed by the placenta from androstenedione supplied by the fetal adrenal glands. The reaction is catalysed by placental aromatase. Heavy smokers have lower placental aromatase activity than nonsmokers.[242] Nicotine, cotinine, and anabasine

(a constituent of cigarette smoke) all inhibit the conversion of androstenedione to estrogen.[240] Subsequently, it was found that 2 other constituents of cigarette smoke and tobacco leaf, N-N-octanoylnornicotine and N-(4-hydroxyundecanoyl) anabasine,[243,244,270] are competitive inhibitors of aromatase and are significantly more potent inhibitors than nicotine. The effect of smoking upon estrogen production illustrates an important general point – multiple chemicals in cigarette smoke may share the same toxic effect.

5.1.2 Immunological Effects

Smoking is associated, in a dose-dependent manner, with premature rupture of membranes, especially when it occurs prior to 33 weeks.[271-273] The mechanisms whereby smoking contributes to preterm rupture of membranes and preterm labour are not known. Numerous lines of evidence indicate that focal damage to the membranes results in their subsequent rupture;[269] and that focal infection and inflammation appear to be common causes of focal damage.[269] Smoking could increase the risk of infection-induced preterm rupture of membranes by lowering local immunity and facilitating the ascent of vaginal micro-organisms into the uterine cavity.[274-276] Smoking has been shown to affect the immune system in the nonpregnant tissues, and may have similar effects in the reproductive tract. Macrophages of smokers have decreased phagocytic capability.[277] In mucosal tissue, such as the vagina, immunoglobulin (Ig)A is an important nonspecific defence mechanism. Salivary IgA is decreased in nonpregnant adult smokers.[275] The secretory component of IgA is 50% higher in the serum of pregnant smokers.[257]

5.1.3 Ascorbic (Vitamin C)

Premature rupture of membranes, especially prior to 32 weeks gestation, is associated with smoking. The tensile strength of the amniochorionic membrane is predominately attributable to the collagen layer of the amnion.[269,278] Ascorbic acid (vitamin C), primarily obtained from amniotic fluid, is required for the biosynthesis of collagen by amnion epithelial cells.[279] Low ascorbic acid levels are associated with premature rupture of membranes.[280] Reduced availability of ascorbic acid may impair the strength of the amnion and facilitate rupture of membranes. The ascorbic acid concentration in amniotic fluid is reduced by 50% among smokers.[281] Reduced ascorbic acid levels in smokers are independent of dietary intake,[282] but are a result of the increased utilisation of ascorbic acid as an antioxidant to detoxify the oxidant gases of cigarette smoke. Additionally, placental villous calcifications are increased among smokers, and this abnormality is lessened by increasing the maternal intake of ascorbic acid, betacarotene and tocopherol (vitamin E).[215] Other smoking-related effects upon collagen can be found in appendix IV. Finally, a relative ascorbic acid deficiency may impair immune function.[281,283] Thus, smoking, via its effect upon ascorbic acid, could facilitate infection during pregnancy.[284,285]

5.1.4 Zinc, Copper and Cadmium

Zinc- and copper-containing enzymes are found in all tissues, including pregnancy-related tissues. Zinc is important for the stabilisation of membranes. Zinc metalloproteinases are found in the amnion and placenta and are important in maintaining the intracellular matrix that contributes to the strength of the amnion. DNA and RNA polymerases are also zinc-containing enzymes. Amniotic fluid zinc is involved in antibacterial activities. A copper enzyme, lysyl oxidase, is required for cross-linking of collagen and elastin which are necessary to produce the tensile strength of the amnion. Deficiencies in both copper and zinc may have a role in the pathogenesis of preterm rupture of membranes. Both lower maternal blood zinc and lower maternal and cord blood copper levels have been found among women with preterm rupture of membranes, and among women with term pregnancies whose membranes have ruptured prior to labour.

Cadmium from cigarette smoking may affect levels of both zinc and copper during pregnancy. Cigarette smoking is the major source of cadmium even among those living near cadmium-emitting industries.[286-290] Cadmium has an elimination half-life of 7 to 30 years, so that cadmium accumu-

lates in the bodies of smokers. Maternal smoking affects umbilical cord blood and placental zinc concentrations.[286,291-293] This may be attributable to the trapping of zinc in the placenta by cadmium[286,294] Smoking during pregnancy appears to adversely affect older women disproportionately, and this might be attributable to the accumulation of cadmium associated with years of smoking.[292] There may also be an interaction between cadmium and copper in the placenta[294] and in amniotic fluid.[295] In cell culture, cadmium inhibits procollagen production,[296] and its effect upon amnion metallothionein may limit the availability of copper to lysyl oxidase, the enzyme that cross-links collagen.[295]

5.1.5 Fibronectin

Fibronectin is a glycoprotein formed by many tissues including the placenta and amnion.[297] It is found in high concentrations in maternal blood and amniotic fluid. It is thought to be important in intracellular adhesion, including the adhesion of the placenta to the decidua basalis.[298] Among pregnant smokers, amnionic fibronectin output is decreased while placental fibronectin output is increased.[219] In cultured fibroblasts, fibronectin production is inhibited by cigarette smoke extract.[299] The extract was less inhibitory after removal of the volatile components. Acrolein and acetaldehyde, 2 volatile components, individually inhibited fibronectin production.[219]

5.1.6 Amino Acid Transport

Amino acid transport across the placenta is disrupted by maternal smoking.[300,224] Nicotine or cotinine may not be the responsible agents, since they do not inhibit transport of a probe amino acid in placental vesicles or perfused placental tissues.[223] In placental vesicles, nicotine was found to inhibit glycine transport on the fetal side but not on the maternal-facing basal membrane.[225] Theoretically, disruption of the transport of essential amino acids may interfere with protein synthesis and contribute to impairment in the strength of the amnionchorionic membrane.[269]

5.1.7 Nitric Oxide

Nitric oxide is a potent myometrial relaxant,[297] but whether or not it has a significant role in preterm labour is unclear.[301] Nitroglycerin, a nitric oxide donor drug,[297,301] is an effective tocolytic.[297] Maternal smoking is associated with decreased placental nitric oxide synthetase activity[222] and decreased production of nitric oxide precursors in umbilical arteries.[221] In vascular endothelial cells, smoking impairs release of nitric oxide and this is reversed by antioxidants, indicating that oxidant stress is responsible for the inhibition.[302] Smoking could contribute to preterm labour by its effect upon nitric oxide.

5.1.8 Eicosanoids and Related Compounds

Platelet activating factor (PAF) and prostaglandins are believed to be involved in the initiation and maintenance of labour at term. Platelet activating factor is secreted by the term fetus into the amniotic fluid. As PAF levels rise, phospholipase A is activated which releases arachidonic acid that results in prostaglandin synthesis. Amniotic fluid levels of prostaglandins rise during labour. Infection is commonly associated with preterm labour. Bacterial contamination of the amniotic fluid will induce release of cytokines from monocytes. Cytokines are known to activate phospholipase A, which releases arachidonic acid which is in turn metabolised to prostaglandin E2 and prostaglandin $F_2\alpha$. Cytokines also stimulate PAF synthesis which stimulates prostaglandin synthesis. As prostaglandin levels rise in amniotic fluid, uterine contractions start. This cascade of events occurs prior to the development of clinical chorioamnionitis. Cigarette smoking may contribute to preterm labour by its effect upon PAF. PAF is inactivated by PAF-acetylhydrolase. Components of cigarette smoke (other than nicotine and cotinine) inactivate PAF-acetylhydrolase.[256] Reduced inactivation of PAF due to smoking would allow more PAF to cross the amniochorionic membrane or placenta and reach the myometrium.[301] Other effects of cigarette smoking upon eicosanoids may be found in appendix I.

5.2 Carbon Monoxide (CO) Effects During Pregnancy and Development

CO merits its own section because it is present in substantial concentrations in tobacco smoke and is a well established reproductive toxin. The dose of CO per cigarette is 10 to 20 times the dose of nicotine. CO is a potent fetal toxin. Accidental symptomatic maternal exposures to CO from which the mother makes a full recovery may result in stillbirth or permanent neurological damage to the fetus.[197,303,304] There is a large body of literature regarding the fetal effects of CO.[197]

5.2.1 Interactions with Haemoglobin

CO tightly binds to maternal and fetal haemoglobin at sites that normally bind oxygen with an affinity that is over 200 times that of oxygen.[305] Carboxyhaemoglobin (COHb), the carbon monoxide-haemoglobin complex, has an elimination half-life of 5 to 6 hours. The COHb concentration in the blood of smokers averages 5 to 10%. At steady state, there is a linear correlation between maternal and fetal carboxyhaemoglobin ($r = 0.8$).[306-308] Fetal haemoglobin binds CO more avidly than adult haemoglobin, and fetal levels of COHb are higher than maternal levels.[307] There is a lag of up to 7 hours before there is full equilibration between fetal and maternal COHb levels.[307] *In vivo* data on fetal and maternal carboxyhaemoglobin give a fetal/maternal ratio of 1.8,[308] although this varies with the time interval between smoking and sample collection.[306] The long elimination half-life of CO in the maternal blood and the lag between maternal-fetal equilibrium means that CO does not clear from the fetus during maternal sleep.

The elevated COHb in smokers results in a functional anaemia and stimulates erythrocyte production. Thus, elevated haematocrits are seen in pregnant smokers and in their fetuses.[226,259,261] The elevated haematocrit increases the viscosity of the blood and places the newborn at risk for stroke and exchange transfusions.[258,309] This effect upon haematocrit and viscosity may adversely affect the placenta.[261] Data indicate that higher maternal blood viscosity is a risk factor for suboptimal placental perfusion.[310]

CO impairs oxygen release from haemoglobin to tissue.[307,311] When oxygen rich blood flows through tissue capillary beds, the low tissue oxygen tension precipitates the release of oxygen from haemoglobin. The fetus functions on the steep part of the oxyhaemoglobin dissociation curve and a small decrease in oxygen tension results in a major decrease in haemoglobin saturation and unloading of oxygen.[312] CO shifts the oxyhaemoglobin dissociation curve to the left, which means that the oxygen tension of the blood decreases to lower than normal values before a given amount of oxygen will be released from haemoglobin. This impairs transfer of oxygen from the mother to the fetus, and from fetal blood to fetal tissue. Thus, CO results in chronic cellular hypoxia in the fetus.[307,311,313] Cellular hypoxia may be a mechanism for fetal growth retardation associated with maternal cigarette smoking. In animals, even mild long term CO exposure with maternal COHb levels in the 4 to 9% range resulted in fetal growth retardation.[314,315] The carboxyhaemoglobin levels associated with smoking are between 5 and 10%.

5.2.2 CNS Effects

The deleterious effects of CO exposure upon the developing CNS are well documented in animals and in humans.[197,303] CNS abnormalities were found among fetuses and pups of pregnant rats chronically exposed to CO concentrations that produced maternal COHb levels (11.5 to 27%) at or slightly above those seen in smokers.[316-322] Similar abnormal findings to the above have been reported after nicotine exposure in the rat (see appendix III). Behavioural studies of animals prenatally exposed to CO have revealed persistent postnatal effects associated with exposure that produced maternal COHb levels in the 6 to 16% range.[319,323-329]

5.2.3 Other Toxicities of CO

In animals, CO exposure is associated with fetal cardiac hypertrophy.[197,330-332] An elevated resting heart rate has been noted among adult rats who were exposed to CO in the early postnatal period.[176] Alterations in levels of various growth

factors have been found among pups after long term exposure to CO.[197] There are 2 other possible general mechanisms whereby CO could affect the developing CNS. First, CO binds to other heme-containing proteins besides haemoglobin.[334] Heme-containing proteins are ubiquitous in the body. Secondly, CO may function as a neurotransmitter, in a manner similar to nitric oxide,[335-337] and might result in inappropriate receptor simulation during fetal development. CO is undoubtedly detrimental to fetal growth and development.

6. Recommendations Regarding Nicotine Replacement Therapy During Pregnancy – Risks versus Benefits

Human and animal data indicate that the risk of cigarette smoking during pregnancy is far greater than the risk of exposure to pure nicotine. However, the use of NRT is not without potential risks, and the magnitude of the risk of NRT to the mother and fetus is unknown. On balance, the use of NRT to aid smoking cessation during pregnancy seems reasonable. However, at this time, our primary recommendation is that NRT be studied in clinical trials as adjunctive therapy for smoking cessation during pregnancy for women who are unable to quit with behavioural therapy alone. Two research questions need to be addressed in clinical trials. First, is NRT efficacious; does it increase smoking cessation? Second, is it safe; what is the true risk of use to the mother and child? NRT could turn out not to be efficacious during pregnancy because the women who would require NRT are the most heavily addicted and the least able to quit even with the assistance of NRT. From an ethical perspective, efficacy and safety should be studied concurrently. No pregnant woman should take NRT unless there is personal benefit. The fetal risk associated with nicotine exposure, as indicated by animal studies, mandates that the safety of long term exposure be studied. Pharmacokinetic studies should be conducted within the context of efficacy studies.

6.1 Efficacy Studies of Nicotine Replacement Therapy During Pregnancy

The efficacy of NRT for smoking cessation should be studied during pregnancy. NRT should be studied within the context of a behavioural smoking programme, which will enhance overall cessation rates. Efficacy studies should collect safety data as an integral component of their design, even if underpowered to detect some adverse outcomes. Efficacy studies should not be delayed awaiting safety study results. Efficacy studies should not be delayed awaiting the completion of pharmacokinetic studies or animal studies.

6.2 Dose and Formulation of Nicotine Replacement Therapy

The goal of NRT is smoking cessation, working primarily through reduction of withdrawal symptoms including craving. An obvious safety goal is to limit the exposure of the mother and fetus to the minimum effective dose of nicotine. The NRT formulation used in efficacy studies should deliver the lowest total dose of nicotine to achieve control of withdrawal symptoms and craving. Although safety considerations will be used to make recommendations regarding selection of a particular formulation and dose of nicotine, ultimately the selection of a formulation and dose must rest on its ability to achieve smoking abstinence in an individual woman.

We suggest that intermittent-use formulations are preferred because they deliver a lower total daily dose of nicotine than formulations that provide continuous drug delivery. Intermittent-use formulations include nicotine gum, nicotine spray, and the nicotine inhaler. The transdermal patch, a continuous-use formulation, is less preferred because it delivers a greater total dose of nicotine than intermittent-use formulations. If used, the patch should initially be worn for only 16 hours a day, unless it is unable to provide control of withdrawal symptoms. Use for 16 hours is reasonable because available clinical data indicate that use of transdermal patches for 16 or 24 hours per day is equally effective.[338] The initial dose of nicotine in NRT

should be matched to the usual dose of nicotine that the mother receives from smoking.

Each study should develop a decision algorithm for continuation or termination of NRT in the event of continued smoking. It is recommended that cotinine, exhaled CO, and/or carboxyhaemoglobin levels be measured frequently to assess compliance with treatment. The duration of nicotine use during pregnancy should be limited to those typically used in smoking cessation programmes (6 to 12 weeks), and the dose should be weaned over time.

6.3 Safety Studies of Nicotine Replacement Therapy During Pregnancy

All efficacy studies should be designed to collect safety data. The statistical power of an efficacy trial and a safety study are very different. Very large safety studies will be required to measure infrequent or rare events like premature delivery or placenta previa. To overcome this limitation, a registry for NRT pregnancy efficacy trials should be set up to pool safety data. A pregnancy registry for NRT should be set up that collects prospective data from efficacy trials and from individual obstetrical care providers who prescribe NRT during pregnancy. Obstetrical standards of practice require that all prenatal care providers and hospital obstetrical services collect specific data on all pregnancies so collection of pregnancy outcome measures would not be an onerous task to include in efficacy studies.

7. Nicotine Replacement Therapy and Breast Feeding

The impact of nicotine and other pharmacotherapies on the infant postpartum is of concern because of the importance of achieving and/or maintaining smoking cessation in mothers of infants. The major issue with maternal drug use postpartum is the potential exposure of their infants to drugs via ingestion of breast milk, and adverse consequences thereof.

7.1 Nicotine and Cotinine Kinetics: Breast Feeding and Infant Exposure

The dose of a drug taken in by an infant during breast feeding is the product of the concentration of the drug in breast milk and the volume of milk consumed by the baby. In neonates, the volume of milk ingested is about 900ml per day, increasing to 1500ml per day as the baby grows. The concentration of a drug in breast milk may vary according to when the drug is taken by the mother relative to when the baby is breastfed. When the mother smokes a cigarette, there is an important difference in the route of nicotine exposure between the mother and the baby. The mother's exposure is via the lungs, while the baby absorbs the nicotine through the gastrointestinal tract. There is considerable first pass metabolism of nicotine after oral administration (metabolism by the liver before entry into the systemic circulation). In adults, the systemic bioavailability of nicotine after oral administration is 25 to 50%.[339] The oral bioavailability of nicotine in the infant is not known, but is likely to be much less than 100%.

Nicotine is both water soluble and lipid soluble, and distributes rapidly to and from breast milk. As maternal plasma concentrations rise, breast milk concentrations rise also and as maternal plasma concentrations fall, the concentration of nicotine in the milk within the breast falls. The systemic dose of nicotine to the mother is 1 to 2mg per cigarette, or 21mg per day per 21mg patch worn for 24 hours, or 15mg per day for a patch worn for 16 hours. Nicotine has a large volume of distribution and only a small fraction of the absorbed dose remains in the plasma. The nicotine in breast milk is acquired from the mother's plasma. There is a high degree of correlation between concentrations of nicotine in maternal serum and in breast milk ranging from $r = 0.7$ to $r = 0.94$.[18,19,340] The concentration of nicotine is substantially higher in breast milk than in serum, with average ratios of 2.5 to 2.9. Nicotine concentrations are higher in breast milk than in plasma because the pH of breast milk is relatively acidic (6.8 to 7.0) compared with serum (7.4). At a breast milk pH of 6.9, the Henderson-Hasselbach

equation predicts a milk-serum ratio of 2.45 and at a breast milk pH of 6.8, a ratio of 3.13, consistent with the observations noted above.

Nicotine leaves the milk within the breast at a similar rate to its elimination from the maternal plasma. This was determined in 5 women who refrained from smoking for 4 hours and gave repeated breast milk samples during the abstinence interval. The mean elimination half-life of nicotine from breast milk was 95 minutes, similar to the elimination half-life in serum (81 minutes). Because of the rapid movement of nicotine from serum into and out of breast milk, the level of nicotine in the milk is highly dependent on how many cigarettes have been smoked since the last breast feeding, and the time of the last cigarette prior to breast feeding.[19]

7.2 The Dose of Nicotine Consumed in Breast Milk

The concentration of nicotine in milk in 21 mothers after smoking a cigarette averaged 55 μg/L,[340] while the average level of nicotine in milk over 24 hours was 18 μg/L in 10 mothers smoking 1 to 10 cigarettes per day; 28 μg/L in 11 mothers smoking 11 to 20 per day, and 48 μg/L in 13 mothers smoking 21 to 40 cigarettes per day.[19] Assuming a breast milk nicotine level associated with smoking greater than 20 cigarettes per day, the dose of nicotine given to the baby would be 45 μg/day (50μg nicotine/L breast milk X 0.9 l/day). For a 4.5kg baby, this dose represents 10 μg/kg/day.

Using the more direct approach of measuring nicotine concentrations in the milk of individual mothers and estimating milk consumption by weighing their infants before and after a feeding, Dahlstrom et al.[340] determined an average daily nicotine dose of 6 μg/kg/day. Since the infant is taking nicotine orally with some degree of first pass metabolism, the systemic dose is probably even less than the 6 or 10 μg/kg/day estimated by these 2 methods. In contrast, the nicotine intake in a 70kg adult smoking 20 cigarettes per day or using a 21mg nicotine patch is about 300 μg/kg/day. Thus, even with maternal exposure consistent with a high level of NRT (i.e. 21mg transdermal patch) the daily exposure normalised for the weight of the infant is more than 50 times less than the exposure of the mother. Serum concentrations of nicotine have been measured in 8 infants of breast-feeding mothers and were found to be quite low (range >0.2 to 1.6 μg/L) with an infant to maternal serum ratio of 0.06 supporting the idea that infant exposure is quite low.[19]

7.3 Recommendations Regarding Breast Feeding and Nicotine Replacement Therapy

Nicotine is present in breast milk and the infant does get exposed to nicotine, but the resulting concentrations are quite low compared to that of an adult smoker or an adult using NRT. It is unlikely that the low level of exposure is hazardous to the infant. In contrast, there is good evidence that exposure to environmental tobacco smoke by the respiratory route is hazardous to the infant. Thus, provision of NRT to the mother, if that results in her not smoking, would be of great potential benefit to the infant. On balance, the benefits of breast feeding and smoking abstinence during the postpartum period greatly outweigh the risks of NRT in the postpartum period.

Breast milk production is lower in smokers compared to nonsmokers. It is unknown what components of cigarette smoke are responsible for the reduced milk production. If something other than nicotine is responsible, then NRT for smoking abstinence postpartum may have an additional benefit of preserving breast milk production. Theoretically, the formulation of NRT may affect the level of nicotine in breast milk. Transdermal nicotine would provide a steady level of nicotine in plasma and thus in breast milk, and the mother could not control the level of nicotine in breast milk except by changing the strength of the patch. Mothers who use NRT intermittently (gum, nasal spray, or inhalation) might minimise the nicotine in their milk by prolonging the duration between nicotine administration and breastfeeding.

8. Conclusion

Nicotine has been shown to be toxic to the developing fetus in animal studies. Cigarette smoke contains thousands of chemicals, many of which are also reproductive toxins. The foremost toxin in cigarette smoke is carbon monoxide, a potent fetal toxin. Nicotine replacement products are not completely without risk, but their risk is much less than that of cigarette smoke. We recommend that the efficacy of nicotine replacement products for smoking cessation be studied during pregnancy. Intermittent delivery formulations of NRT are the preferred formulation during pregnancy because they would be expected to give a smaller daily dose than a continuous delivery formulation such as a patch. Ultimately, the selection of a formulation and dose must rest on its ability to achieve smoking abstinence in an individual woman. We recommend the creation of a pregnancy registry for NRT that collects prospective obstetric outcome data from efficacy trials and from individual prenatal providers who have prescribed the NRT during pregnancy. As regards breastfeeding, we recommend the use of NRT for smoking cessation for women who breastfeed. The dose of nicotine delivered in breast milk from NRT will be very small, the risk to the baby is minimal and the benefit of a smoke-free environment is large.

Appendix I

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Appendix I. Acute effects of cigarette smoking or nicotine on the mother and fetus

No. of patients[a]	Dose and dosage form administered	Maternal nicotine plasma concentration[b] (μg/L)	GA (wk)	Maternal HR	Maternal BP	Fetal HR	Fetal blood flow	Altered uteroplacental blood flow	Comment	Reference
350 nonS			32-40						Results of NST: 13% of nonS were NR and remained NR on a subsequent NST; 21% of smokers were NR ($p < 0.01$ *vs* nonS), but may not have remained NR on subsequent NST	26
128 S			32-40							
292 nonS			18-34	↔		↔	↔	↔	Smoking was not allowed before or during the protocol	27
203 S			18-34	↔		↔	↔	↔		
19 nonS			20-38			Control	Control		Smoking was not allowed before or during the protocol	28
5 S			20-38			↑	Yes			
10	1 cig	19	34-40			↑	Yes			29-31
17	1 cig		33-39	↑		↑	Yes		Effects on fetal aorta: ↑diastolic diameter, ↑pulse amplitude,↑ slope of ascending curve; ↓duration of ascending curve	32

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No. of patients[a]	Dose and dosage form administered	Maternal nicotine plasma concentration[b] (μg/L)	GA (wk)	Maternal HR	Maternal BP	Fetal HR	Fetal blood flow	Altered uteroplacental blood flow	Comment	Reference
30	1 cig	26	33-39	↑	↑	↑	Yes		Effects on fetal aorta: ↑diameter, ↑peak and mean blood velocity, ↑flow; Effects on UV: ↑diameter, ↑mean blood velocity, ↑flow	33
12	1cig	41.3	33-39	↑		↔			Maternal effects: ↑HR correlated with nicotine plasma concentration; Fetal effects: periodically ↑FB and apnoeic episodes (cig only)	13
6	1 herbal	7.9	33-39	↔		↔				
6	2mg gum	10.4	33-39	↑		↔				
6	4mg gum	17.4	33-39	↑		↔				
20	4mg gum			↑	↑	↔	↔	↔	Maximum nicotine plasma concentration = 12 μg/L; Effects on fetal aorta: ↔blood flow or FVW; Effects on UA and UV: ↔blood flow or FVW	34
24	1 cig	17	33-36	↑	↑	↑	Yes		Effects on UV: sig changes in cig groups only	15
24	2 cig	24	33-36	↑	↑	↑	Yes			
12	1 herbal	2.5	33-36	↔	↔	↔	↔			
12	2 herbal	2.5	33-36	↑	↔	↔	↔			
12	4mg gum	7	33-38	↑	↑	↔	↔			
12	8mg gum	12	33-36	↑	↑	↔	Yes			
12	placebo gum	1	33-36	↔	↔	↔	↔			
8	1 cig		22-26	↑	↑	↑			Fetal effects: ↓ long term HR variability	35
8	1 cig		37-41	↑	↑				Fetal effects: ↓short term and long term HR variability	36
8	1 cig		27-32	↑	↑	↑			Fetal effects: variable transient changes in short and long term HR variability	37
8	1 cig		37-40	↑	↑	↔			Fetal effects: transient changes in short and long term HR variability	38
12	1 cig		35-40	↑	↑		Yes		Effects on IBF: ↓ in 7 cases, ↑ in 5 cases; overall ↓ at 5 min, resolved by 15 min after smoking	39
12	1 cig			↑	↑			Yes	Effects on IBF: ↓ in normotensive patients; ↑ IBF in those with hypertension	40
11 hypertensive	1 cig			↑	↑			Yes		

7	1 cig							Yes	Effects on IBF: variable change (↑, ↓ and ↔)	41
47	1 cig		26-39				Yes	↔	Effects on FVW S/D: sig ↑ in UA among those who smoked >10 cig/d	42
35	2mg gum		26-39				Yes	↔		
19	2 cig			↑	↑			Yes	Effects on UA: baseline S/D above 90% in 13 participants, sig ↓S/D; Effects on resistance index: sig ↓	43
25 high risk patients	1 cig		31-44			↑			No participants became NR on NST; Fetal effects: transient ↑HR	44
19	1 cig		29-39	↔		↔	↔		Effects on fetal aorta and UV: ↔blood flow or diameter	33
12	2 cig		39-45	↑	↑	↑				45
40	2 cig							Yes	Abnormal scan in 4 patients	46
>34	2 cig			↑	↑	↑			Maternal effects: NS ↑ in plasma dopamine levels, rapid ↑ in adrenaline plasma levels, slow ↑ in plasma cortisol levels, ↔ in PRL, hPL or E3 levels	47
23	1 cig			↑	↑		↔		Fetal effects: ↔ in fetal cerebral arteries; Effects on UA: ↔ in resistance indices	48
51	1 cig		34-38			↑			Fetal effects: ↓ short term variability; ↓FM on monitor (but not by maternal report); changes in FHR and FM returned to baseline in <1h	49
10	2 cig		34-38						Fetal effects: ↑FB, ↔ in apnoeic episodes; ↓FM (maternal report)	50
10	2 cig		37-40			↔			Fetal effects: some sig changes in HR variability	51
5	cig			↑			Yes		Effects on UA: sig ↑ in pulsatile index	52
6	21mg patch	19	27-38	↑	↔	↔	↔	↔	↔NST; ↔HR accelerations; Effects on UA: ↔blood flow, ↔S/D	11
15	21mg patch	16	24-36	↑	↑	↔	Yes	Yes	Maternal effects: ↑ resistance index in uterine artery; Fetal effects: ↑ resistance index in middle cerebral artery, loss of HR reactivity - 1/15 (patch) *vs* 7/15 (cig; p = 0.058); Effects on UA: ↑ resistance index;	10
15	ad lib cig	19.7	24-36	↑	↑	↔	Yes	Yes		

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Appendix I. Contd

No. of patients[a]	Dose and dosage form administered	Maternal nicotine plasma concentration[b] (μg/L)	GA (wk)	Maternal HR	Maternal BP	Fetal HR	Fetal blood flow	Altered uteroplacental blood flow	Comment	Reference
9	ad lib cig		24-36	↑ cig> gum	↑	↑ cig> gum	Yes	Yes	Maternal effects: drop out rate 1/10 (cig) *vs* 4/19 (gum); ↓ in uterine artery resistance index (gum > cig); Effects on UA: ↓ in resistance index (cig > gum)	16
15	2mg ad lib gum		24-36		↔		Yes	Yes		
15	1 cig			↑	±	↑	Yes	Yes	Maternal effects: ↑ S/D in uterine artery Effects on UA: sig ↑S/D at 5, 10 and 15 min, return to baseline within 30 min	53
21	1 cig			↑	±	↑	Yes/↔		Fetal effects: sig ↑HR (from 146 to 152 bpm) for <10 min, ↔fetal cardiac output and other fetal doppler parameters	54
7	1 cig			↑	↑	↑	↔		Fetal effects: sig ↑HR (from 136 to 142 bpm); fetal aorta: ↔blood flow velocity or systolic or diastolic diameter	55
18	2 cig			23-38					Fetal effects: sig ↑ in duration of apnoeic episodes	56
19 normal	2 cig	15							Fetal breathing effects: cig sig ↑; herbal gradual ↑, 4mg gum no change, 8mg gum sig ↑	14
28 high risk	2 cig	?								
10	2 herbal	low								
7	4mg gum	7.6								
5	8mg gum	16.2								

a There was no nonsmoking control groups in any of the studies except for those by Phelan,[26] Newnham et al.[27] and Eldridge et al.[28] In all other studies, smokers served as their own control; after overnight abstinence, the mother and/or fetus would be assessed and then a cigarette would be smoked, or gum chewed, or a transdermal patch applied, after which the assessment was repeated.

b After smoking a cigarette, chewing gum or wearing a transdermal patch.

bpm = beats per minute; **BP** = blood pressure; **cig** = tobacco cigarette; **E3** = estrogen E3; **FB** = fetal breathing; **FHR** = fetal heart rate; **FM** = fetal movement; **FVW** = flow velocity waveform; **GA** = gestational age in weeks since last menses; **herbal** = herbal cigarette; **hPL** = placental lactogen; **HR** = heart rate; **IBF** = intervillous blood flow; **nonS** = nonsmoker; **NR** = nonreactive; **NS** = nonsignificant; **NST** = nonstress test; **PRL** = prolactin; **S** = smoker; **S/D** = systolic/diastolic ratio; **sig** = statistically significant; **UA** = umbilical arteries; **UV** = umbilical vein; ↑ = increased; ↓ = decreased; ↔ = no change between before and after, or no difference between exposed and control groups, or not altered.

Appendix II. Effects of smoking or acute nicotine infusion in pregnant sheep and monkeys[a]

Toxin	Animal	Route	Dosage	Outcome	Reference
Tobacco smoke	Monkey	IH	Cigarette	Cigarette smoke delivered by sealed helmet over mother's head for 30 min. Animals acted as their own controls and there was a nicotine-free herbal cigarette group. ↔maternal and fetal HR or SBP; both nicotine and non-nicotine cigarette resulted in persistent ↓PaO_2 which was still present 30 min after exposure to smoke	57
Nicotine	Monkey	IV	6.7-11.6mg	Nicotine was infused 1h after surgery in sedated monkeys. Maternal dosages were based on weight; fetal dosages were based on estimated weight. 4 experiments: A - Maternal bolus dose (1 mg/kg or 6.7 mg): immediate ↑maternal SBP and ↓HR with severe arrhythmias and hyperventilation, all returned to baseline in 15-20min; ↔ABG. The fetal response was similar but ↓SBP was persistent; fetuses ≥GD 120 developed acidosis with small sig ↓PaO_2 and ↓%O_2 saturation; 2/9 fetuses died B - Maternal infusion dose (100 μg/kg x 20 min or 11.6mg): maternal ABG changes were consistent with mild hyperventilation. Fetuses ≥GD 120 developed persistent ↓PaO_2 and ↓%O_2 saturation C - Fetal bolus dose (1.1-1.6 mg/kg): fatal haemodynamic changes similar to those seen with maternal administration, but attenuated; slight ↓pH, otherwise no changes in fetal ABG. No change seen in mothers D - Fetal infusion dose (70 μg/kg/min x 30 min): gradual ↑fetal HR and ↓BR. ↑maternal HR and SBP during infusion and slight ↓HR and SBP after infusion	58
Nicotine	Monkey	IV	0.1 mg/kg x 20min	Uterine BF ↓38%; fetus became acidotic and hypoxic	59
Tobacco smoke	Sheep	IH	Cigarette	Pregnant ewes were monitored for 6h after inhalation of 1 cigarette smoked via tracheal tube. Maternal CO-Hb ↑ at 10min (from 2.4 to 8.3%); ↓ to 2.7% after 6h. Fetal CO-Hb ↑ from 5.1 to 7.1% by 3h and was 7% at 6h	60
Nicotine	Sheep	IV	2.5 to 5 mg/kg	Experimental conditions unclear. Nicotine infusion and/or smoking appeared to occur 1-2h after instrumentation of ewe and fetus. 8-9 cigarettes were smoked in 1h by intubated ewes. Probable nicotine bolus dose administered to ewe or fetus: 2.5-5.0 mg/kg. After maternal nicotine infusion: ↓maternal SBP and uterine BF, then sustained ↑ in both. ↔ in fetal HR, SBP or umbilical BF. After fetal nicotine infusion: similar changes noted, but 2-3 times the maternal dose was required. After smoking: ↔ in maternal HR and SBP, fetal HR and SBP, maternal or fetal ABG, umbilical vein BF; sig ↑CO-Hb, but not fetal CO-Hb	61
Tobacco smoke	Sheep	IH	8 to 9 cigarettes		
Nicotine	Sheep	IV	0.14 to 0.85 mg/kg	Nicotine dosages: A = 0.14-0.25 mg/kg by rapid bolus injection in carotid artery (A1) or jugular vein (A2) of ewe. B = 0.27-0.85 mg/kg infused over 30 min in jugular vein of ewe. C = 0.025-1.0mg bolus into fetal carotid artery Results: A: brief ↓ then ↑ for 10-30min in maternal HR; ↑ maternal and fetal SBP, ↓ fetal HR, ↓ fetal PaO_2, ↓ fetal breathing after 10 minutes; ↔ uterine arterial blood pressure or maternal ABG; B: ↔ in fetal or maternal ABG, SBP, HR; C: ↑ fetal SBP, HR and breathing; ↔ in fetal ABG	62
Nicotine	Sheep	IV for 10 min	0.5, 1.0, 1.5mg infused over 10 minutes (or 0.014-0.032 mg/kg/min)	0.5mg dose: ↔ effects; 1.0 and 1.5mg dose: initial ↑ in uterine BF, then prompt ↓ to mean maximum of 44%, ↑ mean uterine VR (from 0.07 to 0.22 mm Hg/ml or 203%), direct infusion of nicotine into uterine artery produced no change in BF	63
Nicotine	Sheep	IV	15mg infused over 10min	Most parameters returned to baseline by 60 min. ↑ maternal HR (from 97 to 116 bpm), SBP (from 91 to 119mm Hg) and pH (from 7.47 to 7.5), ↑ fetal HR (from 197 to 215 bpm); ↓ uterine BF (29%), fetal SBP (from 53 to 50mm Hg); ↔ in fetal PaO_2 or %O_2 saturation	64

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Toxin	Animal	Route	Dosage	Outcome	Reference
Nicotine	Sheep	IV	7.5, 15, 22.5mg infused over 15 minutes	Peak plasma nicotine concentrations were 130, 250, and 350 μg/L respectively. 0.5 mg/min: ↔ in any maternal or fetal haemodynamic parameters or plasma noradrenaline or adrenaline levels; 1.0 or 1.5 mg/min: sig ↓ uterine BF; sig ↑ maternal SBP; 1.5 mg/min: sig ↑ maternal and fetal noradrenaline/adrenaline plasma levels	65
Nicotine	Sheep	IV	0.2 mg/min x 5 min, dose repeated q30min for 4 hours (i.e. 1mg x 8)	Mean nicotine plasma concentrations were 23 μg/L (post-infusion) and 4 μg/L (pre-infusion). ↔ maternal HR, SBP, noradrenaline or adrenaline plasma concentrations and uterine BF	66
Nicotine	Sheep	IV	3, 10, 20, 30 μg/kg/min for 10 min with 5-10 minute intervals between each increase	3μg: ↔; 10μg: minimal effect; As dose ↑ further: ↑ maternal SBP (from 79 to 121mm Hg), ↓ fetal HR (from 174 to 153 bpm) and SBP (from 55 to 69mm Hg), ↓ uterine BF (from 1237 to 719 ml/min or 42%), ↑ uterine VR (from 0.07 to 0.2 mm Hg/min or 344%), and ↔ in maternal HR or fetal ABG (PaO_2 oxygen content, PCO_2, pH); 20 and 30μg: ↓FHR; 30μg: modest ↓ in umbilical BF (from 554 to 449 ml/min) and ↑ umbilical VR (from 0.11 to 0.16mm Hg/ml)	67
Nicotine	Sheep	IM	5mg twice daily for 5 days	Unexposed control and placebo groups were included in the study. Vascular resistance was examined by transcutaneous Doppler on GD 80, 100, 130. Stillbirths: 10% of controls *vs* 62% of nicotine-exposed; premature delivery: 0% in controls *vs* 42% of nicotine-exposed animals; no differences in maternal or fetal HR or uterine resistance index; placental and umbilical resistance indices tended to ↑ late in gestation; at GD 130 ↑ in fetal cerebral resistance index; after birth ↓ vascular response to carbon dioxide challenge	68

a The animals were anaesthetised and the mother and fetus were surgically instrumented and catheterised. Prior to study, there was usually more than 48h recovery from surgery, unless otherwise stated. All values reported are means. In most studies, animals served as their own controls.

ABG = arterial blood gas; **BF** = blood flow; **bpm** = beats per minute; **BR** = breathing rate; **CO-Hb** = carboxy-haemoglobin; **FHR** = fetal heart rate; **GD** = gestational day; **h** = hour; **HR** = heart rate; **IH** = inhalation; **IM** = intramuscular; **IV** = intravenous; **PaO_2** = arterial oxygen tension; **PCO_2** = carbon dioxide tension; **SBP** = systolic blood pressure; **sig** = statistically significant; **VR** = vascular resistance; ↑ = increased; ↓ = decreased; ↔ = no significant change.

Appendix III. Pregnant animal toxicity studies of tobacco smoke and nicotine

Toxin	Animal	Route	Daily dosage mg/kg/d[a]	Outcome[b]	Reference
Blood flow and related studies					
Smk	Monkey	IH	Acute	Both tobacco and herbal cigarettes caused drop in fetal PaO_2 suggestive of CO effect	57
Smk	Mice	IH		Fetal blood vessel abnormalities	69
Nic	Guinea pigs	pb	4.5 or 18 μg/min	Nic plasma concentrations: 72 μg/L (4.5μg/min dosage) and 315 μg/L (18 μg/min dosage); at high dosage only – decreased cardiac output and uteroplacental blood flow, rise in adrenaline but not noradrenaline	70
Nic	Rat	pb	0.5 or 5	40% reduction in uterine blood flow with 5mg dosage and reduced uterine oxygen tension	71
Nic or adrenaline	Rat	mp	2.4, 4.8, 9.6	GD 13-19; severe maternal growth retardation in both groups; fetal weights unaffected; greater than 40% reduction in uterine blood flow; vasoconstriction alone does not affect fetal growth	72
Placental studies					
Nic	Rat	mp	149 μg/h	Poorly developed decidual basalis (placenta); increased incidence of hydrocephaly; no growth retardation or effect upon survival	73
Nic	Rats		1.5, 5	Functional disorders of the placenta	74

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Growth studies (many studies in other section contain growth data)					
Smk	Rat	IH	7 min 16 /d	Fetal growth retardation	75
Smk	Rat	IH	2h/d	Reduced fetal weight compared to pair fed group	76
Smk	Rat	IH	2h/d	Fetal growth retardation, complete catch up by 2 wks old; control groups: pair fed and ad libitum	77
Nic	Rat	pb	30	Maternal and fetal growth retardation	78
Nic	Rat	pb	5	Reduced embryo cell cleavage and cell number, reduced oviduct blood flow	79
Nic	Rat	pb	0.1, 1	No maternal or fetal growth retardation at low dosage, but retardation at 1 mg/kg/day	80
Nic	Rat	Patch	1.75, 3.5	Pregnancy failure 100% at 3.5mg dosage; 25 to 50% pregnancy failure at 1.75 dosage depending on duration of use	81
Behavioural studies					
Smk	Rat	IH	Daily	Altered open field activity; decreased number of litters and survival	82
Nic *vs* Smk *vs* control	Rats	IH pb		Similar learning of avoidance conditioning in all 3 groups; cigarette exposure – growth retardation	83
Nic	Rat	po		Increased motor activity both sexes; maternal growth retardation; fetal growth retardation in males only	84
Nic	Rat	po		Altered behaviour in males; basal cortisol levels decreased in both sexes, no effect on stress cortisol levels; reduced male number and birth weight but no effect on female birth weight	85
Nic	Rat	po	6	Both sexes – impairment in performance in radial arm maze	86
Smkless tob	Rat	po-g	3 or 12	Maternal and fetal growth retardation; increased death in high dosage group; male pups righted faster; poor performance – swimming development; many other behavioural tests – no difference	87
Smkless tob	Rat	po-g	4, 12, 18	At the 2 higher dosages maternal and fetal growth retardation; incidence of death increased in a dose dependant manner; normal pinna and incisor development, earlier eye opening and vaginal patency; numerous behaviour tests – some difference found in higher dosage groups	88
Nic	Rat	pb	6	Altered locomotor activity	89
Nic	Rat	pb	1.5 or 3.0	Altered behaviour in neonates and adults; no maternal or fetal growth retardation but altered organ/body weight ratios including brain	90
Nic EtOH	Rat	pb	6	Behavioural effects with interaction between nic and EtOH; more stillbirths and neonatal death with combined exposure	91
Nic	Guinea pigs	pb	6	Behavioural alterations in neonates and adults	92
Nic	Rats	pb	0.5	Avoidance conditioning: improved in females, reduced in males	93
Nic	Mice	pb	0.9, 1.8, 2.7	Postnatal audiogenic seizures – prolonged latency, delayed onset and extinction	94
Nic	Rat	pb	0.5	Decreased ambulatory activity	95
Nic	Rat	pb	1, 3, 5	Poorer performance on both learned and innate behaviour measures as juveniles and adults	96
Nic	Mice	pb		Delayed postnatal development: bodyweight gain, eye opening, body hair growth, sensory motor reflexes. Motor – persistent hyperactivity	97
Nic	Rat			Altered motor activity, males only	98
Nic	Rat	mp	4	Fetal growth retardation; after propranolol challenge – poorer cognitive function as assessed by a maze	99
Nic	Rat	mp	2	Testing on PND 50; subtle cognitive abnormalities, with sex differences	100
Nic	Rat	mp	2	Increased fetal mortality, decreased weight gain postpartum, normal acquisition of developmental milestones; nic and lobeline challenge on PND 14; in males only – greater locomotor activity s/p nic, and greater stereotypy s/p lobeline	101
Nic	Rat	mp	6	In females – alteration in acoustic startle response	102

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Appendix III. Contd

Toxin	Animal	Route	Daily dosage mg/kg/d[a]	Outcome[b]	Reference
Nic	Rat		1.5	Increased spontaneous locomotor activity	103
Nic	Rat		0.25	Decreased male sexual behaviour, correlated with decreased testosterone levels	104
Nic	Rat			Alterations in motor development, specificity of alteration dependent upon time of gestational exposure to nic	105
Nic	Rat		2	No maternal or fetal growth retardation; subtle alterations in cognitive performances, with sex differences; decreased responsiveness to adrenergic challenge (propranolol)	106
Combined behavioural and central nervous system studies					
Nic	Mouse	pb	0.13	Postnatal nic PND 10-16: behaviour – hypoactivity; alteration in the proportion of high and low affinity receptor binding sites	107
Nic	Rat	pb	2	No behaviour changes in pups; increased susceptibility to electroconvulsive shock.	108
Nic	Mouse	pb	3	Poorer performance in 8-arm and Morris mazes, and in spatial probe tests; altered hippocampal muscarinic receptors	109
Nic	Rat	mp	6	Study of males; brains of exposed hyperactive males had increased nicotinic cortical receptor density without a change in binding affinity, when compared to nonhyperactive males or even to hyperactive controls	110
Nic	Rat	po	1, 4	Postnatal exposure PND 4-12; reduced hippocampal electrophysiological response to auditory stimuli	111
Central nervous system studies					
Smk	Rat	IH	4 h/d	Brain regions analyzed for DNA, protein, and cholesterol content; antenatal exposure – no effect; postnatal exposure – hindbrain reduced DNA with increased protein/DNA ratio indicating reduction in cell number and increase in cell size	112
Nic	Rat	po		Mild increase in dead cells in medulla; normal postnatal respiratory response. Decreased birth weight	113
Nic	Rat	po	6	Male rats only: adrenaline levels elevated in cortex and hypothalamus during infancy; adrenergic receptor binding increased in cortex in adults	114
Nic	Rat	po		PND 14 – reduced M1 and M2 muscarinic sites and corresponding mRNA in selected brain regions, resolved by PND 35	115
Nic	Rat	pb		Desynchronisation of ontogenic patterns of DNA, RNA and proteins, persistent ODC activity, DNA suppression, growth retardation greatest in cerebellum least in brainstem and mid-brain	116
Nic	Rat	pb	3	Growth retardation, including brain; elevated transmitter turnover in noradrenergic pathways; order of magnitude of effect: cerebellum > cerebral cortex > mid-brain and brain stem; noradrenergic pathways more vulnerable than dopaminergic pathways; peripheral sympathetic nervous system – cardiac, renal, adrenal and lungs also affected; CNS affected prior to PNS	117
Nic	Rat	pb	3	Nic caused stimulation of ODC within 1 hr of drug administration with time and region specificity. ODC regulates polyamine biosynthesis and thus regulates cell differentiation; 3 areas of brain differ in time course of maturation and in nicotinic receptor concentration: midbrain + brainstem (earliest and highest), forebrain (intermediate profiles), cerebellum (latest time and fewest receptors)	118
Nic	Rat	pb	3	Inhibition of DNA synthesis by rank order of nic receptors: midbrain, brainstem ≥ cerebral cortex > cerebellum	119

Nic	Rat	pb	3	Transient alteration in somatostatin-like immunoreactivity and in receptor number, that normalised by day 30 of life	120
Nic	Rat	pb	2.5	Fetal and brain growth retardation; cellular ultrastructural changes noted, and reduced thickness of cell layers	121
Nic	Rat	pb	0.3	Ability of nic to cause catecholamine release paralleled the ontogeny of nicotinic cholinergic receptor development	122
Nic	Rat	pb	1	Single dosage given, then sacrifice and brain regions examined for c-fos; GD16, GD18, GD20, PN0 PN2. C-fos induction maximal on GD20 and PN0, minimal on GD18, no expression on GD16 and PN2; expression blocked by mecamylamine, a cholinergic antagonist	123
Nic	Rat	pb	0.4	PND 8-16 critical time – nic exposure resulted in long lasting increase in nicotinic receptors in cortex, hippocampus and striatum in adults; low affinity binding sites were converted to high affinity states; no change in mRNA noted	124
Nic	Rat	mp		Markers of cell number were reduced (DNA, RNA, protein); elevated ODC – indicating altered cellular maturation; half of dams failed to give birth	125
Nic	Rat	mp, pb		Generalized disruption of receptor acquisition which continued after drug exposure. Cerebellum primary target of disruption in CNS	126
Nic	Rat	mp, pb		pb administration caused hypoxic-ischaemic type effects (reactive hyperinnervation); mp – selective depression of CNS maturational increases in noradrenaline and dopamine levels and utilization rates	127
Nic	Rat	mp	1.5	Effects seen in males only; reduced number of male pups with reduced weight gain postpartum; alterations in striatal dopaminergic receptor system; postnatal exposure has greater effect than prenatal exposure	128
Nic	Rat	mp	2	No growth retardation; alterations in nic binding sites, abnormalities in levels of cellular development markers (ODC, DNA); and impaired development of peripheral noradrenergic projections	129
Nic	Rat	mp	6	Persistent alterations in the functional state of noradrenergic and dopaminergic neurons, sex differences noted	130
Nic	Rat	mp		Adverse effect upon nic uptake and choline acetyltransferase activity in the cerebral cortex at time of transition from neurogenesis to synaptogenesis; premature transition from neurogenesis to synaptogenesis; normal choline acetyltransferase activity in projections to adrenals	131
Nic	Rat	mp	6	Postnatal nic challenge; hyperactivity of ODC to postnatal challenge, also occurred earlier than controls	132
Nic	Rat	mp	6	Regional specific and maturational specific alteration in adenylate cyclase activity	133
Nic	Rat	mp	2	30 d postpartum; region specific decrease in noradrenaline levels; nic challenge failed to release noradrenaline	134
Nic	Rat	mp	2, 6	Development of central cholinergic striatum and hippocampal pathways; Regional brain weights and acetyltransferase activity within normal limits, i.e. no growth retardation. Alterations in the presynaptic high affinity cholinergic transporter	135
Nic	Rat	mp	2, 6	No brain growth retardation; altered muscarinic M1 receptor development and receptor regulation mediated by G proteins	136
Nic	Rat	mp		3 groups of nic exposure, early gestation, most of gestation, and gestation + 2 wk postpartum; maternal growth retardation; fetal brain growth retardation significant only in gestation and postpartum exposure group. ODC unchanged in early group, but elevated in other 2 groups – i.e. during times of nic receptor development	137

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Appendix III. Contd

Toxin	Animal	Route	Daily dosage mg/kg/d[a]	Outcome[b]	Reference
Nic	Rat	mp	3, 6	Increased hyperactivity; dopamine concentration increased in substantia nigra, and decreased in ventral tegmentum area and striatum in those with hyperactivity; the reduction in dopamine was associated with reduction in number of dopamine D2 receptors	138
Nic	Rat	mp		Brain regions examined for marker of cell injury or apoptosis (c-fos); rise in c-fos after GD 8, initially confined to regions with concentrated nicotinic cholinergic receptors, but later in gestation more wide spread c-fos expression	139
Nic	Rat	mp, pb bid	6	Nic GD 4-20; sacrifice PND 7, 15, 22; regional brain contents of neurotransmitters and metabolites determined; persistent reduction of dopamine turnover in forebrain PND 15 and 22; persistent reduction in 5HT turnover midbrain, pons-medulla (PND 15), and in forebrain and cerebellum (PND 22); no effect upon noradrenaline found; route of administration differences found for transmitter levels and for behavioural tests	140
Nic	Rat	mp	2	PND 1, 7, 14, 28 analysis of brain regions for various nic receptor mRNA; higher α_4 and β_2 mRNA levels in hippocampal, septal and cortical regions; maximum effect on PND 14, and generally resolved by PND 28	141
Nic	Rat	pb	Single dose	Analgesic effect of single dosage (1 mg/kg) of nic in adult rats who had chronic fetal exposure – shorter analgesic effect, males exhibited shorter effect early in adult hood than females, but by 7 months – no sex differences	142
Nic	Rat			Central noradrenergic development – growth stimulatory effect	143
Nic				Decreased brain protein synthesis and degradation; alterations in various amino acid levels. Increased binding of nic in the brain	144
Nic	Rat		1.5	Enhanced ability of striatal neurons to synthesize dopamine from tyrosine; increased rate of dopamine turnover in the striatum of male rats	145
Nic ACTH	Rat			Pre- and postnatal exposure; effects of postnatal ACTH and nic similar – increased 5HT high affinity uptake. Prenatal nic exposure – reduced 5HT uptake on PND 21; prenatal ACTH – altered 5HT uptake PND 7	146
Nic	Rat			Rat embryos in culture; growth retardation, forebrain and brachial arches (palate formation) abnormalities	147
Nic	Rat			Inhibition of the development of muscarinic receptors in cerebral cortex and cerebellum, and up-regulation of nicotinic receptors in cerebral cortex and brainstem	148
Peripheral nervous system, cardiovascular, respiratory or response to stress studies (studies related to SIDS)					
Smk	Mice	IH		Electron microscopic alterations of sciatic nerves	149
Nic	Rat	pb	10	Single dosage, bolus, subcutaneous. Functional nicotinic receptors are present in adrenal medulla prior to functional splanchnic innervation	150
Nic	Rat	pb		Neonatal rat adrenals secrete catecholamines by a mechanism that does not include nerve stimulation. Hypoxia and nic both appear to simulate secretion by exocytosis.	151
Nic	Rat	pb	0.05	Accelerated maturation of motor end-plate (postnatal administration)	152
Nic	Rat	pb	0.5	Accelerated maturation of developing nerve and muscle. Altered contractile force, speed, isometric twitch, and tetanus half-contraction duration. Increased cellular metabolic activity and hypertrophy of adrenal glands	153
Nic	Rat	pb	2	No difference in dosage dependent response to noradrenaline. Shift to right in chronotropic effect of cumulative dosage response curves; and reduced binding (without change in affinity) of 3H-dihydroalprenolol	154

Nic	Rat	mp	6	Kidney and heart tissue preparation: increased basal levels of membrane adenylate cyclase, supersensitivity to isoproterenol – altered responsiveness without changes in receptor binding	155
Nic	Rat	mp	6	Delayed development of cardiac β-adrenergic receptor binding capabilities and subsensitivity of chronotropic responses to isoproterenol	156
Nic	Rat	mp	5	Transient/reversible alteration in cutaneous axon reflexes	157
Nic	Rat			Decreased sensitivity of peripheral arterial chemoreceptors to hyperoxic test on PN-3	158
Nic	Rat	mp	2, 6	Hypoxic challenge (60 or 75 min) 5% O_2. Mortality increased in 6mg group but not in 2mg or control pups. Neurotransmitter levels studied in 6mg and control pups only. No difference: basal adrenal catecholamine levels, basal CNS noradrenaline levels. Significant differences: decreased adrenal catechlolamine release in response to hypoxic challenge, decreased cardiac β receptor binding capabilities, decreased CNS noradrenaline turnover, but increased CNS noradrenaline release in response to hypoxia	159
Nic	Rat	mp	6	Postpartum response to 10 min hypoxia and hypercarbia (10% O_2, 15% O_2; 5% CO_2). Response unaffected by prenatal nic exposure	160
Nic	Rat	mp	12	Maternal plasma nicotine concentration 134 ± 42 μg/L. Fetal growth retardation. No difference in response to anoxia	161
Nic	Rat	mp		PND 1-2, exposed and controls – similar HR, ECG, and RR in 21% O_2. 10 minutes of 5% O_2 – normal response is tachycardia; exposed pups had rapid decline in heart rate	162
Neuroendocrine					
Smk	Rat	IH		Delay of LH surge during proestrous (dosage-dependent); prolactin unaffected	163
Nic	Rat	po	2.4, 4.5	Disrupted normal pattern of LH release in offspring	164
Nic	Rat	pb	0.1, 6	Stress – restraint, PND 120. male rats who were prenatally exposed to nic; baseline ACTH, prolactin, corticosterone all similar; no difference in corticosterone response; ACTH elevated in 6mg dosage group; prolactin significantly reduced in 6mg dosage group	165
Nic	Rat	pb	0.1, 6	Single nic challenge dosage in PND 120; males – ACTH response blunted in high dosage group only; prolactin response blunted in high dosage group and enhance in low dosage group	166
Nic	Rat	pb	0.1, 6	Complex pharmacological challenges; no augmentation of prolactin or ACTH s/p challenges	167
Nic	Rat	mp	2, 6	Males had CNS aromatase activity similar to females on PND 6, critical time for brain sex differentiation	168
Nic	Rat	mp		Testosterone rise abolished on GD 18 in males; altered normal sex difference preferences for saccharine	169
Morphology					
Smk	Rat	IH	10 min tid	Effect more pronounced with high yield smk exposure: changes in neural plate, neural tube, surface ectoderm, pericardium, and heart	170
Smk	Rat	IH	1 mg/m^3 air	No maternal growth retardation, small but significant fetal growth retardation; no difference in ossification, litter size; no malformations	171
Smk	Rat	IH	Max tolerated dosage	No skeletal malformations	172
Smk	mouse	IH	10 min tid	Exposure during selected gestational periods; dosage-related embryo growth retardation; exposure on GD 0-1 resulted in growth retardation, with a lag prior to catch up growth; delayed ossification; no malformations among genetically normal mice; small increase in malformation among mice with high baseline rate of malformations	137
Smkless tob	mouse	po-g	3.2, 6.4	Maximum nic plasma concentrations were 44 and 79 μg/L. Fetal weight reduction, delayed ossification, increased haemorrhages and lethality	174

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Appendix III. Contd

Toxin	Animal	Route	Daily dosage mg/kg/d[a]	Outcome[b]	Reference
Smkless tob	mouse	po-g	4, 12, 20	Mean plasma nic concentrations were 99, 398, and 623 μg/L; low dosage produced negligible effects on dams and fetuses – precocious ossification in 60% of low dosage fetuses	175
Smkless tob	mouse	po-g	12, 20, 36, 60	Mean nic plasma 368 and 481 μg/L; decreased ossification; no increased malformations; fetal growth retardation and increased resorption	176
Smkless tob	Rat	po-g	4, 18	Plasma nic 283 μg/L (4mg dosage), 846 μg/L (18 mg/dosage); maternal weight gain reduced, fetal bodyweight gain reduced at high dosage only; no difference in malformations; dosage dependent reduction in ossification	177
Nic	mouse	po-g	36	Reduced ossification in 5/19 measurements of bone ossification	178
Nic	Rat	pb	16.7	Significant effect upon morphological development	179
Nic	Rat	mp	3.5	No abnormalities seen in control, nic only or caffeine only groups; but delayed ossification seen in combined nic and caffeine group	180
Nic	Rat	mp	75, 150 μg/h	Embryonic development retardation, no dysmorphogenesis	181
Palate, jaw and teeth formation studies					
Nic	Mouse	pb	1.67	Nic-exposed animals (n = 130): 9.6% cleft palates, fetal growth retardation. Controls (n = 348): 0% cleft palates	182
Nic	Mouse	pb	1.67	Fetal growth retardation, odontogenesis retarded (dentin and enamel formation) among those with cleft palates	183
Nic	Mouse	pb	1.67	Retarded molar development among those with cleft palates	184
Nic	Mouse		1.67	9.6% of nic exposed had cleft palates (n = 130) (controls n = 348); abnormal molar development, type of abnormality dependent upon presence or absence of cleft palate	185
Nic	Mouse	pb	1.67	Among those with cleft palate (9.6%), there was retardation of developing incisors.	186
Nic	Mouse	pb	1.67	Very abnormal tongue development among those with cleft palates; those without cleft palates were similar to controls with some minor abnormalities	187
Nic	Rat			Rat embryos in culture; growth retardation, forebrain and brachial arches (palate formation) abnormalities	147
Miscellaneous and *in vitro* studies					
Smk	mice	IH		Increased number of micronucleated polychromatic erythrocytes in fetal liver	188
Smk	hamster	IH	bid	Daily smoking for 30d prior to mating; abnormalities found in most ovarian and uterine parameters	189
Smk	hamster	*in vitro*		Oviduct explants in chambers with smk dose-dependent inhibition of transport of oocyte complex, gas phase more inhibitory than particulate phase	190
Nic	Rat	*in vitro*		Inhibited iron transport but it required dosages so high that this cannot be postulated as a mechanism in humans	191
Nic	mouse	*in vitro*		Very high dosages adversely affected embryonic development	192
Smk		*in vitro*		Inhibition of ciliary beat frequency; side stream fraction without oxidant gases did not inhibit ciliary beat	193

a Dosage is in mg/kg/day unless other wise specified. Inhaled dosages are in time.

b All studies include unexposed control groups. The outcome is in comparison to the control, unless otherwise specified.

ACTH = adrenocorticotrophic hormone; **bid** = twice daily; **c-fos** = cellular marker (indirect measure of cell death); **CNS** = central nervous system; **d** = day; **ECG** = electrocardiogram; **EtOH** = ethanol; **GD** = gestational day; **IH** = inhalation; **LH** = leutenising hormone; **mp** = implanted osmotic minipump; **Nic** = nicotine; **ODC** = ornithine decarboxylase (sensitive measure of cell injury); **PaO_2** = arterial oxygen tension; **patch** =transdermal nicotine patch; **pb** = parental route (peritoneal or subcutaneous), bolus administration; **PND** = postnatal day; **PNS** = peripheral nervous system; **po** = oral (drinking water); **po-g** = oral-gavage; **RR** = respiratory rate; **SIDS** = sudden infant death syndrome; **Smk** = tobacco smoke; **Smkless tob** = smokeless tobacco extract; **s/p** = status post; **tid** = 3 times daily; **5HT** = serotonin (5-hydroxytriptamine).

Appendix IV. Mechanisms of toxicity: maternal, placental, and fetal studies in humans

Toxic agent	Target	Effect of toxic agent	Reference
Effects on the placenta			
Maternal smoking	Placenta	No difference in weight	203
Maternal smoking	Placenta	No difference in weight	204
Maternal smoking	Placenta	No difference in weight	205
Maternal smoking	Placenta	No difference in weight	206
Maternal smoking	Placenta, all trimesters	Smaller placentas	207
Maternal smoking	Placental volume, GA18 sonogram	Larger placentas with >15 cigarettes/d	208
Maternal smoking	Placenta	No difference in weight	209
Morphological effects			
Maternal smoking	Placenta	Increased subchorionic fibrin deposits, calcifications; thinner, rounder placentas	204
Maternal smoking	Placenta	Atrophic and hypovascular changes in villi	
Maternal smoking	Placenta	No difference in spiral arteries	210
Maternal smoking	Placenta, term	Reduced capillary volume fraction; increased thickness of villous membrane	211
Maternal smoking	Placenta, term	Langhans cells, decreased number	212
Maternal smoking	Umbilical cord	Heavy smoking associated with abnormal vascular cross-sectional profiles	213
Maternal smoking	Placenta, all trimesters	Abnormalities of microvilli, focal syncytial necrosis, decreased syncytial pinocytotic activity, increased mean thickness of basement membranes (trophoblastic layer and fetal capillary), increased collagen in villous stroma, shrinkage endothelial changes in fetal capillaries	207
Maternal smoking	Placenta, early first trimester	Reduction in chorionic villi cell columns, important for attachment of placenta to uterus	214
Maternal smoking	Placenta	Increased surface and villous calcifications; villous calcifications decreased with concomitant maternal intake of tocopherol (vitamin E), betacarotene and ascorbic acid (vitamin C)	215
Cellular effects			
Maternal smoking	Maternal plasma	Lower pregnancy-specific β-1-glycoprotein	216
Maternal smoking	Amniotic fluid	Elevated noradrenaline, vanillyl mandelic acid, and dopamine metabolite levels	217
Maternal smoking	Placenta, term normal	Collagen production approximately doubled	218
Maternal smoking	Placenta, amnion	Placenta – increased fibronectin production; amnion – decreased fibronectin production	219
None	Placenta	HME expressed in normal placental tissue (HME expressed in alveolar macrophages of adult smokers, HME may be affected by smoking during pregnancy)	220
Effects on nitric oxide synthesis			
Maternal smoking	Umbilical artery segments	Reduced production of L-citruline, and, L-arginine (important for nitric oxide synthesis)	221
Maternal smoking	Placental chorionic villi	Decreased nitric oxide synthetase activity	222
Effects on amino acid transport			
Nicotine Nicotine and ethanol	Placental vesicles; perfused placental cotyledon	No decreased transport of probe amino acid	223
Maternal smoking	Placenta	Block of acetylcholine amino acid transport	224

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Appendix IV. Contd

Toxic agent	Target	Effect of toxic agent	Reference
Nicotine	Placental syncytiotrophoblast vesicles	Glycine transport inhibited in fetal facing basal plasma membrane, but not maternal facing membrane	225
Effects on growth factors			
Maternal smoking	Placental	Impaired EGF receptor bioactivity, especially in SGA babies	226
Benzo(a)pyrene	Placental cell culture	Inhibits EGF binding and autophosporylation	227
Benzo(a)pyrene	Human placental trophoblastic chorionic cell culture	Decreased EGF binding; decreased concentration of EGF protein receptors; decreased trophoblast proliferation; reduced hCG secretion	228
Maternal smoking	Maternal serum	Lower VEGF (VEGF positively correlate with GA, serum hCG and serum progesterone)	229
Endocrine effects			
Maternal smoking	Maternal plasma	Lower hPL	230
Maternal smoking	Maternal serum	Elevated hPL, no significant difference in BW	231
Maternal smoking	Maternal serum, newborn serum	Lower maternal prolactin and estradiol levels; lower neonatal estradiol levels; unaffected neonatal prolactin levels	232
Nicotine, cotinine	Granulosa luteal cells from in vitro fertilisation patients	Cultured cells synthesised progesterone; synthesis unaffected by nicotine or cotinine	233
Nicotine, cotinine	Fetal adrenal tissue	Nicotine and continine competitively inhibited 11 β-hydroxylase; nicotine but not cotinine competitively inhibited 12-hydroxylase activity	234
Maternal smoking	Maternal serum	If female fetus – altered hPL, hCG; if male fetus – altered estradiol, prolactin; higher hCG if fetus female than if male	235
Cigarette smoke extract, alkaloids	Granulosa cell cultures MA-10 cell cultures	Cell growth and progesterone synthesis inhibition by CS extract, nicotine, cotinine, or anabasine	236
Nicotine Nicotine and ethanol	Tissue culture, human placenta, rat placenta	No effect on 11 β-hydroxysteroid dehydrogenase; (controls fetal and placental glucocorticoid exposure)	237
Effects on estrogen synthesis			
Maternal smoking	Maternal serum and urine	Lower serum hPL, lower urinary estriol, lower conversion of dehydroepiandrosterone sulfate to estradiol	238
Maternal smoking	Placental tissue	Aromatase activity unaffected	239
Nicotine alkaloids	Choriocarcinoma cell culture	All inhibited androstenedione conversion to estrogen	240
Maternal smoking	Maternal serum	Estrogen levels 10% lower	241
Maternal smoking	Placenta tissue	Lower aromatase, higher aryl hydroxylase activity	242
Cigarette smoke extract, tobacco leaf extract	Enzyme kinetic studies	Aromatase inhibition; N-(N-octanoyl)nornicotine has highest inhibition	243
Acyl analogues of nicotine and anabasine	Placental microsomes breast tumour cells	N-(N-octanoyl)nornicotine, N-(4-hydroxy-undecanoyl) anabasine potent inhibitors of estrogen synthesis	244
Effects on prostaglandins and related compounds			
Nicotine	Umbilical artery segments	Decline in prostacyclin production	245
Maternal smoking	Umbilical artery segments	Reduced prostacyclin production	246
Maternal smoking	Umbilical artery and vein segments	Reduced PGE_2 synthesis in veins	247

Maternal smoking	Cultured endothelial cells from umbilical arteries	Reduced endothelial cell growth in culture; reduced prostacyclin production	248
Maternal smoking	Maternal serum, newborn serum	Newborn prostacyclin-simulating activity reduced; maternal activity reduced but not significantly	249
Nicotine, very high concentrations	Umbilical artery specimens, umbilical cord platelet	Prostacyclin and thromboxane A2 production in arteries unaffected by nicotine; platelet production of thromboxane A2 reduced by 7%; super-physiological concentration of nicotine, 10 to 10 000 mg/L used (physiological nicotine levels <100 μg/L)	250
Maternal smoking	Umbilical cord blood	No effect upon prostaglandin-dependent platelet aggregation (n = 20)	251
Maternal smoking	Umbilical artery segments	Decreased prostacyclin like activity; no correlation with cotinine levels, (n = 38)	252
Maternal smoking	Umbilical artery segments	Decreased prostacyclin like activity (n = 84); weak correlation with cotinine levels	253
Maternal smoking	Maternal urine, newborn urine	No difference in prostacyclin and thromboxane A2 metabolite levels	254
Maternal smoking	Newborn urine	No difference in prostacyclin metabolite levels	255
Maternal smoking	Amniotic fluid	Elevated prostaglandin E_2 and $F_{2\alpha}$ levels	217
Maternal smoking	Umbilical artery segments	Reduced prostacyclin production	221
Nicotine, cotinine	Placental tissue	Activates placental phospholipase-A2-like enzymes	21
Cigarette smoke extract, nicotine, cotinine	Decidual macrophages, maternal blood macrophages, maternal blood monocytes	CS extract inhibited, nicotine and cotinine did not inhibit secretion of platelet activating factor-acetylhydrolase	256
Effects on immunological function			
Maternal smoking	Maternal serum	50% increase in secretory component of immunoglobulin A	257
Effects on haematological parameters			
Maternal smoking	Umbilical cord blood	All higher: haemoglobin, haematocrit, red cell count, mean corpuscular haemoglobin	258
Maternal smoking	Newborn blood	Higher haematocrit levels; haematocrit correlated with maternal smoking; haematocrit correlated with maternal thiocyanate level; inverse correlation between haematocrit and ferritin	259
Maternal smoking	Maternal blood	Elevated haematocrits (41 to 47%) associated with decreased birth weight	260
Maternal smoking	Newborn blood	Haemoglobin increased and O_2 tension for 50% of HbO_2 saturation (P50) decreased with increased maternal smoking; P50 decrease due to increased Hb-F	261
Maternal smoking	Maternal platelets	Platelet aggregation – increased reactivity	262
None	Maternal blood placenta	High maternal haemoglobin (>130 mg/L) associated with low BW, acute infarcts, and syncytial knots of the placenta	263
None	Maternal blood	Negative correlation between haemoglobin level and levels of hCG and hPL	264
Maternal smoking	Maternal serum	Increased ceruloplasmin, without a difference in serum ferroxidase activity, indicating loss of ceruloplasmin ferroxidase activity	265
Maternal smoking	Umbilical cord blood	Elevated erythropoietin, elevated haematocrit	266
Maternal smoking	Maternal blood, birth weight	Among smokers, third trimester haemoglobin levels inversely associated with birth weight	267
Maternal smoking	Umbilical cord blood	Elevated erythropoeitin, positive correlation with number of cigarettes/d (erythropoeitin marker for chronic hypoxia)	268

BW = birthweight; **CS** = cigarette smoke; **EGF** = epidermal growth factor; **GA** = gestational age; **Hb-F** = fetal haemoglobin; **HbO_2** = haemoglobin oxygen; **hCG** = human chorionic gonadotrophin; **HME** = human macrophage metalloelastase; **hPL** = human placental lactogen; **PGE_2** = prostaglandin E_2; **SGA** = small for gestational age; **VEGF** = vascular endothelial growth factor.

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