Chapter 5
Nicotine

Introduction

Nicotine has been addressed in multiple previous reports of the Surgeon General. Most notably, the 1988 Surgeon General's report, *The Health Consequences of Smoking: Nicotine Addiction,* concluded that cigarettes and tobacco products are addicting and that "Nicotine is the drug in tobacco that causes addiction" (U.S. Department of Health and Human Services [USDHHS] 1988, p. 9). The 2010 report, *How Tobacco Smoke Causes Disease,* addressed the mechanisms by which nicotine leads to addiction, providing full coverage of pharmacology, genetic factors, manifestations of addiction, and epidemiologic aspects (USDHHS 2010). The topic of trajectories of addiction and relapse was also addressed and further covered in regard to adolescents and young adults in the 2012 report, *Preventing Tobacco Use Among Youth and Young Adults* (USDHHS 2012).

This chapter addresses the acute toxicity of nicotine and the effects of longer-term exposure on reproductive outcomes, lung growth and development, neurocognitive function and cognitive decline, psychiatric morbidity, immune function, cancer risk, and cardiovascular disease. A number of new noncombustible products (e.g., electronic cigarettes) have been marketed by the tobacco industry and other manufacturers that provide nicotine through the oral and inhaled routes. Use of such products is projected by some to take an increasing market share over the next decade (Citigroup Global Markets 2011). Additionally, nicotine replacement therapy (NRT) remains a mainstay of cessation aids and many former smokers may remain on such therapy for periods of time longer than recommended and approved by the U.S. Food and Drug Administration (West and Russell 1985; Hajek et al. 1988; Hughes et al. 1991; Hughes 1998).

Given the possibility of increasing exposure of the population to nicotine obtained from products other than conventional cigarettes, this chapter considers the acute and longer-term adverse consequences of nicotine. The chapter also provides background for the consideration of future policy directions in Chapter 16, "A Vision for Ending the Epidemic: A Society Free of Tobacco-Related Death and Disease."

Toxicokinetics and Acute Toxicity of Nicotine

Nicotine is the major chemical component responsible for addiction in tobacco products (USDHHS 1988; Stolerman and Jarvis 1995; Royal College of Physicians of London 2000; Balfour 2004). The risk for nicotine addiction depends on the dose of nicotine delivered and the way it is delivered; the potential for addiction increases with the dose delivery rate, the rate of absorption, and the attained concentration of nicotine (Henningfield and Keenan 1993; de Wit and Zacny 1995; Stitzer and de Wit 1998). For an in-depth discussion of the pharmacokinetics of nicotine as related to addiction, see the pharmacokinetics section of Chapter 4 in the 2010 Surgeon General's report (USDHHS 2010). Similarly, the toxicity caused by nicotine is dependent on dose, dose duration and frequency, route of exposure, formulation of the nicotine product, and interpersonal variability as addressed in the 2010 report. This section discusses the toxicokinetics and the acute toxicity of nicotine.

Toxicokinetics

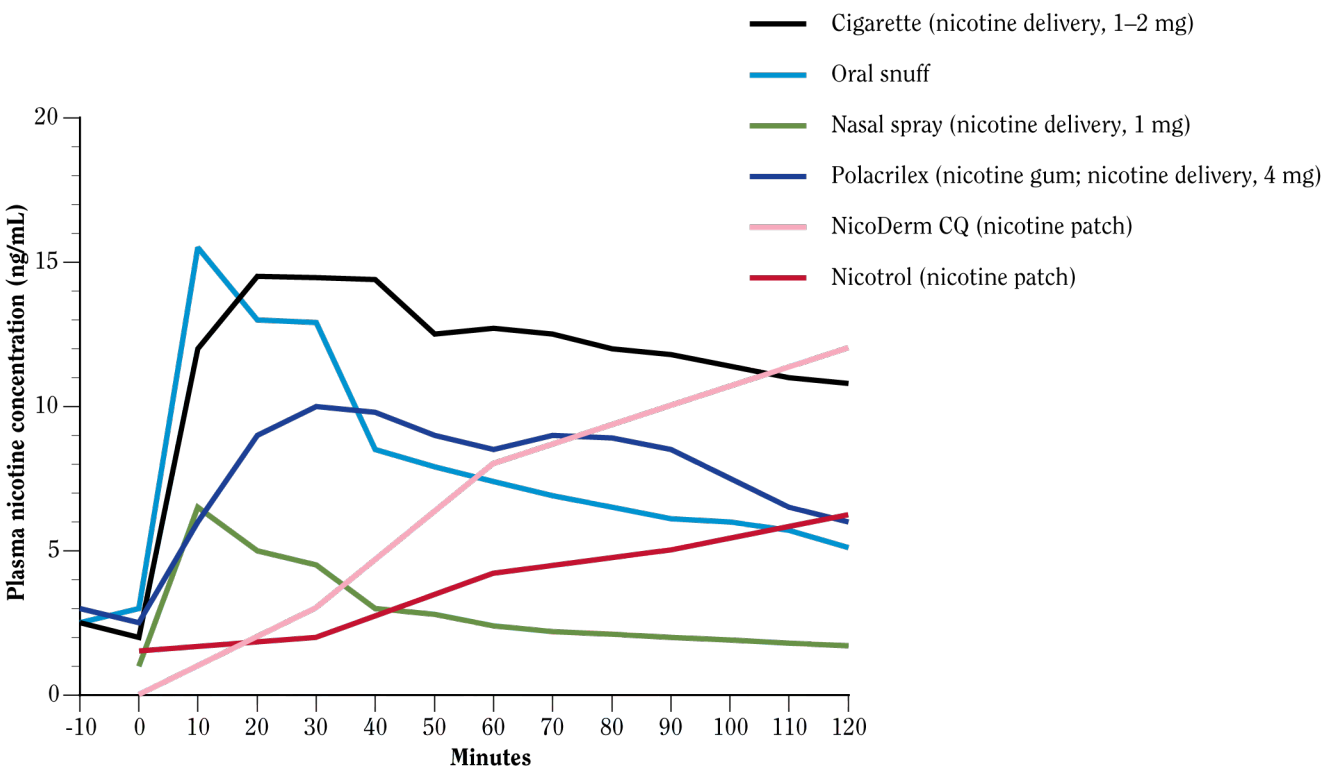
Nicotine, 3-(1-methyl-2-pyrrolidinyl) pyridine, is a volatile alkaloid with a molecular weight of 162.23. The absorption and elimination via renal excretion of nicotine are highly dependent on pH. At a high (alkaline) pH, nicotine (pKa^1 = 8.5) is in the non-ionized state, which passes more easily through lipoprotein membranes than the ionized (charged) state (Stratton et al. 2001). Nicotine in its un-ionized state can be readily absorbed across the epithelium of the lung, the oral mucosa, and the nose, and through the skin. Nicotine in tobacco smoke inhaled into the lung is rapidly absorbed because of the large surface area of the alveoli and small airways and the dissolution of nicotine in the fluid coating the lung's epithelial layer, which has a physiological pH that facilitates absorption. Similarly, nicotine from oral tobacco products that

[1]The logarithmic measure of the acid disassociation constant, which represents the pH of a solution in which half of the acid molecules are ionized.

have an alkaline pH is readily absorbed through the oral mucosa, but more gradually than via the lungs. Nicotine can be well absorbed in the small intestine, because of its more alkaline pH and large surface area. However, nicotine is poorly absorbed from the stomach, because its acidic environment results in greater ionized nicotine. In addition, unlike ingestion, nicotine's bioavailability is greater through the lung or through the oral mucosa, because nicotine reaches the systemic circulation before passing through the liver where it is metabolized (first-pass metabolism). Arterial concentrations of nicotine from smoking are higher than venous concentrations (Figure 5.1). Across studies, the ratios of arterial to venous concentration range from 2.3–10 (Henningfield et al. 1993; Gourlay and Benowitz 1997; Rose et al. 1999). Less than 5% of nicotine is protein-bound in the plasma (Benowitz et al. 1982). It distributes extensively to body tissues, including the liver, kidney, spleen, lung, and brain and also accumulates in gastric juice and saliva, breast milk, skeletal muscle, and fetal serum and amniotic fluid (Dahlstrom et al. 1990; Breese et al. 1997; Perry et al. 1999; Dempsey and Benowitz 2001). The time course of nicotine accumulation in the brain and other body organs, and the resultant pharmacologic effects, are highly dependent on route and rate of dosing. The lag time between a puff on a cigarette until nicotine reaches the brain is 10–20 seconds (Henningfield and Keenan 1993; de Wit and Zachy 1995; Stitzer and de Wit 1998; Rose et al. 1999).

More than 80% of nicotine absorbed into the body undergoes metabolism in the liver, principally by CYP2A6, UDP-glucuronosyltransferase, and flavin-containing monooxygenase (Cashman et al. 1992; Park et al. 1993; Benowitz and Jacob 1994; Benowitz et al. 2009). Several metabolites of nicotine reach the central nervous system (CNS) after acute administration of nicotine (Crooks and Dwoskin 1997). Nornicotine is both a metabolite of nicotine and a minor tobacco alkaloid. Researchers have observed similar behavioral effects from nicotine and nornicotine. However, because nornicotine is present only as a minor metabolite, it is unclear whether it has significant pharmacologic or toxicologic effects in nicotine users. Less data are available on cotinine, a major metabolite of

Figure 5.1 Venous blood concentrations of nicotine over time for various nicotine delivery systems



Source: Adapted from Fant et al. 1999 with permission from Elsevier, ©1999.
Note: **mg** = milligrams; **ng/mL** = nanograms per milliliter.

Table 5.1 Animal studies on acute toxicity of nicotine

Study	Species tested	Route of exposure	Study objective/endpoint
Larson et al. 1945	Mice, rabbits	i.p.	Determine LD50
Hicks and Sinclair, 1947	Rats	i.p.	Determine LD50
Yamamoto et al. 1966	Rats	i.p.	Determine LD50
Lazutka et al. 1969	Mice, rats	Oral, inhalation	Determine LD16, LD50, LD100
Stalhandske and Slanina 1970	Mice	i.p.	Determine difference in response to LD50 between young and old rats
Tepper et al. 1979	Mice	i.p.	Determine LD50 by mouse strain, age, gender; ED50 of onset of tremor
Okamoto et al. 1992	Rats	i.p.	Determine time to convulsions
Okamoto et al. 1994	Rats	i.p.	Determine difference in response to LD50 between young and old rats
Yuen et al. 1995	Rats	Oral (water)	Examine acute hepatotoxicity

Note: **ED50** = median dose where 50% of sample subjects achieve a predefined endpoint; **i.p.** = intraperitoneal; **LD16** = dosage of a given drug required to kill 16% of a test population; **LD50** = dosage of a given drug required to kill 50% of a test population; **LD100** = dosage of a given drug required to kill 100% of a test population.

nicotine (Benowitz and Jacob 1994; Keenan et al. 1994). For discussion of the pharmacodynamics of nicotine in the brain, see the section on "Pathophysiology of Nicotine Addiction" in Chapter 4 of the 2010 Surgeon General's report (USDHHS 2010).

Acute Toxicity of Nicotine

Nicotine exerts its effects via stimulation of the nicotinic acetylcholine receptors (nAChRs), which are located in the CNS, at interganglionic junctions of the autonomic nervous system, and on target organs throughout the body as part of the parasympathetic autonomic nervous system (USDHHS 2010). As a result of the global expression of these receptors, their stimulation causes broad physiologic effects. Although the nicotine intoxication syndrome is not fully characterized, symptoms of mild acute toxicity might include nausea and vomiting, progressing with increased exposure to cholinergic syndrome, which includes diarrhea, increased salivation, increased respiratory secretions, and bradycardia. Severe poisonings can progress further to seizures and respiratory depression. Countering the development of acute toxicity is the relatively rapid development of tolerance with repeated exposure (Benowitz et al. 1987; Okamoto et al. 1992).

Acute toxicologic data on nicotine is limited. Such information comes from three sources: (1) animal studies, (2) studies investigating nicotine as a therapeutic agent (including NRT), and (3) poisonings involving nicotine. A few acute toxicological studies performed on animals are available (Table 5.1). These studies contribute basic LD50 (dose causing 50% lethality) values primarily in rats and mice (Larson et al. 1945; Hicks and Sinclair 1947; Yamamoto et al. 1966; Lazutka et al. 1969; Tepper et al. 1979), as well as examining the effects of age and gender, and endpoints other than lethality, such as hepatotoxicity and time to convulsions. However, the studies available do not adequately characterize acute toxicity. Studies investigating nicotine as a therapeutic agent in humans are limited in predicting the acute toxicity of nicotine. These studies are better at documenting adverse effects rather than overt toxicity, as the doses administered are chosen, in part, because they are considered subtoxic. Mild adverse effects, as defined by the World Health Organization's (WHO's) Collaborating Center for International Drug Monitoring (WHO 1972), of nicotine given as pharmacologic treatment for nicotine addiction have been commonly reported (Barrueco et al. 2005). Studies examining nicotine's potential role to treat ulcerative colitis using nicotine patches or enemas provide similar findings with regard to adverse effects (Nikfar et al. 2010; Lunney and Leong 2012).

Numerous poisonings have been documented in the literature since the use of nicotine as a pesticide became widespread in the early part of the twentieth century. These studies describe patients exposed to doses associated with toxicity via one or more routes of exposure, and a resulting predicted clinical course of acute toxicity as noted previously in this section. However, the literature also notes exceptions, including a rapid progression to near fatal symptoms after a relatively low exposure to a piece of 2 milligrams (mg) nicotine gum that was chewed briefly and discarded – never swallowed (Mensch and Holden 1984), as well as a patient receiving a relatively large dose, 240 mg nicotine, in an accidental subcutaneous administration that proved to be nonfatal (Brady et al. 1979). In both instances, the affected persons were active cigarette smokers. The case report involving the 2 mg gum did not specifically document nicotine intoxication; rather, a clinical diagnosis was made. Yet, despite the abundance of case reports, it appears that there has not been a systematic assessment of the literature to characterize the dose-response relationship. Finally, the human oral fatal dose is commonly reported to be between 50–60 mg for adults, with the fatal dose for youth expected to be lower, but not determined specifically. A study by Lazutka and colleagues (1969), in a Russian language publication, is commonly cited in support of these figures. However, Lazutka and colleagues make no such estimation. Further, a systematic literature search was performed using OVID MEDLINE for nicotine (focusing on 'toxicity' n = 744 and 'poisonings' n = 134), as well as a search of databases such as the Hazardous Substances Data Bank and Haz-Map using Toxnet; however, no study was located as a source for an estimate of the dose that is fatal to humans and the figure of 50–60 mg is poorly documented.

Summary

In its un-ionized state, nicotine readily enters the body, regardless of the mode of administration. It has known acute toxicity, reflecting its pharmacologic activity. There is a potential for poisoning from ingestion of nicotine-containing products.

Pathophysiology of Nicotine Addiction

Summary of Evidence from the 2010 Surgeon General's Report

Dependence on nicotine is characterized by both the persistence of a drug-taking behavior and the emergence of withdrawal symptoms upon the abrupt cessation of nicotine administration (Wikler 1973; Levine 1974; Stewart et al. 1984; Ludwig 1986; O'Brien et al. 1990; Hughes and Hatsukami 1992; Koob et al. 1993; Markou et al. 1993, 1998; American Psychiatric Association 1994; Kenny and Markou 2001; USDHHS 2010). Therefore, both the neurosubstrates (brain structures, pathways, and systems) mediating the reinforcing effects of acute administration of nicotine and those mediating the nicotine withdrawal syndrome are relevant to nicotine addiction. The physiological systems that develop adaptations to repeated nicotine administration, and lead to the emergence of withdrawal signs on cessation of nicotine administration, are likely to intersect with systems that mediate the acute effects of nicotine (Markou et al. 1998; Kenny and Markou 2001). That is, nicotine addiction develops as a neurobiologic adaptation to chronic nicotine exposure. However, all forms of nicotine delivery do not pose an equal risk in establishing or maintaining nicotine addiction. NRT medicines, which are designed to minimize addiction risk, carry a low risk of establishing addiction and are generally substantially easier to discontinue than tobacco products (Henningfield et al. 2011; WHO 2012). Conversely, cigarettes have been researched, designed, and manufactured to increase the likelihood that initiation will lead to dependence and difficulty achieving cessation due to contents and emissions in addition to nicotine (e.g., acetaldehyde, ammonia compounds, and menthol); design features that may increase free-base nicotine and produce larger puffs (filter-tip ventilation); and other factors that reduce the concerns for smokers and increase the attractiveness of the products (USDHHS 2010, 2012).

nAChRs are ligand-gated ion channels composed of five membrane-spanning subunits that combine to form a functional receptor (Lindstrom et al. 1996; Role and Berg 1996; Albuquerque et al. 1997; Lèna and Changeux 1998, 1999; Dani 2000; Gotti et al. 2006). As a result of actions at the nAChR sites, nicotine stimulates the release of most neurotransmitters throughout the brain (Araujo et al. 1988; Toide and Arima 1989; McGehee and Role 1995; Gray et al. 1996; Role and Berg 1996; Wilkie et al. 1996; Albuquerque et al. 1997; Alkondon et al. 1997; Kenny et al. 2000; Grady et al. 2001). Therefore, various transmitter systems are likely to be involved in the rewarding effects of nicotine and in the adaptations that occur in response

to chronic exposure to nicotine, which give rise to dependence and to withdrawal responses.

The positive reinforcing aspects of nicotine addiction primarily results from the release of dopamine in the ventral tegmental area region of the brain (Grenhoff et al. 1986; Nisell et al. 1994a,b, 1997; Pidoplichko et al. 1997; Watkins et al. 2000; Picciotto and Corrigall 2002; Balfour 2004). Nicotine stimulates nAChRs on glutamatergic terminals that release glutamate, an excitatory neurotransmitter, which results in an increased release of dopamine in the nucleus accumbens and the frontal cortex (Gray et al. 1996; Gioanni et al. 1999; Fu et al. 2000; Grillner and Svensson 2000; Mansvelder and McGehee 2000; Reid et al. 2000). Nicotine also excites nAChRs on gamma-aminobutyric acid (GABA)-releasing terminals (Schilström et al. 1998; Mansvelder and McGehee 2000). Thus, levels of GABA, an inhibitory neurotransmitter, are also increased by nicotine. However, the interplay between the quick desensitization of nAChRs on the GABA neuron and the higher doses of nicotine required to desensitize nAChRs on the glutamate neuron results in an increase in dopamine levels (Schilström et al. 1998; Mansvelder and McGehee 2000). A critical role may also be played by nicotine-induced increases in norepinephrine transmission, although the role of this transmitter system in nicotine dependence has not been investigated as extensively as that of the dopamine, glutamate, and GABA systems. The roles of endocannabinoids, serotonin, and endogenous opiates in nicotine addiction are less certain. For further discussion of neurosubstrates, see 'Neurosubstrates of Nicotine Reinforcement' in the "Pathophysiology of Nicotine Addiction" section of Chapter 4 in the 2010 Surgeon General's report.

The neurophysiological mechanisms associated with withdrawal symptoms may vary with the type of symptoms experienced (e.g., somatic vs. affective). The nAChRs appear to be involved in both the somatic and affective components of nicotine withdrawal. Decreased mesolimbic dopaminergic transmission seems to mediate various aspects of the withdrawal syndrome (Fung et al. 1996; Hildebrand et al. 1998, 1999; Carboni et al. 2000). Noradrenergic and serotonergic systems may also play a role in withdrawal. Decreased glutamate transmission appears to mediate the affective aspects of withdrawal, but GABA transmission does not appear to change with withdrawal.

Trajectory of Addiction

The addiction caused by the nicotine in tobacco smoke is critical in the transition of smokers from experimentation to sustained smoking and, subsequently, in the maintenance of smoking for the majority of smokers who want to quit (USDHHS 2010, 2012). Substantial longitudinal research has shown that smoking typically begins with experimental use of cigarettes and that the transition to regular smoking can occur relatively quickly, with the smoking of as few as 100 cigarettes (USDHHS 2012). Longitudinal studies show that there are individual trajectories of smoking as tracked by the index of numbers of cigarettes smoked daily. These trajectories are variable, with some smokers quickly progressing to regular smoking and others doing so more slowly (USDHHS 2010, 2012). Research is in progress on the possible role of genetic factors in determining the trajectory of nicotine use.

The 2012 Surgeon General's report makes clear that addiction can begin in people who begin experimenting with tobacco use during their teenage years (USDHHS 2012). Although the phenotype of addiction is not so well defined as with adults, symptoms of withdrawal occur among youth who become regular smokers. As documented in that report, the longitudinal studies show several different patterns of smoking uptake, with some young people rapidly escalating their use to a typical pattern of regular use and others doing so more slowly. Some adolescents may be able to smoke on an experimental or intermittent basis without becoming addicted (USDHHS 2012).

Health Consequences of Nicotine Exposure

Cancer

Nicotine is a highly bioactive compound with effects ranging from being a natural pesticide in tobacco leaves to causing addiction in tobacco users. For cancer, there is some biological basis for proposing that nicotine may promote cancer based on experimental studies that have limitations in replicating human exposure and on mechanistic studies, but human evidence is lacking (Lee et al. 2005, 2012; Dasgupta and Chellappan 2006; Zheng et al. 2007; Catassi et al. 2008; Chen et al. 2008b, 2010; Egleton et al. 2008). Nicotinic receptors are found not only in the

brain but throughout the body; for example, in muscle, lung, endothelia, kidney, and skin (Improgo et al. 2011; Cardinale et al. 2012; Hurst et al. 2013). These receptors trigger a number of cellular pathways involved in carcinogenesis. The presence of nicotinic cholinergic receptors throughout the normal lung and in lung tumors has been well documented (Schuller 2009; Improgo et al. 2011). This section reviews the current literature that relates to the hypothesis that nicotine may contribute to the carcinogenic process. The evidence comes from experimental cell culture and animal studies, and from human studies including epidemiologic.

The potential for nicotine to contribute to the risk of incident cancer or cancer recurrence is important due to the number of smokers who have quit by using NRT, some of whom use NRT for long durations to remain smoking abstinent, and other smokers who switch to alternate sources of nicotine (e.g., e-cigarettes or smokeless tobacco products). Although using NRT or other noncombusted sources of nicotine is different than smoking in evident ways, the possibility of increased risk in long-term users compared to those who use such products only briefly for cessation merits consideration. Thus, when contemplating the available evidence, coming largely from laboratory experiments, the following questions need to be addressed: (1) What is the cancer risk for those who quit smoking but use long-term NRT or other sources of nicotine compared with those who continue to smoke? (2) What is the cancer risk of a lifetime pattern of repeatedly quitting with NRT and relapsing, but smoking fewer lifetime cigarettes overall? (3) What is the cancer risk of long-term NRT use without relapse to smoking or sustained switching to a noncombusted nicotine source compared with long-term abstinence without NRT or other source of nicotine or relapse to smoking? This section will address these questions.

Genotoxicity

There are mixed data for a genotoxic effect of nicotine. Most studies were negative that used the Ames assay (including urine of rats exposed to nicotine), chromosomal aberration and sister chromatid exchange (SCE) assays in Chinese hamster ovary cells, and the bacterial genotoxicity luminescence test (Mizusaki et al. 1977; Riebe et al. 1982; Doolittle et al. 1991, 1995; Yim and Hee 1995). In contrast, two studies were positive for chromosomal aberration and SCEs (Riebe and Westphal 1983; Trivedi et al. 1990), one was positive for micronuclei formation that was inhibited with antioxidants (Argentin and Cicchetti 2004), one was positive for an *Escherichia coli POLA*$^{+}$/*POLA*$^{-}$ mutation assay (Riebe et al. 1982), and another using nasal mucosal cells was positive by the Comet assay, which is inhibited by antioxidants or nicotinic receptor inhibitors (Ginzkey et al. 2012). One study found that cotinine, and not nicotine, was genotoxic by the bacterial genotoxicity luminescence test, but another was null for the Ames assay and SCE induction (Doolittle et al. 1995; Yim and Hee 1995). Some reports indicate that nicotine can lead to the formation of DNA adducts using the ultrasensitive technique accelerator mass spectroscopy (Cheng et al. 2003). Although cigarette smoke is highly genotoxic, a comparison of Ames mutagenicity for cigarette smoke from cigarettes with differing nicotine yields did not indicate different mutagenic potential, suggesting that there was no additional contribution by nicotine (Chen et al. 2008a).

Effects of Nicotine on Carcinogenic Pathways

There are numerous studies that focus on lung cells and cells from other organs relating to nicotine exposure. A wide range of effects has been reported in cellular systems, including at doses similar to those in the blood of smokers (Cardinale et al. 2012). The presence of nAChRs throughout the lung has been well documented via protein studies and demonstration of the presence of transcripts for both normal tissues and lung tumors (Improgo et al. 2011). These receptors are important for triggering many signaling pathways in lung cells (Schuller 2009). In lung cells, nicotine has been shown to: (1) inhibit apoptosis including apoptosis induced by chemotherapy (Maneckjee and Minna 1990, 1994; Cardinale et al. 2012), which involves the PI3-K-Akt pathway and attendant positive effects on Bcl-2 and negative effects on BAD and BAX (West et al. 2003; Jin et al. 2004; Xin and Deng 2005); (2) affect proliferation by stimulating the release of epidermal growth factor and, therefore, activation of the Ras-Raf-ERK cascade (Dasgupta and Chellappan 2006; Carlisle et al. 2007; Paleari et al. 2008); and (3) stimulating fibronectin production activating ERK, PI3-K, mTOR, and the expression of PPAR-β/δ (Dasgupta et al. 2006). Also, there is evidence that nicotine may promote metastases because of stimulation of cell motility and migration, loss of adhesion, and inducing the transition of a well-differentiated epithelial cell to a highly invasive carcinoma via epithelial-mesenchymal transition (Catassi et al. 2008; Egleton et al. 2008; Cardinale et al. 2012).

An important consideration for cancer survival and metastasis is angiogenesis. A variety of mechanisms are stimulated by nicotine to promote angiogenesis; for example, promoting endothelial cell migration, proliferation, survival, and tube formation (Cardinale et al. 2012; Lee and Cooke 2012). Nicotine directly binding to nicotinic receptors in endothelial cells induced endothelial cell tube migration by stimulating VEGF in lung cancer cells (Conklin et al. 2002; Heeschen et al. 2002; Li and Wang

2006; Ng et al. 2007). Lower doses of nicotine in vitro induce endothelial cell proliferation, while higher doses induce cytotoxicity (Villablanca 1998). These effects also occur via stimulation of nicotinic receptors in the endothelia. The angiogenic effect of nicotine involves MAPK, PI3K/Akt, and NF-κB activation (Heeschen et al. 2002). Angiogenesis has been shown in a variety of tumor cells, such as breast, colon, and lung, implanted in a chick chorioallantoic membrane, and other systems (Heeschen et al. 2002; Mousa and Mousa 2006).

Limited research has addressed whether the nicotine in tobacco smoke somehow alters the toxicity of tobacco smoke. Chen and colleagues (2008a) conducted various in vitro studies comparing cigarettes with differing amounts of nicotine, and where nicotine was added back to the condensate. They found that nicotine attenuated the cytotoxicity of cigarette smoke through inhibition of apoptotic pathways, increased proliferative activity, and increased cell survival. There was no evidence of an effect on the gap junction intracellular communication, which is considered to be a marker of tumor promotion effects.

Experimental Animal Studies for Carcinogenicity

Several studies in experimental animals also did not indicate that nicotine alone is tumorigenic (Martin et al. 1979; Waldum et al. 1996; Hecht 2003; Murphy et al. 2011). These studies have included the inhalational route of exposure, fetal exposure, and exposure through maternal milk. The only exception to the null findings is the report of nicotine inducing sarcomas in the muscle or uterus of exposed A/J mice; other tumors, including those in the lung, were not observed in that same study (Galitovskiy et al. 2012). The A/J mouse model is used for assessing the carcinogenic effects of cigarette smoke in inducing lung tumors. However, the lack of nicotine induction of lung tumors may be related to the dose and route of exposure.

As a tumor promoter, nicotine has been reported to increase the frequency of tumors induced by agents such as nicotine-derived nitrosamine ketone, and 7,12-dimethylbenz(a)anthracene (Chen and Squier 1990), *N*-methyl-N´-nitro-*N*-nitrosoguanidine (Gurkalo and Volfson 1982), and *N*-[4-(5-nitro-2-furyl)-2-thiazolyl] formamide (LaVoie et al. 1985). Other studies showed that nicotine had no effect in promoting tumors related to other *N*-nitrosamines (Habs and Schmahl 1984) and had an antitumor effect in some cases (Zeller and Berger 1989). In a different tumor promotion model, nicotine induced lung tumors in hamsters in the presence of hyperoxia (Schuller et al. 1995). In addition, studies using cancer xenograft models have shown that nicotine promotes tumor growth and metastases (Heeschen et al. 2001; Jarzynka et al. 2006; Al-Wadei et al. 2009; Davis et al. 2009).

Other studies have investigated the potential for nicotine to promote the carcinogenic effects of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Maier and colleagues (2011) conducted a series of studies to determine if nicotine would promote the carcinogenesis induced by NNK. The results were null. The investigators used several models, including a crossed A/J and C57BL/6 mouse, a mutant *k-Ras* animal model prone to develop lung tumors, and a syngeneic lung-cancer graft model with NNK-transformed lung cancer cells. The dosing of nicotine, albeit by drinking water, was specifically intended to be similar to the levels human smokers receive when using NRT. In a separate study, Murphy and colleagues (2011) studied the A/J mouse and did not find a difference in tumorigenesis whether the nicotine was given before or after NNK, compared to NNK alone.

In summary, the findings of animal studies do not support the hypothesis that nicotine is a complete carcinogen. It is a tumor promoter in some experimental models, although not for tobacco-specific nitrosamines. Studies examining other classes of tobacco smoke carcinogens (e.g., polycyclic aromatic hydrocarbons) would need to be performed to better define the potential cancer risk inferred from animal studies.

Human Studies

Very little human data are on human cancer risk relating to nicotine. The Lung Health Study is the only study that provides information about long-term users of NRT (Murray et al. 2009). This study was not designed to directly examine nicotine's potential cancer risk. It was a 5-year randomized trial to assess the effects of smoking cessation and reduction on chronic lung disease and lung function. Among 5,887 persons initially enrolled, the researchers continued to follow them for an additional 7 years (n = 3,220). Study participants were offered NRT without consideration of randomization or study design. Although they were encouraged to use NRT for only 6 months, many continued to use it long term. A total of 75 lung cancers were diagnosed among smokers and quitters of the extended surveillance group, but the use of NRT was not associated with lung cancer (or other cancers). A major limitation was the short follow-up period of only 7 additional years. Notwithstanding the limitations, this study at least does not indicate a strong role for nicotine in promoting carcinogenesis in humans, and clearly the risk, if any, is less than continued smoking.

Another approach to examining whether nicotine could contribute to carcinogenesis would be to consider its delivery in the context of long-term smokeless tobacco

use. Smokeless tobacco products used in northern European countries appear to result in a substantially reduced exposure to many tobacco smoke carcinogens, because smokeless tobacco does not undergo combustion. Epidemiologic studies of smokeless tobacco indicate that it increases the risk of oral cavity, esophageal and pancreatic cancers, (IARC 2012) at least for some forms of smokeless tobacco. The associated risks for these sites are less than the risk of these cancers from smoking; however, high rates of oral cancers in India and Sudan are attributable to the use of smokeless tobacco products (Accortt et al. 2005; Boffetta et al. 2005, 2008; Luo et al. 2007). The risks for many cancers commonly associated with smoking are not elevated by long-term smokeless tobacco use. This pattern of risk suggests that in humans nicotine may not have a strong tumor promoting effect. Further, although levels of nicotine are similar for smokers and smokeless tobacco users, the risk of cancer of the oral cavity, esophagus, and pancreas is less for the smokeless tobacco users, indicating that exposures other than nicotine contribute to the cancer process. This conclusion, however, needs to be tempered by the possibility that there may be a different risk due to route of exposure, because smokeless tobacco use leads to nicotine exposure via the oral mucosa and ingestion, while smoking results in inhalation exposure. Risks inferred from smokeless tobacco studies may not extend directly to inhalation exposures.

There is some evidence that NRT can endogenously lead to the formation of the carcinogenic tobacco-specific nitrosamines, NNK and N-nitrosonornicotine (NNN), at least in rats (Carmella et al. 1997), which conceptually would increase cancer risk if the resultant dose was similar to those which result from smoking or the use of smokeless tobacco. A smoking cessation study by Stepanov and colleagues (2009b) demonstrated that NNK metabolites were not detectable in persons using NRT (Hecht et al. 1999). However, they did find intermittently high levels of NNN similar to baseline smoking levels in 13 of 34 participants using NRT gum or lozenges, and in only 1 of 9 persons using the patch (Stepanov et al. 2009a). Although these data indicate a potential cancer risk to NRT users, especially oral users, it is important to realize that NNN is only one of the tobacco-specific nitrosamines in cigarette smoke. Thus, it will be important to quantify the level of risk from long-term use of NRT or other non-combusted sources of nicotine, particularly if long-term nicotine use from sources other than smoking becomes more prevalent. Although there is a variety of evidence that nicotinic receptor polymorphisms play a role in lung cancer risk and in determining the amount of tobacco use, the genes on chromosome 15 (i.e., *CHRNA3*, *CHRNA4*, *CHRNA5*, *CHRNA6*, *CHRNB2*, *CHRNB3*, and *CHRNB4*), chromosome 1 (i.e., *CHRNB2*), chromosome 8 (*CHRNB3*), and chromosome 20 (*CHRNA4*), it is not known how much of an effect there is, if any, by these genes on carcinogenesis independently of an effect on tobacco use (Thorgeirsson et al. 2008; Bierut 2009, 2010; Johnson et al. 2010; Li et al. 2011; Russo et al. 2011; Sarginson et al. 2011; Sorice et al. 2011; Timofeeva et al. 2011; Wassenaar et al. 2011; Broms et al. 2012; Budulac et al. 2012; Kapoor et al. 2012). Separately, there are data on CYP2A6 genetics and nicotine metabolism that show associations with smoking behavior, nicotine levels, and lung cancer risk (Wassenaar et al. 2011; Gold and Lerman 2012; Liu et al. 2013; Zhu et al. 2013).

Summary

There is insufficient data to conclude that nicotine causes or contributes to cancer in humans, but there is evidence showing possible oral, esophageal, or pancreatic cancer risks. Additionally, there is substantial experimental evidence indicating that nicotine is bioactive for a number of carcinogenic mechanisms in experimental systems. Although in vitro data are suggestive of relevant biological activity, this is not supported overall by the most recent experimental animal studies. In humans, there has been limited research and only one relatively short–term follow-up study on nicotine and cancer.

Cardiovascular Diseases

The potential role of nicotine in atherogenesis and in triggering acute coronary events has been discussed extensively in the medical literature (USDHHS 2010) and reviewed in Chapter 8, "Cardiovascular Diseases," of this volume. It is likely that the sympathomimetic effects of nicotine increase heart rate and myocardial contractility, increase coronary vascular resistance, and reduce insulin sensitivity, contributing to some extent to increasing cardiovascular risk in smokers. However, other mechanisms by which nicotine might contribute to atherogenesis have also been proposed (Lee and Cooke 2011). nAChRs are found not only in neuronal and muscle cells but also in endothelial cells and immune cells. Nicotine has been reported to induce the proliferation of vascular smooth muscle cells and migration of cells into blood vessels (Lee and Cooke 2012). In apoE*deficient mouse models of atherosclerosis, oral nicotine was shown to promote plaque progression and neovascularization. The primary nicotine receptor in endothelial cells is the alpha 7 homomeric nicotine receptor. In mice deficient in this receptor subtype, the effect of nicotine in augmenting angiogenesis

is blunted. Tolerance develops to many of the effects of nicotine with prolonged exposure, both in people and animals. Chronic oral administration of nicotine was shown to abolish the augmenting effect of nicotine on angiogenic responses to limb ischemia (Konishi et al. 2010). Thus, it is unclear whether the short-term effects of nicotine in enhancing angiogenesis persist with long-term exposure, as seen with users of tobacco or other nicotine-delivering products.

A genomewide association study found an association between a gene cluster on chromosome 15 and an increased risk of peripheral arterial obstructive disease (Thorgeirsson et al. 2008). Since this gene cluster is strongly associated with the level of nicotine dependence, it is not clear whether the association indicates a direct role of nicotine in atherosclerosis.

Immune Function and Related Disorders

Nicotine has both stimulatory and suppressive effects on the immune system, and levels of nicotine, inferred from urine markers, have been linked with both induction of and protection from immunologically mediated disease (Cloëz-Tayarani and Changeux 2007). Nicotine exerts its effects via pentameric nicotinic cholinoceptors that vary in their alpha and beta subunit composition (USDHHS 2010). Nicotine can act directly on cells, but in vivo it is also a direct activator of the sympathetic nervous system, which itself can have strong immune-regulatory effects. Aged-smoke extracts that still contain all of the nicotine of fresh smoke but lack reactive intermediates are much less active in immune assays than freshly prepared, oxidant-rich extracts (Laan et al. 2004; Bauer et al. 2008). Nicotine patches or mecamylamine (a full antagonist) or nicotine partial antagonists (e.g., varenicline), which are used as adjuncts in smoking cessation, are not immune-modulatory in humans (Cahill et al. 2008), and snus (the nicotine-rich low nitrosamine smokeless tobacco product that is used widely in Sweden) does not replicate the effects of smoking. This interpretation is consistent with research with macrophages where the effects of smoking on immunity were linked to oxidation (McMaster et al. 2008).

This highly contradictory literature is further reinforced by studies on human immune effector cells linked to atherosclerosis where nicotine was found to stimulate, not suppress, dendritic cells as part of adaptive immune responses (Aicher et al. 2003). However, a large body of evidence suggests that nicotine acting via the alpha 7 subunit that contains neuronal nicotinic cholinoceptors can suppress cellular immunity both in vivo and in vitro. Nicotine suppresses the production of antibodies in B cells, reduces the proliferation of T cells, and induces an anergy-like state where signaling via the T cell receptor is attenuated (Geng et al. 1995, 1996). These effects have been linked to the impaired host defense response to bacteria and viruses in nicotine-treated animals.

In summary, as reviewed here and discussed in more detail in Chapter 10, "Other Specific Outcomes," there is compelling evidence that nicotine affects cellular immunity, either directly by interacting with nicotinic cholinoceptors or indirectly via its effects on the nervous system. Whether these effects contribute to the overall adverse effects of cigarette smoke on immunity is less well-understood.

Reproductive Health Outcomes

Pregnancy is accompanied by a complex series of maternal physiological adjustments to support fetal growth and homeostasis. Basic characteristics of embryologic and fetal development include cell growth, differentiation, interaction, and migration. Teratogenic factors can disturb one or more of these processes, resulting in abnormalities in fetal structure or function, including growth retardation, malformations due to abnormal growth or morphogenesis, and altered CNS performance (Hacker et al. 2010). In addition, there is a growing appreciation that teratogenic substances can have effects throughout the duration of pregnancy, and that those effects can be more subtle than gross anatomic anomalies (Yaffe and Aranda 2011). Thus, for women of reproductive age, a comprehensive exploration of the known and potential harms of the range of available tobacco products, all of which contain nicotine, is needed. The health effects of smoking and of components in tobacco smoke, including nicotine, on reproduction are reviewed in Chapter 9, "Reproductive Outcomes." A focused review of what is known about the effects of nicotine on maternal and fetal health outcomes is presented here.

Cigarette use before and/or during pregnancy remains a major cause of reduced fertility as well as maternal, fetal, and infant morbidity and mortality (see Chapter 9) and over 400,000 live-born infants in the United States are exposed in utero to tobacco from maternal smoking annually (Hamilton et al. 2012; Tong et al. in press). Conditions causally associated with maternal prenatal smoking include preterm delivery and fetal growth restriction, placenta previa, placental abruption, sudden infant death syndrome (SIDS), some congenital anomalies, ectopic

pregnancy, and reduced preeclampsia risk. Maternal prenatal smoking has also been associated with stillbirth and spontaneous abortion (USDHHS 2004).

Much of what can currently be inferred about nicotine and reproductive health comes from studies comparing the effects of prenatal smokeless tobacco use with the effects of prenatal smoking because smokeless tobacco products do not expose users to products of combustion, but all contain nicotine. In addition, some smokeless products such as Swedish snus contain lower levels of certain toxicants when compared with conventional smokeless tobacco products (Stepanov et al. 2008). This differential exposure between snus and other smokeless products allows researchers to study the health effects of smokeless tobacco while reducing the likelihood that adverse outcomes will mistakenly be attributed to nicotine. Studies of health outcomes in randomized trials of nicotine therapy in pregnant women offer additional insights.

Fetal Growth Restriction

It has been believed for decades that in utero exposure to cigarette smoke causes fetal growth restriction through nicotine-mediated vasoconstriction of uteroplacental vessels (Lambers and Clark 1996). However, this hypothesis has been questioned because it is not likely that nicotine's vasoconstrictive effects are sufficient to overcome placental circulatory reserve (Benowitz and Dempsey 2004). Further evidence against a vasoconstrictive mechanism comes from studies of pregnancy outcomes in smokeless tobacco users. These studies have consistently demonstrated only modest contributions of smokeless tobacco to reduced infant birth weight (England et al. 2003, 2012; Gupta and Sreevidya 2004; Juarez and Merlo 2013). Results from a population-based study in Sweden conducted from 1999–2010 suggest that smokeless tobacco (snus) use increases the risk for delivering a small-for-gestational-age infant (for term births, adjusted odds ratio [AOR] = 1.21; 95% confidence interval [CI], 1.02–1.43). The effect was smaller in magnitude for smokeless tobacco than for cigarette smoking (AOR = 2.27; 95% CI, 2.62–2.91) (Baba et al. 2013). In a trial of 250 women randomized to a nicotine patch (15 mg) or to placebo for 11 weeks, the researchers found that there was no difference between the two arms in quit rates or in saliva cotinine, but birth weight was significantly higher in the NRT group (186g [95% CI, 35–336g]), possibly due to reduced cigarette smoking and exposure to products of combustion. Taken together, these studies support a modest role for nicotine in fetal growth restriction.

Preterm Delivery

Maternal smoking is associated with a 27% increase in the risk of preterm delivery (Shah and Bracken 2000) and several studies have also found an increased risk of preterm delivery in smokeless tobacco users (Gupta and Sreevidya 2004; Baba et al. 2012; England et al. 2013). In Sweden, snus use and smoking during pregnancy were both associated with increased risks of preterm birth, and the magnitudes of the associations were similar (Baba et al. 2012). Together, these studies provide evidence that nicotine increases the risk of preterm delivery. The potential roles of nicotine and products of combustion in preterm delivery are discussed in detail in Chapter 9.

Stillbirth, Perinatal Mortality, and Sudden Infant Death Syndrome

Studies of stillbirth have also been conducted among smokeless tobacco users. Studies in India and Sweden showed an increased risk of stillbirth in women using smokeless tobacco (Krishna 1978; Gupta and Subramoney 2006; Wikström et al. 2010). In the study conducted in Sweden, when antenatal bleeding and small-for-gestational-age deliveries were excluded, the smoking-related risk of stillbirth was markedly reduced although the elevated risk for snus users remained the same. These findings suggest that the mechanisms underlying the associations between smoking and stillbirth and between smokeless tobacco use and stillbirth both involve nicotine, but other factors may also contribute to increased risk in smokers (Wikström et al. 2010).

The effects of nicotine on the brainstem, cardiopulmonary integration, fetal and neonatal responses to hypoxic stress, and arousal in early infancy are reviewed in Chapter 9. For example, it has been hypothesized that tobacco-related changes in autonomic function and/or arousal could increase the risk of SIDS, although a mechanistic pathway has not been established (American Academy of Pediatrics Task Force on Sudden Infant Death Syndrome 2011). Studies of human infants have shown an association between prenatal exposure to cigarette smoke and impaired recovery from hypoxia in preterm infants (Schneider et al. 2008) and an association with impaired arousal patterns that correlates with cotinine levels (Richardson et al. 2009). Maternal prenatal cigarette use has also been associated with increased obstructive apnea and decreased arousal in response to apnea events in infants (Sawnani et al. 2004). Additional data suggest that maternal prenatal smokeless tobacco use also increases infants' risk of apnea, of a similar magnitude to that seen with maternal smoking (Gunnerbeck et al. 2011).

Extensive animal research has generated plausible and generalizable models to explain how nicotine could increase the risk of SIDS and perinatal mortality (Slotkin and Seidler 1988); these models are reviewed in Chapter 9. In one such model, the fetal/infant protective response to hypoxia is impaired. During parturition, the fetus normally experiences significant hypoxia, but is able to respond with a massive release of catecholamines from the adrenal medulla (Lagercrantz and Bistolelli 1977; Lagercrantz and Slotkin 1986) in order to maintain blood flow to the brain and heart. In the fetus and neonate, the adrenal gland responds directly to hypoxia, independent of central reflexes, and this direct mechanism persists until chromaffin cells differentiate after the development of splanchnic nerve function (Slotkin 1998). However, prenatal nicotine exposure in rat models causes immature chromaffin cells in the adrenal gland to differentiate prematurely, resulting in loss of the normal direct stimulation of the adrenal gland by hypoxia, complete absence of catecholamine release, and impaired cardiac response in the presence of hypoxia (Slotkin 1998). The effect is a temporary loss of a critical protective response to hypoxia and, theoretically, is accompanied by a temporary increased mortality risk (Slotkin 1998).

Congenital Malformations

In this report, the evidence was determined to be sufficient to support a causal relationship between maternal smoking and orofacial clefts, and to be suggestive of a causal relationship for clubfoot, cryptorchidism, gastroschisis, and some types of congenital heart defects (see Chapter 9). The 2010 Surgeon General's report examined the biological basis for increased risk of congenital defects in infants of mothers who smoke and specifically considered the potential role of nicotine (USDHHS 2010); this report updates that review. A number of potential mechanisms were cited by which nicotine having crossed the placenta, could contribute to defects.

Summary

The evidence supports the hypothesis that nicotine plays a key role in mediating adverse effects of smoking on reproductive health, including preterm delivery and stillbirth. Smoking has been linked to diverse adverse health outcomes for the developing fetus and experimental research and pharmacologic understanding indicate that nicotine specifically has a role in causing them.

Lung Development

The 2004 Surgeon General's report concluded that "the evidence is sufficient to infer a causal relationship between maternal smoking during pregnancy and a reduction of lung function in infants" (p. 27). This conclusion was based on epidemiologic studies that consistently demonstrated an inverse dose-response relationship between the number of cigarettes smoked per day during pregnancy by the mother and the level of lung function and pulmonary compliance in the newborn. The 2006 Surgeon General's report expanded the conclusions of the 2004 report to address the duration of effects after infancy: "The evidence is sufficient to infer a causal relationship between maternal smoking during pregnancy and persistent adverse effects on lung function across childhood" (p. 399). The report further concluded that the "evidence shows that parental smoking *(referring to secondhand smoke exposure and maternal smoking during pregnancy)* reduces the maximum achieved level *(of lung growth),* although not to a degree (on average) that would impair individuals" (p. 400). "Nonetheless, a reduced peak level increases the risk for future chronic lung disease, and there is heterogeneity of the effect so that some exposed children may have a much greater reduction than the mean" (p. 400). This section considers studies on the mechanisms underlying the relationship between maternal smoking and the infant or child's lung development and function and the potential role of nicotine in these mechanisms.

Human lung development begins in the embryonic stage and extends through early adulthood. During fetal lung growth, structural and vascular development take place and major airway branching and mesenchymal proliferation are complete by the end of the second trimester. Alveolarization (marked by septation and multiplication of alveoli) begins in the third trimester of pregnancy and multiplication of alveolar number continues to 2–3 years of age, when lungs reach the full adult quantity of approximately 300 million alveoli. Alveolar size and surface, however, increase until after adolescence as the lungs grow (Joshi and Kotecha 2007). Lung development is tightly regulated, and intrauterine and postnatal environmental factors can interfere with this complicated set of processes. The alveolar phase of development is particularly sensitive to late-pregnancy and postnatal insults (Harding and Maritz 2012).

As reviewed in previous Surgeon General's reports, the clinical and epidemiologic data strongly support that maternal smoking in pregnancy has lasting effects on lung development. Studies of infants exposed in utero to tobacco smoke show evidence of impaired lung function with reduced respiratory compliance, forced expiratory flow, and tidal breathing ratio, consistent with impaired airways development (Hanrahan et al. 1992; Tager et al. 1995; Lødrup Carlsen et al. 1997; Stocks and Dezateux 2003). Maternal prenatal smoking has also been associated with impaired lung function with reduced small airway flow rates in school-age offspring (Cunningham et al. 1994, 1995), even after adjustment for the offspring's current and past exposure to secondhand smoke (Gilliland et al. 2000), and with deficits in measures of airflow among adolescents, especially among those with a history of early-onset asthma (Gilliland et al. 2003). There is also evidence to suggest that exposure to prenatal tobacco smoke could result in an acceleration of lung aging and an increased susceptibility to obstructive lung disease, lasting beyond childhood (Maritz and Harding 2011).

Numerous studies using animal models have been conducted to develop a better understanding of the mechanisms through which maternal smoking affects fetal and infant lungs. These studies are summarized in several review articles (Stocks and Dezateux 2003; Maritz 2008; Maritz and Harding 2011). Studies in primates specifically examining nicotine exposure have demonstrated decreased lung size and volume, increased type I and type III collagens, decreased elastin in the lung parenchyma, increased alveolar volume, and increased airway wall area (Sekhon et al. 1999, 2001, 2002). Animal studies have also demonstrated decreased expiratory flow rates and increased pulmonary resistance with nicotine exposure, similar to findings in human studies (Hanrahan et al. 1992; Cunningham et al. 1995; Tager et al. 1995; Dezateux et al. 1999). Primate studies further suggest that nicotine-induced changes in airway wall thickness or stiffness could be an underlying cause of altered lung function (Pierce and Nguyen 2002; Sekhon et al. 2002). Finally, nicotine exposure in fetal lambs has been associated with accelerated maturation of lung acini and reduced proximal airway conductance (Sandberg et al. 2004), hyperreactive proximal airways, and changes in proximal airway wall composition with associated defects in airflow (Sandberg et al. 2011).

At the molecular level, nicotine crosses the placenta and binds nAChRs in numerous locations in the lung, including bronchial epithelial cells, alveolar epithelial cells, neuroendocrine cells, submucosal glands, airway and vascular smooth muscle cells, fibroblasts, and pulmonary macrophages (Pierce and Nguyen 2002). Nicotine administration to pregnant rhesus monkeys is associated with an increase in nAChRs in the lungs (Sekhon et al. 1999; Fu et al. 2009), increased collagen deposition in airway walls, and increases in the numbers of alveolar type II and neuroendocrine cells (Sekhon et al. 1999, 2002). Coinciding with these changes are alterations in smooth muscle and vascular tension, perhaps explaining the effects of maternal smoking on infant lung function (Stocks and Dezateux 2003). Other hypothesized mechanisms through which nicotine could affect lung development include premature onset of cell differentiation and decreased replication and impaired alveolar development—resulting from altered expression or deposition of elastin (Pierce and Nguyen 2002; Stocks and Dezateux 2003).

Together, these findings indicate that nicotine is a primary mediator of many of the adverse effects of maternal smoking on fetal lung development. However, the mechanisms involved remain incompletely understood.

Summary

Studies reviewed in the 2004 and 2006 Surgeon General's reports and subsequently published data collectively show that prenatal tobacco exposure affects the structure and function of the lung; these effects may have consequences that last into childhood beyond, as lung development and growth are completed. Studies in rhesus monkeys, which have lung development similar to that of humans, and in other animal models consistently show that nicotine may be the primary mediator of many of the adverse effects of maternal smoking on fetal lung development.

Cognitive Function

Researchers have suggested that smoking may have cognition-enhancing properties (West 1993; Heishman et al. 2010), such as improvements in sustained attention, reaction time, and memory (Evans and Drobes 2008; Poorthuis et al. 2009; Heishman et al. 2010). Initial reports of improved cognitive function were based on empirical evidence from smokers (Bell et al. 1999); thus, these observations could reflect the mitigation of cognitive impairment from nicotine withdrawal, enhancement of smokers' cognitive function independent of nicotine's effects on withdrawal symptoms, or both. Interest in the effects of nicotine on cognition has since expanded to include healthy nonsmokers and individuals with underlying neuropsychiatric conditions accompanied by cognitive deficits. Concurrently, there is a growing awareness of the potential harms of nicotine exposure during certain vulnerable stages of brain development, such as during fetal and adolescent growth (Dwyer et al. 2008; Duncan et

al. 2009; Poorthuis et al. 2009; Bublitz and Stroud 2012; Goriounova and Mansvelder 2012). This section reviews the evidence on the effects of nicotine on cognitive function in general (in smokers and nonsmokers), and in potentially vulnerable populations.

Cognitive Function and the Nicotinic Acetylcholine Receptor System

Underlying the purported connection between nicotine and cognitive enhancement is the role of nAChRs in attention, learning, memory, and cortical plasticity (Wallace and Bertrand 2013). nAChRs are receptors that normally bind endogenous neurotransmitter acetylcholine, but are also particularly responsive to nicotine. nAChRs are abundant in brain regions associated with learning and memory, including the prefrontal cortex (Poorthuis et al. 2009), and in primate and rodent models, depletion of acetylcholine in the prefrontal cortex results in impaired attentional performance (Poorthuis et al. 2009; Wallace and Bertrand 2013). β2 nAChRs are especially abundant in the brain and have a high affinity for nicotine (Evans and Drobes 2008; Poorthuis et al. 2009; Herman and Sofuoglu 2010). Recent evidence from animal studies suggests that β2 nAChRs play a critical role in regulating attention (Howe et al. 2010; Poorthuis et al. 2013a). Additional research has demonstrated that nicotine interferes with cholinergic control of β2 nAChRs in the prefrontal cortex in mice, which could result in acute impairment of attention and alterations of the prefrontal cortex network, and lead to long-term effects on attention (Poorthuis et al. 2013a). Mice lacking the β2 nAChR subunit demonstrate deficits in executive function (Granon et al. 2003).

Effects of Nicotine on Cognitive Function in Healthy Adult Smokers and Nonsmokers

In adults, the negative effects of nicotine withdrawal on cognitive function have been documented in both humans and animals, and the administration of nicotine during withdrawal mitigates cognitive impairment (Evans and Drobes 2008). In dependent smokers, abstinence from smoking is associated with reductions in working memory and sustained attention (Evans and Drobes 2008), and adverse effects on attention can be seen as early as 30 minutes after smoking the last cigarette (Hendricks et al. 2006). Nicotine withdrawal is also commonly accompanied by symptoms of negative affect (anxiety and depression) (Edwards and Kendler 2011) and relief of this symptom may be an important element of addiction in smokers (Baker et al. 2004). Because negative affect and attentional control are related, the effects of smoking on these two domains could be interrelated (Evans and Drobes 2008).

Whether there are direct effects of nicotine on cognitive function (positive or negative) in nonabstinent smokers and in healthy nonsmoking adults is less clear. In a recent meta-analysis of double-blind, placebo-controlled studies examining the acute effects of nicotine (administered mainly as nicotine replacement product) on cognitive function in nonsmokers and smokers abstinent for 2 hours or less, nicotine was found to result in cognitive enhancement in six of nine performance domains: fine motor, alerting attention-accuracy and response time (RT), orienting attention and RT, short-term episodic memory accuracy, and working memory RT (Heishman et al. 2010). To separate the effects of nicotine on symptoms of withdrawal versus its direct effects, the results were stratified by smoking status. The effects on alerting attention accuracy and short-term episodic memory accuracy were significant in smokers but not in nonsmokers; effects on alerting attention RT were significant in nonsmokers but not in smokers; effects on working memory RT were significant in both smokers and nonsmokers, and in the remaining outcomes there were insufficient numbers of studies on smokers to conduct stratified analysis. Thus, nicotine may have some positive effects on cognitive performance that are unique to nonsmokers. No studies meeting the inclusion criteria for the review addressed learning or executive function.

Critical Periods of Exposure in the Nervous System

Across the lifespan, there are several developmental windows during which exposure to nicotine may have adverse consequences. In the fetus, nicotine targets neurotransmitter receptors in the brain, potentially resulting in abnormalities in cell proliferation and altering synaptic activity (Slotkin 1998). The effects of prenatal exposure to nicotine on the fetal nervous system are summarized earlier in this chapter and elsewhere in this report (see Chapter 9).

Human brain development continues far longer than was previously realized. In particular, areas involved in higher cognitive function such as the prefrontal cortex continue to develop throughout adolescence (the period during which individuals are most likely to begin smoking) and into adulthood (Poorthuis et al. 2009; Goriounova and Mansvelder 2012). During this extended period of maturation, substantial neural remodeling occurs, including synaptic pruning and changes in dopaminergic input, as well as changes in gray and white matter volume. The density of projections from the amygdala to the prefrontal cortex increases, suggesting that there is substantial development of the connectivity between the emotional and cognitive areas of the brain (Durston et al. 2001; Ernst and Fudge 2009). The cholinergic system, which matures

in adolescence, plays a central role in maturation of cognitive function and reward (Poorthuis et al. 2009).

Smoking during adolescence has been associated with lasting cognitive and behavioral impairments, including effects on working memory and attention, although causal relationships are difficult to establish in the presence of potential confounding factors (Goriunova and Mansvelder 2012). In addition, functional magnetic resonance imaging in humans showed that young adult smokers had reduced prefrontal cortex activation during attentional tasks when compared with nonsmoking controls. Diminished prefrontal cortex activity correlated with duration of smoking, supporting the hypothesis that smoking could have long-lasting effects on cognition (Musso et al. 2007).

Animal studies provide evidence that nicotine exposure during adolescence has effects on the brain that differ from exposure during other periods of development. Studies in rodents show that nicotine induces changes in gene expression in the brain to a greater degree with adolescent exposure than during other periods of development (Schochet et al. 2005; Polesskaya et al. 2007). DNA microarrays in female rats demonstrated that gene expression in response to nicotine was most pronounced around the age of puberty and the effects of nicotine on gene expression were most dramatic in the hippocampus, with upregulation of growth factors and cyclic AMP signaling pathways (Polesskaya et al. 2007). Expression of the *Arc* gene (implicated in synaptic plasticity, learning, memory, and addiction) was upregulated in the prefrontal cortex in adolescent rats exposed to nicotine, and to a much greater extent than in adult rats (Schochet et al. 2005).

Nicotine exposure during adolescence also appears to cause long-term structural and functional changes in the brain (Dwyer et al. 2009). Exposure of adolescent rats to nicotine resulted in upregulation of nAChRs in the midbrain, cerebral cortex, and hippocampus that was still present 4 weeks after the end of the exposure, in contrast to adult rats in which upregulation had disappeared by 4 weeks. Receptor upregulation was more pronounced in male adolescent rats than females (Trauth et al. 1999). Indices of cell damage and size in rats with adolescent nicotine exposure indicate reduced cell number and size in the cerebral cortex, midbrain, and hippocampus (Trauth et al. 2000). Structural changes in prefrontal cortex neurons have also been described, including increased dendritic length and spine density (Brown and Kolb 2001).

Some effects of nicotine exposure appear to be gender-selective. For example, adolescent nicotine exposure resulted in increased membrane protein concentration in the hippocampus, consistent with cell damage and/or cell loss, in female rats, but not in males (Trauth et al. 1999). Male rats with nicotine exposure demonstrated a loss of a dopaminergic response to nicotine more than a month after exposure ended, while females exhibited deficits in hippocampal norepinephrine content and turnover during the month after nicotine exposure (Trauth et al. 2001). Because estrogen regulates hippocampal cell proliferation in an adult rat, there may be interactions between the effects of nicotine and the hormonal milieu in the adolescent (Trauth et al. 1999).

Corresponding behavioral studies of adolescent rats have also shown effects of nicotine exposure. Exposed females exhibited reduced grooming during exposure and reduced locomotion and rearing after cessation of exposure; these results were not seen in exposed adult rats, which show increased grooming in both genders and no decrease in locomotion (Trauth et al. 2000). Adolescent rats, tested 5 weeks after nicotine exposure ended, demonstrated an increase in premature responses and a reduction in correct responses when given a serial reaction time test; this effect was not seen with adult exposure (Counotte et al. 2009).

Thus, adolescents appear to be particularly vulnerable to the adverse effects of nicotine on the CNS. Based on existing knowledge of adolescent brain development, results of animal studies, and limited data from studies of adolescent and young adult smokers, it is likely that nicotine exposure during adolescence adversely affects cognitive function and development. Therefore, the potential long-term cognitive effects of exposure to nicotine in this age group are of great concern.

The effects of nicotine exposure on cognitive function after adolescence and young adulthood are unknown. There are data to suggest that smoking accelerates some aspects of cognitive decline in adults, and that these effects appear to be mediated by an increased risk of respiratory and cardiovascular disease (Swan and Lessov-Schlaggar 2007; Almeida et al. 2011). However, in a cohort study of more than 7,000 men and women, the authors found that current male smokers and recent former smokers had a greater 10-year decline in global cognition and executive function than never smokers (with the greatest adverse effect on executive function); these differences were not explained by other health behaviors or measures, including heart disease and stroke, and measures of lung function. An analysis using pack-years[2] as the exposure measure provided evidence of a dose-response relation-

[2]Pack-years: the number of years of smoking multiplied by the number of packs of cigarettes smoked per day.

ship. The results of the latter study suggest that there may be mechanisms contributing to cognitive decline in addition to and independent of respiratory and cardiovascular disease; however, whether nicotine plays a role in accelerating cognitive decline is unknown.

Other Vulnerable Populations

Although the contribution of nicotine to the effects of smoking on cognitive decline is unclear, there has been a great deal of interest in applications of nicotine as a treatment for several conditions characterized by cognitive deficits, including Alzheimer's disease and Parkinson's disease. These disorders have underlying deficits in the cholinergic system, and it has been hypothesized that nicotine and/or nicotine analogs may be effective in attenuating symptoms or slowing disease progression. This hypothesis is further supported by research (reviewed earlier in this chapter) suggesting that acute administration of nicotine has cognitive-enhancing properties. In addition, some early observational studies showed evidence for a reduced risk of Alzheimer's in smokers, suggesting that components in tobacco smoke, such as nicotine, may have protective properties. A growing body of evidence now links smoking to an increased risk for Alzheimer's disease (Almeida et al. 2002; Anstey et al. 2007; Hernán et al. 2008; Purnell et al. 2009) rather than a reduced risk; however, research on nicotine as a treatment for this condition (and for Parkinson's disease) continues.

Other disorders associated with cognitive and attentional impairment, such as schizophrenia and attention deficit hyperactivity disorder (ADHD), are characterized by a very high prevalence of smoking among those affected. It has been proposed that individuals with these disorders smoke in order to alleviate the symptoms of their disease, and a number of clinical trials using nicotine as a therapeutic agent have been conducted.

Alzheimer's Disease

Alzheimer's disease is a common form of dementia in which individuals experience ongoing deterioration of cognitive abilities. Although smoking is recognized as a risk factor for Alzheimer's disease (Peters et al. 2008; Cataldo et al. 2010), acute nicotine administration has been reported to improve some Alzheimer's symptoms, such as recall, visual attention, and mood (Lopez-Arrieta and Sanz 2001). The plausibility of this effect is supported by studies of Alzheimer's disease patients showing deficits in cholinergic systems and a loss of nicotinic binding sites (Whitehouse et al. 1982). However, evidence from randomized trials to support improvement of Alzheimer's symptoms from nicotine treatment is sparse. In a 2001 Cochrane review updated in 2010, the authors found no double-blind, placebo-controlled, randomized trials of treatment for Alzheimer's disease with nicotine and concluded that there is no evidence to recommend nicotine as a treatment for Alzheimer's disease (Lopez-Arrieta and Sanz 2001).

Parkinson's Disease

Parkinson's disease is a degenerative hypokinetic movement disorder. Most patients with Parkinson's disease will also eventually develop cognitive impairment—with deficits in attention, executive and visual-spatial functions, and memory—and subsequent dementia. In Parkinson's disease, both the dopaminergic and cholinergic systems undergo degeneration, which leads to deficits in dopamine and acetylcholine at synapses; thus, nicotinic mechanisms may play a role in cognitive deficits. In contrast to Alzheimer's, data consistently support that smokers are at reduced risk for developing Parkinson's disease (Ritz et al. 2007; Wirdefeldt et al. 2011), and twin studies have reported a 20–30% reduction of Parkinson's disease risk for ever smoking or regular smoking in monozygotic and dizygotic, same-gender male twin pairs who were discordant for Parkinson's disease (Tanner et al. 2002; Wirdefeldt et al. 2005). This suggests that genetic factors contributing to both Parkinson's disease and smoking are not responsible for the apparent smoking and Parkinson's disease association.

Two studies have examined the association between smokeless tobacco use and risk of Parkinson's disease: a case-control study found a significant inverse association (odds ratio [OR] 0.18; 95% CI, 0.04–0.82, in ever users vs. never users of smokeless tobacco) (Benedetti et al. 2000) and a prospective cohort study that assessed Parkinson's disease mortality as the outcome found a relative risk of 0.22 (95% CI, 0.07–0.67) for current users of smokeless tobacco versus never users (O'Reilly et al. 2005). These studies add support for a protective role for nicotine. However, there are few controlled trials of the effects of nicotine on cognitive function in patients with Parkinson's disease, and results have been inconsistent (Kelton et al. 2000; Vieregge et al. 2001; Lemay et al. 2004; Holmes et al. 2011).

ADHD and Schizophrenia

Several neuropsychiatric disorders characterized by attention-related cognitive defects are characterized by high prevalence of smoking, including ADHD and schizophrenia. It has been suggested that smoking may be particularly reinforcing for individuals with these conditions because of the cognitive-enhancing effects of nicotine. Because cholinergic systems play an important role in functional impairments in certain neurodegenerative

diseases, it also has been suggested that individuals with attention-related cognitive defects may benefit from treatment with nicotine through nicotine's role as a cholinergic agonist (Singh et al. 2004; Kumari and Postma 2005; Evans and Drobes 2008). Some research suggests that nicotine may improve attention performance in individuals with ADHD and schizophrenia (Evans and Drobes 2008).

ADHD is a common disorder of childhood with symptoms of inattention and hyperactivity/impulsivity. Behavioral inhibition and delay aversion deficits are believed to be factors contributing to impulsive behavior. Other features, such as poor planning, and deficits in working memory and cognitive flexibility, are more recently recognized traits. Limited research suggests that nicotine might improve the symptoms and measures of behavioral inhibition, delay aversion, and recognition memory in individuals with ADHD (Gehricke et al. 2006, 2009).

Schizophrenia is a chronic disorder marked by delusions, hallucinations, thought disorder, and negative symptoms such as flattening of affect. The evidence suggests that dysregulation of cholinergic systems is involved in altered sensory physiology and individuals with schizophrenia have decreased dopaminergic activity in the prefrontal cortex (Punnoose and Belgamwar 2006). The prevalence of smoking in individuals with schizophrenia is high, perhaps as the result of an effort of patients to relieve symptoms associated with the disorder (Kumari and Postma 2005). Specifically, it has been suggested that nicotine-induced release of dopamine could improve attention and processing symptoms and sensory-gating deficits in schizophrenia, and that nicotine treatment could attenuate antipsychotic-induced cognitive impairment and extrapyramidal symptoms, through nicotine's effects on dopamine release (Alder et al. 1993; Newhouse et al. 2004; Birkett et al. 2007; Evans and Drobes 2008). However, in a 2012 Cochrane Review update, the authors reviewed all randomized controlled trials in which nicotine or tobacco and placebo were administered to patients with schizophrenia or schizophrenia-like illness and found no studies that met the inclusion criteria. A number of studies were excluded because they were a crossover design, which was determined to be inappropriate because schizophrenia is an unstable condition and nicotine may have carryover effects (Punnoose and Belgamwar 2006).

Tobacco Industry Influence

The tobacco industry has a long-standing interest in nicotine and neurocognitive functioning and psychiatric disease. The tobacco industry has invested in pharmaceutical applications of nicotine and nicotine analogs for decades (Vagg and Chapman 2005). Philip Morris and R.J. Reynolds both developed research programs to explore the potential uses of nicotine and analogs in the treatment of neurological disorders (R.J. Reynolds 1993). In the early 1990s, R.J. Reynolds established both its "Nicotine Pharmacology and Neurodegenerative Disease Program" and later Targacept, a pharmaceutical company, for the purpose of discovering therapeutic uses of nicotinic compounds. Tobacco industry documents indicate that diversification into the pharmaceutical industry was seen not only as potentially profitable but also as a strategy to improve the tobacco industry's corporate image (Vagg and Chapman 2005).

Data from observational studies describing the protective effects of smoking on the risk of Parkinson's disease and Alzheimer's disease and the high prevalence of smoking among individuals with ADHD and schizophrenia are often cited in industry-sponsored and nonindustry-sponsored literature as evidence to support the therapeutic applications of nicotine. However, there is evidence that the tobacco industry influenced many of these epidemiologic studies of smoking and psychiatric disorders. For example, an analysis of publications on the relationship between smoking and Alzheimer's disease that controlled for authors' industry affiliation revealed that pooled ORs for studies without industry funding were neutral or indicated an increased risk with smoking, depending on study design, while industry-affiliated studies indicated a reduced risk (Cataldo et al. 2010). Studies of tobacco industry documents have also revealed that the industry sought to influence scientific attitudes regarding the role of smoking in schizophrenia (Prochaska et al. 2008). Tobacco industry documents indicate that the industry funded research for the specific purpose of perpetuating the belief that smoking improves symptoms in schizophrenic patients, advocated for exceptions for smoking in hospitalized psychiatric patients, and funded studies of medicinal uses of nicotine analogs to treat mental illnesses (Prochaska et al. 2008).

Evidence of the tobacco industry's interest in the cognitive-enhancing properties of nicotine comes from a 1997 review of publications investigating the effects of tobacco and nicotine on cognitive performance. Turner and Spilich (1997) found that authors acknowledging tobacco industry funding were much less likely than nonindustry-funded authors to report a negative effect of nicotine on cognitive performance. Nonindustry-funded authors reported both positive and negative findings, while industry-funded authors reported positive findings almost exclusively (Turner and Spilich 1997). Studies of this type using more recent published articles are needed to better understand current industry influences on the scientific literature.

It is difficult to estimate the extent to which industry-generated research activities have influenced scientific thinking regarding the effects of nicotine on cognitive performance and on nicotine's therapeutic applications. Authors' industry affiliations and potential conflicts of interest reported in publications may go unnoticed by readers, may be difficult to identify, or may not be disclosed at all. Reviews and other articles citing industry-affiliated studies generally did not include author affiliations or potential conflicts of interest at all, leaving the readers unaware of possible industry influences. A growing concern about conflicts of interest resulting from funding through the tobacco industry is reflected in the National Institute on Drug Abuse (NIDA) advice to its grantees that "Receiving funding from the tobacco industry may compromise the perceived objectivity of their research results, which in turn could impact the overall credibility of their research findings, including its interpretation, acceptance and implementation" (NIDA n.d.).

Summary

Evidence shows that acute nicotine administration has some modest cognition-enhancing effects in adult smokers during withdrawal. However, less is known about the acute effects in nonabstinent smokers and in nonsmokers, and about the effects of long-term nicotine exposure on cognitive performance. Human and animal evidence show detrimental effects on cognition from smoking during aging. Evidence also shows that exposure to cigarette smoke and to nicotine has adverse effects on fetal and adolescent brain development, which could result in lasting deficits in cognitive function. Furthermore, withdrawal from tobacco in dependent-users results in cognitive impairment. Among individuals with attention-related cognitive defects, nicotine has been proposed as a potential treatment because of its effect as a cholinergic agonist. However, randomized controlled trials to demonstrate safety and efficacy of nicotine treatment in individuals with these disorders are lacking, and the long-term effects of low-dose, chronic nicotine exposure on individuals with neuropsychiatric disorders are unknown. Because nAChRs are distributed extensively across the central and peripheral nervous systems, studying the effects of nicotine across the behavioral spectrum, rather than on isolated domains, may reveal adverse effects and may help establish whether the potential benefits of nicotine are clinically meaningful (Heishman 1998).

Evidence Summary

This chapter complements reviews in prior reports and in other sections of this report on the potential toxicity of nicotine, a pharmacologically active agent that readily enters the body and is distributed throughout. Nicotine activates multiple biological pathways that are relevant to fetal growth and development, immune function, the cardiovascular system, the CNS, and carcinogenesis. Experimental research documents that nicotine plays a key role in several adverse consequences of maternal smoking for the fetus, including altered lung development, and has effects on the developing brain. Evidence supports that acute nicotine administration has modest cognition-enhancing properties in adult smokers during withdrawal and in adult nonsmokers. However, little is known about the effects of long-term nicotine exposure on cognitive performance and how nicotine withdrawal impairs cognition. Previous reports have reached causal conclusions related to nicotine and addiction (USDHHS 1988, 2010, 2012). Evidence in this chapter considers the particular vulnerability of adolescents and other groups to nicotine. Beyond the use of NRT cessation aids, the therapeutic roles for nicotine have not been established, in spite of clinical research, some carried out by the tobacco industry.

Acute toxicity of nicotine, reflecting its pharmacologic activity, is well established. There is a potential for poisoning from ingestion of nicotine-containing products.

Conclusions

1. The evidence is sufficient to infer that at high-enough doses nicotine has acute toxicity.

2. The evidence is sufficient to infer that nicotine activates multiple biological pathways through which smoking increases risk for disease.

3. The evidence is sufficient to infer that nicotine exposure during fetal development, a critical window for brain development, has lasting adverse consequences for brain development.

4. The evidence is sufficient to infer that nicotine adversely affects maternal and fetal health during pregnancy, contributing to multiple adverse outcomes such as preterm delivery and stillbirth.

5. The evidence is suggestive that nicotine exposure during adolescence, a critical window for brain development, may have lasting adverse consequences for brain development.

6. The evidence is inadequate to infer the presence or absence of a causal relationship between exposure to nicotine and risk for cancer.

Implications

Large numbers of people are exposed to nicotine through products other than conventional cigarettes, including NRT, smokeless tobacco, and new nicotine-containing noncombustible products. The fetus will be exposed to nicotine without other smoke components if the mother uses these products. The number of people exposed to nicotine long-term may grow under a number of potential future scenarios; for example, expanding use of multiple products or the replacement of conventional combustible cigarettes with other nicotine delivery systems (see Chapter 15, "The Changing Landscape of Tobacco Control: Current Status and Future Directions"), or increased appeal and uptake of nicotine product use because of their apparent relative safety in comparison to cigarettes. In considering such scenarios, information will be needed on the risks of long-term exposure to nicotine, including the consequences for reproductive health and adolescent cognitive development, compared with cigarette smoking, and no tobacco products use at all. The evidence reviewed in this chapter, in other chapters in this report, and in previous reports shows that long-term nicotine use may have adverse consequences for those exposed and it clearly harms the developing fetus. The latest U.S. Public Health Service guidelines acknowledge this risk and have not made a specific recommendation on the use of NRT during pregnancy. Pregnant women who smoke should consider and discuss with their health care providers the potential risk to the fetus from continuing to smoke and from using NRT. There is a strong recommendation from the U.S. Preventive Services Task Force for health care providers to ask pregnant women about tobacco use and provide the appropriate counseling.

The possibility of increasing chronic nicotine exposure in the population from various nicotine-containing products for the long-term merits further research. Cancer, cardiovascular, and neurocognitive outcomes are of concern. The evidence is already sufficient to provide appropriately cautious messages to pregnant women and women of reproductive age as well as adolescents about the use of nicotine-containing products such as smokeless tobacco and electronic cigarettes, and newer forms of nicotine-containing tobacco products, as alternatives to smoking.

All tobacco products contain toxicants, so all tobacco product use poses some health risks. Because of the potential for fetal and adolescent nicotine exposure to have long-term detrimental effects on brain development, measures should be taken to ensure that nicotine is not perceived by the public as a cognitive-enhancing substance. It also does not have an established role in the management of people with a severe mental illness.

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