

Chapter 2

Toxicology of Secondhand Smoke

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Introduction

A full range of scientific evidence, extending from the molecular level to whole populations, supports the conclusion that secondhand smoke causes disease. The scope of this evidence is enormous, and encompasses not only the literature on secondhand smoke but also relevant findings on active smoking and on the toxicity of individual tobacco smoke components. The 2004 report of the Surgeon General provides reviews on biologic considerations in relation to active smoking (U.S. Department of Health and Human Services [USDHHS] 2004). The guidelines for causal inference include coherence, which is defined as the extent to which all lines of scientific evidence converge in support of a causal conclusion. Beginning with the 1964 Surgeon General's report on smoking and health (U.S. Department of Health, Education, and Welfare [USDHEW] 1964), reports in this series have comprehensively evaluated the full scope of evidence supporting causal inference with regard to particular associations of smoking with disease. This chapter reviews the evidence relevant to coherence, and includes the mechanisms relevant to the pathogenesis of diseases caused by secondhand smoke.

Studies reviewed for this chapter were selected from Medline and SciFinder literature searches. Search terms included "carcinogens," "environmental tobacco smoke," "DNA adducts," "protein adducts," "urinary metabolites," "tobacco smoke," and the names of specific carcinogens and their metabolites. Recent reviews and cited references in recent papers provided additional sources for this chapter.

This chapter sets out a foundation for interpreting the observational evidence that is the focus of most of the following chapters. The discussion that follows details the mechanisms that enable tobacco smoke components to injure the respiratory tract and

cardiovascular system and to cause nonmalignant and malignant diseases and other adverse effects.

Composition of Tobacco Smoke

The chemical and physical properties of tobacco smoke from mainstream (drawn through the cigarette) and sidestream (released by the smoldering cigarette) smoke have been reviewed in a number of publications (Jenkins et al. 2000; Hoffmann et al. 2001; International Agency for Research on Cancer [IARC] 2004; California Environmental Protection Agency [Cal/EPA] 2005). The IARC (2004) review indicates that some 4,000 mainstream tobacco smoke compounds have been identified (Roberts 1988), and the qualitative composition of the components is nearly identical in mainstream smoke, sidestream smoke, and secondhand smoke. An assessment by the National Research Council (1986) of differences in the composition of mainstream and sidestream smoke indicates that some compounds are emitted at levels up to more than 10 times greater in sidestream smoke compared with mainstream smoke (see also Table III-1 in Cal/EPA 2005). The Cal/EPA (2005) report identified 19 gas-phase and 21 particulate matter compounds in sidestream smoke with known carcinogenic and non-carcinogenic health effects (e.g., pulmonary edema, immune alterations, cardiac arrhythmias, and hepatotoxic and neurologic effects). The National Toxicology Program (USDHHS 2000) estimates that at least 250 chemicals in secondhand smoke are known to be toxic or carcinogenic. Other published reports have additional listings of specific chemical compounds in mainstream and secondhand smoke (Fowles and Dybing 2003; Cal/EPA 2005).

Evidence of Carcinogenic Effects from Secondhand Smoke Exposure

Carcinogens in Sidestream Smoke and Secondhand Smoke

As a result of advances in chemical analytical techniques and an expanded understanding of the mechanisms by which environmental agents are genotoxic, the number of known carcinogens in tobacco smoke increased to 69 in the year 2000 (IARC 2004). Table 2.1 summarizes representative levels of carcinogens found in sidestream and secondhand cigarette smoke, but includes only 30 compounds that have been evaluated by IARC and that have fulfilled certain other criteria: sufficient evidence of carcinogenicity in either laboratory animals or humans and published data on levels found in sidestream or secondhand smoke. Field studies on the carcinogenic composition of secondhand smoke cannot comprehensively evaluate all of the potential carcinogens in secondhand smoke. Some tobacco smoke carcinogens that IARC evaluated were not included in Table 2.1 because there were no published data on their levels in sidestream or secondhand cigarette smoke (Hoffmann et al. 2001). It is likely, however, that these carcinogens (which include some polycyclic aromatic hydrocarbons [PAHs], heterocycles, heterocyclic aromatic amines, nitro compounds, and other miscellaneous organic compounds) are also present in sidestream and secondhand smoke. In addition, there may be carcinogens present that IARC has not yet fully characterized or evaluated.

PAHs are a diverse group of compounds formed in the incomplete combustion of organic material, and are potent, locally acting carcinogens in laboratory animals. PAHs induce tumors of the upper respiratory tract and lung when inhaled, instilled in the trachea, implanted in the lung, or administered by other routes (Shimkin and Stoner 1975), and are found in tobacco smoke, broiled foods, and polluted environments of various types. The best known member of this class of compounds is benzo[*a*]pyrene (B[*a*]P), which induces tumors of the upper respiratory tract and lung when inhaled, instilled in the trachea, implanted in the lung, or administered intraperitoneally, intravenously, subcutaneously, or by other routes (Shimkin and Stoner 1975). When administered systemically, B[*a*]P causes lung tumors in mice but not in rats (IARC 1973, 1983; Culp et al. 1998). Workers in iron and steel foundries and aluminum and coke production plants are

exposed to PAHs. These exposures are considered to be a cause of excess cancers among workers in these settings (IARC 1983, 1984).

N-Nitrosamines are a large group of carcinogens that induce cancer in a wide variety of species and tissues and are presumed to cause cancer in humans (Preussmann and Stewart 1984). These carcinogens can be formed endogenously from amines and nitrogen oxides and are found at low levels in foods (Bartsch and Spiegelhalter 1996). Tobacco smoke contains volatile *N*-nitrosamines such as *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine, as well as tobacco-specific *N*-nitrosamines such as *N*'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (Hoffmann and Hecht 1990). Tobacco-specific *N*-nitrosamines are chemically related to nicotine and other tobacco alkaloids and are therefore found only in tobacco products or related materials (Hecht and Hoffmann 1988). In laboratory animals, many *N*-nitrosamines are powerful carcinogens that display a striking organospecificity and affect particular tissues often independently of the route of administration (Preussmann and Stewart 1984). For example, NNN causes tumors of the esophagus and nasal cavity in rats, while the principal target of NNK in rodents is the lung; NNK is the only tobacco smoke carcinogen that induces lung tumors by systemic administration in all three commonly used rodent models—rat, mouse, and hamster (Hecht 1998).

Among the aromatic amines first identified as carcinogens in dye industrial exposures, 2-naphthylamine and 4-aminobiphenyl are well-established human bladder carcinogens (IARC 1973, 1974). These carcinogens are also found in tobacco smoke. Aromatic amines cause tumors at a variety of sites in laboratory animals. Some members of this class, such as 2-toluidine, are only weakly carcinogenic (Garner et al. 1984).

Formaldehyde and acetaldehyde, weaker carcinogens than PAHs, *N*-nitrosamines, and aromatic amines, have been measured in sidestream and secondhand smoke. When inhaled, formaldehyde and acetaldehyde induce respiratory tract tumors in rodents (Kerns et al. 1983; IARC 1999). Butadiene and benzene are volatile hydrocarbons that also occur in considerable quantities in sidestream and secondhand smoke. Butadiene is a multiorgan carcinogen that is particularly potent in mice; benzene causes leukemia

Table 2.1 Levels of carcinogens in sidestream and secondhand cigarette smoke

Carcinogen	Representative amounts		Study
	Sidestream (per cigarette)	Secondhand (per cubic meter [m ³])	
Polycyclic aromatic hydrocarbons			
Benz[<i>a</i>]anthracene	201 nanograms (ng)	0.32–1.7 ng	Grimmer et al. 1987; Chuang et al. 1991
Benzo[<i>a</i>]pyrene	45–103 ng	0.37–1.7 ng	Adams et al. 1987; Grimmer et al. 1987; Chuang et al. 1991
Benzo[<i>b</i>]fluoranthene Benzo[<i>j</i>]fluoranthene Benzo[<i>k</i>]fluoranthene	196 ng	0.79–2.0 ng	Grimmer et al. 1987; Chuang et al. 1991
Dibenz[<i>a,h</i>]anthracene	NR*	1 ng	Vu-Duc and Huynh 1989
Indeno[1,2,3- <i>cd</i>]pyrene	51 ng	0.35–1.1 ng	Grimmer et al. 1987; Chuang et al. 1991
5-Methylchrysene	NR	35.5 ng	Vu-Duc and Huynh 1989
N-Nitrosamines			
N-Nitrosodiethanolamine	43 ng	NR	Brunnemann and Hoffmann 1981
N-Nitrosodiethylamine	8.2–73 ng	0–20 ng	Brunnemann et al. 1977; Hoffmann et al. 1987
N-Nitrosodimethylamine	143–1,040 ng	4–240 ng	Brunnemann et al. 1977; Hoffmann et al. 1987; Klus et al. 1992
N-Nitrosoethylmethylamine	3–35 ng	NR	Brunnemann et al. 1977; Hoffmann et al. 1987
N'-Nitrosornicotine	110–857 ng	0.7–23 ng	Brunnemann et al. 1983, 1992; Adams et al. 1987; Klus et al. 1992
N-Nitrosopiperidine	4.8–19.8 ng	NR	Adams et al. 1987
N-Nitrosopyrrolidine	7–700 ng	3.5–27.0 ng	Brunnemann et al. 1977; Hoffmann et al. 1987; Klus et al. 1992; Mahanama and Daisey 1996
4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone	201–1,440 ng	0.2–29.3 ng	Brunnemann et al. 1983, 1992; Adams et al. 1987; Klus et al. 1992
Aromatic amines			
2-Naphthylamine	63.1–128 ng	NR	Government of British Columbia Ministry of Health Services 2001
2-Toluidine	3,030 ng	NR	Patrianakos and Hoffmann 1979
4-Aminobiphenyl	11.4–18.8 ng	NR	Government of British Columbia Ministry of Health Services 2001
Aldehydes			
Acetaldehyde	961–1,820 micrograms (μg)	268 μg	Martin et al. 1997; Government of British Columbia Ministry of Health Services 2001
Formaldehyde	233–485 μg	143 μg	Martin et al. 1997; Government of British Columbia Ministry of Health Services 2001

Table 2.1 Continued

Carcinogen	Representative amounts		Study
	Sidestream (per cigarette)	Secondhand (per cubic meter [m ³])	
Miscellaneous organics			
Acrylonitrile	42–109 µg	NR	Government of British Columbia Ministry of Health Services 2001
Benzene	163–353 µg	4.2–63.7 µg	Scherer et al. 1995; Heavner et al. 1996; Martin et al. 1997; Government of British Columbia Ministry of Health Services 2001; Kim et al. 2001
Catechol	98–292 µg	1.24 µg	Sakuma et al. 1983; Martin et al. 1997; Government of British Columbia Ministry of Health Services 2001
Isoprene	668–1,260 µg	657 µg	Martin et al. 1997; Government of British Columbia Ministry of Health Services 2001
1,3-Butadiene	98–205 µg	0.3–40 µg	Heavner et al. 1996; Martin et al. 1997; Government of British Columbia Ministry of Health Services 2001; Kim et al. 2001
Inorganic compounds			
Cadmium	330–689 ng	4–38 ng	Wu et al. 1995; Government of British Columbia Ministry of Health Services 2001
Chromium	57–79 ng	NR	Government of British Columbia Ministry of Health Services 2001
Hydrazine	94 ng	NR	Liu et al. 1974
Lead	28.9–46.6 ng	NR	Government of British Columbia Ministry of Health Services 2001
Nickel	51 ng	NR	Government of British Columbia Ministry of Health Services 2001
Polonium-210	0.091–0.139 picocurie	NR	Ferri and Baratta 1966

*NR = Data were not reported.

Source: Adapted from Hoffmann et al. 2001.

in humans (IARC 1982, 1992, 1999). Metals such as nickel, chromium, and cadmium are human carcinogens that are also present in sidestream smoke (IARC 1990, 1994).

Mainstream cigarette smoke consists of a gas phase and a particulate phase specifically composed of several million semiliquid particles per cubic

centimeter (cm³) within a mixture of combustion gases (Ingebrethsen 1986; Guerin et al. 1992). Sidestream smoke contains free radicals in about the same concentrations as does mainstream smoke (Pryor et al. 1983). Pryor and colleagues (1998) detected reactive yet long-lived radicals in the gas phase; in the particulate phase, these investigators found a free

radical system that is a mixture of semiquinones, hydroquinones, and quinones (Pryor et al. 1998). Whether such agents can induce tumors in laboratory animals is not known.

Carcinogenicity of Sidestream Smoke and Secondhand Smoke

Numerous studies have demonstrated that mainstream cigarette smoke condensate, the solid materials in the smoke, induces tumors on mouse skin and, by implantation, in rat lungs (IARC 1986, 2004). Inhalation experiments with mainstream smoke have demonstrated that cigarette smoke and its particulate phase induce preneoplastic lesions and benign and malignant tumors of the larynx in Syrian golden hamsters (IARC 1986). Studies with rats and mice documented less consistent results (IARC 1986, 2004; Hecht 1999).

The carcinogenicity of sidestream smoke has been less extensively investigated. Sidestream smoke condensate was significantly more carcinogenic than mainstream smoke condensate when tested on mouse skin: mice treated with sidestream smoke developed two to six times more skin tumors than mice treated

with mainstream smoke (Mohtashamipur et al. 1990). In a rat model using implanted sidestream smoke particles, a fraction containing PAHs with four or more rings produced tumors, while a fraction with semi-volatiles and a PAH fraction with fewer rings had little effect (Grimmer et al. 1988). Limited histopathologic changes were observed in rats exposed to cigarette sidestream smoke aged in the chamber for 12 months (Hausmann et al. 1998). Researchers have carried out a series of investigations on the effects of secondhand smoke inhalation in A/J mice (Witschi et al. 1995, 1997a,b,c, 1998, 1999, 2000; Witschi 1998, 2000). Table 2.2 summarizes the data from these studies. Lung tumor multiplicity, the most sensitive indicator of response in this model, increased significantly in all experiments, and lung tumor incidence increased in several experiments. The protocol involved exposing mice to secondhand smoke (89 percent sidestream smoke and 11 percent mainstream smoke) for five months followed by a four-month recovery period in air. Other experiments have demonstrated that to observe an increase in lung tumor multiplicity, there must be a recovery period. These same experiments also showed that the response is due to a gas-phase component of secondhand smoke.

Table 2.2 Inhalation studies of secondhand smoke (89% sidestream smoke and 11% mainstream smoke) in A/J mice

Study	Exposure (mg/m ³ * of total suspended particulates)	Lung tumor multiplicity [†]		Lung tumor incidence [‡]	
		Filtered air control	Smoke	Filtered air control (%)	Smoke (%)
Witschi et al. 1997a	79	0.5 ± 0.1 (24)	1.3 ± 0.3 (26) [§]	42	58
Witschi et al. 1997b	87	0.5 ± 0.2 (24)	1.4 ± 0.2 (24) [§]	38	83 [§]
Witschi et al. 1998	83	0.9 ± 0.2 (29)	1.3 ± 0.2 (33) [§]	69	73
Witschi et al. 1999	132	0.6 ± 0.1 (30)	2.1 ± 0.3 (38) [§]	50	86 ^Δ
Witschi et al. 2000	137	0.9 ± 0.2 (30)	2.8 ± 0.2 (38) [§]	60	100 ^Δ
	137	1.0 ± 0.1 (54)	2.4 ± 0.3 (28) [§]	65	89 ^Δ
Witschi et al., unpublished data	134	1.2 ± 0.2 (25)	2.3 ± 0.3 (26) [§]	60	88 ^Δ

*mg/m³ = Milligrams per cubic meter.

[†]Mean ± standard error (number of animals is in parentheses).

[‡]Percentage of all animals at risk that had tumors.

[§]Significantly different (p < 0.05) compared with air controls by Welsh's alternate test.

^ΔSignificantly different (p < 0.05) compared with air controls by Fisher's exact test.

Source: Adapted from Witschi 2000.

Although these results are of interest, there are some poorly understood features of the model. The animals lose weight during exposure and never weigh as much as the air-treated controls even after the recovery period. The consequences of the weight loss are unknown. The reason for the recovery period requirement also is not clear. In addition, the apparent tumor-inducing effect of the gas phase is inconsistent with most of the earlier work on mainstream smoke inhalation and with the tumor-inducing properties of sidestream smoke condensate described above. Finally, recent data from De Flora and colleagues (2003) somewhat contradict the observations of Witschi and colleagues (1995, 1997a,b,c, 1998, 1999, 2000). De Flora and colleagues (2003) exposed Swiss strain mice to environmental tobacco smoke continuously for a period of nine months without a recovery period and observed a significant increase in the lung tumor response.

Collectively, these studies suggest the potential involvement of multiple carcinogens from sidestream and secondhand cigarette smoke in tumor induction. The results of the implanted mouse skin and rat lung carcinogenicity assays demonstrate the importance of PAHs and other nonvolatile carcinogens. Moreover, sidestream and secondhand smoke contain potent lung carcinogens such as NNK. The results of the mouse inhalation studies indicate that gas-phase constituents of secondhand smoke contribute to tumorigenesis. Prominent among these constituents could be formaldehyde, acetaldehyde, butadiene, and benzene because of their tumorigenic activities and relatively high concentrations in secondhand smoke.

Human Carcinogen Uptake from Secondhand Smoke

Tables 2.3 and 2.4 summarize data from biomarker studies on human uptake of specific secondhand smoke carcinogens. These studies demonstrate that human exposures to secondhand smoke lead to the uptake of carcinogens, a topic that Scherer and Richter (1997) have reviewed.

trans,trans-Muconic acid is a urinary metabolite of benzene, a known cause of leukemia, that has been widely used to estimate benzene uptake (Scherer et al. 1998). Studies on the relationship of this metabolite to secondhand smoke exposure have documented mixed results, with some studies showing somewhat higher levels in persons exposed to secondhand smoke while others found no effect (Scherer et al. 1995, 1999; Weaver et al. 1996; Yu and Weisel 1996; Ruppert et al. 1997; Carrer et al. 2000). The

interpretation of these findings is complicated by differences in excretion rates among participants and by contributions from sources other than benzene, such as sorbate in food, to levels of this metabolite in urine (Yu and Weisel 1996; Ruppert et al. 1997; Scherer and Richter 1997). Benzene itself can be quantified in exhaled breath. Breath measurements of nonsmokers who reported secondhand smoke exposures at work from smokers showed elevated benzene levels, but nonsmokers living with smokers did not have increased levels (Wallace et al. 1987). A second study detected higher levels of exhaled benzene in nonsmokers living with smokers compared with nonsmokers living with nonsmokers (Scherer et al. 1995). Another study documented no difference in levels of exhaled benzene among children living with smokers compared with children living with nonsmokers (Scherer et al. 1999). Collectively, the biomarker data discussed here indicate that benzene uptake in humans is not consistently found to be associated with secondhand smoke exposure, but there are other sources of benzene exposure that complicate efforts to estimate the contribution of secondhand smoke to biomarker levels.

Several methods have been used to estimate PAH uptake by persons exposed to secondhand smoke. 1-Hydroxypyrene and hydroxyphenanthrene are urinary metabolites of pyrene and phenanthrene, respectively. These metabolites are widely used as biomarkers of PAH uptake although the parent compounds, pyrene and phenanthrene, are noncarcinogenic. Exposure to secondhand smoke does not increase 1-hydroxypyrene and hydroxyphenanthrene levels in urine (Hoepfner et al. 1987; Scherer et al. 1992, 2000; Van Rooij et al. 1994; Siwińska et al. 1999). Other factors such as smoking, occupational exposures, and diet are significant contributors to urinary levels of these compounds. Metabolites of B[a]P and other PAHs form covalent binding products (adducts) with hemoglobin and serum albumin and have been measured using a variety of methods, including immunoassay and gas chromatography–mass spectrometry (GC–MS). Studies of adduct formation with hemoglobin and albumin have given mixed results. Using an enzyme-linked immunosorbent technique, one group found increased levels of PAH-albumin adducts in children exposed to secondhand smoke (Crawford et al. 1994; Tang et al. 1999), but two other studies did not find increments in these levels (Autrup et al. 1995; Nielsen et al. 1996). Using GC–MS as the detection method, researchers found no effect of secondhand smoke exposure on B[a]P albumin and hemoglobin adducts (Scherer et al. 2000). Thus, the evidence that

Table 2.3 Representative biomarker studies of carcinogens in persons exposed to secondhand smoke

Carcinogen	Exposure data (if reported)	Biomarker levels	Exposed vs. unexposed: significant difference?	Study
Benzene	11.5 $\mu\text{g}/\text{m}^3$ *, personal exposure (nonsmokers, nonsmoking homes, n = 39)	tt-MA [†] 92 $\mu\text{g}/\text{g}$ creatinine	No	Scherer et al. 1995
	13.6 $\mu\text{g}/\text{m}^3$ (nonsmokers, smoking homes, n = 43)	126 $\mu\text{g}/\text{g}$ creatinine		
Benzene	NR [‡]	tt-MA 3.84 \pm 1.6 ng/ μL [§] in 53 secondhand smoke-exposed children	No	Weaver et al. 1996
		4.02 \pm 1.1 ng/ μL in 26 unexposed children	Yes	
		3.5 \pm 1.4 ng/ μL when urinary cotinine \leq 44 ng/mL [^] (n = 39)		
		4.32 \pm 1.4 ng/ μL when urinary cotinine >44 ng/mL (n = 39)		
Benzene	<0.19–22 $\mu\text{g}/\text{m}^3$, personal exposure, 5 females exposed to secondhand smoke	tt-MA 34–74 μg excreted on nonexposure days	Yes	Yu and Weisel 1996
		42–95 μg excreted on exposure days		
Benzene	2–100 $\mu\text{g}/\text{m}^3$, personal exposure (n = 69 nonsmokers from smoking and nonsmoking households)	tt-MA was not correlated with benzene; marginal difference in tt-MA of nonsmokers from smoking homes vs. those from nonsmoking homes	No	Ruppert et al. 1997
Benzene	11.5 $\mu\text{g}/\text{m}^3$, personal exposure (children, smoking homes, n = 24)	tt-MA 130 $\mu\text{g}/\text{g}$ creatinine	No	Scherer et al. 1999
	19.7 $\mu\text{g}/\text{m}^3$ (children, nonsmoking homes, n = 15)	112 $\mu\text{g}/\text{g}$ creatinine		
Benzene (geometric means)	16.5 \pm 2.3 $\mu\text{g}/\text{m}^3$, personal exposure (nonsmokers, no secondhand smoke, n = 42)	tt-MA 38.9 \pm 2.4 $\mu\text{g}/\text{L}$	Yes	Carrer et al. 2000
	25.4 \pm 2.9 $\mu\text{g}/\text{m}^3$ (nonsmokers, secondhand smoke, n = 27)	54.7 \pm 2.9 $\mu\text{g}/\text{L}$		

Table 2.3 Continued

Carcinogen	Exposure data (if reported)	Biomarker levels	Exposed vs. unexposed: significant difference?	Study
NNK ^q	75–263 ng/m ³ in a 16 m ³ room	Significantly increased levels of NNAL** plus NNAL-Gluc ^{††} in urine of 5 men after secondhand smoke exposure	Yes	Hecht et al. 1993
NNK	NR	Significantly increased levels of NNAL-Gluc in hospital workers (n = 9) exposed to secondhand smoke compared with controls	Yes	Parsons et al. 1998
NNK	2.4–50 ng/m ³ in 19 rooms where smoking took place	NNAL plus NNAL-Gluc levels correlated with nicotine on personal sampler in secondhand smoke-exposed persons	Yes	Meger et al. 2000
NNK	NR	NNAL plus NNAL-Gluc levels were significantly higher in women (n = 23) who lived with male smokers compared with women (n = 22) who lived with male nonsmokers	Yes	Anderson et al. 2001
NNK	NR	34% of 204 children with cotinine >5 ng/mL urine; 52/54 of these samples had detectable NNAL plus NNAL-Gluc; NNAL plus NNAL-Gluc levels were significantly higher in secondhand smoke-exposed vs. unexposed children	Yes	Hecht et al. 2001
Polycyclic aromatic hydrocarbons (PAHs)	NR	5 nonsmokers exposed to secondhand smoke from 100 cigarettes (100–180 µg/m ³ cotinine in the room) over an 8-hour period; no effect on urinary hydroxyphenanthrenes	No	Hoepfner et al. 1987
PAHs	Benzo[a]pyrene (B[a]P), 21.5 ng/m ³ ; phenanthrene, 6.8 ng/m ³ ; pyrene, 17.6 ng/m ³ in an experimental room with 5 smokers and 5 nonsmokers	No effects on urinary hydroxyphenanthrenes (2.0 vs. 2.2 µg/24 hours before and after secondhand smoke exposure); no effects on urinary 1-HOP** (0.24 µg/24 hours before and after secondhand smoke exposure); no effects on ³² P-postlabeling of DNA adducts	No	Scherer et al. 1992
PAHs	NR	No differences in PAH-albumin levels in umbilical cord blood from women exposed to secondhand smoke (n = 49) vs. unexposed women (n = 54)	No	Astrup et al. 1995
PAHs	NR	No effect of secondhand smoke on PAH-albumin adduct levels in 73 persons from Aarhus, Denmark	No	Nielsen et al. 1996

Table 2.3 Continued

Carcinogen	Exposure data (if reported)	Biomarker levels	Exposed vs. unexposed: significant difference?	Study
PAHs	NR	No difference in urinary 1-HOP levels of children exposed to secondhand smoke from their parents' smoking (n = 286) vs. unexposed children (n = 126)	No	Siwińska et al. 1999
PAHs	NR	1-HOP: 0.140 $\mu\text{g}/24$ hours in 19 secondhand smoke-exposed persons (urinary cotinine 12.3 $\mu\text{g}/24$ hours) vs. 0.171 $\mu\text{g}/24$ hours in 23 unexposed persons (urinary cotinine 2.3 $\mu\text{g}/24$ hours) B[a]P-hemoglobin (Hb) adducts: 0.049 fmol/mg ^{ss} Hb in secondhand smoke-exposed persons vs. 0.083 fmol/mg Hb in unexposed persons (same persons as above)	NR No	Scherer et al. 2000
PAH and 4-aminobiphenyl	NR	B[a]P-albumin adducts: 0.021 fmol/mg albumin in secondhand smoke-exposed persons vs. 0.019 fmol/mg albumin in unexposed persons (same persons as above)	NR	
PAH and 4-aminobiphenyl	NR	Significantly higher levels of 4-aminobiphenyl-Hb adducts and PAH-albumin adducts in children whose mothers smoked (n = 23 for 4-aminobiphenyl Hb, n = 44 for PAH albumin) compared with unexposed children (n = 10 for 4-aminobiphenyl Hb, n = 24 for PAH albumin)	Yes	Tang et al. 1999
4-Aminobiphenyl	Estimated weekly average nicotine concentration ranged from <0.5 to ≥ 2.0 $\mu\text{g}/\text{m}^3$	Higher 4-aminobiphenyl-Hb adducts (27.8 pg/g ^{ΔΔ} Hb) in 9 pregnant women with >2.0 $\mu\text{g}/\text{m}^3$ nicotine (personal exposure) than in pregnant women with 0.5–1.9 $\mu\text{g}/\text{m}^3$ (n = 20, 20.8 pg/g Hb) or in pregnant women with <0.5 $\mu\text{g}/\text{m}^3$ (n = 7, 17.6 pg/g Hb)	Yes	Hammond et al. 1993
4-Aminobiphenyl and other aromatic amines	NR	No relationship of aromatic amine-Hb adducts to reported secondhand smoke exposure or cotinine/creatinine ratios in 73 pregnant women	No	Branner et al. 1998

Table 2.3 Continued

Carcinogen	Exposure data (if reported)	Biomarker levels	Exposed vs. unexposed: significant difference?	Study
4-Aminobiphenyl and other aromatic amines	NR	No increase in aromatic amine-Hb adducts among 224 children with increased exposures to secondhand smoke; exposures were confirmed by cotinine testing	No	Richter et al. 2001
Unknown	NR	No effects of secondhand smoke exposure on ³² P-postlabeled DNA adducts in monocytes of 5 nonsmokers exposed for 8 hours	No	Holz et al. 1990
Unknown	5 nonsmokers exposed to secondhand smoke in an unventilated room, 4,091 µg/m ³ respirable suspended particles	A marginal, nonsignificant increase in urinary thioethers was observed	No	Scherer et al. 1992
Unknown	NR	No effect of secondhand smoke exposure on ³² P-postlabeled DNA adducts in women (n = 31 exposed, 11 unexposed)	No	Binková et al. 1995
Unknown	NR	No difference in urinary thioethers between persons exposed to low (n = 23) and high (n = 23) levels of secondhand smoke based on plasma cotinine; no difference in urinary thioethers between persons exposed to low (n = 20) and high (n = 19) levels of secondhand smoke exposures in the home	No	Scherer et al. 1996
Unknown	NR	No difference in placental levels of 8-OH-dG ^{8q} in 10 nonsmokers vs. 9 nonsmokers exposed to secondhand smoke, validated by plasma and urine cotinine; no effects of secondhand smoke on adducts were detected by ³² P-postlabeling	No	Daube et al. 1997
Unknown	NR	Significantly higher (63%) levels of 8-OH-dG in blood DNA of persons exposed to secondhand smoke in the workplace (n = 38) than in unexposed persons, verified by plasma cotinine (n = 36)	Yes	Howard et al. 1998b

Table 2.3 Continued

Carcinogen	Exposure data (if reported)	Biomarker levels	Exposed vs. unexposed: significant difference?	Study
Unknown	NR	No difference in 8-OH-dG levels in leukocytes of unexposed adults (n = 36), adults exposed 1–4 hours/day to secondhand smoke (n = 35), and adults exposed >4 hours/day (n = 21)	No	van Zeeland et al. 1999
Unknown	NR	Among 194 students in Athens and 77 persons in Halkida, Greece, ³² P-postlabeled DNA adducts in lymphocytes showed no relationship to secondhand smoke exposure in the entire group, but did correlate with secondhand smoke exposure measurements in winter in a subgroup living in the Halkida campus area	No/yes	Geordiadis et al. 2001

^{*}µg/m³ = Micrograms per cubic meter.

[†]tt-MA = *trans,trans*-Muconic acid.

[‡]NR = Data were not reported.

[§]ng/µL = Nanograms per microliter.

[^]mL = Milliliter.

[¶]NNK = 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco-specific *N*-nitrosamine.

^{**}NNAL = 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol.

^{††}NNAL-Gluc = A mixture of 4-(methylnitrosamino)-1-(3-pyridyl)-1-(*O*-β-D-glucopyranuronosyl) butane and 4-(methylnitrosamino)-1-(3-pyridyl)-*N*-β-D-glucopyranuronosyl)-1-butanolonium inner salt.

^{‡‡}1-HOP = 1-Hydroxypyrene.

^{§§}fmol/mg = Femtomoles per milligram.

^{ΔΔ}pg/g = Picograms per gram.

^{¶¶}8-OH-dG = 8-Hydroxydeoxyguanosine.

secondhand smoke exposure significantly increases human uptake of PAHs is inconsistent.

Aromatic amines such as 4-aminobiphenyl form adducts with hemoglobin that GC–MS can quantify, but studies of the effects of secondhand smoke on 4-aminobiphenyl–hemoglobin adducts have provided mixed results. Hammond and colleagues (1993) demonstrated that adduct levels were elevated in pregnant women exposed to secondhand smoke. Maclure and colleagues (1989) observed slightly higher levels of 4-aminobiphenyl– and 3-aminobiphenyl–hemoglobin adducts in persons with confirmed secondhand smoke exposures compared with unexposed persons. 4-Aminobiphenyl–hemoglobin adducts were also elevated in children exposed to secondhand smoke (Tang et al. 1999). However, two other studies, including one of pregnant women,

showed no consistent relationship between adduct levels and secondhand smoke exposures (Bartsch et al. 1990; Branner et al. 1998). A recent study of German children also showed no significant increase in aromatic amine–hemoglobin adduct levels with increased secondhand smoke exposures; in fact, there was a significant decrease in ortho- and meta-toluidine adducts (Richter et al. 2001). There is a background level of aromatic amine–hemoglobin adducts in apparently unexposed humans. The origin of this background is unknown, but it could be due in part to the uptake of corresponding nitro compounds from sources such as diesel emissions. Levels of aromatic amines in urine were unaffected by exposures to secondhand smoke in a study of nonsmokers (Grimmer et al. 2000).

Because tobacco-specific nitrosamines are found only in tobacco products or in related

Table 2.4 Relationship of specific biomarkers of carcinogen uptake to secondhand smoke exposure

Carcinogens in secondhand smoke	Biomarker	Association with secondhand smoke exposure	Study
Aromatic amines	Hemoglobin adducts	Mixed results	Maclure et al. 1989; Bartsch et al. 1990; Hammond et al. 1993; Branner et al. 1998; Tang et al. 1999; Richter et al. 2001
Benzene	<i>trans,trans</i> -Muconic acid in urine	Mixed results	Scherer et al. 1995, 1999; Weaver et al. 1996; Yu and Weisel 1996; Ruppert et al. 1997; Carrer et al. 2000
NNK*	NNAL [†] and NNAL-Gluc [‡] in urine	Consistently increased	Hecht et al. 1993, 2001; Parsons et al. 1998; Meger et al. 2000; Anderson et al. 2001
NNK/NNN [§]	Hemoglobin adducts	None	Branner et al. 1998
PAHs ^Δ	1-Hydroxypyrene in urine Hydroxyphenanthrenes in urine Albumin adducts Hemoglobin adducts	None in most studies	Scherer et al. 1992, 2000; Crawford et al. 1994; Van Rooij et al. 1994; Autrup et al. 1995; Nielsen et al. 1996; Siwińska et al. 1999; Tang et al. 1999

*NNK = 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco-specific *N*-nitrosamine.

[†]NNAL = 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol.

[‡]NNAL-Gluc = A mixture of 4-(methylnitrosamino)-1-(3-pyridyl)-1-(*O*- β -D-glucopyranuronosyl) butane and 4-(methylnitrosamino)-1-(3-pyridyl)-*N*- β -D-glucopyranuronosyl)-1-butanolonium inner salt.

[§]NNN = *N'*-Nitrosornicotine.

^ΔPAHs = Polycyclic aromatic hydrocarbons.

Source: Adapted from Scherer and Richter 1997.

nicotine-containing materials, their adducts or metabolites should be specific biomarkers of tobacco exposure. NNK- and NNN-hemoglobin adducts can be hydrolyzed to release 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB), which GC-MS can then quantify. In smokers, levels of HPB-releasing hemoglobin adducts of NNK and NNN are low compared with hemoglobin adducts of several other carcinogens, possibly attributable to the high reactivity of the alkylating intermediate (Carmella et al. 1990; Hecht et al. 1994). Considering the relatively low levels of these adducts in smokers, nonsmokers exposed to secondhand smoke should not have significantly elevated amounts (Branner et al. 1998). However, urinary metabolites of NNK are readily measured in the urine of persons exposed to secondhand smoke. The metabolite 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronide conjugate (NNAL-Gluc) can be quantified using GC with thermal energy analyzer (TEA)

nitrosamine-selective detection (GC-TEA) (Hecht et al. 1993, 2001; Parsons et al. 1998; Meger et al. 2000; Anderson et al. 2001). All studies reported to date show significantly higher amounts of NNAL plus NNAL-Gluc, or NNAL-Gluc alone, in the urine of secondhand smoke-exposed participants than in the urine of unexposed controls (Tables 2.3–2.5). In one study, the uptake of NNK was more than six times higher in women who lived with smokers compared with women who lived with nonsmokers (Anderson et al. 2001). The amount of NNAL plus NNAL-Gluc in these secondhand smoke-exposed women was about 5 percent as great as in their male partners who smoked. Another study found an uptake of NNK in a group of economically disadvantaged schoolchildren, and the range of levels varied approximately 90-fold (Hecht et al. 2001). Most of the studies demonstrate a correlation between levels of cotinine and NNAL plus NNAL-Gluc in urine (Figure 2.1). Cotinine is a

valid biomarker for nicotine uptake in nonsmokers exposed to secondhand smoke. Therefore, NNAL plus NNAL-Gluc is a biomarker for the uptake of the tobacco-specific lung carcinogen NNK in nonsmokers exposed to secondhand smoke. The NNAL plus NNAL-Gluc biomarker is more directly related to cancer risk than cotinine is because NNK (but not nicotine) is carcinogenic. The uptake of NNK by nonsmokers exposed to secondhand smoke thus provides a biochemical link between secondhand smoke exposure and lung cancer risk.

Studies of secondhand smoke exposure have also explored several other less specific markers. 8-Hydroxydeoxyguanosine (8-OH-dG) is a widely used biomarker of oxidative damage to DNA. Two studies observed no increase in 8-OH-dG levels in placentas and leukocytes of persons exposed to secondhand smoke compared with unexposed persons (Daube et al. 1997; van Zeeland et al. 1999). However, in a study of occupational exposure in Reno, Nevada, the average 8-OH-dG level in whole blood DNA of secondhand smoke-exposed workers was

63 percent higher than in unexposed persons; this finding represents a significant difference (Howard et al. 1998b). Urinary 3-ethyladenine is a biomarker of ethylating agents. In one study, exposure to secondhand smoke did not increase urinary concentrations of 3-ethyladenine (Kopplin et al. 1995). ³²P-postlabeling is a technique that can estimate levels of hydrophobic DNA adducts. Four investigations did not find effects of secondhand smoke exposure on levels of ³²P-postlabeled DNA (Holz et al. 1990; Scherer et al. 1992; Binková et al. 1995; Daube et al. 1997). However, a recent study conducted in Greece did find a relationship between secondhand smoke exposure and ³²P-postlabeled DNA adducts in lymphocytes from a subgroup (Georgiadis et al. 2001). Urinary thioethers are conjugates of carbonyl-containing mutagens. Thioethers did not significantly increase as a result of secondhand smoke exposure (Scherer et al. 1992, 1996). 3-Hydroxypropyl mercapturic acid, possibly from acrolein exposure, was identified as a possible secondhand smoke-related product in urine (Scherer et al. 1992). Studies investigating the effects

Table 2.5 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and NNAL-glucuronide (NNAL-Gluc*) in the urine of nonsmokers exposed to secondhand smoke

Study	Population	Analyte	Correlation with cotinine	Mean ± standard deviation pmol/mL [†] (number of samples analyzed)	Range [‡] (fold)
Hecht et al. 1993	Men exposed to secondhand smoke in a chamber	NNAL plus NNAL-Gluc	Yes	0.16 ± 0.10 [§] (n = 7)	0.084–0.296 (4)
Parsons et al. 1998	Hospital workers	NNAL-Gluc	Yes	0.059 ± 0.028 (n = 27)	0.005–0.11 (22)
Meger et al. 2000	Nonsmokers exposed to secondhand smoke	NNAL plus NNAL-Gluc	Yes	0.043 ± 0.044 [‡] (n = 16)	0.0038–0.148 (39)
Anderson et al. 2001	Women married to smokers	NNAL plus NNAL-Gluc	No	0.050 ± 0.068 (n = 23)	0.009–0.28 (31)
Hecht et al. 2001	Elementary school-age children	NNAL plus NNAL-Gluc	Yes	0.056 ± 0.076 (n = 74)	0.004–0.373 (93)

*NNAL-Gluc = A mixture of 4-(methylnitrosamino)-1-(3-pyridyl)-1-(*O*-β-D-glucopyranuronosyl) butane and 4-(methylnitrosamino)-1-(3-pyridyl-*N*-β-D-glucopyranuronosyl)-1-butanolonium inner salt.

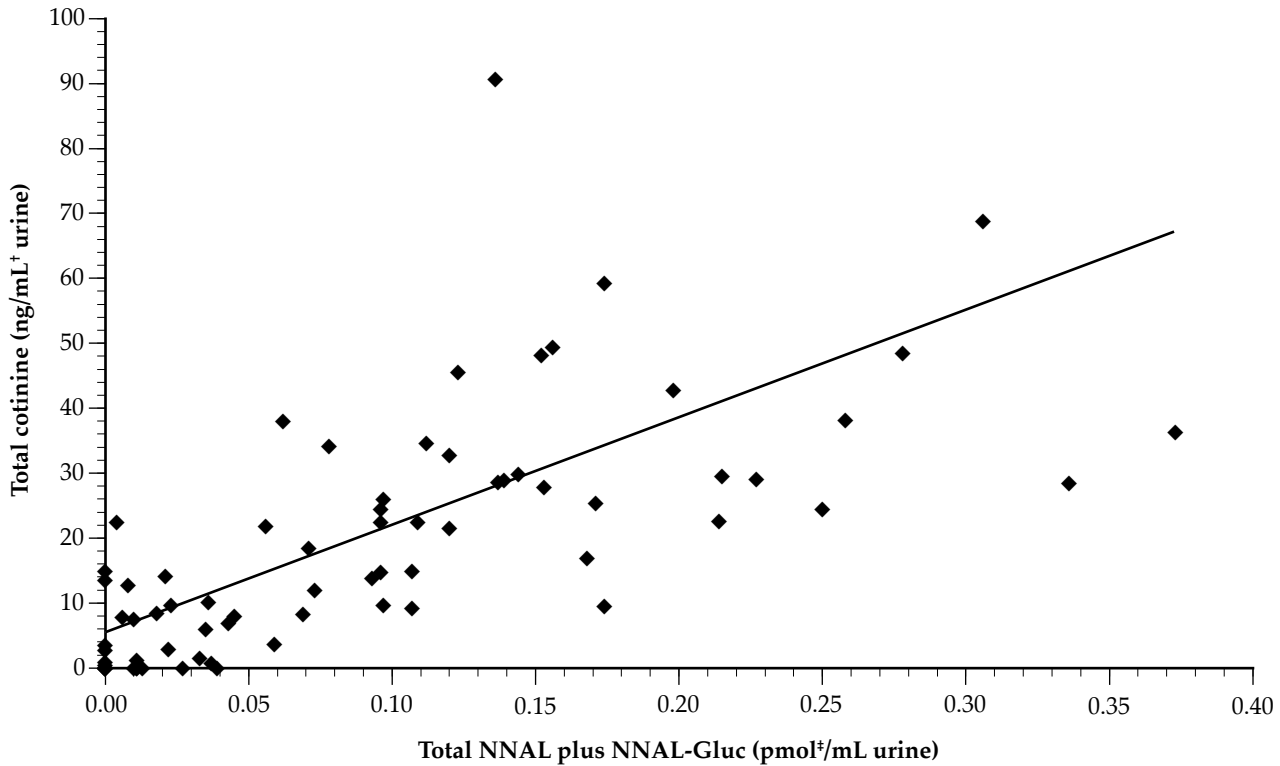
[†]pmol/mL = Picomoles per milliliter.

[‡]Detected values only.

[§]Approximate, based on the assumption of 1,200 mL of urine excreted per day.

Source: Meger et al. 2000.

Figure 2.1 The correlation between levels of cotinine plus cotinine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) plus NNAL-glucuronide (NNAL-Gluc) conjugates in the urine of 74 school-age children exposed to secondhand smoke*



* $r = 0.71$; $p < 0.001$.
[†]ng/mL = Nanograms per milliliter.
[‡]pmol = Picomoles.
 Source: Hecht et al. 2001. Reprinted with permission.

of secondhand smoke on urinary mutagenicity have demonstrated conflicting results (Scherer et al. 1992; Scherer and Richter 1997). In general, there seem to be small and sometimes significant effects of secondhand smoke exposure on urinary mutagenicity when diet is controlled (Scherer et al. 1996; Smith et al. 2000a). In a recent study of 1,249 Italian women whose husbands smoked, there was an inverse dose-response relationship between the intensity of the secondhand smoke and concentrations of plasma beta-carotene and L-ascorbic acid found in the women. There also was a significant inverse association between urinary cotinine and plasma beta-carotene (Farchi et al. 2001).

Mechanisms of Carcinogenesis of Secondhand Smoke

Figure 2.2 presents a framework for considering mechanisms of secondhand smoke carcinogenesis. An analogous scheme proposes how cigarette smoke generally can induce lung cancer (Hecht 1999). The broad mechanisms of cancer induction from exposures to secondhand and mainstream cigarette smoke are probably similar because the same carcinogens are present in both, although in different concentrations. The major difference is the significantly lower carcinogenic dose from inhaling secondhand smoke compared with active smoking.

Exposure to secondhand smoke leads to a small but measurable uptake of NNK and perhaps other carcinogens, as discussed in the previous section. Carcinogens are enzymatically transformed into a series of metabolites as the exposed organism attempts to convert them into compounds that are easily excreted from the body (Miller 1994), a process called metabolic detoxification. An unintended consequence of this detoxification process is that the carcinogen sometimes converts to a form that is reactive with DNA and other cellular macromolecules. These reactive forms usually have an electron-deficient (or electrophilic) center that is reactive with the electron-rich (or nucleophilic) centers in DNA. This process, called metabolic activation, forms adducts in DNA, RNA, and protein.

Because most of the carcinogens in Table 2.1 require metabolic activation to induce cancer, the metabolism of a carcinogen is in most cases a key component of the mechanism of cancer induction. The balance between metabolic activation and detoxification will be important in determining individual risks for cancer upon exposure to carcinogens in secondhand smoke. The initial enzymatic steps are frequently catalyzed by cytochrome P-450 enzymes, which are encoded by the *CYP* family of genes (Guengerich 1997). These enzymes generally oxygenate the carcinogen. Other enzymes, such as cyclooxygenases, myeloperoxidases, lipoxygenases, and monoamine oxidases, may also be involved. The oxygenated intermediates formed in the initial reactions may undergo further transformations by glutathione *S*-transferases, uridine-5'-diphosphate-glucuronosyl-transferases, sulfatases, hydratases, and other enzymes (Armstrong 1997; Burchell et al. 1997; Duffel 1997). All of these enzymes occur in multiple forms with different substrate specificity. Some of the forms are polymorphic in humans (i.e., they occur in variants with different types of metabolic activation). For example,

the glutathione *S*-transferase form M1 (GSTM1) is null in 50 percent of the population.

The complexity of carcinogen metabolism is illustrated for B[a]P and NNK in Figure 2.3 (Hecht 1999). The major metabolic activation pathway of B[a]P is its conversion to 7,8-diol-9,10-epoxide metabolites. One of the four enantiomers produced is highly carcinogenic and reacts with DNA to form an adduct with deoxyguanosine, BPDE-N2-dG. GSTM1 is one of the enzymes competing for the metabolically activated intermediates in this pathway. The major metabolic activation pathways of NNK and NNAL occur by hydroxylating the carbons adjacent to the *N*-nitroso group (α -hydroxylation), resulting in the formation of a variety of DNA adducts including 7-methylguanine, O⁶-methylguanine, and pyridyloxobutyl adducts (Hecht 1998). No specific carcinogen-DNA adducts have been detected in nonsmokers exposed to secondhand smoke, probably because of the low carcinogenic dose. The characterization of such adducts in human tissues is difficult even in smokers, but has been accomplished for a number of different tobacco smoke carcinogens (Hecht 1999). The same adducts probably are present in nonsmokers exposed to secondhand smoke, but at considerably lower levels.

Two studies examined the role of GSTM1 and glutathione *S*-transferase form T1 (GSTT1) variants as modifiers of risk for lung cancer in nonsmokers exposed to secondhand smoke (Bennett et al. 1999; Malats et al. 2000). Neither study found an effect of GSTT1 variants, although opposing results were obtained for GSTM1 null. One study documented an increased risk for lung cancer in secondhand smoke-exposed nonsmoking women (Bennett et al. 1999); the other found no significant effect in secondhand smoke-exposed nonsmokers (Malats et al. 2000).

Figure 2.2 Scheme showing the steps linking secondhand smoke exposure and cancer via tobacco smoke carcinogens

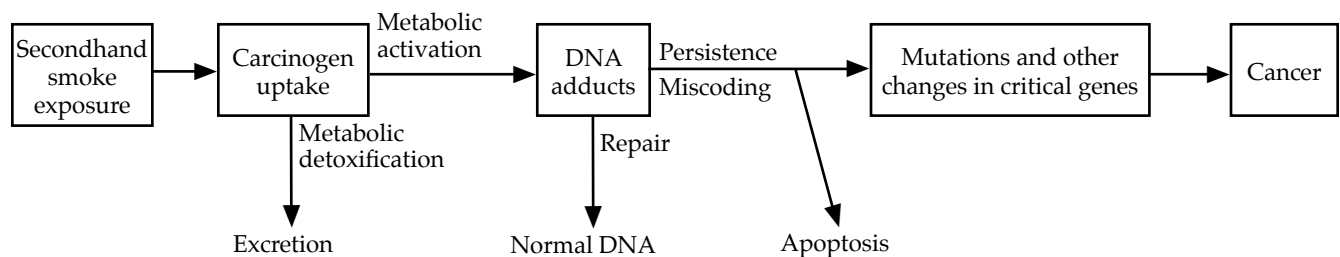
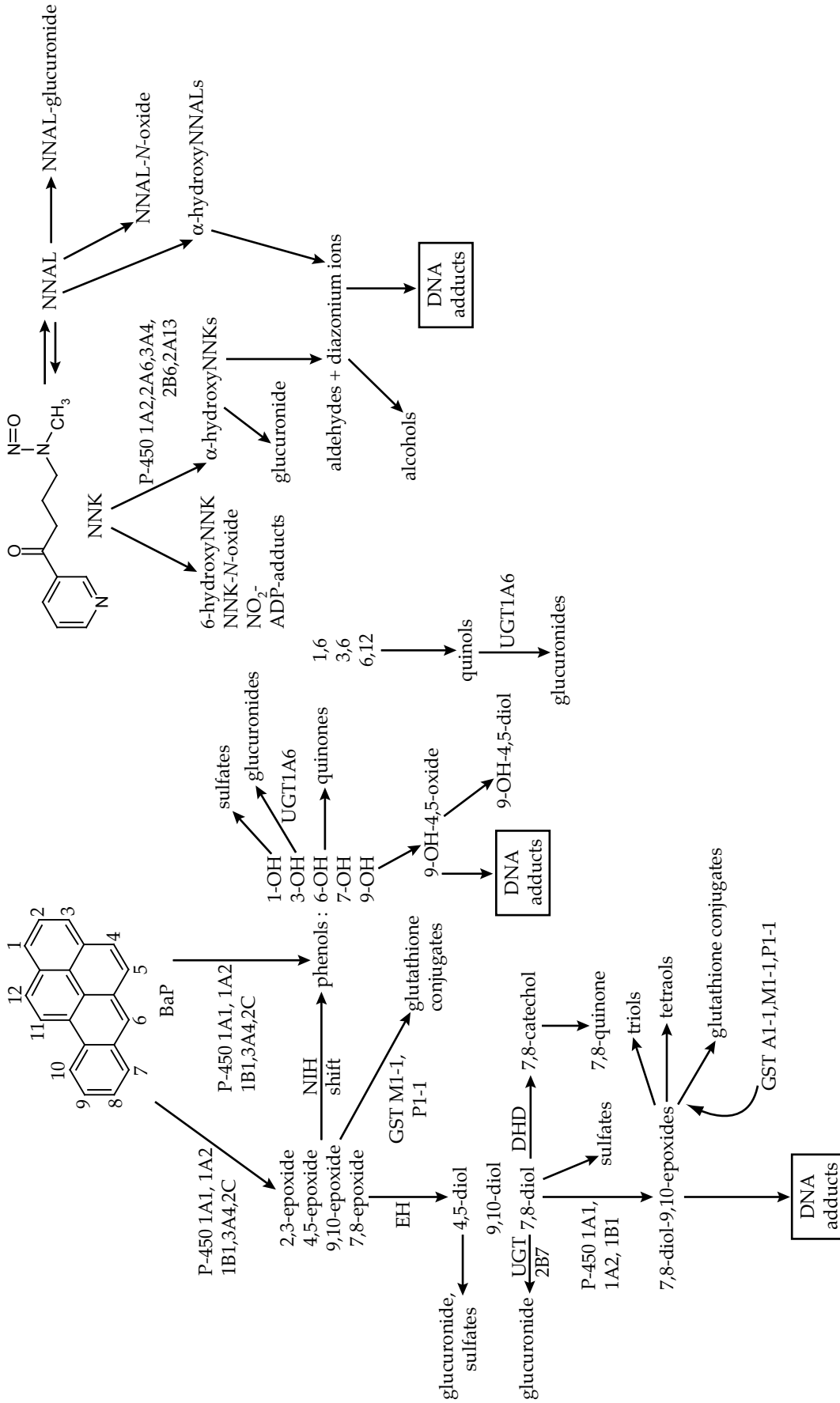


Figure 2.3 Metabolic pathways of benzo[*a*]pyrene (B[a]P) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)



Note: Metabolic pathways of B[a]P and NNK were modified from Cooper et al. 1983 and Hecht 1996, 1998. Some human enzymes involved in the various reactions are indicated (Gelboin 1980; Pelkonen and Nebert 1982; Cooper et al. 1983; Ketterer et al. 1992; Smith et al. 1992; Yamazaki et al. 1992; Yun et al. 1992; Tiano et al. 1993; Conney et al. 1994; Friedberg et al. 1993; Baird and Ralston 1997; Staretz et al. 1997; Sundberg et al. 1997; Kim et al. 1998; Penning et al. 1999). ADP = adenosine diphosphate; DHD = 4 dihydrodiol dehydrogenase; EH = 4 epoxide hydrolase; GST = 4 glutathione S-transferase; NNAL = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NO₂ = nitrogen dioxide; P-450 = 4 cytochrome P-450; UGT = 4 UDP(uridine 5'-diphosphate)-glucuronosyl transferase; 1-OH, 3-OH: 4 1-hydroxy B[a]P, 3-hydroxy B[a]P; National Institutes of Health shift (where the shift [a biochemical process] was first identified) = intramolecular hydrogen migration, which can be observed in enzymatic and chemical hydroxylations of aromatic rings. Source: Hecht 1999. Adapted with permission.

DNA adducts are critical for the induction of tumors by carcinogens. A great deal of mechanistic information is now available about the structures of DNA adducts and their potential to produce mutations (Hemminki et al. 1994; Geacintov et al. 1997). Cellular repair mechanisms exist to protect the DNA from persistent adduction. There are five main mechanisms of DNA repair: direct repair, base excision repair, nucleotide excision repair, mismatch repair, and double-strand break repair (Pegg et al. 1995; Sancar 1996; Singer and Hang 1997). If the adducts are not repaired, cells with damaged DNA may be removed by apoptosis (programmed cell death). When DNA adducts persist they may cause miscoding, resulting in a permanent mutation. Depending on the DNA polymerase involved, the sequence context, and other factors, DNA adducts will typically cause specific mutations. For example, O⁶-methylguanine causes mainly G to A mutations, while BPDE-N²-dG frequently results in G to T mutations (Loechler et al. 1984; Shukla et al. 1997). If a permanent mutation occurs in a critical region of a growth control gene, it can lead to the loss of normal growth control mechanisms and ultimately to cancer. There are six proposed hallmarks of cancer: self-sufficiency in growth signals, evasion of apoptosis, insensitivity to anti-growth signals, sustained angiogenesis, tissue invasion and metastasis, and limitless replicative potential (Hanahan and Weinberg 2000). Virtually all of these processes are controlled by specific genes that can lose their normal function when miscoding occurs. The intricate circuitry of the cell, which involves multiple pathways of signal transduction, can be subverted by inappropriate carcinogen-DNA adduction and miscoding. Multiple events of this type lead to aberrant cells with the loss of normal growth control. For example, lung carcinogenesis involves changes that include the loss of heterozygosity at 3p, 5q, 8p, 9p, 9q, 11p, 11q, 13q, 17p, and 17q loci, which are known or possible sites of tumor suppressor genes such as *p53*, *p16*, and others (Sekido et al. 1998; Vähäkangas et al. 2001).

Although numerous studies describe mutations in the *p53* tumor suppressor gene and *K-ras* oncogene in lung tumors from smokers (Hecht 1999), few investigations include lung tumors from nonsmokers with documented exposures to secondhand smoke, mainly because lung cancer in nonsmokers is relatively uncommon. Two studies have addressed *p53* mutations in nonsmokers. In one study, the risk of mutation in the *p53* gene doubled (odds ratio = 2.0 [95 percent confidence interval (CI), 0.5–8.7]) with

exposure to spousal secondhand smoke only compared with unexposed spouses (Husgafvel-Pursiainen et al. 2000). The risk was 1.5 (95 percent CI, 0.2–8.8) for those ever exposed to spousal or workplace secondhand smoke compared with those who were never exposed. These estimates are statistically unstable because of the small numbers of cases. The findings that G:C to A:T transversions were the most common among lifetime nonsmokers are in agreement with other studies. The second investigation reported a variety of mutations in the *p53* gene from tumors of lifetime nonsmokers exposed to secondhand smoke (Vähäkangas et al. 2001). Mutations in codons 12 and 13 of the *K-ras* gene were also observed. The observed *p53* and *K-ras* gene mutations are plausibly related to DNA adduct formation from carcinogens in secondhand smoke. It is difficult to specify which carcinogen may be responsible for a particular mutation, but the predominance of G mutations observed in these studies is consistent with the generally higher reactivity of G in DNA with metabolically activated carcinogens.

Summary

The evidence indicates that sidestream smoke, the principal component of secondhand smoke, contains carcinogens. Exposure to secondhand smoke results in the uptake by nonsmokers of many of these carcinogens. Although data are sparse on the specific elements in Figure 2.2 linking secondhand smoke exposure and tumor induction in humans via exposure to tobacco smoke carcinogens, substantial data from active smokers support this framework of biologic steps toward cancer. The most plausible mechanisms involved in lung cancer reflect the continuing exposure of the lungs to DNA-damaging material, which leads to multiple genetic changes that culminate in lung cancer. Available evidence points to these same mechanisms as the cause of lung cancer in persons exposed to carcinogens in secondhand smoke.

Conclusions

1. More than 50 carcinogens have been identified in sidestream and secondhand smoke.
2. The evidence is sufficient to infer a causal relationship between exposure to secondhand smoke and its condensates and tumors in laboratory animals.

3. The evidence is sufficient to infer that exposure of nonsmokers to secondhand smoke causes a significant increase in urinary levels of metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). The presence of these metabolites links exposure to secondhand smoke with an increased risk for lung cancer.
4. The mechanisms by which secondhand smoke causes lung cancer are probably similar to those observed in smokers. The overall risk of secondhand smoke exposure, compared with active smoking, is diminished by a substantially lower carcinogenic dose.

Mechanisms of Respiratory Tract Injury and Disease Caused by Secondhand Smoke Exposure

Although attention has centered primarily on secondhand smoke and the risk for lung cancer and coronary heart disease (CHD), extensive epidemiologic data support a broader range of adverse effects, particularly related to respiratory health. Information on the underlying mechanisms of these effects is central to the interpretation of the epidemiologic data and in the understanding of the pathogenesis of the non-malignant related disorders associated with secondhand smoke exposure. This review focuses primarily on pathogenetic mechanisms that likely contribute to secondhand smoke-induced respiratory diseases other than lung cancer. Respiratory effects of secondhand smoke exposure include a higher rate, an earlier onset, and an exacerbation of asthma (Wahlgren et al. 2000); spirometric indicators of lung impairment (Cook and Strachan 1999); an increased risk of lower respiratory tract illnesses in children (Strachan and Cook 1997); sudden infant death syndrome (SIDS) (Cook and Strachan 1999); and possibly chronic obstructive pulmonary disease (COPD) (Jaakkola 2002). This review also briefly discusses mechanisms of nonrespiratory disorders affected by secondhand smoke.

The respiratory system is the portal of entry for secondhand smoke and one of the key systems at risk for damage by secondhand smoke. Its structure and function are relevant to understanding the adverse effects of secondhand smoke. The respiratory tract includes the upper (nose, pharynx, and larynx) and lower (trachea, bronchi, and bronchioles) airways and the alveoli of the lung. Odor and irritant receptors are found primarily in the nose, but there are irritant receptors in the upper and lower airways as well. The airways conduct air to the alveoli where gas exchange occurs across the alveolar-capillary membrane, with

oxygen taken up by red blood cells and carbon dioxide removed from the bloodstream. In addition, the upper and lower airways have defense mechanisms against inhaled particles and gases that impact or are adsorbed onto the airway walls. The upper airways, which clean and condition the inhaled air, prevent most large particles and water-soluble vapors from reaching the airways of the lower respiratory tract. The removal of small particles that reach the lower airways and alveoli is accomplished by mechanisms that include the mucociliary apparatus, macrophages, and epithelial cells. This anatomical framework of the respiratory tract provides a large area for deposition and adsorption of secondhand smoke components.

Secondhand Smoke and Asthma

Extensive data describe an association that connects secondhand smoke exposure, particularly from maternal smoking, with asthma in children (Strachan and Cook 1998) (Chapter 6, Respiratory Effects in Children from Exposure to Secondhand Smoke). Studies also link secondhand smoke exposure with asthma in adults (Dayal et al. 1994; Flodin et al. 1995; Hu et al. 1997; Larsson et al. 2001) (Chapter 9, Respiratory Effects in Adults from Exposure to Secondhand Smoke). This section considers biologic mechanisms that could underlie these associations as they reflect exposures during different points of the life span.

The biologic basis by which maternal smoking during pregnancy increases the risk of asthma is not fully understood, but a number of possible mechanisms have been identified. One mechanism is

the impairment of fetal airway development. A number of studies have reported that infants of mothers who had smoked during pregnancy had abnormal results on lung function tests, including decreased expiratory flow rates (Hanrahan et al. 1992; Cunningham et al. 1994; Tager et al. 1995) and increased airway resistance (Dezateux et al. 1999; Milner et al. 1999). These changes in lung mechanics that result from in utero tobacco smoke exposures persist through late childhood (Cunningham et al. 1994) and perhaps into adulthood (Upton et al. 1998). Also, diminished respiratory function in neonates precedes and is predictive of wheeze in early childhood (Martinez et al. 1988b; Dezateux et al. 1999; Young et al. 2000). Alterations in airway wall structure, particularly increased airway wall thickness, were found in infants exposed to maternal smoking (Elliot et al. 1998). This increased wall thickness could explain a major effect of maternal smoking on expiratory flow rates because it results in a smaller airway lumen, thereby increasing airway resistance. Supporting evidence comes from studies in rats that also indicated that exposure to smoking during pregnancy impaired fetal airway development and function (Collins et al. 1985).

A possible explanation for the impaired airway development, supported by recent data obtained in monkeys, is that the changes in airway structure are attributable to in utero effects of nicotine on extracellular matrix synthesis (Sekhon et al. 1999, 2002). Nicotine readily crosses the feto-placental barrier and attains concentrations in amniotic fluid that are equivalent to or higher than maternal serum nicotine levels (Luck and Nau 1984; Luck et al. 1985). At these concentrations, nicotine can exert profound biologic effects by targeting specific ionotropic channel receptors termed nicotinic acetylcholine receptors (nAChRs). These receptors are a family of ligand-gated, pentameric ion channels. In humans, 16 different subunits have been identified that form a large number of homopentameric and heteropentameric receptors with distinct structural and pharmacologic properties (Leonard and Bertrand 2001). Although the main focus on this receptor family has been to elucidate its role in transmitting signals for the neurotransmitter acetylcholine at neuromuscular junctions, recent interest has included its role in signaling events in nonneural cells. In the developing lung, $\alpha 7$ nAChRs are the most abundant form of nAChRs. Prenatal nicotine exposure strikingly increases $\alpha 7$ nAChR expression and binding. Acting through $\alpha 7$ nAChRs, nicotine markedly affects lung development. For example, prenatal exposure of primates to nicotine significantly alters lung structure (Sekhon et al. 1999). Specifically,

paralleling the increase in $\alpha 7$ expression is a substantial increase in collagen expression surrounding large airways and vessels (Sekhon et al. 1999). Nicotine also increases collagen type I and type III mRNA expressions (i.e., copies of information carried by a gene on the DNA) in airways and alveolar walls (Sekhon et al. 2002). Collectively, these studies suggest that nicotine may be an important component of cigarette smoke responsible for increasing the airway wall thickness in infants of mothers who smoke during pregnancy.

A second mechanism that may cause a predisposition to asthma as a result of secondhand smoke exposure is the induction of bronchial hyper-reactivity (BHR). Secondhand smoke exposure reportedly increases BHR in both children and adults. Martinez and colleagues (1988a) reported an increase in BHR following exposure to secondhand smoke in 70 percent of nine-year-old children whose mothers had smoked regularly during pregnancy. Young and colleagues (1991) reported a modest increase in BHR from inhaled histamine in infants (mean age four and one-half weeks) of parents who smoked compared with unexposed infants. That study was unable to separate the effects of prenatal and postnatal exposure to cigarette smoke. Recent results from the multicenter European Community Respiratory Health Survey demonstrated that secondhand smoke was also significantly associated with BHR in adults (Janson et al. 2001). This analysis included data from more than 7,800 adults who had never smoked. There were also significant dose-related trends between secondhand smoke and BHR. The increase in BHR caused by secondhand smoke may be attributable, in part, to cigarette smoke-induced increases of neuroendocrine cells in the lung. Located in the airway epithelium, neuroendocrine cells synthesize and release bronchoconstrictors, including serotonin, endothelin, and bombesin. Airways of persons with asthma also contained a higher number of neuroendocrine cells (Schuller et al. 2003). In rats, in utero and postnatal secondhand smoke exposure caused BHR and increased the number of neuroendocrine cells in the lungs (Joad et al. 1995). That study exposed pregnant rats to filtered air or to secondhand smoke under controlled conditions from day three of gestation until birth. The female rat pups were then exposed postnatally to either filtered air or secondhand smoke for 7 to 10 weeks. Exposure to prenatal and postnatal secondhand smoke resulted in lungs that were less compliant and more reactive to methacholine, with a 22-fold increase in the number of pulmonary neuroendocrine cells.

Nicotine may also be responsible for this increase in neuroendocrine cells. Sekhon and colleagues (1999) demonstrated that in utero nicotine exposure substantially increased neuroendocrine cells in the lungs of monkeys. Studies also suggest that nicotine may cause the release of bronchoconstrictors. Schuller and colleagues (2003) recently demonstrated that nicotine and its nitrosated carcinogenic derivative NNK bind to $\alpha 7$ nAChRs on pulmonary neuroendocrine cells. This results in the influx of calcium, the release of bronchoconstrictors, and the activation of (1) a mitogenic pathway mediated by protein kinase C, (2) the serine/threonine protein kinase Raf-1, (3) the mitogen-activated protein kinase, and (4) the proto-oncogene *c-myc*. These findings thus identify a possible effector cell for the increased BHR resulting from secondhand smoke exposure and indicate plausible mechanisms.

Researchers have also determined that secondhand smoke exposure affects the neural control of airways. In particular, there are extensive studies on the role of secondhand smoke exposure on the lung C-fiber central nervous system (CNS) reflex. The stimulation of sensory nonmyelinated bronchopulmonary C-fibers can trigger intense respiratory responses through local and CNS reflexes. Responses include bronchoconstriction, mucous secretion, and increased microvascular leakage, which are all hallmarks of asthma (Coleridge and Coleridge 1994). C-fibers are stimulated by components of secondhand smoke including nicotine (Saria et al. 1988), acrolein (Lee et al. 1992), and oxidants (Coleridge et al. 1993). In studies examining the role of secondhand smoke in neural control, Bonham and colleagues (2001) exposed one-week-old guinea pigs to filtered air or secondhand smoke for five weeks. Secondhand smoke exposure increased the excitability of afferent lung C-fibers and neurons in the CNS reflex pathway. This pathway could underline the increased risk for respiratory symptoms attributable to secondhand smoke exposure.

Altered immune responses may also play a role in the increased incidence of asthma in secondhand smoke-exposed children. Active smoking is associated with higher concentrations of total serum immunoglobulin E (IgE) (Sapigni et al. 1998; Oryszczyn et al. 2000). Magnusson (1986) extended these studies and demonstrated that cord blood IgE concentration was elevated significantly in infants whose mothers had smoked during pregnancy and that maternal smoking during pregnancy might predispose infants to subsequent sensitization and allergy. Studies have also associated high serum IgE levels with secondhand smoke exposure in children (Wjst

et al. 1994) and in adults (Sapigni et al. 1998; Oryszczyn et al. 2000), although not all studies observed this association (Janson et al. 2001). Such enhanced IgE values might predict a later development of allergies (Marini et al. 1996).

Cigarette smoke exposure may also modify the balance of immune cells in airways. Studies on immune cells in airways have primarily addressed active smoking, and the effects of secondhand smoke exposure on airway immune cells remain unknown. Hagiwara and colleagues (2001) examined whether cigarette smoking could affect the distribution in the human airway of cells secreting T-helper 1 (Th1) or Th2 cytokines by identifying and quantifying the frequencies of cells spontaneously secreting cytokines in bronchoalveolar lavage fluid (BALF). The researchers collected BALF from nonsmokers or heavy cigarette smokers and performed cytokine assays to quantify cells secreting interleukin-2 (IL-2), IL-4, and interferon gamma (IFN- γ) with or without phorbol 12-myristate 13-acetate stimulation. No cells spontaneously secreting IL-2 were detected in BALF from smokers, whereas the BALF from most nonsmokers had detectable cells secreting IL-2. The number of cells secreting IFN- γ also decreased substantially in smokers compared with nonsmokers. Cells secreting IL-4 were not detected in samples from either group. There were also significant decreases in mitogen-stimulated Th1 cytokine-secreting cells in the airways of smokers. The frequency of cells secreting IL-2 and the lymphocyte CD4/CD8 ratio in BALF had a weak positive correlation. These results indicate that cigarette smoking depletes Th1 cytokine-secreting cells in the human airway and may explain the susceptibility of smokers to certain airway disorders, including allergic diseases.

Nicotine can impair antigen receptor-mediated signal transduction in lymphocytes, possibly contributing further to the asthma phenotype among the huge number of other sensitizing chemicals in tobacco smoke (Geng et al. 1995). Nicotine can inhibit both T cell-dependent and T cell-independent antibody forming cell responses and thus contribute to the immunosuppression that leads to an increased risk of respiratory infections, which are common triggers of BHR.

Nitric oxide (NO) plays an important role in the physiologic regulation of human airways. Changes in its production are implicated in the pathophysiology of airway diseases associated with cigarette smoking (Barnes and Belvisi 1993). Studies show that NO is a mild bronchodilator in persons with asthma when administered exogenously (Hogman et al. 1993). The inhibition of endogenous NO

synthesis by nitro-L-arginine methyl ester, a NO synthase (NOS) inhibitor, increases BHR in response to histamine in persons with asthma (Taylor et al. 1998). This reaction suggests that there are protective effects against bronchoconstriction by the NO that is released within the airways. Of note, inhalation of NG-monomethyl-L-arginine, another NOS inhibitor, increases BHR to bradykinin in patients with mild asthma (Ricciardolo et al. 1996), but not in those with more severe asthma (Ricciardolo et al. 1997), indicating a possible relationship between disease severity and the bronchodilatory role of endogenous NO. Several studies have demonstrated that exhaled NO levels, indicators of endogenous production, were lower in smokers than in nonsmokers (Persson et al. 1994; Schilling et al. 1994; Kharitonov et al. 1995). Those studies were more recently extended to secondhand smoke exposure. Yates and colleagues (2001) demonstrated a rapid (within 15 minutes) fall in exhaled NO levels during secondhand smoke exposure. The decreases in exhaled NO were observed at levels of secondhand smoke exposure frequently experienced in community settings (Yates et al. 1996). The inhibitory effect of cigarette smoke on exhaled NO has also been demonstrated in vitro, where cigarette smoke decreased NO production (Edwards et al. 1999). Thus, the decreased generation of NO in airways provides an additional mechanism for the increased BHR in persons exposed to secondhand smoke.

A number of plausible mechanisms could account for the decrease in exhaled NO associated with secondhand smoke exposure. Cigarette smoke contains high concentrations of oxides of nitrogen, and the reduction in exhaled NO may be attributable to the decreased production of NOS by a negative feedback mechanism (Kharitonov et al. 1995). Other possible mechanisms include an accelerated uptake of NO following tobacco smoke exposure, or an increased breakdown or modification of NO by oxidants in cigarette smoke. NO reacts rapidly with superoxide anion, yielding the harmful oxidant peroxynitrite. This mechanism would be similar to that observed in cystic fibrosis where nitrite levels, indicators of NO oxidative metabolism, are elevated in breath condensate of afflicted persons but exhaled NO is not (Ho et al. 1998).

The induction of BHR following exposure to secondhand smoke might also result from smoke-induced inflammation. Lee and colleagues (2002) demonstrated that airway inflammation markedly increased BHR. Sietta (1999) demonstrated that cigarette smoking caused a profound inflammatory response in airways and lung parenchyma. Cigarette

smokers had increases in total inflammatory cell counts and polymorphonuclear leukocyte (PMN) counts (tested by BAL), and nonsmokers exposed to secondhand smoke for as little as three hours experienced an increase in circulating PMNs, enhanced PMN chemotaxis, and the augmented release of oxidants upon stimulation (Anderson et al. 1991). Airway epithelial cells are likely involved in producing this inflammatory reaction because they line the respiratory tract and interact directly with inhaled cigarette smoke to elaborate proinflammatory cytokines (Yu et al. 2002). Human bronchial epithelial cell cultures exposed to cigarette smoke extract exhibited significantly greater PMN chemotactic activity compared with the control cell cultures (Mio et al. 1997).

Secondhand Smoke and Infection

The topic of active smoking and host defenses against infectious agents has been covered in previous reports of the Surgeon General (USDHHS 1990, 2004). Epidemiologic studies show that secondhand smoke exposure enhances susceptibility to respiratory infections and/or worsens infections in both adults and children (Porro et al. 1992; Strachan and Cook 1997; Jaakkola 2002). Although mechanisms underlying the increased risk of infection associated with secondhand smoke exposure have not been fully evaluated, several studies have identified mechanisms that are likely to be involved. As reviewed earlier (Geng et al. 1995), secondhand smoke can inhibit antibody responses that are either T cell-dependent or T cell-independent, thus contributing to impaired immune responses. Secondhand smoke hinders macrophage responsiveness, further impairing the proper functioning of the immune system (Edwards et al. 1999). It also impairs mucociliary clearance (Wanner et al. 1996), enhances bacterial adherence, and disrupts the respiratory epithelium (Fainstein and Musher 1979; Dye and Adler 1994), a critical host defense barrier. Secondhand smoke exposure may also alter bacterial flora in pharyngeal mucosa of infants, thus providing an additional mechanism for enhanced susceptibility to infection (Kilian et al. 1995).

Secondhand Smoke and Chronic Obstructive Pulmonary Disease

As a slowly progressive condition, COPD is characterized by airflow limitation that is largely irreversible. Characteristic pathologic changes are the accumulation of inflammatory cells in airways and

lung parenchyma and the extensive derangement of the extracellular matrix, resulting in small distinct airspaces that coalesce into much larger abnormal ones (Niewoehner et al. 1974; Cosio et al. 1980; Jeffery 2001). The inflammatory cells are regarded as the source of enzymes (e.g., elastases) that cause the matrix destruction. Oxidative stress is also thought to play an important role in the development of COPD. A number of studies have shown an increased oxidant burden and consequently increased markers of oxidative stress in the airspaces, breath, blood, and urine of smokers and of patients with COPD (MacNee 2001). Sources of the increased oxidative burden in COPD patients include cigarette smoke, which contains abundant amounts of oxygen-based free radicals, peroxides, peroxy nitrates, and phagocytes (Pryor 1992). Alveolar macrophages and PMN from smokers release increased amounts of reactive oxygen species under certain conditions when compared with the same cell types from non-smokers (Hoidal et al. 1981; Ludwig and Hoidal 1982). The consequences of oxidative stress may include oxidative inactivation of antiproteinases, airspace epithelial injury, and expression of proinflammatory mediators (MacNee 2001), which are all elements of the inflammatory process underlying the development of COPD.

Although secondhand smoke clearly causes an increased oxidant burden in the lungs, only a few publications address secondhand smoke and COPD, and the magnitudes of the associations observed are modest. A few studies have suggested an increased risk of COPD with a high level of exposure (Coultas 1998). One approach investigators have taken to determine the potential risk of COPD from secondhand smoke exposure is to examine the relationship between lung function level and secondhand smoke. Although longitudinal data on the effects of active or involuntary smoking and the development of COPD are not available from childhood through adulthood, evidence suggests that COPD in adults may result from impaired lung development and growth, the premature onset of a decline in lung function, and/or an accelerated decline in lung function (Samet and Lange 1996; Kerstjens et al. 1997). As discussed earlier in this chapter (see "Secondhand Smoke and Asthma"), exposure to secondhand smoke in infancy and childhood and active smoking during childhood and adolescence contribute to impaired lung growth (Collins et al. 1985). In general, however, although studies have identified plausible mechanisms, there is a need for additional evidence on the relationship between secondhand smoke and COPD.

Secondhand Smoke and Sudden Infant Death Syndrome

Many epidemiologic studies document that maternal smoking during pregnancy and after birth is a major risk factor for SIDS (Haglund and Cnattingius 1990; Klonoff-Cohen et al. 1995; Taylor and Sanderson 1995). Earlier reports have concluded that maternal smoking during pregnancy causes SIDS (USDHHS 2001, 2004). Research has identified mechanisms in SIDS infants related to arousal failure, inadequate cardiorespiratory compensatory motor responses, and sleep apnea that are attributable to developmental abnormalities in the brainstem and autonomic nervous system (Avery and Frantz 1983; Harper 2000; Slotkin 2004; Spitzer 2005; Adgent 2006). Researchers have studied the potential mechanisms by which prenatal, perinatal, and postnatal exposures to secondhand smoke are related to neurodevelopmental abnormalities. The data suggest that the potent neurotoxic effects of nicotine are important (Slotkin et al. 1997; Önal et al. 2004; Slotkin 2004; Adgent 2006). Children who die from SIDS have higher concentrations of nicotine in their lungs compared with children who die of other causes (Milerad et al. 1998; McMartin et al. 2002). This association holds even when the parents report a nonsmoking environment. The specific role of nicotine and other tobacco smoke constituents in the pathogenesis of SIDS is not known. Research, however, particularly animal exposure models, suggests that many cardiorespiratory control deficiencies are associated with nicotinic receptors within the peripheral and central nervous systems (Neff et al. 1998; Adgent 2006). Animal studies have documented effects that can be related to several potential mechanisms that could cause SIDS, including the effects of perinatal exposure to secondhand smoke on increased nAChR production in brains of monkeys (Slotkin et al. 2002); the disruptions in brain development through cholinergic mechanisms (Slotkin 2004); and adverse effects on brain cell development, synaptic development and function, and neurobehavioral activity. Perinatal exposure to secondhand smoke also has adverse effects on neurobehavioral development (Makin et al. 1991), and recent studies indicate that perinatal exposure to secondhand smoke induces adenylyl cyclase (AC) activity and alters receptor-mediated cell signaling in brains of neonatal rats (Slotkin et al. 2001). In those studies, rats were exposed to secondhand smoke during gestation or during the early neonatal period or both. Brains were examined for alterations in AC activity and for changes in beta-adrenergic and

M2 muscarinic cholinergic receptors and their linkage to AC. Secondhand smoke exposure induced an increase in total AC activity, which was monitored with forskolin, the direct enzymatic stimulant. In the brain, the specific coupling of beta-adrenergic receptors to AC was inhibited in the groups exposed to secondhand smoke despite a normal complement of receptor-binding sites. Because alterations in AC signaling are known to affect cardiorespiratory function, the results provide a possible mechanistic link to the action of secondhand smoke, including postnatal secondhand smoke exposure, in disturbances culminating in SIDS. Secondhand smoke exposure causes the same changes in AC signaling seen previously with prenatal nicotine exposure: increases in AC production and the loss of specific receptor coupling to AC. In a recent independent analysis of perinatal and postnatal exposure to secondhand smoke in rhesus monkeys, researchers observed significant neural cellular effects from postnatal exposures alone, including specific damage in the occipital cortex, in the midbrain, and in temporal cortex cell development. These effects are similar to those previously observed in other animal models for either prenatal nicotine or perinatal secondhand smoke exposure, or for continuous prenatal and postnatal exposures (Slotkin et al. 2006).

A second possible mechanism for the increased incidence of SIDS following secondhand smoke exposure relates to earlier cited evidence from a guinea pig model of postnatal secondhand smoke exposure. That model demonstrated an increase in the production or release of lung C-fiber CNS reflex responses to secondhand smoke (Bonham et al. 2001). The responses invoked by the increased excitability of afferent lung C-fibers and nucleus tractus solitarius (NTS) neurons in the CNS reflex pathway include changes in breathing patterns, such as prolonged expiratory apnea. The findings suggest that an increase in secondhand smoke-induced excitability of NTS neurons augmenting C-fiber reflex output may contribute to SIDS.

Findings of a study that used a piglet model suggest that nicotine interferes with normal autoreuscitation (Frøen et al. 2000). The effect of nicotine was augmented by the additional administration of IL-1B, which is released during infections. Studies with a piglet model also suggest that early involuntary, postnatal nicotine exposure may be responsible for some neuropathologic changes in apoptotic markers that researchers have observed in SIDS infants (Machaalani et al. 2005).

Although investigators have not established a specific role for apnea as a potential cause of SIDS,

one study of human newborns evaluated this theoretical potential of apnea in relation to SIDS (Chang et al. 2003). A controlled sleeping experiment included 10 infants either prenatally or postnatally exposed to tobacco smoke and 10 unexposed control infants. The researchers found that five of the exposed infants did not have a behavioral arousal response to a standard sequence of audiology stimuli, whereas all of the unexposed infants were aroused.

Secondhand Smoke and Nasal or Sinus Disease

Some studies indicate an association, particularly in children, between secondhand smoke exposure and acute or chronic nasal and sinus symptoms (Barr et al. 1992; Moyes et al. 1995; Benninger 1999). In children aged 4 through 11 years, frequent colds and general sinus symptoms were significantly associated with maternal smoking (Barr et al. 1992). Normal healthy persons have also developed nasal congestion, irritation, and increased rhinitis from exposure to moderate levels of secondhand smoke (Willes et al. 1998). Researchers have examined a number of potential mechanisms (Samet 2004). Tobacco smokers have abnormal nasal mucociliary clearance, and a study by Bascom and colleagues (1995) demonstrated differential nasal responsiveness to secondhand smoke. Using the clearance of ^{99m}Tc -sulfur colloid as an indicator of mucociliary function, decreased clearance occurred in 3 out of 12 persons following exposure. Persons with delayed clearances all had a history of secondhand smoke rhinitis (Bascom et al. 1995). In a follow-up study comparing persons who were not sensitive with persons who were sensitive to secondhand smoke, those who were sensitive had more rhinorrhea following the intranasal administration of capsaicin, thus suggesting a role for C-fiber stimulation (Bascom et al. 1991). The researchers observed no changes in nasal vascular permeability or inflammation following secondhand smoke exposure. Studies have also shown secondhand smoke-induced increases in epithelial permeability to environmental allergens, thus enhancing allergic reactions to inhaled allergens (Kjellman 1981; Zetterstrom et al. 1981).

Summary

Cellular, animal, and human studies indicate a number of mechanisms by which secondhand smoke injures the respiratory tract. There is extensive information on the harm from active smoking as well.

There are limitations to many of the cited studies. Most clinical studies base secondhand smoke exposure on self-reports and have not included objective measurements of exposure, such as salivary, serum, or urine cotinine concentrations. An additional limitation is that studies of secondhand smoke exposure frequently use a cross-sectional design and provide little data on the duration of the exposure. In addition, mechanistic studies frequently rely on animal models or *in vitro* studies. Both have limitations, particularly in relation to the level and duration of the exposures and difficulties in simulating human exposures. There is very little information about the concentrations of specific tobacco smoke constituents following secondhand smoke exposure in the alveolar milieu and limited information about the interactions among the various constituents.

Obviously, the closer a model mimics human exposure the more relevant this information will be. In addition to more closely simulating conditions of human exposure, future studies should focus on individual susceptibilities. This approach will lead to the recognition of genetic profiles that influence susceptibility to adverse effects of secondhand smoke and will provide insights into the underlying mechanisms of the health consequences.

Animal and human studies indicate several potential mechanisms by which exposure to secondhand smoke may affect the neuroregulation of breathing, apneic spells, and sudden infant death. The role of nicotine and other tobacco smoke constituents in the pathogenesis of SIDS is not known. However, the neurotoxicity of prenatal and neonatal exposures to nicotine and secondhand smoke in animal models can be related to several potential causal mechanisms, including adverse effects on brain cell development, synaptic development and function, and neurobehavioral activity.

Conclusions

1. The evidence indicates multiple mechanisms by which secondhand smoke exposure causes injury to the respiratory tract.
2. The evidence indicates mechanisms by which secondhand smoke exposure could increase the risk for sudden infant death syndrome.

Mechanisms of Secondhand Smoke Exposure and Heart Disease

When the association of CHD with secondhand smoke was first reported, its plausibility and the magnitude of the observed risk were questioned. The observed risk for involuntary smoking was thought to be relatively strong compared with the well-documented risk of active smoking. In addition, it was uncertain whether the mechanisms underlying the association of active smoking with CHD risk were relevant, considering the lower doses of smoke components associated with typical secondhand smoke exposures. Subsequently, an understanding of the potential mechanisms associating CHD with involuntary smoking has deepened, largely as a result of findings from human and animal experiments involving secondhand smoke exposure.

Clinical and experimental evidence continues to accumulate regarding the mechanisms by which active smoking causes CHD (USDHHS 1990,

1994, 1998, 2001, 2004). Active smoking promotes atherogenesis by unfavorably affecting many elements in the interface of the blood with the arterial wall and the cellular elements of the artery itself. Atherosclerosis is, in part, considered an inflammatory process (Ross 1993, 1999), and smoking results in a potent, systemic inflammatory stimulus (USDHHS 2004). Active smoking is associated with dysfunctional endothelial cells, the cells lining the inner arterial wall that are in contact with the circulating blood. This dysfunction leads to the secretion of inflammatory cytokines, the adhesion of monocytes and lymphocytes and their migration to the endothelium, the proliferation of smooth muscle cells, and the reduction of the normal antithrombotic properties of the endothelium. Compared with nonsmoking controls, smokers also have less endothelium-dependent vasodilatation (Celermajer et al. 1993).

The balance of the tightly regulated coagulation–fibrinolytic system is critical to the prevention of atherothrombotic events such as acute coronary syndromes, which include unstable angina and myocardial infarction (MI) (Corti et al. 2003). Smoking has a prothrombotic effect, tipping this system toward clot formation, which comes from a variety of actions of smoking including impaired endothelial cell functioning, increased platelet aggregation, and reduced fibrinolysis (USDHHS 2004).

Smoking is also associated with an adverse lipid profile (USDHHS 1990, 2004). Smokers tend to have higher concentrations of total low-density lipoprotein (LDL) and very low-density lipoprotein and decreased levels of high-density lipoprotein (HDL). Smoking also increases oxygen demand while reducing oxygen-delivering capacity.

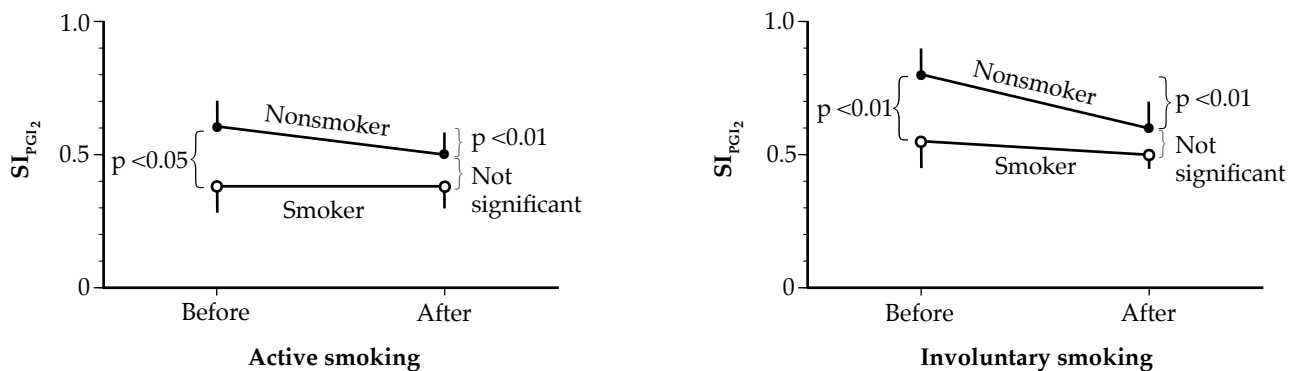
This section reviews mechanisms that are considered to be the basis of the association between exposure to secondhand smoke and CHD. The following section reviews the relevant body of research and covers each of the systems affected unfavorably by active smoking for which there is also research on secondhand smoke exposure. The discussion also provides a foundation for considering the observational evidence in Chapter 8, Cardiovascular Diseases from Exposure to Secondhand Smoke.

Platelets

Exposure to secondhand smoke activates blood platelets (i.e., makes them sticky), and thereby increases the likelihood of a thrombus. These activated platelets can damage the lining of the coronary arteries and may facilitate the development and progression of atherosclerotic lesions (Pittilo et al. 1982; Sinzinger and Kefalides 1982; Burghuber et al. 1986; Davis et al. 1989; Sinzinger and Virgolini 1989; Steinberg et al. 1989). Increased platelet activation is associated with an increased risk for ischemic heart disease (Elwood et al. 1991). Thus, increases in platelet activation observed in persons exposed to secondhand smoke would be expected to have acute adverse effects.

In one experiment, two groups each smoked two cigarettes: individuals who by history were nonsmokers and individuals who were reported smokers (Burghuber et al. 1986). At the beginning of the experiment, the platelets of the chronic smokers were less sensitive to stimulation by exogenous prostacyclin than those of the nonsmokers; platelet sensitivity did not significantly change in the smokers in response to smoking the two cigarettes (Figure 2.4). In contrast to these findings, nonsmokers who smoked just two cigarettes had a significantly ($p < 0.01$) decreased level of response to the same stimulus, reaching a level close

Figure 2.4 Effect of active and involuntary smoking on platelet aggregation in smokers and nonsmokers



Note: The sensitivity index, SI_{pGI_2} , is defined as the inverse of the concentration of prostaglandin I_2 which is necessary to inhibit adenosine diphosphate-induced platelet aggregation by 50 percent. Lower values of SI_{pGI_2} indicate greater platelet aggregation.

Source: Burghuber et al. 1986. Adapted with permission.

to that of the smokers. The findings indicate differing acute responses of platelets of nonsmokers and smokers to the toxins in cigarette smoke.

In an experiment more relevant to involuntary smoking, the same investigators used the same platelet assay in another group of smokers and nonsmokers before and after they sat in a room for 20 minutes where cigarettes had just been smoked (Figure 2.4) (Burghuber et al. 1986). The researchers again found no significant change among smokers, but a significant increase in platelet sensitivity to prostacyclin among nonsmokers brought them to a level similar to that of the smokers. These data, together with findings from other human experiments (Davis et al. 1989), indicate that nonsmokers are sensitive to secondhand smoke, and even very low levels of secondhand smoke exposure can have a major impact on platelet function in nonsmokers. Animal data also show an effect of secondhand smoke exposure. Bleeding time, another measure of platelet function, is significantly shortened by secondhand smoke exposure (meaning more activated platelet activity) in both rabbits (Zhu et al. 1993b; Sun et al. 1994) and rats (Zhu et al. 1994).

With regard to the mechanisms, studies of cigarette smoke extract on platelet function suggest that the toxins in cigarette smoke increase platelet function by interfering with and degrading platelet-activating factor acetylhydrolase (PAF-AH) (Miyaura et al. 1992). Exposure of serum to cigarette smoke extract reduces the effectiveness of PAF-AH and may thus increase the concentration of platelet-activating factor. The reduced efficacy of PAF-AH may explain the increased serum concentration of platelet-activating factor in smokers. Nicotine appears to be one of the active agents in tobacco smoke, but other specific compounds may also contribute to the effects of exposure on platelets (Davis et al. 1985; Miyaura et al. 1992). This *in vitro* finding complements results of clinical studies that compared the effects of smoking and transdermal nicotine on platelets and on hemostatic function. Benowitz and colleagues (1993) carried out a crossover trial that compared the effects of cigarette smoking and transdermal nicotine on eicosanoid formation and hemostatic function. Although both active smoking and transdermal nicotine produced similar nicotine levels, there was an increase in the urinary excretion of several markers of platelet function while smoking cigarettes that was not seen with transdermal therapy (Benowitz et al. 1993).

Some investigators have reported conflicting findings and have questioned whether platelet aggregation is an underlying mechanism of the

association between CHD and secondhand smoke exposure (Smith et al. 2000b, 2001). Smith and colleagues (2001) conducted an observational study that compared secondhand smoke-exposed and unexposed adult nonsmokers and did not find differences in urinary metabolites of thromboxane and prostacyclin.

Endothelial Function and Vasodilation

Arteries are lined by a cell layer known as the vascular endothelium. The endothelium plays a critical role in controlling the ability of arteries to dilate and constrict as they regulate blood flow. In addition, damage to the vascular endothelium facilitates the development of atherosclerosis. Evidence in both animals (Hutchison et al. 1995, 1996, 1997a,b, 1998, 1999; Jorge et al. 1995; Zhu and Parmley 1995; Schwarzacher et al. 1998; Török et al. 2000) and humans (Celermajer et al. 1996; Sumida et al. 1998; Otsuka et al. 2001) shows that secondhand smoke interferes with endothelium-dependent vasodilation. Moreover, these effects can be attenuated by increasing the amount of L-arginine, an amino acid that is a precursor of NO, the mediator of endothelium-dependent vasodilation (Hutchison et al. 1996, 1997a, 1998, 1999; Schwarzacher et al. 1998). Studies in rats have also demonstrated that involuntary smoking reduces NOS in the penis (Xie et al. 1997), indicating that secondhand smoke specifically interferes with the production of NO.

Consistent with other results from animal studies, most human studies indicate that endothelium-dependent vasodilation in nonsmokers is sensitive to secondhand smoke following both chronic (Celermajer et al. 1996; Sumida et al. 1998) and acute (Otsuka et al. 2001) exposures. Indeed, the effects of secondhand smoke on endothelium-dependent vasodilation in human coronary circulation are comparable in magnitude to the effects observed in smokers when compared with nonsmokers (Sumida et al. 1998; Otsuka et al. 2001).

Celermajer and colleagues (1996) studied endothelium-dependent vasodilation in 78 healthy persons aged 15 to 30 years by measuring the extent of reactive hyperemia in the brachial artery after occluding it with a blood pressure cuff (with the flow increase determined by endothelium-dependent vasodilation) before and after administering nitroglycerine (an endothelium-independent vasodilator). Involuntary smokers were classified by self-reported levels of chronic exposure to secondhand smoke. Investigators found similar impairments in flow-mediated

dilation in both involuntary and active smokers when compared with unexposed nonsmoking controls (Figure 2.5). Among those exposed to secondhand smoke, there was an inverse relationship between the intensity of the exposure and flow-mediated dilation ($r = -0.67$, $p < 0.001$). Using similar methods, Woo and colleagues (1997) studied 72 rural Chinese persons and 72 White persons in Australia and England. These researchers did not find a smoking effect among adults living in rural China, but the analysis grouped active with involuntary smokers. An effect of exposure was observed in White participants, but results were also reported with active and involuntary smokers combined.

The adverse effects of chronic secondhand smoke exposure may be partially reversible. In a cross-sectional study of young adults, there was less evidence for arterial endothelial dysfunction in former involuntary smokers compared with current involuntary smokers (Raitakari et al. 1999). Kato and colleagues (1999) experimentally tested whether the reduction in endothelium-dependent vasodilation from secondhand smoke is an acute phenomenon in nonsmokers. The experiment included a brief, acute exposure to secondhand smoke (15 minutes). There were similar responses before and after exposure in the brachial artery flow to acetylcholine, which stimulates endothelium-dependent vasodilation, and to nitroprusside, which stimulates endothelium-independent vasodilation. The investigators concluded that the consequences of exposure to secondhand smoke were attributable to chronic rather than acute effects on the brachial artery.

Two studies document the effects of secondhand smoke on human coronary arteries (Sumida et al. 1998; Otsuka et al. 2001). Sumida and colleagues (1998) studied 38 women aged 40 to 60 years with no known risk factors for CHD other than age and exposure to tobacco smoke. The participants included three groups: nonsmokers who had never smoked and had never been regularly exposed to secondhand smoke, nonsmokers with a self-reported history of exposure for at least an hour a day for at least 10 years, and active smokers. The study examined the changes in the diameter of the epicardial coronary artery (proximal and distal segments of the left anterior descending and left circumflex coronary arteries) in response to an intracoronary injection of acetylcholine. Acetylcholine constricted most coronary arteries in both exposed nonsmokers and active smokers to a similar extent and dilated the coronary arteries in unexposed nonsmokers. This result suggests

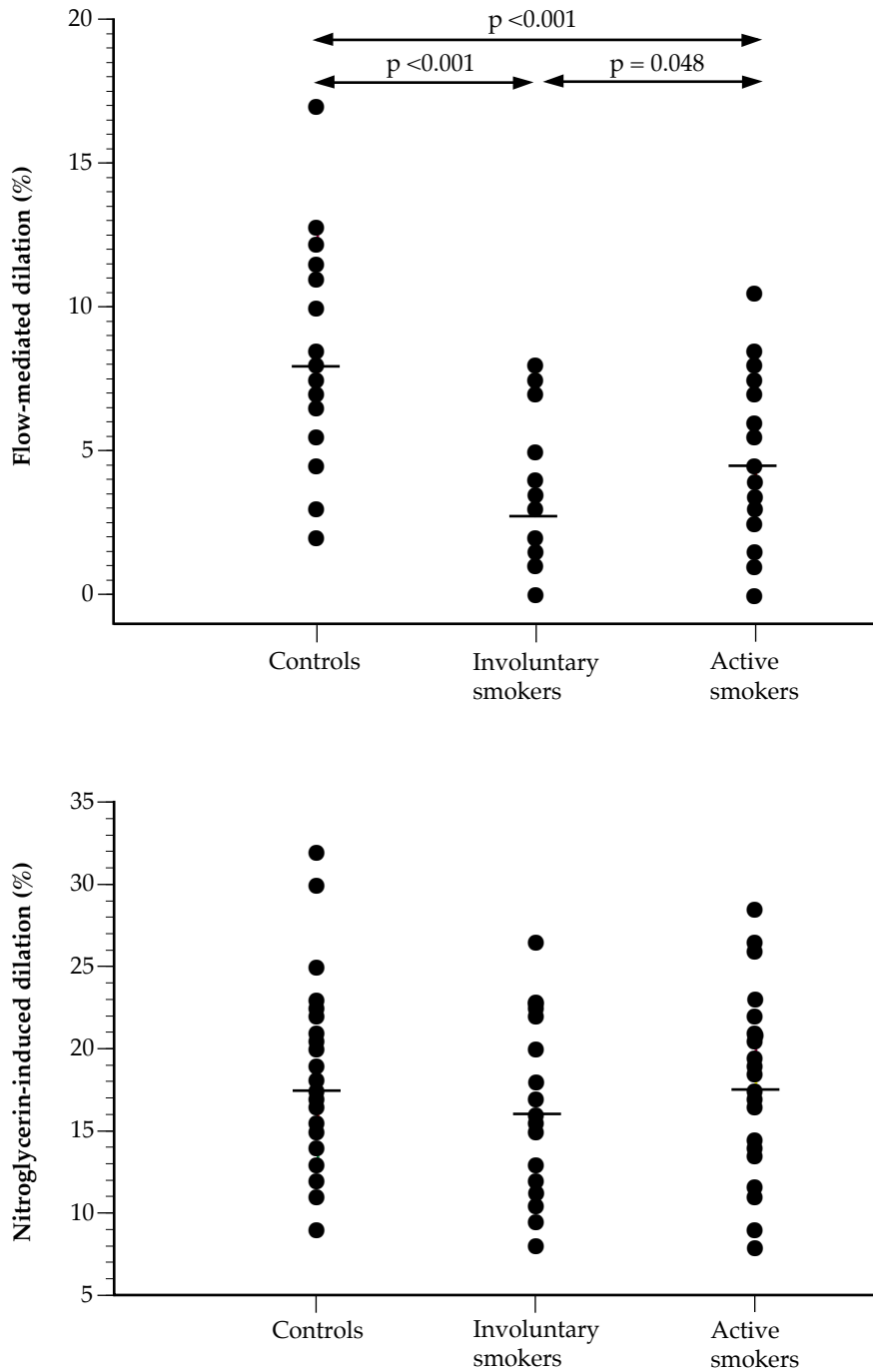
possibly similar levels of coronary endothelial dysfunction among involuntary and active smokers.

Otsuka and colleagues (2001) used ultrasound in healthy young adult nonsmokers and smokers to measure coronary flow velocity changes in response to acetylcholine as a measure of endothelium-dependent vasodilation (quantified as coronary flow velocity reserve). The measurements were made before and 30 minutes after breathing secondhand smoke for 30 minutes in a hospital smoking room in Japan. Before the exposure, nonsmokers had a significantly higher coronary flow velocity reserve compared with smokers (Figure 2.6). The 30 minutes of exposure had no effect on the coronary flow velocity reserve among smokers, but significantly reduced the reserve in nonsmokers to a level that almost equaled the level found in smokers (Figure 2.6). This substantial acute response is similar in magnitude to the effect observed with chronic exposures on brachial (Celermajer et al. 1996) and coronary (Sumida et al. 1998) arteries. However, the finding differs from the lack of effect seen for short-term (15 minutes) acute exposures on the brachial artery (Kato et al. 1999). The different findings in these two studies (Sumida et al. 1998; Otsuka et al. 2001) may be attributable to the duration of the exposure (30 versus 15 minutes) or to differences in the responses of the coronary arteries and the brachial arteries to secondhand smoke exposure.

An experiment in humans also showed that an acute exposure to secondhand smoke reduces the distensibility of the aorta (Stefanadis et al. 1998). In this study, the nonsmokers were exposed to secondhand smoke for five minutes at a mean carbon monoxide (CO) level of 30 parts per million; the smokers smoked one cigarette. The distensibility of the aorta in nonsmokers exposed to secondhand smoke for just five minutes was reduced significantly by 21 percent compared with a 27 percent reduction in the active smokers. There was no change in the sham-exposed patients.

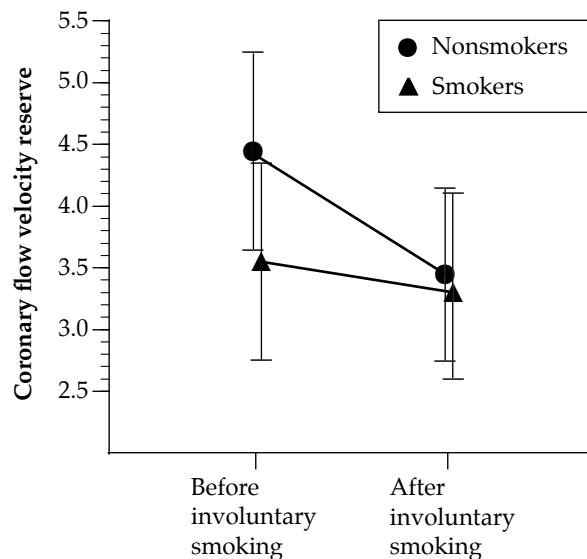
Human experiments have indicated that even short-term exposures to active smoking (Přerovský and Hladovec 1979) or to other tobacco product constituents significantly increase the number of nuclear endothelial cell carcasses in the blood (Davis et al. 1989). The presence of these cell carcasses suggests damage to the endothelium. The number of endothelial cell carcasses (i.e., remains of dead cells) in nonsmokers after they were exposed to secondhand smoke was almost as great as the number of carcasses observed in active smokers.

Figure 2.5 Flow-mediated (endothelium-dependent) and nitroglycerin-induced (endothelium-independent) vasodilation in human brachial arteries



Note: Flow-mediated (endothelium-dependent) vasodilation in human brachial arteries was significantly impaired in chronically exposed involuntary smokers and in active smokers to a similar degree, compared with the controls, whereas nitroglycerine-induced (endothelium-independent) vasodilation was similar in all three groups. Source: Celermajer et al. 1996. Adapted with permission.

Figure 2.6 Coronary flow velocity changes before and after secondhand smoke exposure



Note: Data are mean (standard deviation). Coronary flow velocity reserve (CFVR) before involuntary smoking was significantly higher in nonsmokers than in smokers. However, CFVR after involuntary smoking was reduced significantly in nonsmokers, but only slightly among smokers. Source: Otsuka et al. 2001. Adapted with permission.

Atherosclerosis

Endothelial dysfunction may also contribute to the development of atherosclerosis. Normal endothelial cells promote vasodilation and inhibit atherosclerosis and thrombosis, in part through the release of NO (Harrison 1997). Dysfunctional cells, on the other hand, contribute to vasoconstriction, atherogenesis, and thrombosis. Risk factors contribute collectively to endothelial dysfunction. For example, active smoking interacts with LDL in a way that damages the endothelium (Heitzer et al. 1996). One unifying hypothesis for the effects of cardiovascular risk factors is a combined action to increase damaging oxidative stress (Oskarsson and Heistad 1997). Thus, reducing exposure to risk factors may improve endothelial function and lessen the risk for clinical coronary events. For example, lipid reduction improves endothelial function in patients with hyperlipidemia both acutely (Tamai et al. 1997) and chronically (Treasure et al. 1995).

Platelets are also relevant to the development of atherosclerosis (Ross 1986; Steinberg et al. 1989). Following damage to the arterial endothelium, platelets interact with or adhere to the subendothelial connective tissue and initiate a sequence that leads to the formation of atherosclerotic plaque. When platelets interact with or adhere to subendothelial connective tissue, they are stimulated to release their granule contents.

Endothelial cells normally prevent platelet adherence because of the nonthrombogenic character of their surface and their capacity to form antithrombotic substances such as prostacyclin (Corti et al. 2003). However, platelets can stick to damaged endothelial cells and release mitogens such as platelet-derived growth factor and chemoattractants, which encourage the migration and proliferation of smooth muscle cells in the region of the endothelial injury (Ross 1993). When platelet aggregation increases as a result of exposure to secondhand smoke, platelet accumulation at the injured site is also expected to increase. Tobacco smoke exposure has also been associated with the accumulation of glycosaminoglycans and glycoproteins in vascular tissues of rats, another early event in atherogenesis (Latha et al. 1991).

Effects on Children

Adverse cardiovascular effects of secondhand smoke exposure may begin in childhood. Adolescents and children whose parents smoked exhibited lower HDL levels than children who were not exposed to secondhand smoke (Moskowitz et al. 1990; Feldman et al. 1991). White and Froeb (1991) reported similar results among adults exposed at work. These findings indicate a less favorable lipid profile in persons exposed to secondhand smoke.

Cross-cultural comparisons suggest that genetic differences may influence how children are affected by secondhand smoke. There was a small exposure effect on HDL cholesterol in Japanese children (Misawa et al. 1989) and no effect in Turkish children (Işcan et al. 1996), but the LDL cholesterol level and the ratio of LDL to HDL cholesterol were adversely affected in Turkish children (Işcan et al. 1996). These effects were similar to those found in smokers and may be mediated by inhibiting the activity of the enzyme plasma lecithin: cholesterol acyltransferase in plasma and altered clearance of chylomicron remnants by the liver (Bielićki et al. 1995; Pan et al. 1997). In children with severe hypercholesterolemia, a lower HDL cholesterol level was associated with parental smoking (Neufeld et al. 1997).

Chemical Interactions with Low-Density Lipoprotein Cholesterol

Several animal studies (Albert et al. 1977; Penn et al. 1981, 1996; Majesky et al. 1983; Revis et al. 1984; Penn and Snyder 1993, 1996a,b) demonstrated that PAHs, in particular 7,12-dimethylbenz[*a,h*]anthracene and B[*a*]P, as well as 1,3 butadiene (Penn and Snyder 1996a,b), accelerate the development of atherosclerosis. PAHs, including B[*a*]P and 1,3 butadiene, are constituents of secondhand smoke. PAHs appear to bind preferentially to both LDL and HDL subfragments of cholesterol and may facilitate the incorporation of toxic compounds into the cells lining the coronary arteries. Thus, exposure to PAHs may contribute to both cell injury and hyperplasia in the atherosclerotic process. Adults who inhaled secondhand smoke for only five and one-half hours exhibited compromised antibiochemical defenses and an increased accumulation of LDL cholesterol in macrophages (Valkonen and Kuusi 1998).

Experimental Atherosclerosis

In addition to the studies of single tobacco smoke components, animal experiments have demonstrated that exposure to secondhand smoke for only a few weeks significantly speeds the atherosclerotic process (Table 2.6). These animal models provide an indication of the effect of exposure to more than one component of tobacco smoke.

Zhu and colleagues (1993b) exposed three groups of rabbits to a high-cholesterol diet. Two of the groups were also exposed to 10 weeks of secondhand smoke from Marlboro cigarettes for six hours a day, five days a week. One group was exposed to levels comparable to a smoky bar and the other group was exposed to much higher levels, with a nicotine level 30 times higher. The high-dose group experienced levels comparable to those observed in a car with the windows rolled up while four cigarettes per hour were smoked (Ott et al. 1992). With just 10 weeks of exposure (a total of 300 hours), the fraction of pulmonary artery and aorta covered with lipid deposits was nearly twice as high in the high-exposure group compared with the control animals. There was a smaller increase in the low-exposure group (Figure 2.7) (Zhu et al. 1993b).

This effect appears to be directly attributable to components in the cigarette smoke itself, rather than to an increase in adrenergic tone resulting from the discomfort associated with the forced breathing of secondhand smoke. Sun and colleagues (1994) exposed rabbits to secondhand smoke in an experiment similar to that of Zhu and colleagues (1993b) and gave half

of the rabbits the beta-blocking drug metoprolol. As expected, the animals receiving metoprolol developed fewer lipid deposits than those receiving a placebo (saline), but this effect was independent of whether the rabbits were breathing secondhand smoke. Therefore, increased levels of catecholamines did not mediate the effect of secondhand smoke on the development of atherosclerotic-type lesions in the arteries.

Experiments exposing rabbits to secondhand smoke from standard (Marlboro) and nicotine-free cigarettes produced similar levels of lipid deposits. This finding suggests that nicotine is not the primary atherogenic agent, and there are other combustion products in cigarette smoke that may be responsible for the atherosclerosis (Sun et al. 2001).

Critics have questioned the findings of this rabbit model of atherosclerosis because the animals are fed a high-cholesterol diet in order to develop lesions within a reasonable time (Wu 1993). This experimental model of atherosclerosis has been used since 1908 (Zhu et al. 1993a). Supporting findings come from a different model of plaque development that used young cockerels between the ages of 6 and 22 weeks that were exposed to secondhand smoke for six hours a day, five days a week, for 12 weeks (Penn and Snyder 1993; Penn et al. 1994). The cockerels ate a normal, low-cholesterol diet and were exposed to lower secondhand smoke levels than the rabbits were. The incidence of plaque development was the same in the cockerels breathing secondhand smoke and those breathing clean air. However, the growth rate of the plaques was greater in the exposed animals.

Some specific components have been evaluated in that same model with effects that are not likely to be attributable to the CO in the smoke because exposure of cockerels to high doses of CO (Penn et al. 1992), to tobacco-specific nitrosamines (Penn and Snyder 1996b), or to the tar fraction of the smoke (Penn et al. 1996) did not produce similar effects. Thus, agents in the vapor phase of the smoke appear to be the atherogenic agents; 1,3 butadiene (Penn and Snyder 1996a,b) and 7,12-dimethylbenz[*a*]anthracene (Penn et al. 1981) did increase the amount of atherosclerotic plaque in this experimental model.

Gairola and colleagues (2001) studied the effects of secondhand smoke on apolipoprotein E -/- mice that were on a high-cholesterol diet, which is another model for human atherosclerosis. After exposure to secondhand smoke from University of Kentucky 1R4F research cigarettes for six hours a day, five days a week, for up to 14 weeks, there was a dose-dependent increase in the fraction of the aorta that was covered with atherosclerotic lesions. The exposed

mice had significant increases compared with control animals on the same diet who had breathed clean air for just seven days, with the effect increasing over time. The exposed mice had lesions that were about twice the size of those found in the clean-air controls; there were similar increases in the cholesterol content of the aortas in the exposed mice.

Elements in the smoke rapidly affect the process of incorporating LDL cholesterol into the linings of arteries. Roberts and colleagues (1996) used isolated perfused carotid arteries from rats exposed to secondhand smoke for two or four hours. The researchers demonstrated a synergistic effect between secondhand smoke and LDL that facilitated the binding of oxidized LDL to the vessel wall (Roberts et al. 1996). Rats exposed to secondhand smoke for just two hours had higher rates of incorporation of LDL cholesterol into their carotid arteries.

Secondhand smoke exposure induces atherosclerotic-like changes in four different species of experimental animals after only a few weeks of exposure to secondhand smoke at levels similar to those experienced by people in normal day-to-day life. These findings provide strong support for the epidemiologic evidence that exposure to secondhand smoke causes heart disease. The experimental studies on rabbits, cockerels, mice, and rats were not affected by potential confounding and support a causal conclusion by showing that atherosclerosis can be induced in experimental animals exposed to secondhand smoke.

Oxygen Delivery, Processing, and Exercise

Secondhand smoke reduces the ability of the blood to deliver oxygen to the myocardium. The CO in secondhand smoke competes with oxygen for binding sites on hemoglobin and thus displaces oxygen (USDHHS 1983, 1986; Leone et al. 1991; U.S. Environmental Protection Agency 1991). Children of smoking parents have elevated levels of 2,3-diphosphoglycerate, a compound that increases in red blood cells to compensate for reduced oxygen availability (Moskowitz et al. 1990, 1993) and is associated with serum thiocyanate levels, a measure of secondhand smoke exposure (Moskowitz et al. 1990).

Evidence from animal studies shows that in addition to reducing the ability of the blood to deliver oxygen to the heart, secondhand smoke may reduce the ability of the heart muscle to convert oxygen into the "energy molecule" adenosine triphosphate (ATP). In a rabbit model, there was an approximate 25 percent reduction in cytochrome oxidase activity after a

single 30-minute exposure to secondhand smoke, and the activity continued to drop with a prolonged exposure; after eight weeks of exposure for 30 minutes per day, its activity was 50 percent of the level found in controls (Gvozdják et al. 1987). Thus, not only does secondhand smoke exposure reduce the ability of the blood to deliver oxygen to the myocardium, it may also reduce the ability of the myocardium to effectively use the oxygen it receives (Gvozdjaková et al. 1984, 1985, 1992; Gvozdják et al. 1987).

Secondhand smoke also significantly increases the amount of lactate in venous blood with an exercise challenge (McMurray et al. 1985). Eight women with and without exposure to tobacco smoke through a mouthpiece (concentration not given) engaged in exercises. Compared with the unexposed group, the exposed group documented a lower maximum oxygen uptake and a higher blood lactate. People with CHD cannot exercise as long or reach a level of exercise as high after breathing secondhand smoke, even relatively briefly, compared with breathing clean air (Aronow 1978; Khalfen and Klochkov 1987; Leone et al. 1991). Another study showed that 10 persons with a past MI were more likely to develop increased arrhythmias from exercise following secondhand smoke exposure (Leone et al. 1992).

Free Radicals and Ischemic Damage

Free radicals are highly reactive oxygen products (Church and Pryor 1985; Ferrari et al. 1991) that are destructive to the heart muscle cell membrane as well as to other processes within the cell. Tobacco smoke contains high levels of activated oxygen species, and the inflammatory consequences of tobacco smoke components in various organs are thought to be a critical path of injury. Antioxidants provide protection against the free radicals, but levels of antioxidants, such as beta-carotene and vitamin C, tend to be lower in active smokers (USDHHS 2004) and possibly in involuntary smokers (Farchi et al. 2001).

Experiments have demonstrated that exposure to secondhand smoke worsens the outcome of an ischemic event in the heart through the activity of free radicals during reperfusion injury. Animal studies indicate that low exposures to nicotine or to other cigarette smoke constituents significantly worsen reperfusion injury. Intravenous administration of the amount of nicotine delivered by just one cigarette doubled the reperfusion injury in a dog model of MI (Przyklenk 1994). This dose was low and had no effect on heart rate, blood pressure, regional myocardial shortening,

Table 2.6 Studies of experimental atherosclerosis in animals exposed to secondhand smoke

Study	Species	Secondhand smoke exposure			
		Source	Duration	Measure	
Penn and Snyder 1993	Cockerel	1R4F research cigarettes	6 hours/day, 5 days/week for 16 weeks	Nicotine: CO: [†] Particulates:	365–414 $\mu\text{g}/\text{m}^3$ [*] 35 ppm [‡] 8 mg [§] /m ³
Zhu et al. 1993a	Rabbit	Marlboro	6 hours/day, 5 days/week for 10 weeks	<u>Low exposure</u>	
				Air nicotine: CO: Particulates:	30 $\mu\text{g}/\text{m}^3$ 19 ppm 4 mg/m ³
				<u>High exposure</u>	
				Air nicotine: CO: Particulates:	1,000 $\mu\text{g}/\text{m}^3$ 60 ppm 33 mg/m ³
Penn et al. 1994	Cockerel	1R4F research cigarettes	1 cigarette/day, 5 days/week for 16 weeks	Nicotine: CO: Particulates:	90–130 $\mu\text{g}/\text{m}^3$ 4 ppm 2.5 mg/m ³
Sun et al. 1994	Rabbit	Marlboro	6 hours/day, 5 days/week for 10 weeks	Air nicotine: CO: Particulates:	1,100 $\mu\text{g}/\text{m}^3$ 60–70 ppm 38 mg/m ³
Roberts et al. 1996	Rat	Data were not reported	2 or 4 hours	Nicotine: CO: Particulates:	615 $\mu\text{g}/\text{m}^3$ 18 ± 2 ppm 3 $\mu\text{g}/\text{m}^3$
Gairola et al. 2001	Mouse	1R4F research cigarettes	6 hours/day, 5 days/week for 7, 10, and 14 weeks	Blood CO hemoglobin: Particulates:	10% in secondhand smoke-exposed mice 25 mg/m ³
Sun et al. 2001	Rabbit	Standard or nicotine-free research cigarettes	6 hours/day, 5 days/week for 10 weeks	CO: Particulates:	45–54 ppm 24–35 mg/m ³

* $\mu\text{g}/\text{m}^3$ = Micrograms per cubic meter.

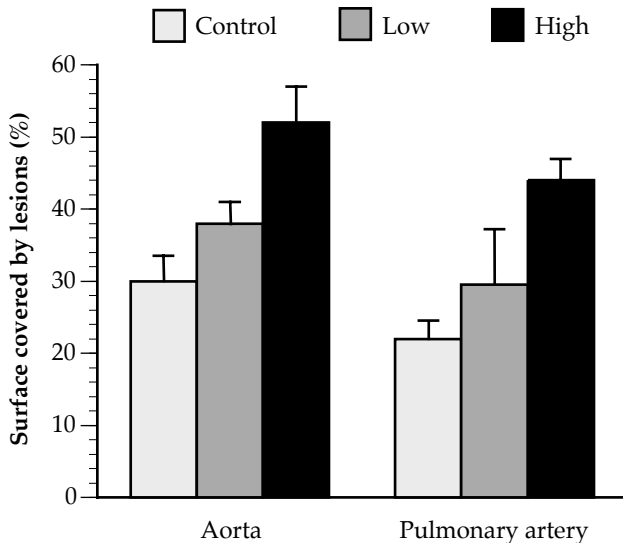
[†]CO = Carbon monoxide.

[‡]ppm = Parts per million.

[§]mg = Milligram.

[¶]LDL = Low-density lipoprotein.

End point	Findings
Number and size of plaques in aortic segments	<ul style="list-style-type: none"> • Exposure had no effect on the number of plaques • Plaques in exposed animals were significantly larger (median size about 1.5 times larger in each aortic segment) than in unexposed animals
Area of atherosclerotic lesions by planimetry in aorta and pulmonary artery; bleeding time (to measure platelet activity)	<ul style="list-style-type: none"> • High-exposure secondhand smoke group had dose-dependent lipid deposits with lesion size about 1.7 times larger than those in the low-exposure group • Low-exposure group was between the high-exposure and control groups • Bleeding times were shorter in rabbits that breathed secondhand smoke • No differences between high-dose and low-dose exposures for serum triglycerides, cholesterol, and high-lipoprotein cholesterol
Number and size of plaques in aortic segments	<ul style="list-style-type: none"> • Exposure had no effect on the number of plaques • Plaques in exposed animals were significantly larger (median size about 1.5 times larger in each aortic segment) than those in unexposed animals
Area of atherosclerotic lesions by planimetry in aorta and pulmonary artery; bleeding time (to measure platelet activity)	<ul style="list-style-type: none"> • Secondhand smoke exposure was associated with greater lipid deposits and shorter bleeding times • Metoprolol did not block these effects, indicating that they are not mediated by increased circulating catecholamines
Uptake of LDL ^A cholesterol in isolated perfused carotid artery	<ul style="list-style-type: none"> • Rate of LDL uptake more than quadrupled
Area of atherosclerotic lesions at several places in aorta measured by planimetry; cholesterol content of aortic segments	<ul style="list-style-type: none"> • Increasing lesion size and cholesterol content over time in both groups • Secondhand smoke-exposed mice had approximately twice the level of atherosclerosis as controls at any given time
Area of atherosclerotic lesions by planimetry in aorta and pulmonary artery	<ul style="list-style-type: none"> • Secondhand smoke increased the area of arteries with lipid deposits by about 50% • There was no significant difference between nicotine and nicotine-free cigarette smoke

Figure 2.7 Secondhand smoke exposure and lipid deposits in rabbits

Note: Exposure to secondhand smoke increased lipid deposits in arteries of rabbits in a dose-dependent manner. Bars are for controls (clear air), and low doses and high doses of secondhand smoke exposures. Error bars represent standard error of the mean.

Source: Zhu et al. 1993b. Reprinted with permission.

or on other hemodynamic measures of cardiac function that are commonly affected by nicotine in active and involuntary smokers (Benowitz 1991). After an ischemic episode from ligation of the left anterior descending coronary artery for 15 minutes, the regional shortening during reperfusion was reduced by 50 percent of the pre-ischemic values. When the dog was exposed to nicotine from just a single cigarette, the regional shortening during reperfusion was reduced by 25 percent of control values. When the dog was given a free radical scavenger along with the nicotine, this effect was obliterated. Thus, exposure to a very low dose of nicotine doubled the impact of the reperfusion injury on the myocardium.

The effects of free radicals induced by secondhand smoke have been explored at the cellular level (van Jaarsveld et al. 1992a,b). Rats exposed to secondhand smoke from two cigarettes a day for two months exhibited severely damaged mitochondrial function during reperfusion injury. Thus, the ability of cardiac mitochondrial cells to convert oxygen into ATP

was more compromised during reperfusion injury among rats exposed to these low doses than among control rats.

Secondhand smoke exposure is associated with lower levels of antioxidant vitamins in nonsmoking women (Farchi et al. 2001). Despite a similar dietary intake of beta-carotene, retinol, L-ascorbic acid, and alpha-tocopherol, women whose husbands smoked exhibited a dose-dependent relationship between the extent of exposure and plasma concentrations of beta-carotene and L-ascorbic acid. These associations persisted even after controlling for daily beta-carotene and vitamin C intake and for other potential confounders (vitamin supplementation, alcohol consumption, and body mass index). A similar dose-response relationship was observed when urinary cotinine was used as the measure of exposure.

In a mouse model, a 30-minute exposure to secondhand smoke also produced evidence of oxidative DNA damage in the myocardium assessed by increased levels of 8-OH-dG (Howard et al. 1998a). There are also parallel human data. In a cross-sectional study, persons exposed to secondhand smoke at work exhibited increased levels of 8-OH-dG (Howard et al. 1998b). The plasma cotinine levels were 65 percent higher in the exposed group compared with controls, and increases in 8-OH-dG levels were similar. In workers exposed to secondhand smoke, 8-OH-dG levels fell after 60 days of antioxidant supplementation (Howard et al. 1998c).

There is also evidence that smokers are less sensitive to free radical damage from cigarette smoke than nonsmokers are because of changes in the levels of enzymes that control free radicals (McCusker and Hoidal 1990). When hamsters were exposed to secondhand smoke from six cigarettes a day for eight weeks, the activity of antioxidant enzymes in their lungs nearly doubled. Similar changes found in the lungs of smokers compared with nonsmokers provide further evidence that secondhand cigarette smoke may affect smokers and nonsmokers differently. Chronic exposures to cigarette smoke appear to increase the capacity of free radical scavenging systems in smokers.

In addition, human exposures to secondhand smoke sensitize lung neutrophils (Anderson et al. 1991). As with platelets, neutrophils are an important element of the body's defenses against infection and damage. Inappropriately activated neutrophils, however, release oxidants that can play a role in tissue damage. In a group of nonsmokers exposed to three hours of sidestream smoke at relatively high levels

(respirable particles $>2,000$ micrograms/m³), there were significant increases in circulating leukocyte counts, in stimulated neutrophil migration, and in the release of reactive oxidants by neutrophils.

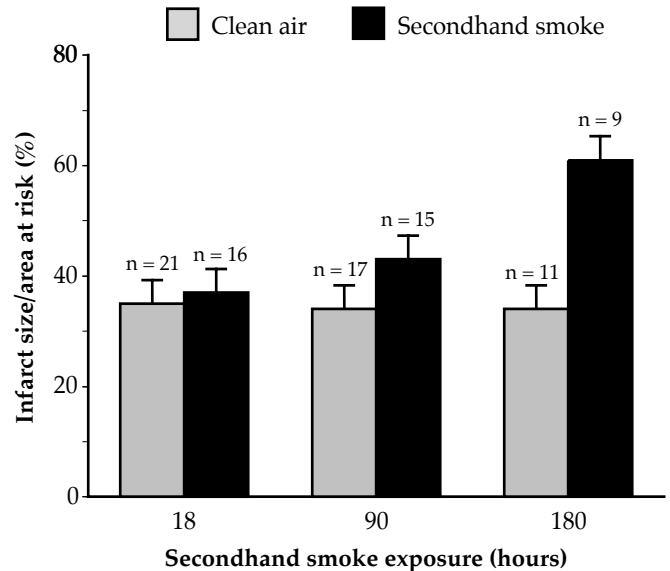
Myocardial Infarction

Several of the effects discussed above would lead to the expectation that exposure to secondhand smoke would increase the severity of MIs. Direct animal data show that secondhand smoke increases tissue damage following a MI. Dogs exposed to secondhand smoke for one hour daily for 10 days and then subjected to a coronary artery blockage developed MIs that were twice as large as those found in controls breathing clean air (Prentice et al. 1989). This effect was not due to elevated circulating levels of nicotine or carboxy-hemoglobin, because the infarcts were created the day after the last day of secondhand smoke exposure. Zhu and colleagues (1994) conducted an experiment in rats to investigate the effects of secondhand smoke exposure on infarct size. Rats were exposed to secondhand smoke six hours a day for three days, three weeks, or six weeks, and then subjected to a left coronary artery occlusion for 35 minutes followed by reperfusion. There was a dose-dependent increase in infarct size, with the longest exposure of 180 hours yielding infarcts nearly twice as large as in the control group that breathed clean air (Figure 2.8). This effect could be countered by feeding the animals L-arginine (Zhu et al. 1996). This finding suggests that the effect of secondhand smoke in producing an MI comes from interference with the vascular endothelium. There is no evidence indicating a threshold level of exposure that is needed to produce this effect.

Heart Rate Variability

Alterations in heart rates are caused by the opposing effects of the sympathetic and parasympathetic nervous systems on the sino-atrial node (the pacemaker of the heart) through the elevation of catecholamines. The sympathetic nervous system tends to oppose the rate-slowness effects of the parasympathetic (vagus) nervous system, and sympathetic activation reduces heart rate variability. If sympathetic tone is reduced and vagal activity enhanced, heart rate variability increases. Clinically, decreased heart rate variability predicts a higher risk of cardiac death or arrhythmic events after an acute MI, presumably reflecting the adverse effects of increased sympathetic tone (Kleiger et al. 1987; Singh et al. 1996).

Figure 2.8 Secondhand smoke exposure and infarct size in rats



Note: Exposure to secondhand smoke increased infarct size in rats subjected to a 35-minute occlusion of the left coronary artery in a dose-dependent manner. There is no evidence of a threshold effect.

Source: Zhu et al. 1994. Adapted with permission.

Activation of the sympathetic nervous system would tend to reduce heart rate variability. One experimental study has tested this hypothesis. Pope and colleagues (2001) measured heart rate variability in healthy young adults for two hours in the smoke-free areas of a U.S. airport, followed by two hours in the smoking area, and then repeated this protocol. When the experimental participants were in the smoking area, heart rate variability was 12 percent lower. The levels of secondhand smoke were not high enough to affect mean heart rate or blood pressure, but the secondhand smoke exposure was associated with altered cardiac autonomic function in a direction consistent with an increased risk of a cardiac event.

Summary

A source of uncertainty in interpreting evidence on secondhand smoke exposure and heart disease has been the apparently large size of the effect compared with active smoking. Active smoking delivers doses

of the toxins in secondhand smoke that are markedly greater than the doses received by a nonsmoker, and active smoking approximately doubles, depending on the amount smoked, the risk of heart disease (USDHHS 1983). Thus, the effect of secondhand smoke may appear large for the associated doses of cigarette smoke components, particularly since secondhand smoke exposure generally does not produce changes in systemic physiologic measures such as heart rate or blood pressure (Celermajer et al. 1996; Hausberg et al. 1997; Sumida et al. 1998; Otsuka et al. 2001). However, findings of a wide variety of clinical and experimental studies of various designs demonstrate that the effects of secondhand smoke on the cardiovascular system occur at low doses in nonsmokers, with some of the effects (on platelets and vascular function) similar to those in active smokers. For this reason, it is not appropriate to scale from the effects of active smoking in a linear, dose-dependent approach to estimate the effects of exposure to secondhand smoke based on comparative doses of smoke components (Howard and Thun 1999).

Secondhand smoke interferes with the normal functioning of the heart, blood, and vascular systems in ways that increase the risk of a cardiac event. For some of these effects (changes in platelet and vascular function), the immediate effects of even short exposures to secondhand smoke appear to be as large as

those seen in association with active smoking of one pack of cigarettes a day. Some evidence indicates lower levels of circulating antioxidants associated with secondhand smoke exposure. The experimental and observational evidence reviewed in this chapter supports the plausibility of the findings of the epidemiologic studies reviewed in Chapter 8 (Cardiovascular Diseases from Exposure to Secondhand Smoke). The large body of evidence documenting that secondhand smoke produces substantial and rapid effects on the cardiovascular system demonstrates that even a brief exposure to secondhand smoke has adverse consequences for the heart, blood, and blood vessels (Glantz and Parmley 2001; Barnoya and Glantz 2005).

Conclusions

1. The evidence is sufficient to infer that exposure to secondhand smoke has a prothrombotic effect.
2. The evidence is sufficient to infer that exposure to secondhand smoke causes endothelial cell dysfunctions.
3. The evidence is sufficient to infer that exposure to secondhand smoke causes atherosclerosis in animal models.

Evidence Synthesis

This chapter reviews the substantial amount of data from cellular, animal, and human studies supporting the overall conclusion that exposure to secondhand smoke causes a broad range of adverse effects in both children and adult nonsmokers. These data provide a strong foundation for the biologic plausibility of causal conclusions related to specific diseases and other adverse health effects that are reviewed in Chapters 5 through 9. This chapter provides substantial additional evidence on the underlying pathogenic mechanisms for major adverse health outcomes associated with exposure to secondhand smoke.

Secondhand smoke is a complex mixture of thousands of chemicals emitted from burning tobacco. The toxicologic profiles of a large number of these specific chemicals and compounds are well

established (<http://www.atsdr.cdc.gov/toxpro2.html>). This chemical mixture includes more than 50 carcinogens, and both IARC (2004) and the National Toxicology Program (USDHHS 2000) have classified this mixture as a known human carcinogen. Researchers have thus concluded that exposure to secondhand smoke can cause DNA damage and genetic mutations. For DNA-damaging carcinogens, the occurrence of permanent mutations implies that there is no level of exposure that does not pose a risk.

The complex mixture of chemicals in secondhand smoke also contains a large number of toxicants harmful to the respiratory and cardiovascular systems. Evidence from both animal and human studies indicates that exposures to secondhand smoke can produce substantial and rapid adverse effects on the

functioning of the heart, blood, and vascular systems in ways that increase the risk of a cardiac event. Furthermore, many of these acute and chronic changes in blood and vascular function appear to be as large as those seen in active smokers. The immediate effects in some measures of blood and vascular functioning among nonsmokers from even brief exposures (i.e., 30 minutes or less) to secondhand smoke are comparable in magnitude to the effects observed in active smokers. Thus, the evidence reviewed in this chapter supports the biologic plausibility of adverse cardiovascular health outcomes that are associated with exposure to secondhand smoke, which are reviewed in Chapter 8.

As the portal of entry for secondhand smoke, the respiratory system is the initial site of deposition for the particulate and gaseous compounds found in secondhand smoke. This chapter identifies the multiple mechanisms by which secondhand smoke exposure can induce both acute and chronic adverse health effects within the respiratory tract that affect infants, children, and adults. The evidence for underlying mechanisms of respiratory injury from exposure to secondhand smoke suggests that a safe level of

exposure may not exist, thus implying that any exposure carries some risk. For infants, children, and adults with asthma or with more sensitive respiratory systems, even very brief exposures to secondhand smoke can trigger intense bronchopulmonary responses that could be life threatening in the most susceptible individuals.

Animal and human studies indicate that prenatal and postnatal exposure to nicotine and other toxicants in tobacco smoke may affect the neuroregulation of breathing, apneic spells, and sudden infant death. Experimental data on the neurotoxicity of prenatal and neonatal exposure to nicotine and secondhand smoke in animal models can be related to several potential causal mechanisms for SIDS, including adverse effects on brain cell development, synaptic development and function, and neurobehavioral activity. Finally, studies have documented that exposure to tobacco smoke from active smoking has a broad effect on immune function and host defenses against infectious agents. Evidence indicates that exposure to secondhand smoke appears to also impair immune function in both children and adult nonsmokers, which increases susceptibility to infection.

Conclusions

Evidence of Carcinogenic Effects from Secondhand Smoke Exposure

1. More than 50 carcinogens have been identified in sidestream and secondhand smoke.
2. The evidence is sufficient to infer a causal relationship between exposure to secondhand smoke and its condensates and tumors in laboratory animals.
3. The evidence is sufficient to infer that exposure of nonsmokers to secondhand smoke causes a significant increase in urinary levels of metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). The presence of these metabolites links exposure to secondhand smoke with an increased risk for lung cancer.

4. The mechanisms by which secondhand smoke causes lung cancer are probably similar to those observed in smokers. The overall risk of secondhand smoke exposure, compared with active smoking, is diminished by a substantially lower carcinogenic dose.

Mechanisms of Respiratory Tract Injury and Disease Caused by Secondhand Smoke Exposure

5. The evidence indicates multiple mechanisms by which secondhand smoke exposure causes injury to the respiratory tract.
6. The evidence indicates mechanisms by which secondhand smoke exposure could increase the risk for sudden infant death syndrome.

Mechanisms of Secondhand Smoke Exposure and Heart Disease

7. The evidence is sufficient to infer that exposure to secondhand smoke has a prothrombotic effect.

8. The evidence is sufficient to infer that exposure to secondhand smoke causes endothelial cell dysfunctions.

9. The evidence is sufficient to infer that exposure to secondhand smoke causes atherosclerosis in animal models.

Overall Implications

The biologic mechanisms reviewed in this chapter underlie a wide range of acute and chronic adverse health effects in infants, children, and adults examined in Chapters 5 through 9. This broadly reaching body

of evidence on the toxicology of secondhand smoke and on these biologic mechanisms indicates that any exposure to secondhand smoke will increase risk for adverse health outcomes.

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