4. Other Data Relevant to an Evaluation of Carcinogenicity and its Mechanisms

The Working Group attempted to provide comprehensive coverage of the published literature on other data relevant to the evaluation of the carcinogenic hazards of secondhand smoke (since 1985), in some cases referring to recent reviews.

4.1 Absorption, distribution, metabolism and excretion

For a description of the absorption, distribution, metabolism and excretion of components of tobacco smoke, the reader is referred to the monograph on tobacco smoke.

4.1.1 *Humans*

(*a*) *Enzyme activities and metabolism*

In a study of human placental monooxygenase activity, as measured by in-vitro oxidation of 7-ethoxyresorufin, *O*-deethylase activity was significantly inhibited ($p < 0.05$) by 7,8-benzoflavone with placental microsomes from women passively exposed to cigarette smoke, but not with those from women who had not been exposed (Manchester & Jacoby, 1981).

A pharmacokinetic study reported a significantly faster clearance of theophylline in a group of seven nonsmokers exposed to secondhand tobacco smoke, as determined by questionnaire data and cotinine levels, than in a matched group of non-exposed individuals, as determined by clearance rate, terminal elimination half-time and mean residence time ($T_{½}$ = 6.93 h versus 8.69 h, $p < 0.05$) (Matsunga *et al*., 1989). Conversely, no changes in theophylline clearance rate were observed in five male subjects who were heavily exposed to secondhand tobacco smoke for 3 h/day on 5 consecutive days under controlled conditions (Casto *et al*., 1990).

(*b*) *Tobacco smoke carcinogen biomarkers*

(i) *Urinary compounds*

The use of urinary compounds as biomarkers of carcinogen uptake from environmental tobacco smoke was reviewed by Scherer and Richter (1997).

4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides (NNAL-Gluc) are metabolites of the tobacco-specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). The use of the assay for NNAL and NNAL-Gluc in urine for investigations of exposure to secondhand tobacco smoke offers several advantages. Firstly, it has the sensitivity required to measure relatively low concentrations (typically about 0.05 pmol/mL urine). Secondly, because NNK is a tobacco-specific compound, the detection of NNAL and NNAL-Gluc in urine specifically signals exposure to tobacco smoke. All studies reported to date have found significantly higher concentrations of NNAL plus NNAL-Gluc, or NNAL-Gluc alone, in the urine of nonsmokers exposed to secondhand tobacco smoke than in the urine of unexposed controls, and a good correlation between urinary levels of cotinine and NNAL plus NNAL-Gluc (Table 4.1). In one study, the uptake of NNK was more than six times higher in women who lived with smokers than in women who lived with nonsmokers (Anderson *et al*., 2001). In another investigation, widespread uptake of NNK was demonstrated in a group of economically disadvantaged school-children heavily exposed to tobacco smoke at home (Hecht *et al*., 2001). Correlations have been consistently observed between levels of urinary cotinine and NNAL + NNAL-Gluc in people exposed to secondhand tobacco smoke (Hecht, 2002). Because NNAL is a metabolite of the lung carcinogen NNK, these data imply that there is elevated carcinogen uptake in subjects with raised concentrations of urinary cotinine.

Mixed results have been obtained in studies on the relationship between *tt*-muconic acid, a metabolite of benzene, and exposure to secondhand tobacco smoke. Some studies have shown significantly increased concentrations in people exposed to secondhand tobacco smoke (Yu & Weisel, 1996; Taniguchi *et al.*, 1999; Carrer *et al*., 2000) whereas others found no effect (Scherer *et al*., 1995; Weaver *et al*., 1996; Ruppert *et al*., 1997; Scherer *et al*., 1999), the levels being primarily dependent on whether the subject's home is in the city or the suburbs and on dietary intake of sorbic acid rather than on exposure to secondhand tobacco smoke. The levels of 1-hydroxypyrene and hydroxyphenanthrenes in urine are generally not increased by exposure to secondhand tobacco smoke (Hoepfner *et al*., 1987; Scherer *et al*., 1992; Scherer *et al*., 2000), although significant increases have been reported under some high exposure conditions (Van Rooij *et al*., 1994; Siwinska *et al*., 1999).

The concentrations of aromatic amines (Grimmer *et al*., 2000) and 8-hydroxy-2′-deoxyguanosine (8-OHdG) (Pilger *et al*., 2001) in urine were also unaffected by exposure to secondhand tobacco smoke. The concentration of urinary 5-(hydroxymethyl)uracil was significantly elevated in nonsmokers exposed to high levels of secondhand tobacco smoke, in a dose-dependent manner (Bianchini *et al*., 1998). Exposure to secondhand tobacco smoke did not affect urinary concentrations of 3-ethyladenine (Kopplin *et al*., 1995). Elevated concentrations of thioethers, in particular of 3-hydroxypropylmercapturic acid, were observed under controlled, high exposure conditions (Scherer *et al*., 1990, 1992), but not in a field study (Scherer *et al.*, 1996).

Lackmann *et al.* (1999) first reported the presence of NNAL and NNAL-Gluc in the urine of newborns of women who smoked (Table 4.1). The available data indicate that

Table 4.1. Urinary NNAL and its glucuronides (NNAL-Gluc): biomarkers of NNK uptake in studies of involuntary exposure to tobacco smoke

Study group	Main conclusions[a]	Reference
Exposure of adults to secondhand smoke		
5 men exposed to secondhand smoke	Significantly increased levels of NNAL + NNAL-Gluc after exposure in a chamber: 127 ± 74 pmol/day (approx. 0.16 ± 74 pmol/mL urine)	Hecht *et al*. (1993)
5 men, 4 women exposed to secondhand smoke 5 unexposed controls	Significantly increased levels of NNAL-Gluc in exposed workers compared to unexposed controls: 0.059 ± 0.028 pmol/mL urine	Parsons *et al*. (1998)
29 nonsmokers (13 women)	NNAL + NNAL-Gluc levels correlated with nicotine levels from personal samplers. NNAL, 20.3 ± 21.8 pmol/day; NNAL-Gluc, 22.9 ± 28.6 pmol/day in exposed nonsmokers	Meger *et al*. (2000)
45 nonsmoking women, 23 exposed to secondhand smoke in the home, 22 non-exposed	NNAL + NNAL-Gluc significantly higher in exposed women: 0.050 ± 0.068 pmol/mL urine	Anderson *et al*. (2001)
204 nonsmoking elementary school-aged children	34% with total cotinine ≥ 5 ng/mL; 52/54 of these samples had detectable NNAL or NNAL-Gluc, 93-fold range. NNAL + NNAL-Gluc, 0.056 ± 0.076 pmol/mL urine	Hecht *et al*. (2001)
In-utero exposure to mother's smoking		
31 newborns of mothers who smoked; 17 newborns of mothers who did not smoke	NNAL-Gluc detected in 71%, NNAL in 13% of urine samples of newborns of smokers; neither detected in urine of newborns of nonsmokers ($p < 0.001$); NNAL + NNAL-Gluc in urine of newborns of smoking mothers, 0.13 ± 0.15 pmol/mL urine	Lackmann *et al*. (1999)
21 smokers and 30 nonsmokers	NNAL detected in amniotic fluid of 52.4% of smokers and 6.7% of nonsmokers ($p = 0.0006$). NNAL concentration in amniotic fluid of smokers, 0.025 ± 0.029 pmol/mL	Milunsky *et al*. (2000)
12 smokers and 10 nonsmokers	NNAL and NNAL-Gluc not detected in follicular fluid	Matthews *et al*. (2002)

[a] Values represent mean ± SD.

NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone

NNK, a transplacental carcinogen, is taken up by the fetus and metabolized to NNAL and NNAL-Gluc by fetal enzymes (Lackmann *et al*., 1999). Consistent with these results, NNAL was detected in the amniotic fluid of pregnant smokers (Milunksy *et al*., 2000). However, neither NNAL nor NNAL-Gluc could be detected in follicular fluid (Matthews *et al*., 2002).

Urinary excretion of 8-OHdG by newborn babies (n = 12) whose mothers were exposed to secondhand tobacco smoke was significantly higher (p = 0.047) than that of babies whose mothers were not exposed (n = 8). In both groups, the concentration of 8-OHdG excreted was also significantly higher for babies whose mothers had the *GSTM1* null genotype (Hong *et al*., 2001).

(ii) *Protein adducts*

To determine whether involuntary smoking increased the levels of aromatic amine-haemoglobin adducts, a group of 14 volunteers who reported negligible exposure to secondhand tobacco smoke was compared with a group of 15 nonsmokers who reported exposure to at least one pack of cigarettes per day smoked by others, and one of 15 non-smokers with unknown levels of exposure to secondhand tobacco smoke. No measurable quantities of cotinine were detected in the blood of members of any of the three groups (see Section 4.1.1.(d) for measurement of cotinine). A further group of 13 nonsmokers, including six bartenders who were heavily exposed to secondhand tobacco smoke, had measurable levels of cotinine in their blood. Background levels of adducts from 4-amino-biphenyl (ABP) and 3-aminobiphenyl were detected in all subjects, but higher levels were found in subjects with detectable cotinine levels (p = 0.05 and 0.027, respectively) (Maclure *et al.*, 1989). In another study, the levels of 4-ABP-haemoglobin adducts in 15 nonsmokers who reported being exposed to secondhand tobacco smoke were not significantly higher than those in 35 nonsmokers who were not exposed to secondhand tobacco smoke (87.9 ± 19 [SE] pg/g haemoglobin and 69.5 ± 7 pg/g, respectively). Only four out of 15 of those subjects who reported exposure to secondhand tobacco smoke and four out of 35 of those who reported no exposure had measurable levels of urinary cotinine (Bartsch *et al.*, 1990). The level of exposure of 40 nonsmoking women to secondhand tobacco smoke was determined by questionnaire, use of a diary and a personal air monitor and was stratified by average nicotine concentration. There was a significant correlation between the concentration of 4-ABP–haemoglobin and exposure category (p = 0.009) (Hammond *et al.*, 1993).

Exposure of pre-school children to secondhand tobacco smoke from their mothers was investigated by measuring plasma cotinine levels and PAH–albumin adducts in peripheral blood, the latter detected by ELISA. The study involved 87 mother–child pairs; 31 mothers smoked and 56 did not. Not only did the mothers who smoked have higher levels of adducts (see monograph on tobacco smoke), but the levels in their children were also significantly higher ($p < 0.05$). There was also a significant correlation between adduct levels in the mothers and in their children (p = 0.014) (Crawford *et al.*, 1994). In a subsequent study, PAH–albumin and 4-ABP–haemoglobin adducts were also found to be

significantly higher in children whose mothers smoked or who lived with other smokers ($p < 0.05$) (Tang *et al.*, 1999). In a study in children from three different-sized cities, levels of 4-ABP adducts and other aromatic amines correlated with the size of the city. Exposure to secondhand tobacco smoke was associated with a non-significant increase in levels of 4-ABP adducts and a significant decrease in adducts of *ortho*- and *m*-toluidine ($p < 0.05$) (Richter *et al*., 2001).

In a study of 69 adults, 27 were smokers, and 19 of the 42 nonsmokers were classified as passive smokers as determined by self-report and cotinine levels. The levels of benzo-[*a*]pyrene adducts with albumin and haemoglobin were similar for nonsmokers and passive smokers (Scherer *et al.*, 2000).

The levels of *N*-hydroxyethylvaline in haemoglobin were reported to be similar in nonsmokers who did not live or work with a smoker ($n = 74$) and in those who did ($n = 28$). Cotinine levels in passive smokers were not higher than in nonsmokers (Bono *et al.*, 1999).

There was a significantly higher level ($p = 0.02$) of nitrated proteins in blood plasma of nonsmokers who were exposed to secondhand tobacco smoke ($n = 30$) than in non-smokers who were unexposed ($n = 23$) (Pignatelli *et al*., 2001).

Measurements of maternal–fetal exchange of 4-ABP in pregnant women who smoked ($n = 14$) and in nonsmoking ($n = 38$) pregnant women (see monograph on tobacco smoke) showed consistently lower levels of haemoglobin adducts in cord blood than in maternal blood, with an average maternal to fetal ratio of 2. A significant correlation was found between maternal and fetal levels for all subjects ($p < 0.001$) and for smokers only ($p = 0.002$), but not for nonsmokers only ($p = 0.06$) (Coghlin *et al.* 1991). Another study also found that adduct levels were lower in fetal blood than in maternal blood and were correlated with the smoking status of the mothers (subjects included 74 smokers and 74 nonsmokers) (Myers *et al.*, 1996). Another study of 73 nonsmoking pregnant women whose cotinine:creatinine ratios correlated with self-reported exposure to secondhand tobacco smoke, found no association with levels of haemoglobin adducts formed by any of nine aromatic amines (Branner *et al*., 1998).

Blood samples from smoking and nonsmoking mothers and cord blood from their newborns were analysed for *N*-hydroxyethylvaline in haemoglobin. The average adduct concentration in newborns of mothers who smoked ($n = 13$; 147 ± 68 [mean ± SD] pmol/g) was significantly higher ($p < 0.01$) than in those from mothers who were nonsmokers ($n = 10$; 42 ± 18 pmol/g). There was also a significant correlation ($p < 0.01$) between adduct levels in the newborns and in their mothers (Tavares *et al.*, 1994). The same samples showed a strong correlation between the concentration of *N*-(2-cyanoethyl)valine (CEVal) adducts in the mothers who smoked and in their newborns ($p < 0.001$). The adduct levels in the babies were also strongly correlated with the numbers of cigarettes smoked per day by their mothers ($p = 0.009$). The levels of CEVal in babies of mothers who did not smoke were below the limit of detection of the assay (1 pmol/g) (Tavares *et al.*, 1996).

(iii) *DNA adducts*

Although many studies have investigated the levels of DNA adducts or other measures of DNA damage in the tissues of smokers, ex-smokers and nonsmokers (see monograph on tobacco smoke), relatively few studies have investigated the use of these biomarkers to monitor the exposure of nonsmokers to secondhand tobacco smoke, probably because they may not distinguish the effects of exposure to secondhand tobacco smoke from those of exposure to other sources of environmental carcinogens.

In a study in which declining DNA adduct levels in the white blood cells of smokers enrolled in a smoking cessation programme were measured by ELISA, the levels of PAH–DNA adducts both at baseline and 10 weeks after cessation were significantly associated with number of hours of exposure to secondhand tobacco smoke at home ($p = 0.009$ and $p = 0.02$, respectively) and were also higher if the subject lived with another smoker ($p = 0.02$). However, there was no observable influence of exposure to secondhand tobacco smoke at the workplace (Mooney *et al.*, 1995).

Using the prevalence of serum antibodies to benzo[*a*]pyrene diol epoxide (BPDE)–DNA adducts as a biomarker of exposure to environmental PAHs in an Italian population, no association between the percentage of subjects with DNA adducts and passive smoking was found (Petruzzelli *et al.*, 1998). In a study in which significant differences were observed between the levels of BPDE–DNA adducts in the peripheral lymphocytes of smokers ($n = 40$) and nonsmokers ($n = 35$), as determined by a flow cytometric method using BPDE–DNA antibodies, the mean value for nonsmokers with no or low exposure to secondhand tobacco smoke ($n = 17$) was marginally *higher* than that of exposed nonsmokers ($n = 18$), but the difference was not statistically significant (Shinozaki *et al.*, 1999). Using antibodies to BPDE–DNA adducts, immunohistochemical staining of ovarian granulosa-lutein cells from women undergoing in-vitro fertilization showed a strong correlation between smoking status and adduct levels in nonsmokers ($n = 11$), passive smokers ($n = 7$) and active smokers ($n = 14$); all pairwise comparisons were highly significant ($p < 0.0001$) (Zenzes *et al.*, 1998).

Oxidative damage caused by exposure to secondhand tobacco smoke was assessed by measuring the concentration of 8-OHdG in the blood of 74 nonsmokers. The levels were, on average, 63% higher in the subjects exposed to secondhand tobacco smoke in the workplace ($n = 27$) than in the unexposed group ($n = 29$) and this difference was statistically significant ($p < 0.05$) (Howard *et al*., 1998a). However, in another study, the levels of 8-OHdG in leukocytes were significantly *lower* in smokers than in nonsmokers (see monograph on tobacco smoke) and no association was observed with exposure to secondhand tobacco smoke (van Zeeland *et al*., 1999).

Five nonsmokers were exposed to secondhand tobacco smoke under controlled conditions (exposure to gas phase only for 8 h, followed by exposure to whole secondhand tobacco smoke for 8 h, 40 h later). When their monocyte DNA was analysed by ^{32}P-post-labelling, no changes in the adduct patterns were seen after either exposure period, when compared with the samples obtained before exposure (Holz *et al.*, 1990). In a study of biomarkers of exposure to air pollution in three Greek populations, one urban, one rural and

one on a university campus, ^{32}P-postlabelling analysis of lymphocyte DNA revealed the presence of DNA adducts that significantly ($p < 0.001$) paralleled the level of exposure to secondhand tobacco smoke, as determined by self-report, plasma cotinine concentrations and profiles of personal exposure to PAHs that were characteristic of secondhand tobacco smoke, rather than other environmental sources (Georgiadis *et al.*, 2001).

DNA adducts of PAHs were detected by ELISA in 6/14 placentas and in 5/12 matched fetal lung samples from spontaneous abortions. None of the samples were from women who reported smoking during pregnancy, suggesting that the adducts are due to some other source of hydrocarbon exposure (Hatch *et al.*, 1990). In another study using ELISA, BPDE–DNA adducts were detected in 13/15 placental samples from smokers and 3/10 from nonsmokers. There was a strong correlation between concentrations of both adducts and urinary cotinine for both placental DNA and umbilical cord DNA, the former tissue having the higher adduct levels (transfer coefficient = 0.37–0.74) (Arnould *et al.*, 1997). When placental DNA was analysed both for bulky DNA adducts by ^{32}P-postlabelling and for 8-OHdG by electrochemical detection, neither method showed a difference between 11 smokers, ten nonsmokers and nine nonsmokers exposed to passive smoking (Daube *et al.*, 1997). Using ^{32}P-postlabelling with nuclease P1 digestion, DNA adducts were detected in placental and umbilical cord DNA regardless of the smoking status of the mothers and were significantly higher in maternal tissue than in fetal tissue (maternal/fetal ratio = 2.0). When considered separately, tissues showed only marginally increased adduct levels in smokers, but total DNA adduct levels in all tissues combined were significantly higher in smokers ($n = 8$) than in nonsmokers ($n = 11$) (Hansen *et al.*, 1992, 1993).

(*c*) *Other biomarkers*

(i) *Breath compounds*

Carbon monoxide

Carbon monoxide (CO) in expired air has been reported to be an indirect measure of passive smoking both in adults and children. Given its very short half-life, CO concentration must be measured shortly after exposure.

After a 9-h period of exposure to secondhand tobacco smoke at the workplace, 100 nonsmoking waiters exhaled on average 5.0 ppm CO (the pre-exposure CO concentration was 2.0 ppm) compared to a concentration of 2.5 ppm CO exhaled by 100 medical students who spent the day in a nonsmoking environment ($p < 0.001$; Laranjeira *et al.*, 2000). A study in Japan found that mean concentrations of exhaled CO in passive smokers were also significantly higher ($p < 0.001$) than those of nonsmokers who were not exposed to secondhand tobacco smoke (Taniguchi *et al.*, 1999).

The concentrations of exhaled CO were also measured in 235 healthy and 54 asthmatic children (Ece *et al.*, 2000). Regardless of the parents' smoking habits, CO concentrations were higher in asthmatic than in healthy children (1.32 ppm versus 0.86 ppm; $p = 0.028$). Significant relationships were found between the number of cigarettes smoked

in the house and concentrations of exhaled CO in both healthy ($p = 0.003$) and asthmatic children ($p = 0.01$).

Both women exposed to secondhand smoke during pregnancy and their newborns exhaled higher concentrations of CO than non-exposed women (1.95 ppm versus 1.33 ppm and 2.51 versus 1.74 ppm, respectively) (Seidman *et al*., 1999).

Nitric oxide

Fifteen nonsmoking subjects were exposed to secondhand tobacco smoke or asked to smoke for 60 min in a ventilated chamber. Within 15 min of exposure, the concentrations of exhaled nitric oxide (NO) had fallen by 23.6% or 30.3%, respectively, and remained low during the entire time of exposure (Yates *et al*., 2001). Similarly, newborns exposed prenatally to cigarette smoke ($n = 7$) exhaled significantly less NO than non-exposed ($n = 13$) newborns (Hall *et al*., 2002).

Benzene

The concentrations of exhaled benzene in children exposed to secondhand tobacco smoke were not significantly related to the smoking status of the household members, but depended primarily on the location of the home (Scherer *et al*., 1999). Passive smokers not exposed at home, but exposed for more than 50% of their time at work had significantly higher levels of benzene, ethylbenzene, *meta*-xylene + *para*-xylene and *ortho*-xylene in their breath than did non-exposed nonsmokers (Wallace *et al*., 1987) .

(ii) *Blood compounds*

Carboxyhaemoglobin

Measurements of the concentration of carboxyhaemaglobin in subjects exposed to secondhand tobacco smoke and in subjects who were not, are consistent with measurements of CO in the environment (Russell *et al.*, 1973; Hugod *et al.*, 1978; Jarvis *et al.*, 1983). However, carboxyhaemoglobin measurements may be largely confounded by endogenous formation and environmental factors, and are thus not a reliable means for monitoring passive smoking (Scherer & Richter, 1997).

Thiocyanate

It is not possible to distinguish between nonsmokers who are exposed to secondhand smoke and those who are not by measuring serum thiocyanate concentrations (Robertson *et al*., 1987; Scherer & Richter, 1997).

(iii) *Particles*

Experimental deposition of particulate matter with a diameter of < 1 μm from secondhand tobacco smoke within the human respiratory tract was evaluated in 15 nonsmokers and three regular smokers (Morawska *et al*., 1999). On average, smokers had a higher rate of deposition than nonsmokers (65.3 ± 24.1% versus 56.0 ± 15.9% for nose breathing and 66.1 ± 17.6 % versus 48.7 ± 11.6% for mouth breathing). The large variations observed

between individuals indicate that deposition of environmental tobacco smoke is governed by an individual's airway anatomy and breathing patterns.

(*d*) *Nicotine and its metabolites as biomarkers*

Many of the biological markers other than nicotine or cotinine that are used as indicators of exposure and uptake in smokers (e.g. carboxyhaemoglobin and thiocyanate; see monograph on tobacco smoke) are not suitable for accurate measurement of exposure to secondhand tobacco smoke because of potential confounding exposure from diet and environment (US National Research Council, 1986; US Environmental Protection Agency, 1992; California Environmental Protection Agency, 1997; Benowitz, 1999).

In addition to nicotine from use of tobacco products, pharmaceutical products for nicotine replacement therapy or exposure from secondhand tobacco smoke, small quantities of nicotine may enter the body from dietary sources, mainly from consumption of tea and some solanaceous plants such as aubergine, potato peel and tomato (Castro & Monji, 1986; Sheen, 1988; Davis *et al*., 1991; Domino *et al*., 1993; reviewed in Leyden *et al*., 1999). The contribution from dietary sources has, however, been estimated to be minimal and is generally thought not to influence the concentrations of nicotine or cotinine in body fluids significantly enough to affect their use as a biomarker for exposure to secondhand tobacco smoke (Tunstall-Pedoe *et al*., 1991; Henningfield, 1993; Benowitz, 1996), although there has been some disagreement on the subject (Davis *et al.*, 1991). It has been calculated that even very high consumption of these nicotine-containing products would equal, at most, about 10% of the amount of nicotine generally taken up by nonsmokers exposed to secondhand tobacco smoke (Jarvis, 1994; Repace, 1994; Pirkle *et al*., 1996).

Measurements of nicotine and/or cotinine in body fluids of smokers have demonstrated that nicotine and cotinine are biomarkers of high sensitivity (96–97%) and specificity (99–100%) of exposure to tobacco smoke (Jarvis *et al*., 1987; see monograph on tobacco smoke). Owing to its longer half-life, cotinine measured in the blood, saliva or urine of nonsmokers is presently the most widely used biomarker for assessment of exposure to secondhand tobacco smoke (IARC, 1986; US National Research Council, 1986; US Environmental Protection Agency, 1992).

(i) *Adults*

Numerous studies have investigated the dependence of concentrations of cotinine in the serum, saliva and urine on exposure to secondhand tobacco smoke (concentration and duration of exposure) in experimental conditions as well as in nonsmokers exposed to secondhand tobacco smoke, as reviewed in the reports of IARC (1986), US National Research Council (1986), the US DHHS (1986), the US Environmental Protection Agency (1992) and the California Environmental Protection Agency (1997). Studies involving several thousands of subjects have demonstrated that cotinine concentrations measured in the blood, saliva or urine of nonsmokers exposed to secondhand tobacco smoke at home or at work are significantly higher than the concentrations in non-exposed

nonsmokers (Coultas *et al*., 1987; Cummings *et al*., 1990; Riboli *et al*., 1990; Tunstall-Pedoe *et al.*, 1991; Pirkle *et al*., 1996; Wagenknecht *et al*., 1993; Fontham *et al*., 1994; Jarvis *et al*., 2001). In nonsmokers exposed to secondhand tobacco smoke, cotinine levels are typically 0.6–2% of those detected in smokers (Hackshaw *et al.*, 1997; Jarvis *et al*., 1987, 2001; Etzel *et al*., 1990; Benowitz, 1999; Etter, 2000), and they correlate well with self-reported exposure (Jarvis *et al*., 1985; Haley *et al*., 1989; Coultas *et al*., 1987; Cummings *et al*., 1990; Riboli *et al*., 1990; Jarvis *et al*., 1991, 2001, and also discussed in the reviews of the US Environmental Protection Agency (1992) and the California Environmental Protection Agency (1997)).

Cut-points have been introduced in these studies to distinguish occasional smokers from nonsmokers exposed to secondhand smoke. The cut-off values used in the various studies are typically in the range of 10–30 ng/mL for salivary cotinine, 10–15 ng/mL for cotinine in serum, 20–40 ng/mL for cotinine in plasma, and 50–90 ng/mL for cotinine in urine (reviewed in Etzel, 1990; Pérez-Stable *et al.*, 1992; California Environmental Protection Agency, 1997), but higher values may sometimes be applied (Riboli *et al*., 1995).

The relationships between exposure to secondhand tobacco smoke and cotinine concentrations in body fluids have been investigated. Cummings and co-workers (1990) found a clear association between concentrations of urinary cotinine and the number of reported exposures to secondhand tobacco smoke in the 4 days before sampling in 663 nonsmokers. Another study that investigated almost 200 nonsmokers who were exposed to secondhand tobacco smoke at home or at work showed that concentrations of urinary cotinine increased with increasing duration of exposure (Thompson *et al*., 1990). Every additional 10-h period of exposure was found to result in an increase in urinary cotinine of 44% (95% CI, 23–67%; $p < 0.001$).

In a large multicentre, multinationality study conducted among 1300 nonsmoking women, a clear linear increase in mean concentrations of urinary cotinine was observed from the group of women not exposed to secondhand smoke either at work or at home (mean, 2.7 ng/mg creatinine) to those exposed both at work and at home (mean, 10.0 ng/mg creatinine) (Riboli *et al*., 1990). Cotinine concentrations have been demonstrated to be dependent both on the duration of exposure and the number of cigarettes smoked by others. When the number of cigarettes was corrected for duration of exposure and room volume, it was estimated that a similar increase in concentration of cotinine (5 ng/mg) is predicted with 7.2 cigarettes smoked at home versus 17.9 cigarettes smoked at the workplace. Based on the measured cotinine concentrations, the number of cigarettes smoked by the spouse was found to be the best estimate for domestic exposure, and duration of exposure provided the best estimate for occupational exposure (Riboli *et al*., 1990).

Cotinine concentrations associated with occupational exposure to secondhand tobacco smoke vary somewhat more than those related to domestic exposure. This is because occupational exposure is subject to larger variations in several variables including the number of smokers present, ventilation conditions and variation in physical workload of the nonsmokers. Studies of flight attendants and workers in restaurants, bars, casinos

and other similar public settings have found that these occupations lead to greater exposure to secondhand tobacco smoke than that of the average population, with cotinine concentrations generally reflecting those of tobacco smoke concentrations measured in the workplace (Mattson *et al*., 1989; Jarvis *et al*., 1992; Siegel, 1993; Dimich-Ward *et al*., 1997; Trout *et al*., 1998; Maskarinec *et al*., 2000). One study showed that in addition to exposure in the workplace, exposure of bartenders to secondhand smoke at home further elevated their cotinine concentrations (Maskarinec *et al*., 2000). Jarvis *et al*. (1992) reported a median salivary cotinine concentration of 7.95 ng/mL and a maximum concentration of 31.3 ng/mL among 42 nonsmoking staff working in a bar. Thus, under certain circumstances of exposure, peak values detected in people exposed to secondhand tobacco smoke may exceed the cut-points used to distinguish smokers from nonsmokers in many studies (Pérez-Stable *et al.*, 1992; California Environmental Protection Agency, 1997).

(ii) *Children*

The reports of the US Environmental Protection Agency (1992) and the California Environmental Protection Agency (1997) summarize studies that have reported increased concentrations of cotinine in children exposed to secondhand tobacco smoke at home. Many more recent studies have also found a significant correlation between cotinine concentrations in children and the amount of smoking by the parent(s) (Jarvis *et al.*, 1985; reviewed in Hovell *et al*., 2000a). A cross-sectional survey of secondary-school children conducted in 1998 found that salivary cotinine concentrations were correlated with parental smoking, but that the concentrations had halved since the late 1980s (Jarvis *et al*., 2000). Counselling, during 3 months, of non-employed mothers who smoked and who had children under school age significantly reduced the children's urine cotinine concentration at 12 months (Hovell *et al.*, 2000b).

(iii) *Newborns*

Higher concentrations of cotinine were found in amniotic fluid than in maternal urine in both smokers and nonsmokers (Jordanov, 1990). Studies of isolated perfused human placental cotyledon indicated that less than 1% of nicotine is metabolized to cotinine by the placenta. Rather, after rapid transfer across the placenta, nicotine is metabolized to cotinine by fetal tissues (Pastrakuljic *et al*., 1998; Sastry *et al*., 1998). The elimination kinetics of nicotine, cotinine, trans-3′-hydroxycotinine and their conjugates in the urine of newborns were first reported by Dempsey *et al*. (2000). The results indicated that the half-life of nicotine in newborns was 3–4 times longer than that in adults, whereas the half-life of cotinine was essentially the same in newborns as in adults. The data indicate that newborns are capable of metabolizing nicotine to cotinine and of conjugating nicotine, cotinine and 3′-OH cotinine. However, it is not known what percentage of cotinine is formed by the fetus and what percentage is acquired transplacentally, and which P450 isozymes are involved in fetal metabolism of nicotine (Dempsey *et al*., 2000).

Etzel *et al*. (1985) used a radio-immunoassay to detect cotinine in the 1-day urine of infants born to self-identified smokers and nonsmokers. The median concentration of urinary cotinine for newborns of smokers was 1233 ng/mg creatinine as opposed to 14.5 ng/mg for newborns whose mothers were nonsmokers.

A study of 31 mothers and their newborns was conducted in Bulgaria (Jordanov, 1990). Analysis of 1-day urine by a direct colorimetric method found a mean urinary cotinine concentration of 13 ± 3 μmol/L for the newborns of nonsmokers not exposed to tobacco smoke, 18 ± 4 μmol/L for the newborns of passive smokers and 44 ± 18 μmol/L for the newborns of active smokers who smoked an average of 15 cigarettes per day; all differences were statistically significant. First-day urine of newborns was analysed in a large study of 429 mothers in Barcelona, Spain (Pichini *et al*., 2000). Concentrations of urinary cotinine higher than the cut-off value of 50 ng/mL were measured in 17% of samples from newborns of nonsmoking mothers exposed to secondhand smoke, versus 2% for nonsmoking mothers who were not exposed to secondhand smoke. The concentrations of cotinine in the urine and cord serum of newborns of nonsmoking women with a calculated daily exposure to nicotine of more than 4 mg were significantly higher than the levels in newborns of nonsmoking mothers who were not exposed (30.9 versus 6.2 ng/mL; $p < 0.05$), after adjustment for creatinine, maternal age and sex. Daily intake of nicotine for active smokers was stratified into ≤ 3.6 mg nicotine per day, 3.6–9 mg per day, and > 9 mg per day. Urinary and cord serum cotinine concentrations were 515 ng/mL for newborns of mothers with intermediate daily nicotine intake and 568 ng/mL for the newborns of mothers with high daily nicotine intake and were statistically different ($p < 0.05$) from the concentrations in newborns of mothers with a low daily nicotine intake (161 ng/mL).

Nicotine has recently been demonstrated to occur in newborn urine. Lackmann *et al*. (1999) used gas chromatography–mass spectrometry with selective ion monitoring to detect nicotine in first voided urine samples in newborns of mothers who smoked an average of 12.4 cigarettes per day. The average concentration of nicotine in 18/31 (58%) samples was 0.63 nmol/mL. Nicotine was not detected in the urine of 17 newborns of women who did not smoke ($p < 0.001$). In the same study, cotinine was detected in 28/31 (90%) of the urine samples from newborns of mothers who were smokers, at a mean concentration of 0.87 nmol/mL. The newborns of women who were nonsmokers had a mean urine cotinine concentration of 0.049 nmol/mL. The difference was statistically significant ($p < 0.001$). Similarly, using high-performance liquid chromatography, Köhler *et al*. (2001) were able to detect nicotine and cotinine in the urine of newborns of active smokers (mean ± SD, 374 ± 765 nmol/L for nicotine and 500 ± 572 nmol/L for cotinine), but not in the urine of the newborns of nonsmokers, whether or not they were exposed to secondhand tobacco smoke ($p < 0.05$ and $p < 0.001$ for nicotine and cotinine, respectively). They also observed a strong correlation between the nicotine and cotinine concentrations in the mothers and in their newborns.

(iv) *Alternative nicotine-related measures of exposure*

The analysis of nicotine in hair has been suggested as an alternative and non-invasive measure of exposure to tobacco smoke, particularly in children. This method may allow past exposure to be measured over a longer time period than is possible using measurements of nicotine in blood, saliva or urine. Several studies have shown a strong correlation between nicotine levels in hair and self-reported exposure to tobacco smoke or exposure in experimental chambers (Zahlsen *et al*., 1996; Nafstad *et al*., 1997; Dimich-Ward *et al*., 1997; Al-Delaimy *et al*., 2000). In fact, this has been proposed to be a more precise indicator of exposure to secondhand tobacco smoke than concentrations of urinary cotinine (Al-Delaimy *et al*., 2002).

The concentrations in the urine of minor nicotine-related tobacco alkaloids not present in nicotine medications, such as anabasine or anatabine, have been proposed as indicators of exposure to tobacco smoke in individuals undergoing nicotine replacement therapy (Jacob *et al*., 1999).

In summary, cotinine and its parent compound nicotine have a very high specificity and sensitivity for exposure to secondhand tobacco smoke, and, as such, cotinine is presently the best suited biomarker for assessing exposure to secondhand smoke and its uptake and metabolism in adults, children and newborns.

4.1.2 *Experimental systems*

The studies in which experimental animals were exposed to sidestream smoke alone or to simulated environmental tobacco smoke are reviewed below (see Section 3.1 for definitions).

In most studies the amount of smoke administered to the animals was monitored by measuring its total particulate matter (TPM), carbon monoxide (CO) and/or nicotine content. Internal dose measurements include those of carboxyhaemoglobin (COHb) adducts and/or cotinine in blood or urine.

(*a*) *Effects of tobacco smoke on enzyme activities*

Studies in animals on the effects of sidestream smoke on enzyme concentrations have evaluated changes in phase I and phase II enzymes in liver, lung and trachea (Table 4.2). A few studies have looked at changes in enzyme activities in brain and heart.

(i) *Phase I enzymes*

The ability of sidestream smoke to induce hepatic P450 activity was investigated in male Wistar rats (Kawamoto *et al*., 1993). Animals were exposed for 8 h/day to the smoke from 1, 3 or 5 cigarettes/h for 5 days (6–500 ppm CO). Total cytochrome P450 and nicotinamide-adenine dinucleotide phosphate (reduced form; NADPH) cytochrome c reductase activities were not affected, but cytochrome b_5 was increased 1.6-fold and aryl hydrocarbon hydroxylase (AHH) activity was significantly decreased in the highest exposure

Table 4.2. Effect of sidestream or simulated environmental tobacco smoke on enzyme concentration or activity

Species	Strain/sex	Exposure conditions (mg/m^3 TPM)	Enzyme affected	Effect (tissue)	Reference
Rat	S-D/M	n.g.	Ornithine decarboxylase	+ (trachea); 0 (lung)	Olson (1985)
			S-Adenosyl-methionine decarboxylase	– (trachea, lung)	
Mouse	C57BL/M	n.g.	Aryl hydrocarbon hydroxylase	+ (lung)	Gairola (1987)
Rat	S-D/M		Aryl hydrocarbon hydroxylase	+ (lung)	
Guinea-pig	Hartley/M		Aryl hydrocarbon hydroxylase	0 (lung)	
Mouse	C57BL/F	5.21 mg/kg bw	Aryl hydrocarbon hydroxylase	+ (lung)	Gairola *et al*. (1993)
	DBA/F	7.05 mg/kg bw	Aryl hydrocarbon hydroxylase	0 (lung)	
Rat	Wistar/M	6–500 ppm CO	Total P450s	0 (liver)	Kawamoto *et al*. (1993)
			P450 1A1, 1A2, 2B1	+ (liver)	
			NADPH cytochrome C reductase	0 (liver)	
			Cytochrome b_5	+ (liver)	
			Aryl hydrocarbon hydroxylase	– (liver)	
Rat	S-D/M	1	P450 1A1	+ (lung)	Ji *et al*. (1994)
			NADPH reductase	+ (lung)	
			P450 2B	0 (lung)	
Rat	S-D/M	1	P450 1A1	0 (trachea, liver); + (lung)	Gebremichael *et al*. (1995)
			P450 2B1	0 (lung, liver)	
Ferret	European/M&F	38; 381	P450s	– (liver)	Sindhu *et al*. (1995)
			P450 reductase	– (liver)	
			7-Ethoxycoumarin *O*-deethylase	– (liver)	
			P450 1A	– (liver)	
	European/F		Cytochrome b_5	– (liver)	
			Cytochrome b_5 reductase	– (liver)	

Table 4.2 (contd)

Species	Strain/sex	Exposure conditions (mg/m^3 TPM)	Enzyme affected	Effect (tissue)	Reference
Mouse	C57BL/6N/M	1	P450 1A1	+ (lung)	Gebremichael *et al.* (1996)
	DBA/2N/M		P450 1A1	0 (lung)	
Mouse	AJ/M	87.3	P450 1A1	+ (trachea, lung)	Witschi *et al.* (1997a)
			P450 2B1, 2E1	0 (lung)	
Rat	Wistar/M	n.g.	P450s	0 (liver)	Kurata *et al*. (1998)
Rat	Sprague-Dawley/n.g.	73–93	Aryl hydrocarbon hydroxylase	+ (lung)	Izzotti *et al*. (1999)
			Glutathione-*S*-transferase	+ (lung)	
Rat	Wistar/M	n.g.	Protein kinase C	+ (lung)	Maehira *et al*. (1999)
Rat	Wistar/M	10	Inducible nitric oxide synthase*	+ (alveolar macrophages)	Morimoto *et al*. (1999)
Rat	Sprague-Dawley/ M&F	1	P450 1A1	+ (lung)	Lee *et al*. (2000)
			P450 1B1, 2B1	0 (lung)	
			NADPH reductase	0 (lung)	
Rat	Sprague-Dawley/F	1	Adenylyl cyclase	+ (brain, heart)	Slotkin *et al*. (2001)
Rat	Wistar/M	90	P450 1A1	+ (lung)	Nadadur *et al*. (2002)

TPM, total particulate matter; M, male; F, female; bw, body weight; +, significant increase; 0, unchanged; –, significant decrease; n.g., not given
* With mineral fibre treatment

group. Although total cytochrome P450s did not change, P450 1A1, P450 1A2, and P450 2B1 were elevated by the high exposure regimen.

The effect of sidestream smoke on bronchiolar epithelial cell expression of P450 1A1 was studied in postnatal male Sprague-Dawley rats that were exposed to aged and diluted sidestream smoke (1 mg/m^3 TPM; 6 ppm CO; 350 μg/m^3 nicotine) for 6 h/day, 5 days/week from birth until 7, 14, 21, 50 or 100 days of age. Exposure to sidestream smoke significantly increased the expression of P450 1A1 in Clara cells of the proximal and distal airways and in alveolar Type II cells in the lung parenchyma at all times, with a maximal expression occurring at 50 days of age. NADPH reductase was increased in bronchiolar epithelial cells at 21 and 50 days, but not at 7 or 100 days. Cytochrome P450 2B expression was not affected by sidestream smoke in any airway epithelial cells during this study (Ji *et al.*, 1994).

Sindhu *et al*. (1995) studied hepatic cytochrome P450s after the exposure of ferrets to simulated environmental tobacco smoke. Six-week old male and female European ferrets were exposed to simulated environmental tobacco smoke (38 mg/m^3 TPM (low-dose) and 381 mg/m^3 TPM (high-dose)) for 2 h/day, 5 days/week for 8 weeks. In both male and female animals, there was a significant decrease in P450 content, and in P450 reductase and 7-ethoxycoumarin *O*-deethylase activities after exposure to both high and low concentrations. Immunoblot analysis revealed a decrease in P450 1A in exposed animals compared with controls. In addition, cytochrome b_5 content and the activity of its reductase were decreased in females.

The expression of P450 1A1 and 2B1 was evaluated in newborn male rats which were exposed to aged and diluted sidestream smoke for 6 h/day, 5 days/week (1 mg/m^3 TPM). Sidestream smoke induced pulmonary P450 1A1 activity as early as day 7 after birth, whereas it was not detected in controls. Pulmonary P450 1A1 activity remained significantly (3- to 4-fold) elevated until 100 days, whereas pulmonary P450 2B1 activity did not change at any age. Hepatic P450 1A1 and P450 2B1 were generally unchanged following exposure to sidestream smoke, except that P450 2B1 activity was decreased by 30% at 100 days. The effects of short-term exposure were studied in 47-day-old rats exposed for 6 h/day for 4 days, to either filtered or unfiltered sidestream smoke (0.03 and 1 mg/m^3 TPM, respectively). Whole, but not filtered, sidestream smoke increased pulmonary P450 1A1 more than threefold; P450 2B1 was unchanged by either type of exposure (Gebremichael *et al.*, 1995).

The role of the Ah receptor in response to exposure to sidestream smoke was evaluated (Gebremichael *et al*., 1996). Male C57BL/6N and DBA/2N mice were exposed for 6 h/day for 4 days to aged and diluted sidestream smoke (1 mg/m^3 TPM; 3.4 ppm CO; 703 μg/m^3 nicotine). Sidestream smoke induced ethoxyresorufin-*O*-dealkylase activity in the lungs of C57BL/6N mice, but had no effect in mice of the DBA/2N strain, which has a reduced AhR functionality.

The induction of pulmonary tumours and P450 1A1 after exposure to simulated environmental tobacco smoke was examined in male A/J mice (Witschi *et al*., 1997a). Mice, 12 weeks of age, were exposed to simulated environmental tobacco smoke (87.3 mg/m^3

TPM) for 6 h/day, 5 days/week for 5 months. The expression of P450 1A1 was significantly increased in airway epithelium and lung parenchyma of the smoke-exposed mice after 5 months of exposure; however, after a 4-month recovery period, no expression of P450 1A1 could be detected. P450 2B1 and 2E1 were not affected by exposure to tobacco smoke. No enhanced expression of P450 1A1 was detected in lung tumours. Filtered smoke containing 0.1 mg/m^3 TPM did not induce P450 1A1 expression in female A/J mice under the same conditions (Witschi *et al.*, 1997b).

The effect of sidestream smoke on the expression of pulmonary cytochrome P450 mRNAs in rats has been examined following in-utero and postnatal exposure (Lee *et al*., 2000). Gestating Sprague-Dawley rats were exposed to aged and diluted sidestream smoke (1 mg/m^3 TPM; 7.3 ppm CO; 250 µg/m^3 nicotine) beginning on gestational day 5. None of the P450 isozymes analysed were increased in fetal lungs when evaluated at 17, 19 or 21 days of gestation. In contrast, postnatal exposure to sidestream smoke induced P450 1A1 expression as early as 1 day after birth. No induction of P450 1B1, 2B1 or NADPH cytochrome P450 reductase was observed following continuous in-utero and postnatal exposure to sidestream smoke.

Total liver content of P450 remained unchanged in male Wistar rats following exposure to sidestream smoke for 2 h/day for 25 days [no information on TPM or other measurements of smoke concentration was given] (Kurata *et al.*, 1998).

Spontaneously hypertensive (SH) rats exhibit heritable risk factors similar to those found in patients with chronic obstructive pulmonary disease, and are more susceptible to lung injury and inflammation, to oxidative stress resulting from exposure to combustion by-products and to induction of pulmonary diseases in general. Nadadur *et al*. (2002) used this model to examine the differential gene expression following exposure to sidestream smoke. Male SH rats were exposed to sidestream smoke (90 mg/m^3 TPM) for 6 h/day on 2 consecutive days. Total RNAs were isolated from lungs on the third day and cDNA was examined by gene-expression array filters containing 588 genes. Exposure to sidestream smoke resulted in a differential expression of 16 genes, including P450 1A1.

The effect of sidestream smoke on AHH activity was investigated in different species and strains. Male C57BL mice, Sprague-Dawley rats and Hartley guinea-pigs [ages not stated] were exposed to sidestream smoke once or twice daily, on 7 days/week for 16 weeks. AHH levels were significantly increased in mice and rats (3.7-fold and 2.7-fold, respectively), but remained unchanged in guinea-pigs (Gairola, 1987). In a later study by Gairola *et al.* (1993), female C57BL and DBA mice, 8–9 weeks old, were exposed to sidestream smoke daily [duration of exposure not stated] for 65–70 weeks (average TPM intake, 5.21 and 7.05 mg/kg body weight, respectively). Exposure to sidestream smoke induced pulmonary AHH activity two- to threefold in C57BL mice, but no effect was found in DBA mice. In a later study, male Sprague-Dawley rats were exposed to simulated environmental tobacco smoke (a mixture of 89% sidestream smoke and 11% mainstream smoke) for 6 h/day, 5 days/week for up to 5 weeks (73–93 mg/m^3 TPM; 350 ppm CO). Exposure to simulated environmental tobacco smoke resulted in a significant induction of AHH activity in lung microsomal fractions, which increased over the first 4

weeks of exposure; however, within 1 week after termination of the exposure, AHH activity decreased to the same levels as those measured in sham-treated rats (Izzotti *et al*., 1999).

(ii) *Phase II enzymes*

Male Sprague-Dawley rats were exposed to simulated environmental tobacco smoke for 6 h/day, 5 days/week for up to 5 weeks (73–93 mg/m^3 TPM; 350 ppm CO) (Izzotti *et al.*, 1999). This exposure resulted in the concentrations of GSH in lung post-mitochondrial (S12) fractions undergoing a progressive and consistent decrease, which became significant after 4 weeks. After 5 weeks, GSH levels in exposed animals were 67% of the levels in controls. The activity of glutathione-*S*-transferase (GST) in lung cytosolic fractions from exposed animals increased steadily and became significantly elevated after 5 weeks.

(iii) *Other enzymatic alterations*

The effect of chronic exposure to sidestream smoke on ornithine decarboxylase and *S*-adenosyl-methionine decarboxylase activity was determined in the rat trachea and lung. Male Sprague-Dawley rats were exposed for 10 min daily, 7 days/week, for 4 or 8 weeks to 25% sidestream smoke, or for 20 weeks to either 50%, 25% or 10% sidestream smoke (Olson, 1985). Ornithine decarboxylase activity in the lung was elevated in the group exposed for 8 weeks, but not in the group exposed for 20 weeks at any dose. Ornithine decarboxylase in the trachea was significantly elevated by all concentrations of sidestream smoke at all times. None of the treated rats showed any significant increase in *S*-adenosyl-methionine decarboxylase activity at any concentration or duration of exposure.

In 8-week-old male Wistar rats exposed to sidestream smoke [concentration not reported] for 1-h periods, twice daily, for 8, 12 or 20 weeks, protein kinase C activity in lung was increased by 120% at 8 weeks, and by 86% and 81% at 12 and 20 weeks, respectively (Maehira *et al*., 1999).

The synergistic effects of sidestream smoke and mineral fibres were investigated by Morimoto *et al*. (1999). Ten-week-old male Wistar rats were first given an intratracheal instillation of chrysotile or ceramic fibres and subsequently exposed to sidestream smoke for 4 h/day, 5 days/week for 4 weeks (10 mg/m^3 TPM; 79 ppm CO). Control groups included animals exposed to saline only, chrysotile only, ceramic fibres only and sidestream smoke only. Both exposure to mineral fibres and/or to sidestream smoke increased the number of cells recovered from bronchoalveolar lavage; alveolar macrophages accounted for > 95% of the total cells. Levels of IL-1α mRNA were significantly increased ($p < 0.05$) in all exposed groups (i.e. those exposed to sidestream smoke, mineral fibres, and sidestream smoke plus mineral fibres) in alveolar macrophages, but not in lung (when compared to saline-treated controls). Increased expression of IL-6 mRNA was only seen in the lung when sidestream smoke was combined with chrysotile, but neither exposure alone was sufficient to induce expression of IL-6 mRNA. No such

increase was observed in alveolar macrophages. Similarly, inducible nitric oxide synthase (iNOS) was not increased in the alveolar macrophages of rats treated with mineral fibres or sidestream smoke alone, but was significantly increased in animals that received combination treatments ($p < 0.01$). iNOS was not induced in the lungs by any treatment.

To mimic fetal and childhood exposure to secondhand smoke, gestating Sprague-Dawley rats were exposed to mainstream smoke (29 mg/m^3 TPM; 93 ppm CO; 4.6 mg/m^3 nicotine) for 6 h/day, 7 days/week, from gestational day 5 to day 20. One or two days after parturition, dams and pups were exposed to sidestream smoke (1 mg/m^3 TPM; 5.6 ppm CO; 117 μg/m^3 nicotine) until postnatal day 21. Animals were exposed either prenatally or postnatally or both. Adenylyl cyclase (AC) activity was evaluated under four different conditions in brain and heart tissues: basal AC, after isoproterenol or forskolin stimulation, and after forskolin stimulation followed by carbachol inhibition. In the brain, both prenatal and postnatal exposure were effective in upregulating AC when measured by forskolin response, but not when measured by the other methods. In the heart, AC activity as measured by all methods was significantly elevated after prenatal exposure, postnatal exposure, or both. The authors concluded that postnatal exposure to sidestream smoke elicited changes similar to, or more severe than, those observed during prenatal exposure from maternal smoking (see also Section 4.3.2(iii)) (Slotkin *et al.*, 2001).

Male SH rats were exposed to sidestream smoke (90 mg/m^3 TPM) for 6 h/day for 2 days (Nadadur *et al.*, 2002; see Section 4.1.2(*a*)(i) for details). A two- to threefold increase was observed in expression of macrophage inflammatory protein-2, suggesting the potential for lung inflammation. Over-expression of matrix metalloproteinase-7 was also observed; this may play a role in cell migration and invasion.

(*b*) *Tobacco smoke carcinogen biomarkers*

Animal studies on the formation of carcinogen biomarkers following exposure to sidestream smoke have evaluated protein and DNA adducts, including those in lung and liver. These studies are summarized in Table 4.3; only the main studies are described in detail in the text.

(i) *Urinary compounds*

Urine samples from male and female Fischer 344 rats exposed to sidestream smoke for 15 min four times/day for 5 days elicited DNA adducts in a plasmid assay *in vitro* (Takenawa *et al.*, 1994).

(ii) *DNA adducts*

Lee *et al*. (1992, 1993) evaluated the formation of DNA adducts in various tissues following 14-day and 90-day periods of exposure to sidestream smoke. In these studies, 7-week-old male Sprague-Dawley rats were exposed to aged and diluted sidestream smoke for 6 h/day for 14 or 90 days at target exposure concentrations of 0, 0.1, 1.0 and 10 mg/m^3 TPM. DNA adducts were observed only in the lung and heart of animals that received the highest dose. Adducts in lung were observed after 7 and 14 days and were

Table 4.3. Effects of sidestream or simulated environmental tobacco smoke on DNA adducts and other biomarkers

Species	Strain/sex	Biomarker	Effect (tissue)	Reference
		DNA adducts		
Rat	S-D/*	Smoke-related (14-day exposure)	+ (lung, heart/high dose) – (lung, heart/low dose)	Lee *et al*. (1992)
Rat	S-D/M	Smoke-related (90-day exposure)	+ (lung, heart, larynx) – (liver, bladder)	Lee *et al*. (1993)
Mouse	Parkes/M	Smoke-related	+++ (skin, lung, bladder) ++ (heart, kidney)	Carmichael *et al.* (1993)
Mouse	C7BL/F DBA/F	Smoke-enhanced	+ (lung); – (liver) + (lung); – (liver)	Gairola *et al*. (1993)
Rat	F344/M&F	Smoke-related	+ (bladder, kidney) – (testis)	Takenawa *et al.* (1994)
Mouse	BALB/c/F BALB/c/F	Smoke-related 8-Hydroxy-2′-deoxyguanosine	+ (lung, liver, heart) + (lung, liver, heart)	Howard *et al.* (1998b)
Rat	S-D/M	Smoke-related 8-Hydroxy-2′-deoxyguanosine	+ (lung, heart, bladder, trachea, bronchi) 0 (liver), ± (testes) + (lung)	Izzotti *et al*. (1999)
Rat	Wistar/M	8-Hydroxy-2′-deoxyguanosine	+ (lung)	Maehira *et al*. (1999)
	S-D/F	Smoke-related	+++ (lung), ++ (trachea, heart) + (bladder)	Arif *et al*. (2000)
Rat	S-D/M	Smoke-related 8-Hydroxy-2′-deoxyguanosine	+ (lung, trachea, heart) + (lung)	Izzotti *et al*. (2001)
Mouse	SKH-1	Smoke-related 8-Hydroxy-2′-deoxyguanosine	+ (skin), ++ (lung) + (lung)	De Flora *et al*. (2003)

Table 4.3 (contd)

Species	Strain/sex	Biomarker	Effect (tissue)	Reference
		Other biomarkers and metabolites		
Ferret	EUR/M&F	(+)-*Anti*-B*a*P 7,8-dihydrodiol-9,10-epoxide		Sindhu *et al*. (1995)
		- glutathione	– (liver) (female only)	
		- glucuronide	0 (liver)	
		- sulfate	0 (liver)	
		total B*a*P	– (liver)	
		(-)-7R *trans*-B*a*P-7,8-dihydrodiol-9,10-epoxide	0 (liver)	
Rat	Wistar/M	L-Ascorbic acid	+ (urine, plasma, tissues)	Kurata *et al*. (1998)
Rat	n.g./n.g.	Cotinine	+ (urine)	Oddoze *et al*. (1998)
Rat	S-D/M	8-Iso-prostaglandin-$F_{2\alpha}$	+ (urine)	Visioli *et al.* (2000)
Rat	S-D/M	B*a*P-7,8-diol-9,10-epoxide haemoglobin	+ (blood)	Izzotti *et al*. (2001)

M, male; F, female; S-D, Sprague-Dawley; +, significant increase; 0, unchanged; –, significant decrease; n.g., not given
* Sex not stated, but likely to be males (see Lee *et al.*, 1993)

still present after 14 days of recovery. Adducts in heart tissue were first seen after 14 days and persisted through the 14-day recovery period. Neither liver nor larynx exhibited exposure-related adducts at any time period or any dose. In the 90-day study, animals were killed at 28 and 90 days, and after a 90-day recovery period. After 28 and 90 days, a significant elevation in adducts in lung, heart and larynx was seen only in the animals exposed to 10 mg/m^3 TPM, and liver and bladder were unaffected by exposure at any time or any dose. After a 90-day recovery period, adduct levels in all organs in which there had been a response to exposure decreased, but were still elevated compared to the levels in controls [no statistical test performed]. These data establish a no-observed-effect-level of at least 1.0 mg/m^3 TPM for DNA adducts.

Sidestream smoke condensate was applied topically on mouse skin and DNA adducts formed from the condensate in several organs were quantified by ^{32}P-postlabelling techniques. When compared with unexposed controls, sidestream smoke condensate was found to induce approximately five- to sevenfold higher levels of adducts in skin, lung and bladder and two- to threefold higher levels in heart and kidney (Carmichael *et al*., 1993).

Long-term studies of exposure of female C57BL and DBA mice to sidestream smoke were conducted by Gairola *et al*. (1993). DNA adducts were assayed in lung and liver after 65–70 consecutive weeks of exposure (average TPM intake, 5.21 and 7.05 mg/kg body weight, respectively). Sidestream smoke enhanced DNA adducts in lung in both strains of mice; the increase was about 16-fold in C57BL mice and 8-fold in DBA mice (the difference between the two strains was not statistically significant. Adduct maps showed no qualitative difference between strains, or between treated and control mice. No increase in adduct levels was observed in the liver.

Male and female Fischer 344 rats were exposed to sidestream smoke for 15 min four times/day for 5 days (Takenawa *et al*., 1994). [No monitoring of exposure was reported.] A significant increase in DNA adducts in bladder and kidney was seen in exposed animals when compared to control samples, but not in testicular tissues; this suggests that the DNA adducts were formed in the tissues along the urinary tract.

Adult female BALB/c mice were exposed to a regimen of 30-min exposures to sidestream smoke followed by a 90-min recovery, for three consecutive cycles. The level of 8-OHdG adducts was increased by exposure to sidestream smoke in heart, lung and liver (about 1.6-fold) and remained elevated in lung and heart after the recovery period [limited statistical analysis was performed] (Howard *et al*., 1998b).

In a study to evaluate the inhibitory effect of indole-3-carbinol on cigarette smoke-related formation of DNA adducts in target organs, Arif *et al*. (2000) found that whole-body exposure of female Sprague-Dawley rats to sidestream smoke (6 h/day, 7 days/week for 4 weeks; 27 mg/m^3 TPM) induced smoke-related adducts in all tissues examined, including (in descending order) lung, heart, trachea and bladder. The adducts were qualitatively similar in all organs, but were present in different proportions.

Male Sprague-Dawley rats were exposed to simulated environmental tobacco smoke for 6 h/day, 5 days/week for up to 5 weeks (73–93 mg/m^3 TPM; 350 ppm CO) (Izzotti *et al.*, 1999, 2001). The exposure continued for 1, 2, 3, 4 or 5 weeks and rats were killed

16 h after the last exposure. Samples of heart, lung, liver, testes, bladder, bronchial alveolar macrophages and tracheal epithelium were analysed for adducts. Examination of the autoradiograph patterns revealed the existence of four major and two minor spots in tracheal epithelium, three major spots in macrophages, and one major and one minor spot in lung, heart and bladder. All organs showed a time-related increase in adducts during the first 4 weeks, and the trachea and macrophages continued to accumulate adducts through the fifth week. When animals were allowed 1 week of recovery after 4 weeks of exposure, levels of adducts decreased significantly in all tissues except heart, but remained significantly higher than in control animals. There was a slight but significant increase in 8-OHdG adducts in lung of animals exposed to smoke for 4 weeks when compared with sham-treated rats (Izzotti *et al.*, 2001).

In a preliminary experiment reported by De Flora *et al*. (2003), SKH-1 hairless mice were exposed to simulated environmental tobacco smoke for 28 days [exposure concentrations not given]. Whole-body exposure resulted in bulky DNA and 8-OHdG-adducts in the skin and lungs of treated animals. A potential synergistic effect was noted between exposure to simulated environmental tobacco smoke and exposure to sunlight-simulating lamps with respect to the induction of bulky DNA adducts in the lung.

(*c*) *Other biomarkers and metabolites*

The results of animal studies that have reported changes in metabolites and carboxyhaemoglobin levels in various tissues are summarized below and in Table 4.3.

(i) *Blood compounds*

Serum concentrations of carboxyhaemoglobin, together with nicotine and/or cotinine, are commonly used as biomarkers of exposure in experimental models as they are strongly correlated with the estimated total particulate matter (von Meyerink *et al.*, 1989; Coggins *et al.*, 1992, 1993; Zhu *et al.*, 1994; Sun *et al.*, 2001).

(ii) *Particles*

A comparison of the deposition of environmental tobacco smoke particles in human and rat tracheobronchial tree and pulmonary region was performed by Oberdörster and Pott (1986). Their calculations showed that the relative deposition of particles (mass median aerodynamic diameter of 0.2 μm) was about the same in the tracheobronchial tree of rats and humans, and was less in the pulmonary region in rats than in humans. However, the rate of deposition in the transitional region of the lung was about twice as high in rats as in humans. These data should be taken into consideration when using the results of experiments in rats to predict the results of human exposure.

(iii) *Urinary compounds including cotinine*

L-Ascorbic acid is a potential scavenger of free radicals under normal conditions and following most carcinogenic insults, including the free radicals contained in and generated by cigarette smoke. To evaluate the effect of sidestream smoke on the metabolism and excretion of L-ascorbic acid, Kurata *et al*. (1998) exposed 7-week-old male Wistar

rats to the sidestream smoke generated by two cigarettes every 30 min, four times/day, for 25 days [concentrations of TPM, CO and nicotine not stated]. The excretion of L-ascorbic acid into urine increased steadily with duration of exposure to sidestream smoke, and became significantly higher than in controls after day 12. At 25 days, L-ascorbic acid in liver, adrenal glands, lungs and kidneys in exposed animals was higher than in controls.

The exposure of rats to sidestream smoke produces a smoke-related oxidative stress, resulting in lipid peroxidation, that can be monitored by the urinary excretion of F_2 isoprostanes (e.g. 8-*iso*-prostaglandin $F_{2\alpha}$), produced from arachidonic acid by free radical-catalysed mechanisms. Visioli *et al*. (2000) evaluated the antioxidant effect of olive oil on the excretion of 8-*iso*-prostaglandin $F_{2\alpha}$. Male Sprague-Dawley rats were exposed to sidestream smoke for 20 min/day for 4 days (2600 ppm CO). The excretion of 8-*iso*-prostaglandin $F_{2\alpha}$ increased from 237 to 319 pg/mg creatinine after 2 days of exposure, an increase of 44%. After four exposures, the excretion of 8-*iso*-prostaglandin $F_{2\alpha}$ was 55% higher than in control rats. Treatment with olive oil reduced the excretion to pre-exposure levels and to a 34% increase over pre-exposure levels, after 2 and 4 days of exposure, respectively.

Oddoze *et al*. (1998) developed a rapid and sensitive assay for measuring urinary metabolites in human nonsmokers and rats. In rats exposed to sidestream smoke for 4 days [strain and sex of rats and conditions of exposure not stated], 24-h urine samples were collected before the exposure began and after the last exposure. No cotinine was found in sham-exposed samples, but the amount of cotinine in the urine of exposed rats (n = 5) ranged from 525 to 675 ng/mL and one sample had a cotinine concentration of 1587 ng/mL.

4.2 Toxic effects

Exposure to secondhand tobacco smoke is a cause of cardiovascular and respiratory disease. The studies reviewed here add to the knowledge of the adverse effects of exposure to secondhand tobacco smoke on the health of adult humans.

4.2.1 *Humans*

(*a*) *Nicotine addiction*

No data on nicotine addiction resulting from involuntary exposure to tobacco smoke were available to the Working Group.

(*b*) *Cardiovascular system*

A causal association between active smoking and coronary heart disease (CHD) is well established (US Department of Health and Human Services, 1983, 1990). Since 1984, some 20 studies have examined the association between exposure to secondhand tobacco smoke and risk of CHD in nonsmokers. The available literature was first reviewed in 1986 in a report from the US National Research Council (US National

Research Council, 1986) and a report of the Surgeon General (US DHHS, 1986). Both reviews concluded that an association between exposure to secondhand tobacco smoke and CHD was biologically plausible, but that the epidemiological evidence was inconclusive. Since then, numerous reviews and reports have become available (Wells, 1988; Wu-Williams & Samet, 1990; Glantz & Parmley, 1991; Steenland, 1992; Wells, 1994; Glantz & Parmley, 1995; Kritz *et al*., 1995; Law *et al*., 1997; Wells, 1998; He *et al*., 1999; Thun *et al*., 1999; US National Cancer Institute, 1999). Nine of these reviews included a meta-analysis to calculate a pooled relative risk for CHD in relation to exposure to secondhand tobacco smoke (Wells, 1988; Glantz & Parmley, 1991; Wells, 1994 ; Glantz & Parmley, 1995; Kritz *et al*., 1995; Law *et al*., 1997; Wells, 1998; He *et al*., 1999; Thun *et al*., 1999).

(i) *Epidemiological studies*

The results of three recent meta-analyses (Law *et al*., 1997; He *et al*., 1999; Thun *et al*., 1999) are summarized in Tables 4.4 and 4.5.

Law *et al.* (1997) carried out five sets of meta-analyses using published data (Table 4.4). In the first analysis, which included 19 studies of exposure to secondhand tobacco smoke and ischaemic heart disease (IHD), it was estimated that never-smokers living with a smoker have a 30% increased risk of IHD. In the second analysis, which included five large cohorts of men, it was estimated that the risk for CHD in nonsmokers living with a smoker was similar to the excess risk from smoking one cigarette per day. The third analysis, which included three cohorts, estimated that almost all the excess risk reversed after cessation of smoking; the residual excess risk was 6%. The fourth analysis was conducted on 18 studies to estimate the potential effect of confounding attributable to differences in diet between passive smokers and nonsmokers. People exposed to secondhand smoke were more likely than nonsmokers not exposed to tobacco smoke to consume diets with few vegetables and fruits and were less likely to take antioxidant vitamin supplements. However, clinical trials have indicated that taking β-carotene and vitamin E supplements does not reduce the risk for CHD in persons with no history of myocardial infarction (Alpha-Tocopherol β Carotene Cancer Prevention Study Group, 1994; Hennekens *et al.*, 1996). It was estimated that nonsmokers living with smokers eat a diet that gives them a 6% increased risk for IHD. The relative risk of exposure to secondhand smoke for ischaemic heart disease adjusted for diet was 1.2 (95% CI, 1.1–1.3). In the fifth analysis, which was based on eight studies, the increase in risk for IHD attributable to secondhand tobacco smoke-related platelet aggregation was estimated. It was concluded that the increase in experimentally produced platelet aggregation caused by exposure to secondhand tobacco smoke would be expected to have acute effects increasing the risk for IHD by 34%.

In the meta-analysis conducted by He *et al.* (1999), passive smoking was consistently associated with an increased relative risk for CHD. This association was observed in cohort studies, in case–control studies, in men, in women and in those exposed to

Table 4.4. Relative risk for coronary (or ischaemic) heart disease (and/or death from coronary heart disease) in never-smokers exposed to secondhand tobacco smoke in meta-analyses

Focus of meta-analysis	Relative risk (95% CI)	Exposure to tobacco smoke	Number of studies included and references
Secondhand tobacco smoke and IHD	1.30 (1.22–1.38; $p < 0.001$)	Never-smokers living with a smoker	Meta-analysis of 19 studies (Garland *et al.*, 1985; Lee *et al.*, 1986; Svendsen *et al.*, 1987; He, 1989; Hole *et al.*, 1989; Sandler *et al.*, 1989; Hirayama, 1990; Humble *et al.*, 1990; Dobson *et al.*, 1991; Lee, 1992; La Vecchia *et al.*, 1993; He *et al.*, 1994; Layard, 1995; LeVois & Layard, 1995; Muscat & Wynder, 1995; Tunstall-Pedoe *et al.*, 1995; Steenland *et al.*, 1996; Kawachi *et al.*, 1997; Ciruzzi *et al.*, 1998)
Smoking at low doses and IHD	1.39 (1.18–1.64) 1.78 (1.31–2.44)	Active smoking of 1 cig/day Active smoking of 20 cig/day	Five large cohorts of men (660 IHD events) (Hammond & Horn, 1958; Doll & Hill, 1964; 1966; Hammond, 1966; Kahn, 1966; Hammond & Garfinkel, 1969; Pooling Project Research Group, 1978)
Smoking cessation and reversibility of excess risk of IHD	1.06 (1.02–1.10)	Former smokers (smoking cessation)	Meta-analysis of 3 studies (Hammond & Garfinkel, 1969; Rogot & Murray, 1980; Doll & Peto, 1976)
Dietary differences between nonsmokers living with a smoker and nonsmokers living with a nonsmoker and IHD	1.06	Diet of nonsmokers living with a smoker	Meta-analysis of 18 studies (Keith & Driskell, 1980; Fehily *et al.*, 1984; Stryker *et al.*, 1988; Sidney *et al.*, 1989; Larkin *et al.*, 1990; Subar *et al.*, 1990; Cade & Margetts, 1991; Le Marchand *et al.*, 1991; Nuttens *et al.*, 1992; Bolton-Smith *et al.*, 1993; Margetts & Jackson, 1993; Midgette *et al.*, 1993; Tribble *et al.*, 1993; Järvinen *et al.*, 1994; McPhillips *et al.*, 1994; Thornton *et al.*, 1994; Emmons *et al.*, 1995; Zondervan *et al.*, 1996)
Secondhand tobacco smoke-related platelet aggregation and risk of IHD	1.34 (1.19–1.50)	Platelets experimentally exposed to second-hand tobacco smoke	Meta-analysis of 8 studies (cohort of 2398 men) (Davis & Davis, 1981; Davis *et al.*, 1982; Schmidt & Rasmussen, 1984; Davis *et al.*, 1985a,b, 1986; 1989; Blache *et al.*, 1992)

CI, confidence interval; IHD, ischaemic heart disease; cig, cigarettes

Table 4.5. Relative risk for coronary heart disease (and/or death from coronary heart disease) in never-smokers exposed to secondhand tobacco smoke in meta-analyses

Meta-analyses	Number of studies analysed	Number of cases of CHD	Relative risk (95% CI)	References
He *et al.* (1999)	10 cohort studies 8 case–control studies	6813	1.25 (1.17–1.32) in all studies 1.21 (1.14–1.30) in cohort studies 1.51 (1.26–1.81) in case–control studies 1.22 (1.10–1.35) in men 1.24 (1.15–1.34) in women 1.17 (1.11–1.24) at home 1.11 (1.00–1.23) at work 1.26 (1.16–1.38) pooled adjusted relative risk[a] *Intensity of exposure to secondhand smoke* 1–19 cig/day, 1.23 (1.13–1.34) ≥ 20 cig/day, 1.31 (1.21–1.42) (*p* for linear trend = 0.006) *Duration of exposure to secondhand smoke* 1–9 years, 1.18 (0.98–1.42) 10–19 years, 1.31 (1.11–1.55) ≥ 20 years, 1.29 (1.16–1.43) (*p* for linear trend = 0.01)	Cohort studies: Hirayama (1984); Garland *et al.* (1985); Svendsen *et al.* (1987); Butler (1988); Hole *et al.* (1989); Sandler *et al.* (1989); Hirayama (1990); Humble *et al.* (1990); Steenland *et al.* (1996); Kawachi *et al.* (1997) Case–control studies: Lee *et al.* (1986); He (1989); Jackson (1989); Dobson *et al.* (1991); La Vecchia *et al.* (1993); He *et al.* (1994); Muscat & Wynder (1995); Ciruzzi *et al.* (1998)
Thun *et al.* (1999)	9 cohort studies 8 case–control studies	7345	1.25 (1.17–1.33) in all studies 1.23 (1.15–1.31) in cohort studies 1.47 (1.19–1.81) in case–control studies 1.24 (1.15–1.32) in men 1.23 (1.15–1.32) in women 1.22 (1.13–1.30) in USA 1.41 (1.21–1.65) in other countries 1.22 (1.14–1.30) for fatal CHD 1.32 (1.04–1.67) for non-CHD	Cohort studies: Hirayama (1984); Garland *et al.* (1985); Svendsen *et al.* (1987); Butler (1988); Hole *et al.* (1989); Sandler *et al.* (1989); Hirayama (1990); Humble *et al.* (1990); Steenland *et al.* (1996); Kawachi *et al.* (1997) Case–control studies: Lee *et al.* (1986); He (1989); Jackson (1989); Dobson *et al.* (1991); La Vecchia *et al.* (1993); He *et al.* (1994); Muscat & Wynder (1995); Lam & He (1997); Ciruzzi *et al.* (1998)

CHD, coronary heart disease; IHD, ischaemic heart disease; CI, confidence interval; cig, cigarettes

[a] Analysis confined to 10 studies that adjusted for age, sex, blood pressure, body weight and serum cholesterol

smoking at home or in the workplace. Positive dose–response relationships for intensity and duration of exposure were observed (Table 4.5).

Thun *et al.* (1999) found that never-smokers married to smokers had an increased relative risk for fatal or non-fatal coronary events when compared with never-smokers married to nonsmokers. The increase in relative risk was similar in men and women, in cohort and case–control studies, in the USA and other countries and in studies of fatal and non-fatal coronary events (Table 4.5).

(ii) *Other human data*

Several mechanisms may increase the risk of CHD in nonsmokers exposed to secondhand tobacco smoke (US DHHS, 1990; Wells, 1994; He *et al*., 1999). The acute effects of passive smoking include alterations in heart rate (Pope *et al*., 2001), blood pressure, concentrations of carboxyhaemoglobin and carbon monoxide in the blood, in the blood's ability to use oxygen in the formation of adenosine triphosphate (ATP), and reduced exercise capability in people breathing secondhand smoke (Glantz & Parmley, 1995). An increase in the ratio of serum total cholesterol to high-density lipoprotein cholesterol (HDL-C), a decrease in the serum level of HDL-C (Feldman *et al*., 1991), an increase in platelet aggregation (Davis *et al*., 1989) and endothelial cell dysfunction (Otsuka *et al*., 2001) have also been described. Exposure to secondhand tobacco smoke may also contribute to atherosclerosis by priming and sensitizing neutrophils, resulting in their activation and subsequent oxidant-mediated tissue damage (Anderson *et al*., 1991).

(*c*) *Respiratory system*

The relationship between exposure to secondhand tobacco smoke and a variety of non-malignant respiratory health endpoints has been examined extensively in epidemiological and experimental studies. When this topic was first raised in the 1972 Report of the Surgeon General (US DHHS, 1972), the handful of studies that had addressed this issue had provided only limited information.

Since then, several reviews of the literature on secondhand tobacco smoke have addressed some aspects of the effects of secondhand tobacco smoke on the risk for non-neoplastic respiratory diseases in adults (Weiss *et al*., 1983; US DHHS, 1984, 1986; US National Research Council, 1986; Crawford, 1988; Eriksen *et al*., 1988; Spitzer *et al*., 1990; Trédaniel *et al*., 1994; Jinot & Bayard, 1996; California Environmental Protection Agency, 1997; Coultas, 1998; Weiss *et al*., 1999; US National Academy of Sciences, 2000).

A variety of adverse respiratory health outcomes in children have been causally linked to exposure to secondhand tobacco smoke or there is suggestive evidence of a causal association (see Table 4.6). For a detailed discussion of the relevant studies, the reader is referred to the recent reviews of the California Environmental Protection Agency (1997) and the US National Academy of Sciences (2000).

In adults, irritation of the eyes and nasal irritation have been causally associated with exposure to secondhand tobacco smoke and other annoyance has been described in

Table 4.6. Respiratory effects associated with exposure to secondhand tobacco smoke in children

Effects causally associated with exposure to secondhand tobacco smoke	Effects for which there is suggestive evidence of a causal association with exposure to secondhand tobacco smoke
Acute infections of the lower respiratory tract (e.g. bronchitis and pneumonia) Induction and exacerbation of asthma Chronic respiratory symptoms Middle-ear infections	Exacerbation of cystic fibrosis Decreased pulmonary function

Modified from California Environmental Protection Agency (1997)

several studies (US DHHS, 1999). For decreased pulmonary function, especially in combination with other exposures (e.g. prior exposure to occupational irritants) and for exacerbation of asthma, there is suggestive evidence of a causal association (California Environmental Protection Agency, 1997; US National Academy of Sciences, 2000). Some of the studies on exposure to secondhand tobacco smoke and respiratory health effects are briefly summarized below; for more comprehensive details, the reader is referred to some recent reviews (California Environmental Protection Agency, 1999; US National Academy of Sciences, 2000).

(i) *Acute effects of sensory irritation and annoyance*

The determination of the acute effects of secondhand tobacco smoke is difficult, because the observed reactions, although immediate, are largely subjective (Speer, 1968). A review of the irritation and annoyance attributable to exposure to secondhand tobacco smoke was published by the California Environmental Protection Agency (1997). The chemical constituents of secondhand tobacco smoke thought to be responsible for sensory irritation include organic acids (acetic acid and propionic acid), aldehydes (formaldehyde and acrolein), nicotine, ammonia, pyridine, toluene, sulfur dioxide and nitrogen oxides, among others (Ayer & Yeager, 1982; Triebig *et al*., 1984; US DHHS, 1986).

Nonsmokers seem to react significantly more than smokers (Weber, 1984a). The most common effect is tissue irritation, especially of the eyes (Speer, 1968; Basu *et al*., 1978; Shephard *et al*., 1979a; Bascom *et al*., 1991; White *et al*., 1991), but also of the nose, throat and airways (Bascom *et al*., 1991; Willes *et al*., 1992, 1998). The complaints are especially marked among aircraft passengers (US National Institute for Occupational Safety and Health, 1971; US National Academy of Sciences, 1986; Mattson *et al*., 1989).

Weber and co-workers (Weber *et al*., 1976; Weber, 1984b; Weber & Grosjean, 1987) and Muramatsu *et al*. (1983) conducted experiments in which volunteers were exposed to progressively increasing concentrations of secondhand tobacco smoke; as duration and intensity of exposure increased, subjects began to report subjective eye irritation, and blink rate also increased.

Lebowitz *et al*. (1992) found an increased prevalence of acute respiratory symptoms as levels of indoor secondhand tobacco smoke increased, especially in the households of subjects with lower socioeconomic status.

(ii) *Chronic respiratory symptoms*

In an early study, 25% of 10 320 nonsmoking office workers reported exacerbation of pre-existing pulmonary conditions when working with a smoker (Barad, 1979).

Other studies have shown no association (or a weak and statistically non-significant association) between the frequency of major respiratory symptoms and exposure to secondhand tobacco smoke from family members or spouse (Lebowitz & Burrows, 1976; Schilling *et al.*, 1977; Comstock *et al.*, 1981; Kauffmann *et al.*, 1983; Gillis *et al.*, 1984; Hole *et al.*, 1989; Kauffmann *et al.*, 1989).

Since 1990, however, several investigations have demonstrated a significant increase in risk for many respiratory symptoms (including cough, phlegm, breathlessness, wheeze, chest illness and dyspnoea) in subjects exposed to secondhand smoke at home and/or at work (Schwartz & Zeger, 1990; White *et al.*, 1991; Ng *et al.*, 1993; Leuenberger *et al.*, 1994; Janson *et al.*, 2001).

(iii) *Lung function testing*

A number of studies have been published that have examined the effects of secondhand tobacco smoke on pulmonary function in adults. These investigations were often initiated within the framework of research projects not primarily concerned with secondhand tobacco smoke; as a result, certain limitations apply regarding the validity of some of the findings, because of the low sensitivity and low power of these studies.

Acute exposure

Many studies have shown that exposure of nonsmoking adults to secondhand tobacco smoke is associated with a decrease in maximum expiratory flow at 25% (MEF25), FVC, FEV_1 and FEF25–75 and a decrease in dynamic lung volume (Pimm *et al.*, 1978; Shephard *et al.*, 1979a,b,c; Bascom *et al.*, 1991; Smith *et al.*, 2001).

Chronic exposure

A number of studies failed to detect any association between exposure to secondhand tobacco smoke and ventilatory parameters of lung function (Schilling *et al*., 1977; Comstock *et al.*, 1981; Jones *et al.*, 1983; Lebowitz, 1984a,b; Kentner *et al.*, 1984; Laurent *et al.*, 1992; Jaakkola *et al.*, 1995; Frette *et al.*, 1996).

Other investigators have reported an association between exposure to secondhand tobacco smoke and pulmonary function determined using different test parameters. In numerous studies, FEV_1 and/or FVC were reported to be significantly decreased (Brunekreef *et al.*, 1985; Svendsen *et al.*, 1987; Hole *et al.*, 1989; Kauffmann *et al.*, 1989; Masjedi *et al.*, 1990; Xu & Li, 1995; Carey *et al.*, 1999; Chen *et al.*, 2001). Other studies reported a significant decrease in the ventilatory parameters FEF25–75, PEF or FEF75–85 (Kauffmann & Perdrizet, 1981; Kauffmann *et al.*, 1983; Salem *et al.*, 1984; Masi *et al.*,

1988; White & Froeb, 1980; Masjedi *et al.*, 1990; Lebowitz *et al.*, 1992). Decreases in MEF50 and/or MEF75 were reported to occur only in nonsmoking men exposed at home (Masi *et al.*, 1988) or nonsmoking women exposed at home (Brunekreef *et al.*, 1985).

(iv) *Chronic obstructive pulmonary disease*

Few studies have examined the possible association between exposure to secondhand tobacco smoke and development of chronic obstructive pulmonary disease (COPD). With the exception of two studies that reported a negative association (Hirayama, 1981; Lee *et al.*, 1986), most of them found an increased risk for COPD including emphysema and bronchitis, airways obstructive disease (AOD) and obstructive respiratory disease associated with exposure to secondhand smoke (Euler *et al.*, 1987; Kalandidi *et al.*, 1987; Sandler *et al.*, 1989; Kalandidi *et al.*, 1990; Robbins *et al.*, 1993; Dayal *et al.*, 1994).

(v) *Asthma*

Many patients regard secondhand tobacco smoke as a major factor in the exacerbation of asthma (Cockcroft, 1988).

Symptoms and lung function. Many studies have shown that patients with allergies and/or asthma experienced more nasal symptoms, headache, cough, wheezing, sore throat, hoarseness (Speer, 1968), eye irritation (Weber & Fisher, 1980), aggravation of the asthma (Dales *et al.*, 1992) and restrictions in activity (Ostro *et al.*, 1994) in response to secondhand smoke. Other studies have reported a statistically significant association between the new onset of asthma, asthma ever diagnosed by a physician or current asthma and exposure to secondhand tobacco smoke at the workplace (Greer *et al.*, 1993), in the home environment and among young adults exposed to parental smoking (Hu *et al.*, 1997; Thorn *et al.*, 2001).

Two studies have found no statistically significant change in dynamic lung volume of asthmatic subjects exposed for 1 or 2 h to tobacco smoke (Shephard *et al.*, 1979b; Wiedeman *et al.*, 1986), whereas other studies have reported a statistically significant decrease in FEV_1, FVC and FEF25–75 in asthmatic subjects exposed to smoke in a chamber study (Dahms *et al.*, 1981), to secondhand tobacco smoke from the spouse and/or other close contacts (Jindal *et al.*, 1994) or in the workplace, particularly in asthmatic women (Künzli *et al.*, 2000).

Chamber studies. Chamber studies have been used to investigate potential relationships between controlled exposure to secondhand smoke and lung function and airway reactivity in asthmatic subjects. The principal advantage of this methodology over epidemiological studies is that the exposure to secondhand tobacco smoke can, in theory, be measured precisely.

Most of the studies of exposure to secondhand tobacco smoke in inhalation chambers reported slight-to-moderate transient effects on lung function in at least some of the study subjects. In several studies, some participants experienced decreases in lung function of more than 20% and a marked increase in bronchial reactivity to inhaled histamine or methacholine. These changes in lung function are considered clinically significant, parti-

cularly when they occur in conjunction with lower respiratory symptoms such as chest tightness, dyspnoea and cough (Dahms *et al.*, 1981; Knight & Breslin, 1985; Stankus *et al.*, 1988; Menon *et al.*, 1991, 1992; Nowak *et al.*, 1997). However, these results were not confirmed by Magnussen and colleagues (Jörres & Magnussen, 1992; Magnussen *et al.*, 1992) who exposed adults with mild and moderate asthma to secondhand tobacco smoke for a short period (1 h) and then conducted a bronchoprovocation test with methacholine.

Suggestion can induce an attack of asthma (Spector *et al.*, 1976). Most of the above-mentioned studies were unable to exclude the possibility that the changes reported in asthmatic subjects were emotionally related to cigarette smoke which might result in psychological suggestion being the cause of the observed symptoms, such as changes in lung function and others (Witorsch, 1992). Urch *et al.* (1988) argued that, if physiological responses were dominant, changes in pulmonary function should show a dose–response relationship to secondhand tobacco smoke, whereas, if psychological reactions were dominant, correlations between functional changes and specific measures of suggestibility would be expected. Sixteen nonsmoking asthmatic subjects were exposed to high or low concentrations of secondhand tobacco smoke or to ambient air for 65 min. Cigarette smoke was generated by a machine located outside the exposure chamber, but visible to the subjects; during sham-exposure, the smoke from the cigarettes was diverted from the study chamber. Subjects with asthma showed significant dose–response relationships for MEF50 at 5 min, and for FVC and FEV_1 at 30 min of exposure; these results support a physiological rather than psychological explanation of the findings. In the study by Danuser *et al.* (1993), the subjects wore noseclips and were exposed to secondhand tobacco smoke administered by a mouthpiece, thus blinding them to the differences in the concentrations of secondhand tobacco smoke delivered. In these conditions subjective airway symptoms were weak, but most of the symptomatic responses of the subjects with airway hyperresponsiveness appeared to be dose-related.

4.2.2 *Experimental systems*

Studies in which experimental animals were exposed to sidestream smoke alone or to simulated environmental tobacco smoke were reviewed, and the results of studies on adult animals are summarized in Tables 4.7 and 4.8 (see also Witschi *et al.*, 1997c) (see Section 3.1 for definitions).

(*a*) *Exposure of adult animals*

(i) *Effects on the respiratory tract*

Rats and hamsters were exposed to sidestream smoke (4 mg/m^3 TPM; 25–30 ppm CO) for 10 h/day, 5 days/week for 90 days. Hyperplasia and metaplasia in the epithelium of the dorsal nasal turbinates were the only changes seen in the rats. The changes partially receded after 30 days and had completely reversed 60 days after exposure. No signs of toxicity were observed in the hamster respiratory tract (Von Meyerinck *et al.*, 1989).

Table 4.7. Toxicity of sidestream or simulated environmental tobacco smoke on respiratory tract in adult animals

Species	Strain/sex	Exposure concentration (mg/m^3 TPM)	Exposure duration; conditions	Effects	Reference
Rat	F344/CrlBr/M+F	4	90 days; 10 h/d; 5 d/wk	Hyperplasia/metaplasia of the dorsal nasal epithelium	von Meyerinck *et al.* (1989)
Hamster	Syrian golden/M+F			No effect	
Rat	SD/M+F	0.1; 1; 10	14 d; 6 h/d	Slight-to-mild hyperplasia and inflammation in rostral nasal cavity at high dose only	Coggins *et al.* (1992)
Rat	SD/M	0.1; 1; 10	4 d; 28 d; 90 d; 6 h/d; 5 d/wk	Slight-to-mild hyperplasia and inflammation in rostral nasal cavity at high dose only	Coggins *et al.* (1993)
Rat	SD/F	1	3 h; 4 d	No effect	Joad *et al.* (1993)
Mouse	A/J/M	1	3 d; 5 d; 6 h/day	Increased cell proliferation in airways	Rajini & Witschi (1994)
Mouse	C57BL/6/M		5 d	No effect	
Hamster	Syrian golden/M	1	1 wk; 6 h/d; 7 d/wk	Increased cell proliferation in respiratory epithelium of nasal septum; increase after 1-week recovery period in terminal bronchioles	Witschi & Rajini (1994)
Rat	Wistar/M+F	35 ppm CO	3 mths; 90 min/d; 5 d/wk	Emphysema in lungs	Escolar *et al.* (1995)
Mouse	A/J/M	83.5	20 wks; 6 h/d; 5 d/wk	Increased cell proliferation in airways during the first 2 wks. No changes in lung parameters (volume and cell number)	Witschi *et al.* (1997a)

TPM, total particulate matter; M, male; F, female; h, hour; d, day; wk, week; mths, months; SD, Sprague-Dawley

Table 4.8. Toxicity of sidestream or simulated environmental tobacco smoke on cardiovascular system in adult animals

Species	Strain/sex	Exposure concentration (mg/m^3 TPM)	Exposure duration; conditions	Effects	Reference
Cockerel	–	8	16 wks; 6 h/d; 5 d/wk	Increase in size of arteriosclerotic plaques, but not in number or distribution	Penn & Snyder (1993)
Rabbit	New Zealand/M	4 or 33	10 wks; 6 h/d; 5 d/wk	Dose-dependent increase in formation of arteriosclerotic plaques in cholesterol-fed animals	Zhu *et al.* (1993)
Cockerel	–	2.5	16 wks; 6 h/d; 5 d/wk	Increase in size of arteriosclerotic plaques, but not in number or distribution	Penn *et al.* (1994)
Rat	SD/not given	60	3 d; 3 wks; 6 wks; 6 h/d; 7 d/wk	Time-dependent increase in infarct size	Zhu *et al.* (1994)
Mouse	Apolipoprotein $E^{-/-}$/F	25	7, 10, 14 wks; 6 h/d; 5 d/wk	Increased percentage of atherosclerotic lesions in aortic intimal surface	Gairola *et al.* (2001)
Rabbit	New Zealand White/M	24	10 wk; 6 h/d; 5 d/wk	Increased percentage of surface lipid lesions in aorta and pulmonary artery	Sun *et al.* (2001)

TPM, total particulate matter; h, hour; d, day; wk, week; M, male; F, female; SD, Sprague-Dawley

Rats were exposed, nose-only, for 6 h/day for 4, 14, 28 or 90 days to sidestream smoke at concentrations of 0.1, 1 or 10 mg/m^3 TPM. The only pathological response observed was slight to mild epithelial hyperplasia and chronic active inflammation in the most rostral part of the nasal cavity, in the group exposed to the high dose (10 mg/m^3 TPM). No time-dependent increase in the severity of the lesions was observed. After a 14-day recovery period, the changes had completely reversed (Coggins *et al*., 1992, 1993).

Male A/J and C57BL/6 mice were exposed for 6 h/day for up to 5 days to aged and diluted sidestream smoke (1 mg/m^3 TPM; 5.9 ppm CO; 549 μg/m^3 nicotine). Labelling indices in the epithelium of the large intrapulmonary airways and terminal bronchioles, but not in the alveoli, were significantly increased after 3 and 5 days of exposure in A/J mice. No signs of increased cell proliferation in the respiratory tract were seen in C57BL/6 mice (Rajini & Witschi, 1994).

Hamsters were exposed to aged and diluted sidestream smoke containing 1 mg/m^3 TPM for 6 h/day for 1–3 weeks, after which some subgroups were allowed a 1-week recovery period. Increased cell proliferation was observed in the respiratory epithelium of nasal septum after 1 week of exposure, but not at later time points. After 1 week of exposure and 1 week of recovery, cell proliferation in the terminal bronchioles was significantly increased when compared to concomitant controls and to the levels observed before recovery (Witschi & Rajini, 1994).

A similar initial increase in cell proliferation was seen in alveoli and terminal bronchioles in male A/J mice during the first 2 weeks of exposure to simulated environmental tobacco smoke (83.5 mg/m^3 TPM; 233 ppm CO; 18.9 mg/m^3 nicotine) (Witschi *et al.*, 1997a).

Wistar rats were exposed for 90 min/day, 5 days/week, for 3 months to sidestream smoke containing 35 ppm CO. Emphysematous changes (decreased number of distal airspaces and increase in alveolar chord) were observed in the lungs. These changes were accompanied by decreases in tissue density, internal alveolar perimeter, wall thickness and density and perimeter of elastic fibres (Escolar *et al.*, 1995).

Exposure of female rats to sidestream smoke (1 mg/m^3 TPM; 6.5 ppm CO) either on 1 day for 3 h or for 6 h/day on 4 days had no effect on dynamic compliance, lung resistance, lung weight/body weight, pulmonary artery pressure, or airway reactivity to methacholine (all $p > 0.4$) (Joad *et al.*, 1993).

(ii) *Cardiovascular effects*

Cardiovascular changes resulting from exposure to sidestream smoke have been demonstrated in several animal models.

Male New Zealand rabbits, fed a cholesterol-rich diet, were exposed to low-dose or high-dose sidestream smoke (4 and 33 mg/m^3 TPM, respectively) for 6 h/day, 5 days/week for 10 weeks. A dose-dependent significant increase in the size of arteriosclerotic plaques was found in the aorta and the pulmonary artery when compared with control rabbits receiving the same diet but exposed to clean air (Zhu *et al*., 1993).

Sprague-Dawley rats were exposed to sidestream smoke (60 mg/m^3 TPM; 92 ppm CO; 1103 µg/m^3 nicotine) 6 h/day, 5 days/week for 3 days, 3 weeks or 6 weeks. Infarct sizes increased in a time-dependent manner ($p = 0.023$) (Zhu *et al.*, 1994).

Exposure of 6-week old cockerels for 6 h/day, 5 days/week to sidestream smoke containing 8 mg/m^3 TPM (Penn & Snyder, 1993) or 2.5 mg/m^3 TPM (Penn *et al.*, 1994) resulted in a significant increase in the size of arteriosclerotic plaques in the aorta.

Roberts *et al.* (1996) developed a model to measure the rate of accumulation of low-density lipoproteins (LDL) in rat carotid arteries. First, rats were exposed to simulated environmental tobacco smoke for 4 h (3.3 mg/m^3 TPM; 18 ppm CO; 615 µg/m^3 nicotine) to obtain simulated tobacco smoke-plasma). Second, carotid arteries from unexposed rats were perfused with control plasma containing fluorescently labelled LDL and subsequently with tobacco smoke-plasma containing fluorescently labelled LDL. Photometric measurements were made during perfusion with labelled LDL. Perfusion with tobacco smoke-plasma increased the rate of LDL accumulation measured as fluorescence intensity (6.9 ± 1.8 mV/min (mean ± SEM)) when compared with control animals (1.6 ± 0.40 mV/min, $p < 0.01$). The maximal increase was observed after 40–60 min perfusion. LDL accumulation was primarily dependent on the interaction of tobacco smoke-plasma with LDL, which occurred before perfusion, rather than interaction with the artery wall. It was also noted that LDL accumulation resulted from its increased binding to artery wall rather than an increase in its permeability. Perfusion with tobacco smoke-plasma increased the lumen volume measured as fluorescence intensity (43.3 ± 5.1 mV versus 35.1 ± 4.4 mV; $p < 0.05$) in treated and untreated animals, respectively.

Rabbits receiving a 0.5% cholesterol diet and exposed for 6 h/day, 5 days/week for 10 weeks, to sidestream smoke (24 mg/m^3 TPM; 45 ppm CO) were compared with control animals. There was no difference in serum lipids between cholesterol fed and control animals. Exposure to sidestream smoke significantly increased the percentage of surface lipid lesions in the aorta (54 ± 5% versus 39 ± 4%; $p = 0.049$) and in the pulmonary artery (66 ± 4% versus 43 ± 3%; $p < 0.001$). Exposure to nicotine-free cigarettes (35 mg/m^3 TPM; 53 ppm CO) had the same effects as standard cigarettes. Vascular tension was measured in intact aortic rings. Endothelium-dependent and endothelium-independent relaxation were measured with acetylcholine and the calcium ionophore A23187, and nitroglycerin, respectively. There were no significant differences with any treatment between exposed and control animals (Sun *et al*., 2001).

Female ApoE-deficient mice, which are used as a mouse model of human atherosclerosis, were exposed to sidestream smoke (25 mg/m^3 TPM) 6 h/day, 5 days/week for 7, 10 and 14 weeks. There were no consistent differences in serum concentrations of cholesterol between control mice and those exposed to sidestream smoke. In exposed mice, atherosclerotic lesions in the aorta covered a larger part of the intimal area at all time points than in non-exposed mice. Also the total affected area increased at a higher rate than in controls. The increase was most evident in the thoracic region. Lesions appeared thicker, as reflected by increased amounts of esterified and unesterified cholesterol in the aortic tissues of mice exposed to sidestream smoke (Gairola *et al*., 2001).

(iii) *Immunological effects*

BALB/c mice, sensitized with aluminium hydroxide-precipitated ovalbumin (OVA/AL) antigen, were exposed for 6 h/day, 5 days/week to simulated environmental tobacco smoke (1 mg/m^3 TPM; 6.1 ppm CO; 269 µg/m^3 nicotine), from days 15 to 58 after sensitization. Sensitized mice, with or without smoke exposure, had elevated levels of IgE. Exposure to simulated environmental tobacco smoke enhanced and prolonged the IgE response in sensitized mice, and the levels were significantly increased at all time points at which measurements were made (day 19 to day 58); the concentrations of OVA-specific IgG1 were elevated in the smoke-exposed group from days 34 to 54. For both IgE and IgG1 the increase was strongest at 54 days. The numbers of eosinophils were increased in the blood and lungs of smoke-exposed, pre-sensitized mice. The total number of bronchoalveolar lavage cells was increased ($p = 0.016$); about 90% of the increase was due to alveolar macrophages. The concentrations of cytokines IL-4 and IL-10 were significantly higher in the smoke-exposed group than in the control animals. The demonstration of an exaggerated inflammatory response in sensitized mice may have relevance to the early events in carcinogenesis where an inflammatory response often precedes mild hyperplasia (Seymour *et al*., 1997).

(iv) *Effects on gastric ulceration*

A smoke chamber was designed to investigate the effects of exposure to secondhand smoke on gastric ulceration. Different concentrations of cigarette smoke (0%, 1%, 2% and 4%) were perfused during one hour into a chamber in which male Sprague-Dawley rats were placed. This exposure potentiated ethanol (70% v/v, oral administration)-induced gastric mucosal damage and increased serum nicotine concentrations, but did not affect the pH, pCO_2 or pO_2 and the concentration of HCO_3 in blood, or the systemic blood pressure and heart rate. Under these experimental conditions, exposure to cigarette smoke produced no significant changes in the blood acid/base balance or stress in the animals, but significantly potentiated ethanol-induced gastric mucosal damage. This experimental model is suitable for studying adverse interactions between passive smoking and alcohol drinking in gastric ulcer formation in rats (Chow *et al*., 1996).

(*b*) *Effects of perinatal exposure*

(i) *Effects on lung development and lung function*

Exposure of Sprague-Dawley rats to aged and diluted sidestream smoke from birth (1 mg/m^3 TPM; 6 h/day, 5 days/week) significantly reduced the labelling index of epithelial cells in distal airways at 7 and 14 days of age, but not at later times or in proximal bronchi (Ji *et al.*, 1994).

Gestating Sprague-Dawley rats were exposed to aged and diluted sidestream smoke (1 mg/m^3 TPM; 4.9 ppm CO) from gestational day 5 until gestational day 14, 18 or 21. Maternal exposure to sidestream smoke significantly increased fetal expression of Clara cell secretory protein and mRNA in the terminal bronchioles at gestational day 21, but not at gestational day 14 or 18 (Ji *et al*., 1998).

A series of studies was designed to determine the effects of perinatal exposure to sidestream smoke on airway reactivity in Sprague-Dawley rats. Female rats were exposed 6 h/day, 5 days/week from day 2 of life to week 8 or week 15 of age (1 mg/m^3 TPM; 6.5 ppm CO). Exposure to sidestream smoke did not change the ratio of lung weight/body weight or the baseline values for lung resistance, dynamic compliance or pulmonary artery pressure. Airway reactivity to methacholine was also unaffected at either time-point (all $p > 0.2$). In animals exposed from day 2 of life to week 11 of age, sidestream smoke reduced airway ($p = 0.004$), but not pulmonary artery ($p = 0.63$) reactivity to serotonin (Joad *et al*., 1993). In a further study, Joad *et al*. (1999) exposed rats to aged and diluted sidestream smoke (1 mg/m^3 TPM; 6.9 ppm CO) for 4–6 h/day from gestational day 3 until 21 days of age. The airway responsiveness of one female pup from each litter was assessed at 8 weeks of age. Perinatal exposure to sidestream smoke did not affect baseline lung function, but enhanced methacholine-induced changes in lung resistance (three-fold increase; $p = 0.02$), dynamic compliance ($p = 0.004$), and pulmonary pressure ($p = 0.007$). These changes occurred in the absence of any increase in pulmonary neuroendocrine cells, neuroepithelial bodies or mast cells. In another study, rats were exposed prenatally and/or postnatally to sidestream smoke (1 mg/m^3 TPM; 4.9 ppm CO; 344 µg/m^3 nicotine) for 4 h/day, 7 days/week from gestational day 3 until 7–10 weeks of age. Pulmonary pressure was not affected by any type of exposure. Prenatal or postnatal exposure alone did not affect baseline values or metacholine-induced changes in lung responsiveness. Prenatal followed by postnatal exposure to sidestream smoke reduced dynamic lung compliance at baseline ($p = 0.0006$) and increased lung responsiveness to methacholine ($p = 0.0001$). This reaction was accompanied by an increase in the number of neuroendocrine cells and neuroepithelial bodies (Joad *et al.*, 1995a).

Male guinea-pigs were exposed 6 h/day, 5 days/week from age 8 days to age 37–48 days (1 mg/m^3 TPM; 5.6 ppm CO; 586 µg/m^3 nicotine). Exposure to sidestream smoke did not change lung morphology, collagen or elastin deposition, lung volume, surface area or mean linear intercept length of alveolar airspace. Baseline dynamic lung compliance ($p = 0.05$), but not lung resistance ($p = 0.61$) was increased by exposure to sidestream smoke (see also Section 4.2.2(*b*)(iii)) (Joad *et al.*, 1995b).

(ii) *Cardiovascular effects*

Sprague-Dawley rats were exposed to filtered air or sidestream smoke (33 mg/m^3 TPM; 60 ppm CO) for 6 h/day, 5 days/week, for 3 weeks before birth and/or for 12 weeks in the neonatal to adolescent period. Exposure to sidestream smoke postnatally increased endothelin-1 levels in plasma ($p = 0.001$) independently of in-utero exposure. Infarct size (infarct mass/risk area × 100) was greater in all animals exposed postnatally than in unexposed controls ($p = 0.005$), and was greater in males than in females ($p < 0.001$) (Zhu *et al*., 1997).

In rats exposed under the same conditions, aortic rings were excised and isometric force responses to phenylephrine, acetylcholine, the calcium ionophore A23187 and nitroglycerin were studied in organ baths. In-utero exposure to sidestream smoke increased the

sensitivity of aortic rings to phenylephrine ($p < 0.0005$) and reduced the half-maximal contraction (EC_{50}; $p = 0.04$). It reduced the maximal endothelium-dependent relaxation response to acetylcholine ($p = 0.04$) and increased its half-maximal contraction value ($p = 0.05$). Finally, in-utero exposure decreased the sensitivity to the endothelium-independent vasodilator nitroglycerin ($p = 0.003$). The sensitivity of aortic rings to phenylephrine was reduced after neonatal exposure ($p = 0.01$) (Hutchison *et al*., 1998).

(iii) *Neurological effects*

Female Sprague-Dawley rats were exposed to filtered air or sidestream smoke for 4 h/day, 7 days/week from day 3 of gestation until birth and/or for 9 weeks postnatally (1 mg/m^3 TPM; 4.9 ppm CO; 344 μg/m^3 nicotine). Postnatal exposure to sidestream smoke increased the mortality of the pups during the first 18 days of life ($p < 0.001$) and significantly reduced body weights at 9 weeks of age ($p = 0.016$). In-utero exposure had no effect on DNA, protein, or cholesterol concentration or on the weight of forebrain or hindbrain. Postnatal exposure reduced DNA concentration in the hindbrain, an indicator of cellular density, by 4.4% ($p < 0.001$) and increased the hindbrain protein/DNA ratio, an index of cell size, by 8.4% ($p = 0.001$). The weight of the hindbrain was not affected by exposure to sidestream smoke (Gospe *et al*., 1996).

Rhesus monkeys were exposed to aged and diluted sidestream smoke (1 mg/m^3 TPM; 5.3 ppm CO; 190 μg/m^3 nicotine) from gestational day 100 until 70–80 days after birth. Expression of beta-adrenergic and m2-muscarinic cholinergic receptors in heart and lungs of the offspring were not changed by exposure to smoke. Whereas there were no changes in the heart, a strong induction of adenylyl cyclase was observed in the lungs (Slotkin *et al*., 2000).

To mimic fetal and childhood exposure to secondhand smoke, gestating Sprague-Dawley rats were exposed to mainstream smoke (29 mg/m^3 TPM; 94 ppm CO; 4600 μg/m^3 nicotine) for 6 h/day, 7 days/week from gestational days 5 to 20. One to two days after delivery, dams and pups were exposed to sidestream smoke (1 mg/m^3 TPM; 5.6 ppm CO; 117 μg/m^3 nicotine) until postnatal day 21. Animals were exposed either prenatally, postnatally, or both. Prenatal and/or postnatal exposure significantly increased total adenylyl cyclase activity in brain and heart when monitored with the direct enzymatic stimulant forskolin (see Section 4.1.2(*a*)(iii) for details). In the brain, the specific coupling of beta-adrenergic receptors to adenylyl cyclase was inhibited in all exposed animals, despite normal expression of beta-receptors. In the heart, a decrease in m2-receptor expression was observed after postnatal or continuous exposure, but no inhibition of beta-adrenergic receptors was seen. In both tissues, and for all parameters, the effects of combined prenatal and postnatal exposure were equivalent to those seen in response to postnatal exposure alone (Slotkin *et al*., 2001).

In a series of studies, guinea-pigs were exposed to sidestream smoke (1 mg/m^3 TPM; 6.2 ppm CO; 224 μg/m^3 nicotine) for 6 h/day, 5 days/week from age 1 to 6 weeks (age equivalent of human childhood). Sidestream smoke reduced capsaicin-induced changes in lung resistance ($p = 0.02$) and lung dynamic compliance ($p = 0.04$), indicating a down-

regulation of the lung C-fibre reflex response (Joad *et al.*, 1995b). Primary bronchopulmonary C-fibres were tested for their responsiveness to chemical and mechanical stimuli. Exposure to sidestream smoke had no effect on baseline activity of C-fibres but augmented the responsiveness to left atrial injection of capsaicin ($p = 0.047$) and to lung hyperinflation ($p = 0.03$) (Mutoh *et al.*, 1999). A study on the impulse activity of bronchopulmonary C-fibre-activated nucleus tractus solitarii neurons showed that exposure to sidestream smoke significantly augmented the peak ($p = 0.02$) and duration ($p = 0.01$) of the neuronal response to C-fibre activation, and prolonged the expiratory time (apnoea) ($p = 0.003$), at the higher dose of capsaicin (2.0 µg/kg). Exposure to sidestream smoke did not alter baseline values or capsaicin-induced changes in tracheal pressure, arterial blood pressure or heart rate (Mutoh *et al*., 2000). A recent study presented data to suggest that actions of the neuropeptide substance P in the nucleus tractus solitarius may contribute to these effects (Bonham *et al.*, 2001).

In summary, exposure of experimental animals to sidestream smoke can produce changes that are similar to those observed in response to exposure of humans to secondhand tobacco smoke, such as inflammatory changes in the airways and accelerated formation of arteriosclerotic plaques. The results obtained from studies of perinatal exposure may provide a potential mechanism to explain the association between exposure to secondhand smoke and sudden infant death syndrome.

4.3 Reproductive, developmental and hormonal effects

4.3.1 *Humans*

(*a*) *Reproductive effects*

There are inherent ambiguities in the interpretation of data on reproductive effects: if involuntary smoking in women is defined in terms of household exposure to secondhand smoke, reproductive effects could be due either to the exposure to secondhand smoke of the female or to a direct effect of active smoking on the fertility of the male partner. In most of the published studies, the effects of secondhand smoke have been estimated on the basis of paternal smoking. A possible direct effect of smoking on the father's sperm cannot be ruled out when the father has been the source of exposure to secondhand smoke (Lindbohm *et al.*, 2002).

(i) *Fertility and fecundability*

The available data regarding the effects of passive smoking by women on fertility and fecundity are conflicting (US DHHS, 2001): some studies have reported an increased risk of delayed conception (Hull *et al*., 2000), whereas others have found no association (US DHHS, 2001). The results of investigations of the association between passive smoking during the prenatal period or childhood and later fertility have also been inconsistent: in some studies such exposure has been associated with reduced fecundability (in the case of prenatal exposure) or an *increased* fecundability (in the case of childhood exposure),

whereas others have reported no association (Weinberg *et al.*, 1989; Wilcox *et al.*, 1989; Jensen *et al.*, 1998; US DHHS, 2001; Lindbohm *et al.*, 2002). These investigations are particularly hampered by potential exposure measurement error, confounding factors and other biases.

(ii) *Pregnancy outcomes*

The data regarding the association between maternal exposure to secondhand smoke and preterm birth are not entirely consistent, but in aggregate they suggest a modestly increased risk associated with high exposure (Lindbohm *et al*., 2002).

(iii) *Birth outcomes*

The adverse effect of cigarette smoking on birth weight is well-established; on average, women who smoke cigarettes deliver term infants that weigh about 150–250 g less than those of nonsmokers (Andres & Day, 2000; US DHHS, 2001). When expressed as relative risks, mothers who smoke have more than a doubled risk for having low-birth-weight babies (US DHHS, 2001). There is a similar association between maternal smoking and delivery of small-for-gestational-age infants (US DHHS, 2001). The association is characterized by a dose–response relationship that persists after adjustment for possible confounding factors, and seems to be more pronounced for older mothers (US DHHS, 2001).

Numerous studies have also investigated the association between fetal growth and maternal passive smoking (US DHHS, 2001). On average the birth weight of infants born to nonsmoking mothers exposed to secondhand smoke after adjustment for important potential confounders seems to be about 25–50 g lower than that of babies born to mothers who were not exposed (California EPA, 1997; Windham *et al.*, 1999a; US DHHS, 2001; Lindbohm *et al.*, 2002). However, some studies have not found such an adverse effect (Sadler *et al*., 1999; US DHHS, 2001).

There has been little investigation of the association between maternal exposure to secondhand smoke and spontaneous abortion or stillbirth. The available data are inconsistent, although some studies have shown effects as large as those seen in investigations of the effects of active smoking (Windham *et al*., 1999b; US DHHS, 2001; Lindbohm *et al*., 2002).

The association between maternal smoking during pregnancy and the risk of birth defects has been extensively investigated (US DHHS, 2001). When the focus is on major defects as a single end-point, generally no association has been found. When separate classes of malformations are considered individually, there are indications that maternal smoking during pregnancy is associated with oral clefts, limb reductions, and perhaps malformations of the urinary tract (US DHHS, 2001).

(*b*) *Body weight*

In contrast to the extensive and consistent literature describing the effects of active smoking on body weight, findings on the effects of passive smoking on weight are sparse.

The available data suggest that women exposed to secondhand smoke weigh more than women who are not exposed. However, this association may be confounded by a more sedentary and less healthy lifestyle being adopted by nonsmokers exposed to secondhand smoke (Cress *et al.*, 1994; Thornton *et al.*, 1994; Bernstein, 1996).

(*c*) *Hormones*

No data regarding involuntary exposure to tobacco smoke and levels of estrogens, androgens or vitamin D were available to the Working Group. In one study cord blood from mothers who smoke seemed to contain higher concentrations of insulin-like growth factor 1 (IGF-1) than specimens obtained from nonsmoking mothers (Beratis *et al.*, 1994). In other studies, a decreased concentration of IGF-1 in cord blood has been reported (Heinz-Erian *et al.*, 1998; Coutant *et al.*, 2001).

(*d*) *Menopause*

Three studies addressed the association between exposure to secondhand smoke and age at menopause. One study reported that passive smoking was associated with an advancement in the age at menopause similar to that reported for active smoking, but the study population was small (Everson *et al*., 1986). More recent investigations reported no association between exposure to secondhand smoke at home and age at menopause (Cramer *et al*., 1995; Cooper *et al*., 1999).

4.3.2 *Experimental systems*

A few studies have reported the effects of exposure of gestating female animals to sidestream smoke on embryo implantations, size of litters, mortality rate or body weight of pups in the first weeks of life.

Female hamsters were exposed to the smoke of 1, 2 or 3 cigarettes, twice a day, 7 days/week, from 14 days before mating until the third day of pregnancy. Transport of pre-implantation embryos through the hamster oviduct was retarded in exposed females at all three doses. In a study of exposure to a single dose of smoke, the rate of oviductal muscle contraction decreased significantly within 15 min of exposure and failed to return to baseline rates during a 25-min recovery period. Both pre-implantation embryo transport and muscle contraction were more sensitive to sidestream smoke than to mainstream smoke (see monograph on tobacco smoke, Section 4.3) (DiCarlantonio & Talbot, 1999).

Rats were exposed for 2 h/day, from days 1 to 20 of gestation, to the smoke of 10 king-size cigarettes/2 h [exposure concentrations not reported]. Exposure to smoke significantly reduced food consumption of gestating dams. The average fetal weight ($n = 8$) in the animals exposed to sidestream smoke was reduced to 91% of the pair-fed control values ($p < 0.05$). Litter size and proportion of resorptions were not significantly affected. Combination of smoke with alcohol had a synergistic effect that led to significantly smaller litter size and fetal weight than in pair-fed animals [no comparison was made with smoke exposure alone] (Leichter, 1989). In another study, gestating Sprague-Dawley rats

were exposed to aged and diluted sidestream smoke (1 mg/m^3 TPM; 5.5 ppm CO; 405 µg/m^3 nicotine) for 6 h/day on days 3, 6–10 and 13–17 of pregnancy and killed on day 20. Maternal body weight gain, average daily food consumption and the number of fetuses and of implantation sites per litter were comparable between smoke-exposed and pair-fed controls. However, there was a small, but significant reduction in mean pup weight ($p < 0.05$). This was not accompanied by any significant decrease in fetal ossification, an index of gestational age (Rajini *et al*., 1994). In a similar study, animals were exposed for 6 h/day to aged and diluted sidestream smoke (1 mg/m^3 TPM) from day 3 to day 11 of gestation. Average pup weight per litter was not affected by exposure to smoke, but the average number of implantations and of live pups per litter were significantly lower ($p < 0.05$) in the smoke-exposed animals (Witschi *et al.*, 1994).

Gestating rats were exposed for 3 weeks before delivery to sidestream smoke, 4 cigarettes/15 min, 6 h/day, 5 days/week. Mortality at birth was higher in the exposed animals than in controls (11.9% versus 2.8%; $p < 0.001$), and body weights at 3 and 4 weeks of age were lower than those of controls ($p < 0.001$) (Zhu *et al.*, 1997). In a later study under the same conditions, mortality at birth was also greater in rats exposed *in utero* than in those not exposed (12% versus 3%, $p < 0.001$), but in-utero exposure did not reduce body weight at 4 weeks (Hutchison *et al.*, 1998). Other studies found that exposure of rats to aged and diluted sidestream smoke (1 mg/m^3 TPM) *in utero* did not affect fetal body weight at any gestational age (Ji *et al.*, 1998) or at birth (Joad *et al.*, 1999).

4.4 Genetic and related effects

4.4.1 *Humans*

(*a*) *Mutagenicity, sister chromatid exchange and* HPRT *mutations*

(i) *Urinary mutagenicity*

Mutagenicity of urine from smokers has been detected by use of the *Salmonella* (Ames) mutagenicity test in a large number of studies ever since the first report by Yamasaki and Ames (1977). Urinary mutagenicity correlates significantly with the number of cigarettes smoked daily and with urinary nicotine and/or cotinine concentrations (e.g. Bartsch *et al*., 1990; Vermeulen *et al*., 2000).

Urine voided by nonsmokers exposed to secondhand tobacco smoke or to diluted sidestream smoke also shows bacterial mutagenicity (Bos *et al.*, 1983; Sorsa *et al*., 1985; IARC, 1986; Kado *et al*., 1987; Bartsch *et al*., 1990; Smith, C.J. *et al*., 2000; Vermeulen *et al*., 2000). This is in accordance with the mutagenicity of sidestream smoke and samples of airborne particulate matter or the vapour-phase of the air collected from environments contaminated with secondhand tobacco smoke (see Section 4.4.2). However, the increase in urinary mutagenicity was small in many of the studies (Sorsa *et al*., 1985; Husgafvel-Pursiainen *et al*., 1987; Kado *et al*., 1987; US Environmental Protection Agency, 1992; Smith C.J. *et al*., 2000), and no increase in mutagenicity was found in the urine of volunteers (nonsmokers) after 8 h of exposure to the gaseous phase of second-

hand tobacco smoke or to whole secondhand tobacco smoke under experimental conditions (Scherer *et al*., 1990). The small increases in urinary mutagenicity that have been detected are subject to confounding from dietary, occupational and environmental exposures. Such confounding factors affect the sensitivity and specificity of the assay for secondhand tobacco smoke exposure in the same manner as they do in smokers (Sasson *et al*., 1985; Malaveille *et al*., 1989; US Environmental Protection Agency, 1992; Scherer *et al*., 1996; Vermeulen *et al*., 2000).

Increased urinary mutagenicity was clearly associated with exposure to secondhand tobacco smoke in two studies that used urinary cotinine concentrations to indicate exposure to tobacco smoke in smokers and nonsmokers (Bartsch *et al*., 1990; Vermeulen *et al*., 2000). Bartsch *et al.* (1990) found urinary mutagenicity to be a specific indicator of exposure to secondhand tobacco smoke. In the study by Vermeulen *et al.* (2000), an increase in urinary mutagenicity was found to follow an increase in urinary cotinine in a manner similar to that seen in smokers. In fact, cotinine-adjusted urinary mutagenicity levels showed an almost identical increase for both nonsmokers exposed to secondhand smoke and smokers (Vermeulen *et al*., 2000).

(ii) *Sister chromatid exchange*

Significantly higher levels of sister chromatid exchange, chromosomal aberrations and micronuclei have been found in cultured peripheral lymphocytes of smokers than in nonsmokers (IARC, 1986). However, studies on nonsmokers exposed to secondhand tobacco smoke in experimental or field conditions, where cotinine measurements were used as indicators of exposure and uptake, have shown predominantly negative results for sister chromatid exchange in cultured lymphocytes of peripheral blood (Sorsa *et al*., 1985; Collman *et al*., 1986; Husgafvel-Pursiainen, 1987; Husgafvel-Pursiainen *et al*., 1987). A study of 106 adult nonsmokers who were divided into two groups according to whether they experienced high or low levels of exposure to secondhand tobacco smoke as determined from plasma cotinine levels, found no difference in sister chromatid exchange frequencies between the two groups (Gorgels *et al*., 1992). More recently, sister chromatid exchange was investigated in 109 preschool children, aged 1–6 years, whose mothers or other persons living in the same household smoked. Exposure to secondhand tobacco smoke at home, based on interview data and plasma cotinine measurements, was found to be associated with an almost significant increase in sister chromatid exchange ($p = 0.076$) when compared with the level measured in children living in nonsmoking households. The increase paralleled statistically significant increases ($p < 0.05$) in 4-ABP-haemoglobin and PAH-albumin adducts in the children exposed to secondhand smoke (Tang *et al*., 1999).

The frequencies of sister chromatid exchange in cord blood lymphocytes from mothers who smoked or who were exposed to secondhand tobacco smoke were not elevated when compared with those in non-exposed mothers (Sorsa & Husgafvel-Pursiainen, 1988). Chromosomal aberrations were not increased in nonsmoking waitresses and

waiters exposed to secondhand tobacco smoke who had increased cotinine levels (Sorsa *et al*., 1989).

In summary, studies on sister chromatid exchange have found marginal effects in nonsmokers exposed to secondhand tobacco smoke. However, the lack of sensitivity of the assay for exposure to low doses of this complex mixture needs to be taken into account.

(iii) HPRT *gene mutations*

Smokers have been found to have higher frequencies of *HPRT* mutant lymphocytes than nonsmokers in most of the populations studied (Ammenheuser *et al*., 1997; Curry *et al*., 1999; see monograph on tobacco smoke). A set of studies has been conducted on *HPRT* mutations in the newborns of mothers who were exposed or not exposed to secondhand tobacco smoke. After an initial study that found no difference between *HPRT* mutant frequencies in T lymphocytes from the cord blood of infants born to mothers exposed to secondhand tobacco smoke and to non-exposed mothers (Finette *et al*., 1997), the same authors carried out another study, in which the types of *HPRT* mutations were investigated (Finette *et al*., 1998). Maternal exposure was based on self-reported smoking status, interview data on exposure to secondhand tobacco smoke at home or at work and on measured concentrations of cotinine in cord blood plasma. Analysis of 30 *HPRT* mutants from 12 infants whose mothers were classified as not exposed and 37 mutant isolates from 12 infants born to mothers who were exposed found a significant difference between the mutation spectra in these groups. The difference was attributed to *HPRT* exon 2–3 deletions, which are mutational events presumably mediated by illegitimate combinatorial rearrangement of multiple V (variable), D (diversity) and J (junctional) coding gene segments (V(D)J) recombinase activity (Finette *et al*., 1998).

In another study, cord blood T lymphocytes from 60 newborns were investigated and were found not to show an independent effect of (self-reported) maternal exposure to secondhand tobacco smoke on *HPRT* mutant frequencies. However, the exon 2–3 deletions comprised 26.3 and 28.6% of all mutants in cord blood of infants of mothers who were not exposed or were passively exposed to secondhand smoke, respectively. In infants born to mothers who smoked, this percentage was 85.7% (Bigbee *et al*., 1999).

(iv) *Other*

A study of lift workers conducted in China examined DNA damage in lymphocytes with the single-cell gel electrophoresis (comet) assay. It was found that in 255 never-smokers, the tail moment in the assay was significantly increased by any reported exposure to secondhand tobacco smoke at home or at work. Analysis of covariance showed a significant, independent effect of domestic, but not of occupational, exposure to secondhand tobacco smoke, measured by the number of smokers nearby, on the comet tail moment (Lam *et al*., 2002).

(*b*) *Mutations in* TP53*,* KRAS *and related genes*

(i) TP53 *gene mutations*

The frequency of mutations of the *TP53* gene is higher in lung tumours from smokers than in those from nonsmokers (as reviewed in Hussain & Harris, 1998; Hernandez-Boussard *et al*., 1999; see monograph on tobacco smoke) and this correlates with lifetime cigarette consumption or duration of smoking (Takeshima *et al*., 1993; Wang *et al*., 1995; Kondo *et al*., 1996; Husgafvel-Pursiainen *et al*., 1999). In addition, a significant difference has been observed between mutation spectra in smokers and nonsmokers (see monograph on tobacco smoke).

Frequencies of *TP53* mutations found in lung cancer tissues from lifetime nonsmokers vary between 10 and 35% (Huang *et al*., 1998; Marchetti *et al*., 1998; Takagi *et al*., 1998; Gealy *et al*., 1999; Husgafvel-Pursiainen *et al*., 2000; Vähäkangas *et al*., 2001). A few studies have investigated lung tumours from patients who, as determined from interview data, were lifetime nonsmokers who had experienced long-term exposure to secondhand tobacco smoke at home and compared the mutation frequencies with those recorded in lifetime nonsmokers without exposure to secondhand smoke (Husgafvel-Pursiainen *et al*., 2000; Vähäkangas *et al*., 2001). Life-long nonsmokers studied as a single group (i.e. irrespective of exposure to secondhand tobacco smoke) were found to have a significantly lower prevalence of *TP53* mutations than smokers (odds ratio, 2.9; 95% CI, 1.2–7.2; n = 91 for never-smokers) (Husgafvel-Pursiainen *et al*., 2000) or ex-smokers (odds ratio, 9.1; 95% CI, 2.1–40.0; n = 117 for never-smokers) (Vähäkangas *et al*., 2001). When the prevalence of mutations in the lifetime nonsmokers who reported exposure to secondhand tobacco smoke from spousal smoking was compared with that in cases who reported no exposure to secondhand tobacco smoke at home, mutations were more common in exposed cases who reported exposure from spousal smoking (odds ratio, 2.0; 95% CI, 0.5–8.7; based on six exposed cases with mutation and 42 exposed cases without mutation) (Husgafvel-Pursiainen *et al*., 2000). In addition, the predominant type of mutation detected in nonsmokers was GC→AT transition, but the number of mutations was too small to allow comparisons to be made between the exposure groups (Husgafvel-Pursiainen *et al*., 2000; Vähäkangas *et al*., 2001).

(ii) KRAS *mutations*

Mutations of the *KRAS* gene (codons 12, 13 or 61) occur in approximately 30% of lung adenocarcinomas obtained from smokers (Rodenhuis *et al*., 1988; Slebos *et al*., 1991; Husgafvel-Pursiainen *et al*., 1993; Westra *et al*., 1993; Gealy *et al*., 1999). Studies that have looked for *KRAS* mutations in lung tumours from nonsmokers (typically codon 12) have found low frequencies of mutation: 0% (0/35) (Marchetti *et al*., 1998), 5% (2/40) (Rodenhuis & Slebos, 1992), 7% (2/27) (Westra *et al*., 1993), 9% (2/23) (Gealy *et al*., 1999) and 11% (13/117) (Vähäkangas *et al*., 2001). Only Vähäkangas *et al.* (2001) studied *KRAS* mutations in lifetime nonsmokers exposed to secondhand tobacco smoke: of the 13 nonsmokers with a *KRAS* mutation in codon 12, seven had been exposed to secondhand smoke and six had not.

(*c*) *Polymorphisms in xenobiotic metabolizing genes*

Many studies have investigated smokers for associations between polymorphisms of genes involved in xenobiotic metabolism, proposed as markers of susceptibility, and various end-points of genotoxicity and related effects (Vineis & Malats, 1999). However, many of the data from such studies are contradictory and were frequently based on small numbers. Few studies have addressed the influence of genetic polymorphisms on nonsmokers exposed to secondhand tobacco smoke, and no firm conclusion can be drawn regarding the influence of polymorphisms on smoking-associated biomarkers.

4.4.2 *Experimental systems*

(*a*) *In-vitro studies on genotoxicity*

The genotoxicity of whole sidestream smoke or fractions of sidestream smoke or secondhand tobacco smoke has been investigated in many studies. Sidestream smoke or secondhand tobacco smoke collected from indoor environments has been shown to be mutagenic in the *Salmonella* (Ames) mutagenicity assay (Husgafvel-Pursiainen *et al*., 1986; Löfroth & Lazaridis, 1986; Ling *et al*., 1987; Claxton *et al*., 1989; Doolittle *et al*., 1990) as reviewed by Sorsa and Löfroth (1989). Condensates of mainstream smoke and cigarette smoke were mutagenic in the presence of S9 activation systems (IARC, 1986; see monograph on tobacco smoke), and some studies found that sidestream smoke or secondhand tobacco smoke also induced bacterial mutagenicity in the absence of S9 (Ling *et al*., 1987; Claxton *et al*., 1989). One study that found that secondhand tobacco smoke condensate induced mutations in the *Salmonella* assay also observed a genotoxic response in the SOS chromotest with *Escherichia coli* (Chen & Lee, 1996). Another study investigated the particulate matter of secondhand tobacco smoke collected near the breathing zone of nonsmoking individuals and detected mutagenicity that correlated with the concentrations of nicotine in air (Kado *et al*., 1991).

Several studies have shown sidestream smoke, secondhand tobacco smoke and their fractions to be potent inducers of sister chromatid exchange in Chinese hamster ovary cells in the presence and absence of metabolic activation (Husgafvel-Pursiainen *et al*., 1986; Salomaa *et al*., 1988; Doolittle *et al*., 1990). Other studies have reported smaller effects (e.g. Chen & Lee, 1996). Sidestream smoke has also been found to induce chromosomal aberrations, but not *Hprt* gene mutations, in Chinese hamster ovary cells (Doolittle *et al*., 1990).

(*b*) *In-vivo studies on genotoxicity*

Studies in rodents have indicated in-vivo genotoxicity of sidestream smoke, or of the combination of sidestream smoke and mainstream smoke, as reviewed by IARC (1986). Various studies have been conducted on the clastogenic effects of sidestream cigarette smoke in mice or rats under whole-body or nose-only exposure conditions. In mice exposed to sidestream smoke in an exposure chamber (whole-body exposure), a signifi-

cant increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow was observed (Mohtashamipur *et al*., 1987). Similarly, sidestream smoke and mainstream smoke condensates injected either separately or in a mixture increased the formation of micronuclei in polychromatic erythrocytes in treated mice in a dose-dependent manner (Mohtashamipur *et al*., 1988). In agreement with chemical analyses showing that the concentrations of several genotoxic and carcinogenic substances are higher in sidestream smoke than in mainstream smoke, sidestream smoke condensate induced significantly more micronuclei than mainstream smoke condensate. This difference was more pronounced in animals pretreated with the enzyme inducer Arochlor 1254 (Mohtashamipur *et al*., 1988).

Aged and diluted sidestream smoke was not found to induce chromosomal aberrations in alveolar macrophages in rats after nose-only exposure for 7 days (Lee *et al*., 1992), or after 28 days or 90 days (Lee *et al*., 1993). More recently, whole-body exposure of rats to a mixture of mainstream (11%) and sidestream (89%) cigarette smoke for 28 consecutive days was found to induce DNA adducts and cytogenetic damage in all tissues examined. The frequencies of micronucleated and polynucleated pulmonary alveolar macrophages as well as those of micronucleated polychromatic erythrocytes in bone marrow were significantly increased in animals exposed to sidestream smoke when compared with sham-exposed animals (Izzotti *et al*., 2001).

4.5 Mechanistic considerations

Biological measurements have demonstrated uptake and metabolism of tobacco smoke constituents in nonsmokers who reported regular exposure to secondhand tobacco smoke. In particular, cotinine concentrations measured in the body fluids of nonsmokers have provided both qualitative and quantitative evidence of exposure to secondhand tobacco smoke. In addition, the presence of tobacco-specific nitrosamines and their metabolites in the urine of nonsmokers exposed to secondhand tobacco smoke, with a correlation between the metabolites and cotinine concentration in the urine, provides clear evidence of the exposure of nonsmokers to carcinogenic constituents of tobacco smoke. The results of current studies on individual variation due to environmental or genetic factors are insufficient to permit conclusions regarding the influence of these factors on the response of people to exposure to secondhand tobacco smoke.

Evidence is provided in this monograph for the genotoxicity of secondhand tobacco smoke in humans. Exposure of nonsmokers to secondhand tobacco smoke has often been demonstrated by measurements of both cotinine and protein adducts. Studies analysing somatic mutations in the *TP53* and *KRAS* genes in lung tumours from life-long nonsmokers have suggested that the mutation burden in nonsmokers who are exposed to secondhand tobacco smoke may be higher than that in nonsmokers who have not been exposed. These observations in humans are supported by the findings from animal studies and other experimental systems that have demonstrated the genotoxicity of sidestream smoke (a major component of secondhand tobacco smoke), of a mixture of mainstream

and sidestream smoke, and of secondhand tobacco smoke collected in indoor environments.

The evidence from studies of nonsmokers exposed to secondhand tobacco smoke, supported by other data from experimental systems, is compatible with the current concept of tobacco-related carcinogenesis. According to this concept, tobacco smoke carcinogens, regardless of the type of smoke in which they occur, are associated with genetic effects that disrupt crucial biological processes of normal cellular growth and differentiation in smokers as well as in nonsmokers (see monograph on tobacco smoke).

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