RESEARCH PAPER

Carcinogen derived biomarkers: applications in studies of human exposure to secondhand tobacco smoke

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Objective: To review the literature on carcinogen derived biomarkers of exposure to secondhand tobacco smoke (SHS). These biomarkers are specifically related to known carcinogens in tobacco smoke and include urinary metabolites, DNA adducts, and blood protein adducts.
Method: Published reviews and the current literature were searched for relevant articles.
Results: The most consistently elevated biomarker in people exposed to SHS was 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronides (NNAL-Gluc), urinary metabolites of the tobacco specific lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). The tobacco specificity of this biomarker as well as its clear relation to an established lung carcinogen are particularly appropriate for its application in studies of SHS exposure.
Conclusion: The results of the available carcinogen derived biomarker studies provide biochemical data which support the conclusion, based on epidemiologic investigations, that SHS causes lung cancer in non-smokers.

The International Agency for Research on Cancer (IARC) will soon publish a report on secondhand tobacco smoke (SHS).[1] It concludes that SHS causes lung cancer in humans. This conclusion agrees with the evaluations of other groups which have previously examined this issue.[2–6] It is based on over 50 epidemiologic studies of involuntary smoking and lung cancer risk in never smokers. These studies are bolstered by biochemical data demonstrating carcinogen uptake in non-smokers exposed to SHS. These biomarker studies are the subject of this review.

SHS, also known as environmental tobacco smoke, is produced mainly by the release of smoke from the burning tip of a cigarette between puffs (sidestream smoke, or SS) and the smoke exhaled by the smoker (exhaled mainstream smoke). Small additional contributions come from the tip of the cigarette and through the cigarette paper during a puff, and through the paper and mouth end of the cigarette between puffs.[7] Similar considerations apply to other forms of tobacco smoking such as cigars and pipes.

Figure 1 presents a framework for considering mechanisms of lung cancer induction by SHS. An analogous scheme has been proposed as an outline of lung cancer induction in smokers.[8] Carcinogens are responsible for the cancer causing effects of tobacco smoke. It is very likely that the broad mechanisms of cancer induction by SHS and mainstream cigarette smoke are similar because the same carcinogens are present in both, although in different relative concentrations. The major difference is that the carcinogen dose from SHS exposure is significantly less than that from smoking.

CARCINOGENS IN SS AND SHS

Constituents of cigarette mainstream smoke and SS have been discussed in a number of publications.[7 9 10] Table 1 summarises representative levels of carcinogens in SS and SHS.[10] Structures of the organic compounds are shown in fig 2. Table 1 includes only compounds that have been evaluated by IARC and for which there is sufficient evidence of carcinogenicity in either laboratory animals or humans. Many of these compounds have also been evaluated by the US National Toxicology Program.[3] It also includes only compounds for which there are published data on levels in

SS or SHS. The amounts of each constituent are taken from representative publications. A number of other tobacco smoke carcinogens which have been evaluated by IARC are not included in table 1 because there are no published data on their levels in SS or SHS. It is likely, however, that these compounds are also present. In addition, there may be carcinogens present that have not been fully characterised or evaluated by IARC.

Polycyclic aromatic hydrocarbons (PAH) are a diverse group of carcinogens formed in the incomplete combustion of organic material. These carcinogens are found in tobacco smoke, broiled foods, and polluted environments of various types. Workers in iron and steel foundries and aluminium production plants are exposed to PAH. These exposures are thought to be the cause of excess cancers in these settings.[11 12] Benzo[*a*]pyrene (BaP) is the best known member of this class of compounds. PAH are potent locally acting carcinogens in laboratory animals. They induce tumours of the upper respiratory tract and lung when administered by inhalation, instillation in the trachea, or implantation in the lung.[13 14] When administered orally, BaP does not generally cause lung tumours in mice and rats, but rather causes tumours of the digestive tract.[15–18]

N-Nitrosamines are a large group of carcinogens which induce cancer in a wide variety of species and tissues. There is no reason to assume that humans should be resistant to the effects of these carcinogens.[19] They are present at low concentrations in foods and can be formed endogenously from amines and nitrogen oxides.[20] Tobacco smoke contains volatile *N*-nitrosamines such as *N*-nitrosodimethylamine and *N*-nitrosopyrrolidine as well as tobacco-specific *N*-nitrosamines

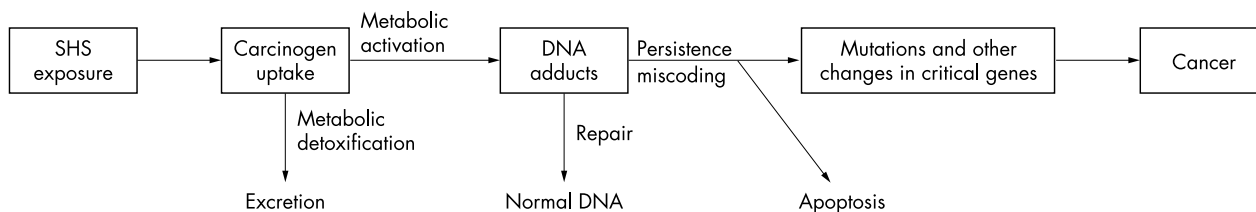...

Figure 1 Scheme showing the steps which would link secondhand smoke (SHS) exposure and cancer via tobacco smoke carcinogens.

such as N′-nitrosonornicotine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK).[21] Tobacco specific N-nitrosamines are chemically related to nicotine and other tobacco alkaloids and are therefore found only in tobacco products or related materials.[22] Many N-nitrosamines are powerful carcinogens in laboratory animals. They display striking organospecificity, affecting particular tissues, often independent of the route of administration.[19] For example, N′-nitrosonornicotine causes tumours of the oesophagus and nasal cavity in rats, while the principal target of NNK in
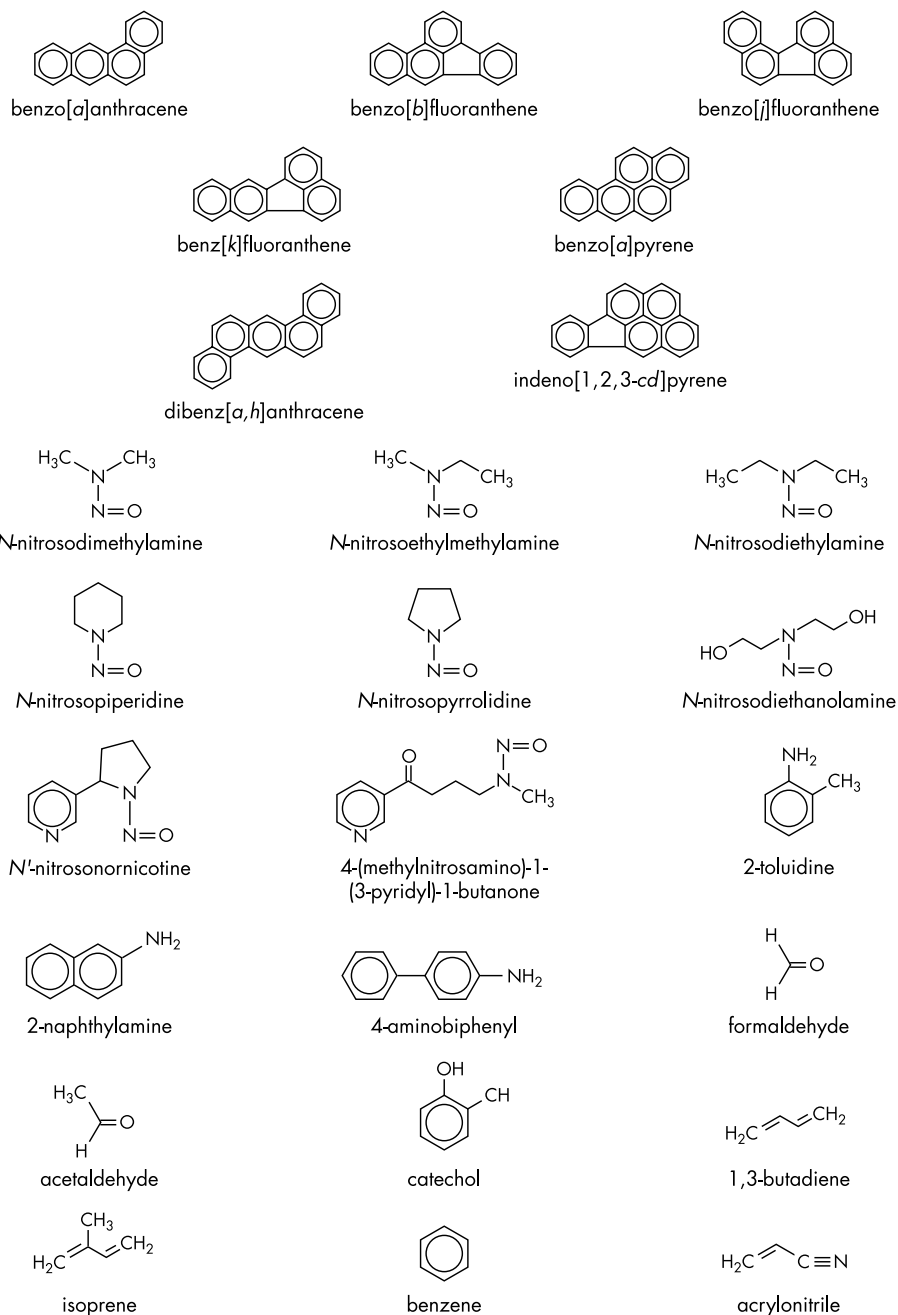

Figure 2 Structures of the organic carcinogens in sidestream smoke and secondhand tobacco smoke (see table 1).

Table 1 Carcinogens in sidestream smoke (SS) and secondhand smoke (SHS) of cigarettes*

Carcinogens	Representative amounts		
	SS (per cigarette)	SHS (per m^3)	References
Polycyclic aromatic hydrocarbons (PAH)			
benz[a]anthracene	201 ng	0.32–1.7 ng	84, 85
Benzo[b]fluoranthene			84, 85
Benzo[j]fluoranthene }	196 ng	0.79–2.0 ng	84, 85
Benzo[k]fluoranthene			84, 85
Benzo[a]pyrene	45–103 ng	0.37–1.7 ng	84–86
Indeno[1,2,3-cd]pyrene	51 ng	0.35–1.1 ng	84, 85
Dibenz[a,h]anthracene		1 ng	87
N-nitrosamines			
N-nitrosodimethylamine	143–1040 ng	4–240 ng	88–90
N-nitrosoethylmethylamine	3–35 ng		88, 90
N-nitrosodiethylamine	8.2–73 ng	20–200 ng	88, 90
N-nitrosopiperidine	4.8–19.8 ng		86
N-nitrosopyrrolidine	7–700 ng	3.5–27.0 ng	88–91
N-nitrosodiethanolamine	43 ng		92
N'-nitrosonornicotine (NNN)	110–857 ng	0.7–23 ng	86, 89, 93, 94
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)	201–1440 ng	0.2–29.3 ng	86, 89, 93, 94
Aromatic amines			
2-toluidine	3030 ng		95
2-naphthylamine	63.1–128 ng		96
4-aminobiphenyl	11.4–18.8 ng		96
Aldehydes			
Formaldehyde	233–485 μg	143 μg	96, 97
Acetaldehyde	961–1820 μg	268 μg	96, 97
Miscellaneous organics			
Catechol	98–292 μg	1.24 μg	96–98
1,3-butadiene	98–205 μg	0.3–40 μg	96, 97, 99, 100
Isoprene	668–1260 μg	657 μg	96, 97
Benzene	163–353 μg	4.2–63.7 μg	68, 96, 97, 99, 100
Acrylonitrile	42–109 μg		96
Inorganic compounds			
Hydrazine	94 ng		101
Nickel	51 ng		96
Chromium	57–79 ng		96
Cadmium	330–689 ng	4–38 ng	96, 102
Lead	28.9–46.6 ng		96
Polonium-210	0.091–0.139 pCi		103

*Adapted from Hoffmann *et al.*[10]

rodents is the lung. NNK is the only tobacco smoke carcinogen that induces lung tumours systemically in all three commonly used rodent models—rat, mouse, and hamster.[23]

Aromatic amines were first identified as carcinogens as a result of industrial exposures that occurred in the dye industry. Among these, 2-naphthylamine and 4-aminobiphenyl are well established human bladder carcinogens.[24 25] Aromatic amines cause tumours at a variety of sites in laboratory animals. Some members of this class such as 2-toluidine are only weakly carcinogenic.[26]

Formaldehyde and acetaldehyde induce respiratory tract tumours in rodents when administered by inhalation.[27 28] They are weaker carcinogens than PAH, N-nitrosamines, and aromatic amines, but their levels in SS and SHS are thousands of times higher. Butadiene and benzene are volatile hydrocarbons which also occur in considerable quantities in SS and SHS. Butadiene is a multi-organ carcinogen, with particular potency in mice, while benzene causes leukemia in humans.[29–31] Metals such as nickel and cadmium are human carcinogens which are also present in SS.[32 33]

There are also reports that SS contains free radicals in about the same concentration as mainstream smoke.[34] The gas phase is reported to have reactive yet long lived radicals while the particulate phase apparently has a free radical system which is an equilibrium mixture of semiquinones, hydroquinones, and quinones.[35] It is not known whether such agents can induce tumours in laboratory animals.

CARCINOGEN DERIVED BIOMARKERS: APPLICATIONS IN SHS STUDIES

Figure 1 shows that carcinogens undergo metabolism which may lead either to detoxification and excretion or to activation to a more reactive form that can bind to DNA. Most carcinogens in SHS require metabolism for binding to DNA, although some will react directly. Covalent binding to DNA results in production of ''DNA adducts'' in which the carcinogen metabolite is chemically bound to one of the DNA bases or to phosphate. This binding is critical to the carcinogenic process. There are cellular repair mechanisms which can remove these adducts and return the DNA to its normal form, but these are not always efficient. If the adducts persist during DNA replication, miscoding can occur leading to permanent mutations. Apoptosis, or programmed cell death, removes some mutated cells. If the mutations occur in critical genes such as oncogenes and tumour suppressor genes, loss of normal cellular growth control processes can result and, ultimately, cancer occurs. The constant barrage of DNA damaging carcinogens experienced upon exposure to SHS is completely consistent with the multiple genetic changes known to occur in lung cancer and other cancers. These genetic changes are known to be associated with six proposed ''hallmarks of cancer'': self sufficiency in growth signals; evasion of apoptosis; insensitivity to anti-growth signals; sustained angiogenesis; tissue invasion and metastasis; and limitless replicative potential.[36]

Carcinogen derived biomarkers, which are analytes directly related to specific carcinogens, are produced during the

Table 2 Representative carcinogen derived biomarker studies of people exposed to SHS

Entry No.	Carcinogen and exposure data, if available	Biomarker concentrations	Significant difference? (SHS exposed v non-exposed)	Reference
1	PAH	1-HOP 0.140 µg/24 h in 19 SHS exposed people (urinary cotinine 12.3 µg/24 h) v 0.171 µg/24 h in 23 non-exposed (urinary cotinine 2.3 µg/24 h)	No	40
2	PAH	BaP, 21.5 ng/m³, phenanthrene, 6.8 ng/m³, pyrene 17.6 ng/m³ in an experimental room with 5 smokers and 5 non-smokers	BaP-Hb adducts 0.049 fmol/mg Hb in SHS exposed, 0.083 fmol/mg Hb in non-exposed (same individuals as above) BaP-albumin adducts 0.021 fmol/mg in SHS exposed, 0.019 in non-exposed (same individuals as above) No effects on urinary hydroxyphenanthrenes (2.0 v 2.2 µg/24 h before and after SHS exposure); no effects on urinary 1-HOP (0.24 µg/24 h before and after SHS exposure); no effects on ^{32}P-postlabelling of DNA adducts	No	41
3	PAH	No differences in PAH-albumin levels in umbilical cord blood from women exposed to SHS (n=49) v non-exposed (n=54)	No	48
4	PAH	No effect of SHS on PAH-albumin adduct levels in 73 individuals from Aarhus, Denmark	No	47
5	PAH	5 non-smokers exposed to SHS from 100 cigarettes (100–180 µg/m³ nicotine in the room) over an 8 h period. No effect on urinary hydroxyphenanthrenes	No	44
6	PAH	No difference in 1-HOP levels in urine of children exposed to SHS (n=286) or not exposed (n=126) from their parents' smoking	No	42
7	PAH and 4-amino-biphenyl	Significantly higher levels of 4-aminobiphenyl-Hb adducts and PAH-albumin adducts in children whose mothers smoked (n=23 for 4-aminobiphenyl-Hb, n=44 for PAH-albumin) compared to unexposed children (n=10 for 4-aminobiphenyl-Hb, n=24 for PAH albumin)	Yes	46
8	NNK	75–263 ng/m³ in a 16 m³ room	Significantly increased levels of NNAL plus NNAL-Gluc in urine of 5 men after exposure to SHS	Yes	52
9	NNK		Significantly increased levels of NNAL-Gluc in hospital workers (n=9) exposed to SHS compared to controls	Yes	54
10	NNK	2.4–50 ng/m³ in 19 rooms where smoking took place	NNAL+NNAL-Gluc levels correlated with nicotine on personal sampler in SHS exposed subjects	Yes	56
11	NNK		NNAL+NNAL-Gluc levels significantly higher in women (n=23) who lived with men who smoked compared to women (n=22) who lived with men who did not smoke	Yes	55
12	NNK		34% of 204 children with cotinine >5 ng/ml urine; 52/54 of these samples had detectable NNAL+NNAL-Gluc. Levels of NNAL+NNAL-Gluc significantly higher in SHS exposed than in non-exposed children	Yes	53
13	4-Aminobiphenyl		Higher 4-aminobiphenyl-Hb adducts (27.8 pg/g Hb) in 9 pregnant women with >2.0 µg/m³ nicotine (personal exposure) than in 0.5–1.9 µg/m³ (n=20, 20.8 pg/g Hb) than in <0.5 µg/m³ (n=7, 17.6 pg/g Hb)	Yes	57
14	4-Aminobiphenyl and other aromatic amines		No relation of aromatic amine-Hb adducts to reported SHS exposure or cotinine/creatinine ratios in 73 pregnant women	No	51
15	4-Aminobiphenyl and other aromatic amines		No increase of aromatic amine-Hb adducts with increased exposure to SHS, confirmed by cotinine, in 224 children	No	60
16	Benzene (geometric means)	16.5 (2.3) µg/m³, personal exposure (non-smokers, no SHS) 25.4 (2.9) µg/m³, (non-smokers, SHS)	tt-MA 38.9 (2.4) µg/l (n=42) 54.7 (2.9) µg/l (n=27)	Yes	65
17	Benzene	2–100 µg/m³, personal exposure (n=69 non-smokers from smoking and non-smoking households)	tt-MA not correlated with benzene. Marginal difference in tt-MA, non-smokers from smoking homes compared to non-smoking homes	No	67
18	Benzene	11.5 µg/m³, personal exposure (non-smokers, non-smoking homes, n=39) 13.6 µg/m³ (non-smokers, smoking homes, n=43)	tt-MA 92 µg/g creatinine 126 µg/g creatinine	No	68
19	Benzene	11.5 µg/m³, personal exposure (children, smoking homes, n=24) 19.7 µg/m³, (children, non-smoking homes, n=15)	tt-MA 130 µg/g creatinine 112 µg/g creatinine	No	66

Table 2 Continued

Entry No.	Carcinogen and exposure data, if available	Biomarker concentrations	Significant difference? (SHS exposed v non-exposed)	Reference
20	Benzene	t-MA 3.84 (1.6) ng/µl in 53 SHS exposed children 4.02 (1.1) ng/µl in 26 non-exposed 3.53 (1.4) ng/µl when urinary cotinine ≤44 ng/ml (n=39) 4.32 (1.4) ng/µl when urinary cotinine ≥44 ng/ml (n=39)	No	64
21	Benzene	<0.19–22 µg/m³, personal exposure, in 5 females exposed to SHS	t-MA 34–74 µg excreted on non-exposure days 42–95 µg excreted on exposure days	Yes	63
22	Ethylene oxide		Ethylene oxide-Hb adducts not different in 28 subjects exposed to SHS v 74 non-exposed	No	70
23	Unknown		No difference in 8-OHdG levels in leucocytes of adults unexposed (n=36), exposed 1–4 h/day to SHS (n=35), and exposed >4 h/day (n=21)	No	71
24	Unknown		No difference in placental levels of 8-OHdG in 10 non-smokers v 9 non-smokers exposed to SHS, validated by plasma and urine cotinine. No effects of SHS on adducts detected by ^{32}P-postlabelling	No	72
25	Unknown		Significantly higher (63%) levels of 8-OHdG in blood DNA of subjects exposed to SHS (n=38) in the workplace, by plasma cotinine than in unexposed (n=36)	Yes	73
26	Unknown		5 non-smokers exposed to SHS in an unventilated room, 4091 µg/m³ RSP. Marginal, non-significant increase in urinary thioethers observed	No	41
27	Unknown		No difference in urinary thioethers between low SHS (n=23) and high SHS (n=23) exposed individuals based on plasma cotinine. No difference in urinary thioethers between low SHS (n=20) and high SHS (n=19) exposure in the home	No	80
28	Unknown		No effects of SHS exposure on ^{32}P-postlabelled DNA adducts in monocytes of 5 non-smokers exposed for 8 h	No	77
29	Unknown		No effect of SHS exposure on ^{32}P-postlabelled DNA adducts in white blood cells of women (n=31 exposed, 11 non-exposed)	No	76
30	Unknown		194 students in Athens and 77 subjects in Halkida; ^{32}P-postlabelled DNA adducts in lymphocytes showed no relation to SHS exposure in entire group but did correlate with SHS exposure measurements in winter in a subgroup living in Halkida campus area	No/Yes	78
31	Unknown +PAH	total PAH 42.3 ng/m³	No significant increase in ^{32}P-postlabelled adducts in sputum or lymphocytes of 15 non-smokers who spent time in smoky pubs	No	79
32	Unknown		No effect of SHS exposure on oxidised plasma proteins and decreased levels of nitrated plasma proteins	No	74

1-HOP, 1-hydroxypyrene; 8-OHdG, 8-hydroxydeoxyguanosine; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNAL-Gluc, a mixture of 4-(methylnitrosamino)-1-(3-pyridyl)-1-(O-β-D-glucopyranuronosyl)butane and 4-(methylnitrosamino)-1-(3-pyridyl)-1-(3-pyridyl)-1-(O-β-D-glucopyranuronosyl)-1-butanolonium inner salt; t-MA, trans, trans-muconic acid.
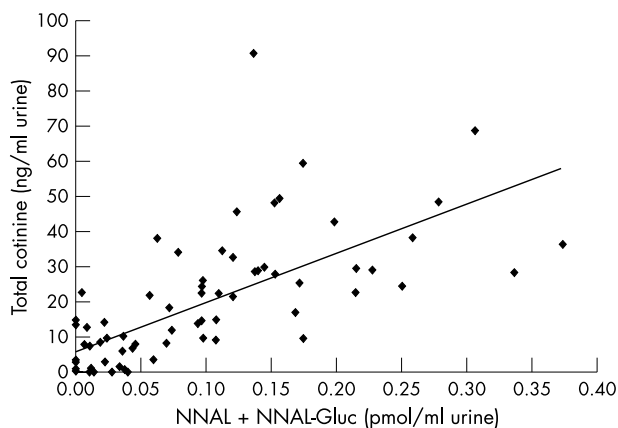
Figure 3 Correlation between levels of cotinine plus cotinine-glucuronide and NNAL plus NNAL-Gluc in the urine of 74 school aged children exposed to SHS ($r = 0.69$, $p < 0.0001$).[53]

events outlined in fig 1. These biomarkers have the potential to provide information on cancer risk.[37] Three main types of carcinogen derived biomarkers have been measured in humans exposed to SHS: carcinogens and their metabolites in urine; DNA adducts in white blood cells and sputum; and adducts to haemoglobin or albumin in blood. Carcinogens and their metabolites in urine provide information about carcinogen dose. DNA adduct measurements give an indication of dose to DNA, the critical target in carcinogenesis. Haemoglobin and albumin adducts are not directly involved in the carcinogenic process, but they are often used as surrogates for DNA adducts, because they are more readily measured and in many cases their levels correlate with those of DNA adducts. This review will discuss carcinogen derived biomarkers as applied to studies of SHS exposure. Studies on cotinine, a major metabolite of the non-carcinogen nicotine widely used in investigations of SHS exposure, will not be discussed here except as they relate to carcinogen derived biomarkers.[38]

Table 2 summarises data on human uptake of specific carcinogens from SHS, as determined by measurement of carcinogen derived biomarkers. These studies provide a link between SHS exposure and uptake of actual carcinogens to which people are exposed. This topic has been reviewed previously.[39]

Several methods have been used to estimate PAH uptake in humans exposed to SHS. 1-Hydroxypyrene and hydroxyphen-anthrenes are urinary metabolites of pyrene and phen-anthrene, respectively. These metabolites are widely used as biomarkers of PAH uptake although the parent compounds, pyrene and phenanthrene, are non-carcinogenic. Levels of 1-hydroxypyrene and hydroxyphenanthrenes in urine are not increased by exposure to SHS.[40–44] Other factors such as smoking, occupational exposures, and diet are significant contributors to the levels of these compounds in urine.

Metabolites of BaP and other PAH form adducts with haemoglobin and serum albumin. These adducts have been measured by a variety of methods, including immunoassay and gas chromatography-mass spectrometry (GC-MS). Using a relatively non-specific immunoassay technique, one group has found increased levels of PAH-albumin adducts in SHS exposed children,[45 46] but an increment in levels of this marker with SHS exposure was not found in two other studies.[47 48] An effect of SHS exposure on albumin and haemoglobin adducts of BaP was not found in a recent study which used GC-MS as the detection method.[40] Thus, the evidence that SHS exposure significantly increases human uptake of PAH is inconsistent.

Since tobacco specific *N*-nitrosamines are found only in tobacco products or related nicotine containing materials, their adducts or metabolites should be specific biomarkers of tobacco exposure. Haemoglobin adducts of NNK and NNN can be hydrolyzed to release 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB), which is quantified by GC-MS. In smokers, levels of HPB releasing haemoglobin adducts of NNK and NNN are low compared to haemoglobin adducts of several other carcinogens, possibly because of the high reactivity of the alkylating intermediate.[49 50] Considering the relatively low levels of these adducts in smokers, one would not expect to find significantly elevated amounts in non-smokers exposed to SHS, as reported by Branner et al.[51] Metabolites of NNK are readily measured in the urine of people exposed to SHS. The metabolites 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and its glucuronide conjugates NNAL-Gluc- are quantified by GC with nitrosamine-selective detection (GC-TEA).[52–56] All studies reported to date show significantly higher amounts of NNAL plus NNAL-Gluc, or NNAL-Gluc, in the urine of SHS exposed humans than in unexposed controls (table 2). In one study, uptake of NNK was over six times higher in women who lived with smokers compared to women who lived with non-smokers.[55] In another investigation, widespread uptake of NNK was demonstrated in a group of economically disadvantaged schoolchildren, and the range of levels varied over 90-fold.[53] Most studies to date demonstrate a correlation between levels of cotinine and NNAL plus NNAL-Gluc in urine (fig 3). Cotinine is a reliable biomarker for nicotine uptake by non-smokers exposed to SHS, and has been widely used in studies of SHS exposure.[38] NNAL plus NNAL-Gluc is a biomarker for uptake of the tobacco specific lung carcinogen NNK by non-smokers exposed to SHS. The NNAL plus NNAL-Gluc biomarker is more directly related to cancer risk than cotinine because NNK but not nicotine is carcinogenic. The uptake of NNK, a potent lung carcinogen, by non-smokers exposed to SHS provides a biochemical link between SHS exposure and lung cancer.

Aromatic amines such as 4-aminobiphenyl form adducts with haemoglobin that can be quantified by GC-MS. Mixed results have been obtained in studies of the effects of SHS on 4-aminobiphenyl haemoglobin adduct levels. Hammond et al demonstrated that adduct levels were elevated in pregnant

Table 3 Relation of carcinogen derived biomarkers to SHS exposure*

Carcinogen in SHS	Biomarker	Association with SHS exposure	References
PAH	1-Hydroxypyrene in urine Hydroxyphenanthrenes in urine PAH-albumin adducts PAH-haemoglobin adducts	None in most studies	40–43, 45–48
NNK	NNAL and NNAL-Gluc in urine	Consistently increased	52–56
Aromatic amines	Haemoglobin adducts	Mixed results	46, 51, 57–60
Benzene	*trans, trans*-Muconic acid in urine	Mixed results	63–68

*Modified from Scherer and Richter.[39]

women exposed to SHS.[57] Maclure *et al* observed slightly higher levels of haemoglobin adducts of 4-aminobiphenyl and 3-aminobiphenyl in persons with confirmed SHS exposure compared with unexposed persons.[58] 4-Aminobiphenyl haemoglobin adducts were also elevated in children exposed to SHS.[46] However, two other studies, including one of pregnant women, showed no consistent relation between adduct levels and SHS exposure.[51 59] A study in German children also showed no significant increase in aromatic amine haemoglobin adduct levels with increasing SHS exposure; in fact there was a significant decrease in *ortho*- and *meta*-toluidine adducts.[60] There is a background level of aromatic amine haemoglobin adducts in apparently unexposed humans. The origin of this background is unknown, but it could be due in part to uptake of the corresponding nitro compounds from sources such as diesel emissions. Levels of aromatic amines in urine were unaffected by exposure to SHS.[61]

trans,trans-Muconic acid (tt-MA) is a urinary metabolite of benzene which has been widely used to estimate benzene uptake.[62] Mixed results have been obtained in studies on the relation of this metabolite to SHS exposure, with some studies showing somewhat higher levels in people exposed to SHS while others found no effect.[63–68] Interpretation of these findings is complicated by differing excretion rates among individuals and contributions of sources other than benzene, such as sorbate in food, to its levels in urine.[39 63 67] Benzene itself has been quantified in exhaled breath. Breath measurements of non-smokers who reported exposure to smokers at work showed elevated benzene levels, but levels in non-smokers living in homes with smokers were not increased.[69] In a second study, increased levels of exhaled benzene were detected in non-smokers living in homes with smokers compared to non-smokers living with non-smokers.[68] There was no difference in exhaled benzene among children living in homes with smokers or non-smokers.[66] Collectively, the biomarker data discussed here indicate that benzene uptake in humans is not consistently associated with SHS exposure.

Haemoglobin adducts of ethylene oxide can be quantified by GC-MS of terminal *N*-hydroxyethylvaline. There was no difference in levels of these adducts between non-smokers who did not live or work with a smoker compared to those who did.[70]

Several other less specific markers have been explored in studies of SHS exposure. 8-Hydroxydeoxyguanosine (8-OH-dG) is a widely used biomarker of oxidative damage to DNA. In two studies, no increase in 8-OH-dG levels in placenta and leucocytes of individuals exposed to SHS was observed.[71 72] However, in a study of occupational exposure in Reno, Nevada, the average 8-OH-dG level in whole blood DNA of SHS exposed workers was 63% higher than that in non-exposed individuals, a significant difference.[73] Levels of nitrated proteins in blood plasma of non-smokers exposed to SHS were significantly lower than in unexposed non-smokers, and there was no effect of SHS on levels of oxidised proteins.[74] Urinary 3-ethyladenine is a biomarker of ethylating agents. In one study, concentrations of 3-ethyladenine in urine were not increased by exposure to SHS.[75] ^{32}P-Postlabelling is a technique which can estimate levels of hydrophobic DNA adducts. Four investigations did not find effects of SHS exposure on levels of ^{32}P-postlabelled DNA.[41 72 76 77] However, a study conducted in Greece did find a relation between SHS exposure and ^{32}P-postlabelled DNA adducts in lymphocytes in a subgroup of the subjects examined.[78] A recent study demonstrated no significant increase in levels of ^{32}P-postlabelled adducts in induced sputum of individuals exposed to SHS in a pub compared to pre-exposure levels. However, one of the DNA adducts found in the SHS exposed individuals may have been derived from BaP.[79]

What this paper adds

Epidemiologic data support the conclusion that exposure to secondhand tobacco smoke causes lung cancer in non-smokers. Measurement of carcinogen derived biomarkers can further strengthen this conclusion and can provide insights pertinent to mechanisms of cancer induction and modes of cancer prevention.

A review of the use of carcinogen derived biomarkers to assess uptake and metabolism of tobacco smoke carcinogens in people exposed to secondhand tobacco smoke is presented.

Urinary thioethers are conjugates of carbonyl-containing mutagens. Thioethers were not significantly increased as a result of SHS exposure.[41 80] 3-Hydroxypropyl mercapturic acid, possibly from acrolein exposure, was identified as a possible SHS related product in urine.[41] Conflicting results have been obtained in studies of urinary mutagenicity as affected by SHS exposure (reviewed in[41] and[39]). In general, there seem to be small and sometimes significant effects of SHS exposure on urinary mutagenicity when diet is controlled.[80 81] In a study of 1249 Italian women, there was an inverse dose–response relation between intensity of current husband's smoke and concentrations of plasma β carotene and L-ascorbic acid. There was a significant inverse association between urinary cotinine and plasma β carotene.[82]

SUMMARY

An overview of the carcinogen derived biomarkers most widely applied in studies of SHS exposure is presented in table 3. Analyses of biomarkers of PAH uptake and metabolic activation have produced mainly mixed results, probably because there are significant exposures to these carcinogens through the diet and general environment. Similarly, mixed results have been reported in studies of benzene and aromatic amine uptake in people exposed to SHS. In contrast to these mixed results, studies which measured the tobacco specific nitrosamine metabolites NNAL and NNAL-Gluc in the urine of people exposed to SHS have shown consistently elevated levels of these biomarkers. The assay for urinary NNAL and NNAL-Gluc is highly specific to carcinogen exposure from SHS because NNK, the parent compound of these metabolites, is found only in tobacco products. The contribution of non-tobacco sources to all other biomarkers discussed here confounds their validity in SHS studies, where carcinogen exposure is generally relatively low.

Studies of NNAL and NNAL-Gluc levels in non-smokers exposed to SHS have provided some potentially significant insights on the role of SHS as a lung carcinogen. Prominent among these are the results of studies of non-smoking women who lived with men who smoked.[6] The risk for lung cancer in these women is about 20% greater than in non-exposed non-smoking women. The risk for lung cancer in smokers is 15–20 times (1500–2000%) greater than in non-smokers.[83] Therefore, the risk for lung cancer in these non-smoking women exposed to SHS is about 1–2% as great as that of smokers.[6 83] The level of NNAL plus NNAL-Gluc in the urine of the SHS exposed women was about 5.6% as great as that of their smoking partners, consistent with their comparative 1–2% risk for lung cancer compared to smokers.[55] Other studies show a mean of about 0.05 pmol/ml NNAL plus NNAL-Gluc in non-smokers exposed to SHS. This is about 1.6% of the typical levels found in smokers, which is also consistent with the results just discussed. These

carcinogen uptake data provide biochemical support for the role of SHS as a lung carcinogen in non-smokers. The results are particularly relevant because of the established carcinogenicity of NNK to the lungs of rodents, where adenocarcinoma are commonly observed in treated animals, consistent with observations in SHS exposed women.

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