ELSEVIER

Food and Chemical Toxicology 42S (2004) S53–S83

Food and Chemical Toxicology

www.elsevier.com/locate/foodchemtox

An overview of the effects of tobacco ingredients on smoke chemistry and toxicity

Richard R. Baker*, Eian D. Massey, Graham Smith

British American Tobacco, Research and Development Centre, Regent's Park Road, Southampton SO15 8TL, UK

Received 15 August 2003; accepted 9 January 2004

Abstract

This paper presents an overview of a series of studies designed to assess the influence of 482 tobacco ingredients on cigarette smoke chemistry and toxicity. The studies are: pyrolysis of the ingredients; influence of the ingredients on smoke constituents believed by regulatory authorities to be relevant to smoking-related diseases ("Hoffmann analytes"); influence of the ingredients on in vitro genotoxicity and cytotoxicity of smoke partiulate matter; and influence of the ingredients on the inhalation toxicity of smoke. The present paper brings the salient features of these studies together. A pyrolysis technique has been developed which, as far as practicably possible, mimics the combustion conditions inside a burning cigarette. The results from 291 single-substance ingredients indicate that almost a third would transfer out of the cigarette burning zone at least 99% intact (i.e. less than 1% pyrolysis), and almost two thirds would transfer at least 95% intact. Of the ingredients that underwent some degree of pyrolysis, a few "Hoffmann analytes" were detected amongst the pyrolysis products of 19 ingredients. Taking into account maximum use levels, their maximum pyrolysis levels were generally small and often insignificant compared to the levels typically present in smoke. Possible exceptions were acetaldehyde and benzene from the pyrolysis of malic acid. However, subsequent smoke chemistry studies indicated that the maximum levels predicted from pyrolysis of this involatile substance were overestimated, suggesting that malic acid does not undergo complete pyrolysis in the burning cigarette and/or generates acetaldehyde and benzene at similar rates to that of tobacco on a per weight basis. When added to tobacco, many of the ingredient mixtures produced no significant effect on the levels of many of the "Hoffmann analytes" in smoke, while some produced increases or decreases relative to the relevant control cigarettes. The study has concentrated on the increases. Many of the differences were found to be not significant when the long-term variability of the analytical methodology was taken into account. However, even taking this into account, the smoke formaldehyde levels in two of the test cigarettes were significantly increased relative to their controls, by up to 26 μg (73%). These increases are likely to be due to the pyrolysis of sugars, cellulose and other polysaccharide materials. The activity of smoke particulate matter from cigarettes containing tobacco ingredients has been determined with three in vitro bioassays, two for genotoxicity and one for cytotoxicity. These were the Ames test, the mammalian cell micronucleus assay, and the neutral red uptake cytotoxicity assay. Within the sensitivity and specificity of these bioassays, the specific activity of the cigarette smoke particulate matter was not changed by the addition of ingredients to the cigarette. Three 90-day sub-chronic inhalation studies have been undertaken and histopathological and histomorphometric assessments made within the respiratory tracts of animals exposed to smoke from cigarettes containing the various ingredient mixtures and their control cigarettes. The response due to tobacco smoke exposure was not distinguishable between the test and control cigarettes, indicating that the presence of the ingredients had made no discernable differences to the type and severity of the treatment-related changes.
© 2004 Elsevier Ltd. All rights reserved.

Keywords: Ames assay; Casing; Chemical analysis; Cigarette; Constituent; Cytotoxicity; Flavour; Genotoxicity; 'Hoffmann analyte'; Ingredient; Inhalation; In vitro toxicology; In vivo toxicology; Mammalian cell micronucleus assay; Mutagenicity; Neutral red uptake assay; Pyrolysis; Smoke; Sub-chronic inhalation; Tobacco; Toxicity

Abbreviations: BALT, bronchus-associated lymphoid tissue; CAS, Chemical Abstract Service; COHb, carboxyhaemoglobin; CORESTA, Co-operation Centre for Research Relative to Tobacco, Paris; DNA, deoxyribosenucleic acid; EEC, European Economic Commission; IC_{50}, inhibiting concentration that results in a reduction in viability to 50% that of the untreated control cell viability; ICH, International Committee on Harmonisation of technical requirements for the registrations of pharmaceuticals for human use; ISO, International Organisation for Standardisation; *n*, number of replicates; NFDPM, nicotine-free dry particulate matter (commonly called 'tar'); NCI, National Cancer Institute (U.S.A.); NIH, National Institute of Health (U.S.A.); OECD, Organisation for Economic Co-operation and Development; ppm, parts per million; S.D., standard deviation; TPM, total particulate matter; UKEMS, United Kingdom Environmental Mutagen Society; uv, ultra-violet; WHO, World Health Organisation.

* Corresponding author. Tel.: +44-23-8079-3739; fax: +44-23-8079-3076.
E-mail address: Richard_Baker@bat.com (R.R. Baker).

0278-6915/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2004.01.001

1. Introduction

In some parts of the world e.g. Germany and the U.S.A., tobacco companies add ingredients to tobacco products, either to impart a specific taste, flavour or aroma to the product, or for a specific technological purpose such as increasing the moisture-holding capacity of the tobacco. In other countries, e.g. the U.K., Australia and Canada, flavour ingredients are not generally used on cigarettes. Since the 1960s, it has been suggested that the use of such ingredients might increase the toxicity of tobacco smoke. In their classic 1967 book on tobacco smoke, Wynder and Hoffmann (1967) hypothesised that the use of tobacco ingredients might potentially either increase or decrease the toxicity of tobacco smoke. This has been repeated several times since, generally emphasising the possibility of increasing the toxicity of smoke (LaVoie et al., 1980; Hoffmann and Hoffmann, 1997, 2001; World Health Organisation, 2001). In the context of low 'tar' cigarettes, it has also been assumed in the 1979 and 1981 U.S. Surgeon General's reports, as well as by the World Health Organisation, that flavour ingredients are more likely to be used (United States Public Health Service, 1979, 1981; World Health Organisation, 2001). It is emphasised that in these discussions of the potential changes in smoke toxicity through the use of tobacco ingredients, no evidence was presented to support the hypotheses. Furthermore, the increased use of flavour ingredients with low 'tar' cigarettes was an assumption. Low 'tar' cigarettes are developed with a variety of cigarette design options: filtration, filter ventilation, paper permeability, tobacco blend, reconstituted tobacco sheet and expanded tobacco. In general, within British American Tobacco, flavour ingredients are not used any more on low 'tar' cigarettes than on higher yield cigarettes; to do so would produce an 'unbalanced' taste. One exception to this generalisation is in mentholated cigarettes, where menthol usage tends to increase as 'tar' yields decrease.

The hypothesis of increased toxicity has been refuted by work recently published in several papers. Three major reviews were published in 2002 that examined studies on the chemical and biological effects of tobacco ingredients on smoke properties conducted over the last 50 years (Paschke et al., 2002; Rodgman, 2002a, b). Paschke et al. gave an overview of 189 published papers that covered the effects of well over 300 ingredients, not all of which are used commercially. They concluded that the results from most studies clearly indicate that ingredients do not increase the biological activity of cigarette smoke (carcinogenicity, mutagenicity and cytotoxicity) but found many gaps in knowledge on the chemical effects of ingredients. Rodgman examined the extensive laboratory data on the effects of tobacco ingredients on smoke chemistry and various bioassays, in particular previously unpublished information from the R.J. Reynolds Tobacco Company undertaken from the 1950s to the 1970s (Rodgman, 2002a,b). His conclusions were similar to those of Paschke et al., namely that ingredients added to tobacco during commercial cigarette manufacture in the U.S.A. do not increase the toxicity of cigarette smoke.

Also during 2002, Carmines and co-workers published a series of four papers that described a comprehensive study on the evaluation of 333 ingredients used in Philip Morris products. This study included smoke chemistry, in vitro genotoxicity and cytotoxicity, and animal sub-chronic inhalation toxicity (Carmines, 2002; Rustemeier et al., 2002; Roemer et al., 2002; Vanscheeuwijck et al., 2002). Taking into account all the smoke chemistry and biological data, they concluded that the addition of the ingredients to tobacco did not increase the toxicity of the smoke.

Gaworski et al. have also recently published details of studies in which the biological effects of single ingredients, or mixtures of ingredients, added to cigarettes were evaluated (Gaworski et al., 1997, 1998, 1999; Heck et al., 2002). They have assessed the effects of menthol (Gaworski et al., 1997), glycerol (glycerine) and propylene glycol (Heck et al., 2002) singly, and 172 flavour ingredients in six combinations (Gaworski et al., 1998) in various 13-week sub-chronic smoke inhalation toxicity studies with rats. They concluded that the addition of the flavour mixtures or menthol, glycerol and propylene glycol, as single ingredients at typical application levels to cigarettes, had no discernable effect on the character or extent of the biological responses normally associated with inhalation of cigarette mainstream smoke in rats. In 1999 Gaworski et al. also reported the results of a series of SENCAR mouse skin painting bioassays that were aimed at assessing the potential of six combinations of 150 flavour ingredients to affect the tumour-promoting activity of cigarette smoke particulate matter (Gaworski et al., 1999). No indications of any increase in tumour-promoting activity were found for any of the ingredient combinations evaluated.

In 2002 Seeman et al. published a comprehensive review of the formation of acetaldehyde in mainstream cigarette smoke and its bioavailability in the smoker (Seeman et al., 2002). They covered all known literature on the subject, including the effects of sugars as a tobacco ingredient on smoke acetaldehyde levels. They concluded that sugars do not produce greater yields of acetaldehyde in mainstream smoke than are produced from tobacco itself on a weight-for-weight basis. They also concluded that natural tobacco polysaccharides, including cellulose, were the primary precursors of acetaldehyde in mainstream smoke.

Thus, the results from numerous studies have indicated that the addition of either single ingredients, or various combinations of ingredients used commercially,

to tobacco does not adversely change the smoke chemistry or toxicity as measured in specified assays. In spite of this extensive research, the present study was undertaken to assess the effect of cigarette tobacco ingredients at or above typical maximum levels used by British American Tobacco on smoke chemistry and toxicity.

Potentially, ingredients can undertake one or more of the following processes during tobacco combustion in the smoking process:

(a) Distil directly into smoke
(b) Pyrolyse or oxidise and their products enter smoke
(c) Pyrolyse or oxidise and the reaction products react with tobacco and smoke constituents and affect their yields or generate other smoke products.

Because of the possibilities of processes (b) and (c), justification for the use of tobacco ingredients based solely on their approved use in food may not always be appropriate. There are no regulatory requirements for testing tobacco ingredients in the U.S.A. although a few individual states have specific requirements of disclosure. In the European Union there is a requirement for all available information on ingredients relevant to smoke toxicity to be submitted to the individual member states.

In the present paper, an overview is given on studies undertaken to investigate the potential chemical changes and biological activity of smoke from cigarettes with added ingredients used by British American Tobacco. The detailed results of the individual chemical studies have been published elsewhere, as indicated below. This present paper brings the salient features together and includes the biological studies. This covers the following aspects:

1. Determination of the relative amounts of distillation and pyrolysis/oxidation of the ingredients during the combustion of tobacco (Baker and Bishop, 2004).
2. Evaluation of the effect of the ingredients on the generation of known toxic chemical constituents of cigarette smoke (Baker et al., 2004a,b).
3. Evaluation of the effect of ingredients on the potential of cigarette smoke particulate matter to damage DNA (genotoxicity) as measured by:
   (a) the Ames test, an in vitro bacterial mutation assay (Massey et al., 2002)
   (b) the in vitro micronucleus test, a mammalian cell assay for chromosome damage.
4. Evaluation of the effect of ingredients on the in vitro cytotoxicity of cigarette smoke particulate matter, as measured by the neutral red uptake assay, a mammalian cell viability test. Cytotoxicity is regarded as having a potential role in several chronic disease processes associated with smoking, including carcinogenesis and emphysema (Bombick, 2002).
5. Evaluation of the effect of ingredients on the potential sub-chronic inhalation toxicity of cigarette smoke.

Individually, each of these assessments may have some limitations. However, collectively they provide comprehensive information on the potential effects of tobacco ingredients on the chemical and toxicological profile of cigarette smoke. Most of the assessments are recommended in the U.K. Guidelines for the assessment of new tobacco ingredients (Report of the Scientific Committee on Tobacco and Health, 1998). Two of the three in vitro bioassay studies and the sub-chronic inhalation toxicity studies were done at external laboratories, as indicated in Sections 2.4 and 2.5 below. These laboratories operated in compliance with the Good Laboratory Practice Regulations as set forth by the U.S. Environmental Protection Agency, Code of Federal Regulations, 40, Part 792 (Code of Federal Regulations, 2001).

2. Materials and methods

2.1. Pyrolysis studies

For the pyrolysis experiments, 200 μg of the flavour ingredient was pyrolysed, usually in solution in ethanol, in the pyroprobe of a CDS Pyrolyser 2000. The products of pyrolysis were separated by passage through a Hewlett Packard 6890 gas chromatograph and identified with a Hewlett Packard 5973 mass spectrometer. The pyrolysis conditions were carefully chosen to simulate the *average* conditions inside the burning zone of the cigarette during smoking. Much is known about these conditions: extensive work conducted in the 1970s and 1980s showed that the cigarette burning zone consists of two regions (Baker, 1975, 1977, 1981a,b, 1999). The exothermic combustion region has depleted oxygen levels, at or near zero throughout much of the combustion zone, temperatures varying from about 700 to 950 °C, and tobacco heating rates up to 500 °C/s. Immediately downstream of the combustion region, the endothermic pyrolysis/distillation region has temperatures of about 100 to 600 °C, heating rates of about 10 to 50 °C/s, and oxygen levels of 5 to 20% v/v. Thus, the pyrolysis conditions used in the present study included a flowing atmosphere of 9% oxygen in nitrogen through the pyrolysis unit (the mean oxygen concentration inside the cigarette burning zone), initially holding the pyroprobe at 300 °C for 5 s (to simulate the interpuff smoulder period), then heating at 30 °C s^{-1} to 900 °C (mean heating rate and temperature range of cigarette burning zone during a puff), and finally holding the temperature at 900 °C for 5 s (maximum duration of

the high burning zone temperature during a puff under extreme human smoking conditions).

2.2. Experimental cigarettes

Mixtures of ingredients were added to typical U.S. blended tobacco by normal manufacturing techniques. The tobaccos were then made into non-ventilated filter cigarettes. The design features of the cigarettes were deliberately chosen to produce relatively high 'tar' yields, approximately 13 mg under the standard ISO machine smoking conditions given in ISO 3308 (2000). This was so that any effects of the ingredients would be maximised and readily observed. A total of 482 ingredients was added in various mixtures to the tobacco at or above their typical maximum levels used on cigarettes sold by British American Tobacco. In order to keep the tobacco mixtures as authentic as possible, three series of cigarettes were used to assess the ingredients on the smoke chemical and biological properties.

(i) A series of cigarettes [coded A1 (control) to A8] in which the control tobacco blend was a typical U.S. blend, including typical reconstituted tobacco sheet containing some ingredients. This was then cased. Flavourings were added to this typical cased blend. The use of this series allowed the effects of flavouring ingredient mixtures to be assessed relative to a typical cased tobacco blend containing reconstituted sheet.
(ii) A series of cigarettes [coded B1 (control) to B5] in which the control tobacco blend contained control tobacco only (no reconstituted sheet or casings). Flavourings and casing ingredients were added to this tobacco blend, as well as specific additives. The use of this series allowed the effects of flavourings, casing ingredients and additives to be assessed relative to a control blend, which contained only tobacco.
(iii) A series of cigarettes [coded C1 (control) to C9] in which the control tobacco blend was a typical U.S. blend containing lamina and reconstituted tobacco sheet containing some ingredients. Casing ingredients were added to this tobacco. The use of this series allowed the effects of casing ingredients to be assessed relative to a typical control blend containing reconstituted tobacco.

A summary of the ingredients used in this study, and which experimental cigarettes they were added to, is given in Table 1. The table also lists the CAS number of the ingredient, its function (flavour, humectant, preservative etc.), the typical maximum level used on cigarettes sold by British American Tobacco on a dry-weight of tobacco basis, and the actual level of the ingredient in the test cigarette. (The minimum quantity of ingredient used in this study was 12 ppm although in some cases the typical maximum application level of some flavouring ingredients in commercial products is as low as 0.05 ppm.) The cigarettes were manufactured in the R&D Centre of British American Tobacco in the U.K. Further details of the experimental cigarettes are given in previous papers (Baker et al., 2004a,b). Additional information on the chemical structures, molecular weights and boiling and/or melting points of many of the flavour ingredients are reported by Baker and Bishop (2004). The combinations of ingredients used in these experimental cigarettes were for experimental convenience. The combinations are not necessarily those used in any commercial cigarette.

2.3. Smoke chemistry studies

The analyses of selected smoke constituents, outlined in Section 3.1 below, were undertaken in the R&D Division of the British American Tobacco company in Rio de Janeiro, Brazil (Souza Cruz). Following conditioning of the cigarettes for 48 h at 22 °C and 60% relative humidity, the cigarettes were machine smoked under the standard ISO smoking regime of one 35-ml puff of 2-s duration taken every minute to a butt length equal to the filter length + 8 mm, under ambient conditions of 22 °C and 60% relative humidity (ISO 3308, 2000). A series of smoking runs was undertaken, the smoke collected and groups of constituents measured in the smoke. TPM (total particulate matter), NFDPM (nicotine-free dry particulate matter, or 'tar'), nicotine and carbon monoxide in the mainstream smoke were all determined by the relevant ISO standard method [ISO Standards 4387 (2000), 10315 (2000), 10362-1 (1999) and 8454 (1995)]. For these smoke analytes, the quoted means and standard deviations are based on eight replicate smoking runs, with 5 cigarettes of each type smoked in each replicate, randomised across the smoking ports. There are no internationally recognised standard methods for the determination of the other "Hoffmann analytes" in smoke. The analytical methodology used for these smoke constituents has been described elsewhere (Baker et al., 2004a). For these smoke analytes, the quoted means and standard deviations are based on five replicate smoking runs, with 2–20 cigarettes of each type smoked in each replicate, dependent on the analyte. The various smoke constituents in the three series of cigarettes, (A, B and C) were measured on separate occasions, approximately 2–3 months apart. Within each cigarette series, various smoking runs were undertaken and groups of smoke analytes (phenols, tobacco-specific nitrosamines, carbonyl compounds, etc.) were measured following each run. For a given smoke constituent, each test cigarette was always measured at the same time as its control cigarette.

Table 1
Ingredients tested in experimental cigarettes

Ingredient Name	CAS Number[a]	Function	Level (ppm)		Test cigarette
			Max. cig. level[b]	Tested[c]	
Acacia gum	9000-01-5	Flavour	15,000	15000	B5
Acetanisole	100-06-1	Flavour	40	47	B2
Acetic acid	64-19-7	Flavour	500	9000	C3
Acetoin	513-86-0	Flavour	140	164	A6
Acetophenone	98-86-2	Flavour	45	53	A2
2-Acetylpyrazine	22047-25-2	Flavour	15	18	A8
2-Acetylthiazole	24295-03-2	Flavour	1	12	A8
Alfalfa extract	84082-36-0	Flavour	34	100	C8
Ambroxide	3738-00-9	Flavour	5	12	A4
Ammonium glycyrrhizinate	53956-04-0	Flavour	52	100	B3
n-Amyl butyrate	540-18-1	Flavour	15	18	A3
α-Amylcinnamaldehyde	122-40-7	Flavour	1	12	B3
Amyl hexanoate	540-07-8	Flavour	0.5	12	A3
Amyris oil	8015-65-4	Flavour	5	12	B2
Angelica root oil	8015-64-3	Flavour	2.5	12	A5
Angelica root tincture/extract	84775-41-7	Flavour	105	234	B3
Anisyl acetate	104-21-2	Flavour	12	23	A7
Anisyl alcohol	105-13-5	Flavour	10	12	A7
Anisyl formate	122-91-8	Flavour	5	12	A7
Anisyl phenylacetate	102-17-0	Flavour	0.5	12	A7
Anisyl propionate	7549-33-9	Flavour	1	12	A7
Apple extract	85251-63-4	Flavour	200	234	B3
Apricot extract	68650-44-2	Flavour	560	600	C5
Ascorbic acid	50-81-7	Process Aid	0.05	12	A3
Balsam oil, Peru	8007-00-9	Flavour	56	66	A6
Banana extract	97281-11-3	Flavour	10	12	B3
Benzaldehyde	100-52-7	Flavour	60	351	A3, B2
Benzaldehyde glyceryl acetal	1319-88-6	Flavour	40	58	B3
Benzoic acid	65-85-0	Preservative	140	164	A5
Benzoin resinoid, Sumatra	84929-79-3	Flavour	45	53	A5
Benzophenone	119-61-9	Flavour	30	35	A7
Benzyl alcohol	100-51-6	Flavour	1700	1990	A7
Benzyl benzoate	120-51-4	Flavour	500	585	A7
Benzyl butyrate	103-37-7	Flavour	20	23	A7
Benzyl cinnamate	103-41-3	Flavour	80	94	A7
Benzyl formate	104-57-4	Flavour	0.5	12	A7
Benzyl isobutyrate	103-28-6	Flavour	10	12	A7
Benzyl isovalerate	103-38-8	Flavour	1	12	A7
Black currant buds, absolute	68606-81-5	Flavour	5	12	A7
Bois de rose oil	8015-77-8	Flavour	1	12	A3
Boronia, absolute	91771-36-7	Flavour	10	12	A7
Brandy, absolute	N/A	Flavour	40	47	B2
Buchu leaf oil	68650-46-4	Flavour	2.5	12	A7
n-Butyl acetate	123-86-4	Flavour	15	18	A3
n-Butyl alcohol	71-36-3	Flavour	2.5	12	A3
n-Butyl butyrate	109-21-7	Flavour	7.5	18	A3
n-Butyl isovalerate	109-19-3	Flavour	10	12	A3
n-Butyl valerate	591-68-4	Flavour	0.5	12	A3
n-Butyric acid	107-92-6	Flavour	110	129	A3
Calcium carbonate	471-34-1	Filler	9000	9000	B5
Cananga oil	68606-83-7	Flavour	2.5	12	A4, B2
Caramel	8028-89-5	Flavour	500	16,000	B3
Caramel sugar syrup	N/A	Flavour	2.5	12	B2
Caraway seed oil	8000-42-8	Flavour	2.5	12	A4
Carboxymethyl cellulose	9000-11-7	Binder	1000	1000	B5
Cardamon oleoresin	977090-82-6	Flavour	10	23	B2
Cardamon seed oil	8000-66-6	Flavour	76	94	A5
Carob bean extract	84961-45-5	Flavour	3400	5000	C8
Carob bean extract (roasted)	84961-45-5	Flavour	3400	7000	B3
Carob bean powder	9000-40-2	Flavour	7000	7000	B3

(*continued on next page*)

Table 1 (*continued*)

Ingredient Name	CAS Number[a]	Function	Level (ppm)		Test cigarette
			Max. cig. level[b]	Tested[c]	
Carvacrol	499-75-2	Flavour	10	12	A5
4-Carvomenthenol	562-74-3	Flavour	1	12	A4
d-Carvone	2244-16-8	Flavour	5	12	B3
l-Carvone	6485-40-1	Flavour	1	12	A4
β-Caryophyllene	87-44-5	Flavour	30	292	A5
Caryophyllene oxide	1139-30-6	Flavour	1	18	A5
Cascarilla bark oil	8007-06-5	Flavour	10	82	A5
Cassia bark oil	8007-80-5	Flavour	30	35	A5
Cassia leaf oil	8007-80-5	Flavour	30	35	B3
Cassia, absolute	89958-31-6	Flavour	0.5	12	A7
Catechu powder	8007-76-1	Flavour	1	12	B3
Celery seed oil	8015-90-5	Flavour	10	12	A5
Celery seed oleoresin	8015-90-5	Flavour	10	12	B2
Cellulose fibre	65996-61-4	Binder	17,000	28,400	B5
Chamomile extract, Roman	8015-92-7	Flavour	530	702	B3
Chamomile oil, Hungarian (charmonile blue)	8002-66-2	Flavour	75	88	B2
Chamomile oil, Roman	8015-92-7	Flavour	78	94	A4
Cherry juice/concentrate	89997-53-5	Flavour	10	12	B3
Chicory extract	68650-43-1	Flavour	30	100	C8
Chocolate	N/A	Flavour	2700	7000	B3, C7
Cinnamic acid	621-82-9	Flavour	2.5	12	B2
Cinnamon bark oil	8015-91-6	Flavour	30	64	A5
Cinnamon bark powder	84961-46-6	Flavour	30	35	B3
Cinnamyl acetate	103-54-8	Flavour	10	12	A7
Cinnamyl alcohol	105-54-1	Flavour	10	12	A7
Cinnamyl cinnamate	122-69-0	Flavour	40	47	A7
Cinnamyl isobutyrate	103-59-3	Flavour	2.5	12	A7
Citral	5392-40-5	Flavour	10	23	A3
Citric acid	77-92-9	Flavour	500	11,400	C5
Citronella oil	8000-29-1	Flavour	2	12	A7
Citronellal	106-23-0	Flavour	1	12	A3
dl-Citronellol	106-22-9	Flavour	10	12	A3
Citronellyl acetate	150-84-5	Flavour	13	18	A3
Clary sage infusion	84775-83-7	Flavour	10	12	B3
Clary sage oil	8016-63-5	Flavour	10	70	A4
Cocoa distillate	84649-99-2	Flavour	100	117	B3
Cocoa extract	84649-99-3	Flavour	20,000	20,000	C7
Cocoa infusion (ethanol extract)	84649-99-1	Flavour	500	585	B3
Cocoa powder	84649-99-0	Flavour	20,000	37,700	B4, C2
Cocoa oleoresin	N/A	Flavour	20	23	B3
Cocoa shell extract	8002-31-2	Flavour	12,000	20,000	B3
Coffee extract (roasted)	93348-12-0	Flavour	100	117	B3
Coffee extract	93348-12-0	Flavour	100	2700	C8
Cognac oil, green	8016-21-5	Flavour	10	88	A6
Cognac oil, white	977050-49-9	Flavour	7.5	88	A6
Coriander oleoresin	N/A	Flavour	20	23	B2
Coriander seed oil	8008-52-4	Flavour	560	795	A5
Corn syrup	68131-37-3	Flavour	62,000	62,000	C7
Corn syrup, high fructose	8029-43-4	Flavour	62,000	62,000	C2
Cumin seed powder	8014-13-9	Flavour	10	12	B3
Cumin seed oil	8014-13-9	Flavour	2.5	12	A5
Cuminaldehyde	122-03-2	Flavour	5	12	A5
Cyclamen aldehyde	103-95-7	Flavour	0.05	12	B2
β-Damascenone	23696-85-7	Flavour	30	35	A7
β-Damascone	35044-68-9	Flavour	15	18	A7
Davana oil	8016-03-3	Flavour	10	12	A7
γ-Decalactone	706-14-9	Flavour	15	18	A7
δ-Decalactone	705-86-2	Flavour	15	18	A7
Decanal	112-31-2	Flavour	5	12	B3
Decanoic acid	334-48-5	Flavour	15	18	B2
Dextrin	9004-53-9	Flavour	1	12	B3

(*continued on next page*)

Table 1 (*continued*)

Ingredient Name	CAS Number[a]	Function	Level (ppm)		Test cigarette
			Max. cig. level[b]	Tested[c]	
Diacetyl	431-03-8	Flavour	15	18	A6
Diammonium hydrogen phosphate	7783-28-0	Flavour	10,000	10,000	B2
Diethyl malonate	105-53-3	Flavour	7.5	12	B3
2,3-Diethylpyrazine	15707-24-1	Flavour	0.5	12	A8
Dill herb oil	8006-75-5	Flavour	10	12	A4
Dill seed oil	8016-06-6	Flavour	1	12	A4
m-Dimethoxybenzene	151-10-0	Flavour	7.5	12	B3
p-Dimethoxybenzene	150-87-7	Flavour	22	29	A5
2,6-Dimethoxyphenol	91-10-1	Flavour	7.5	12	A5
3,4-Dimethyl-1,2-cyclopentadione	13494-06-9	Flavour	15	18	A6
2,5-Dimethyldihydrofuranolone	3658-77-3	Flavour	20	35	A3, B2
4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one	28664-35-9	Flavour	7.5	12	A6
3,7-Dimethyl-6-octenoic acid	502-47-6	Flavour	0.05	12	A3
2,3-Dimethylpyrazine	5910-89-4	Flavour	15	18	A8
2,5-Dimethylpyrazine	123-32-0	Flavour	15	18	A8
2,6-Dimethylpyrazine	108-50-9	Flavour	1	18	A8
γ-Dodecalactone	2305-05-7	Flavour	10	12	A7
δ-Dodecalactone	713-95-1	Flavour	140	164	A7
Ethanol	64-17-5	Solvent	As needed	23,400	A3–A8, B2–B4
p-Ethoxybenzaldehyde	10031-82-0	Flavour	0.05	12	A5
Ethyl acetate	141-78-6	Flavour	220	643	A6
Ethyl acetoacetate	141-97-9	Flavour	7.5	12	A6
4-Ethylbenzaldehyde	4748-78-1	Flavour	5	12	A7
Ethyl benzoate	93-89-0	Flavour	10	12	A7
Ethyl *trans*-2-butenoate	10544-63-5	Flavour	8	12	A8
Ethyl butyrate	105-54-4	Flavour	250	292	A6
Ethyl cinnamate	103-36-6	Flavour	15	18	A7
Ethyl decanoate	110-38-3	Flavour	5	47	B3
2-Ethyl-3,[5 or 6]-dimethylpyrazine	13925-07-0	Flavour	15	18	A8
4-Ethylguaiacol	2785-89-9	Flavour	2.5	12	A5
Ethyl heptanoate	106-30-9	Flavour	20	64	A6
Ethyl hexanoate	123-66-0	Flavour	10	64	A6
3-Ethyl-2-hydroxy-2-cyclopenten-1-one	21835-01-8	Flavour	0.5	12	A6
5-Ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone	698-10-2	Flavour	2.5	12	B2
2-Ethyl-4-hydroxy-5-methyl-3(2*H*-)-furanone	27538-09-6	Flavour	1	12	A6
Ethyl isobutyrate	97-62-1	Flavour	0.5	140	B3
Ethyl isovalerate	108-64-5	Flavour	10	18	A6
Ethyl lactate	97-64-3	Flavour	5	12	B3
Ethyl laurate	106-33-2	Flavour	7.5	187	A3, B2
Ethyl maltol	4940-11-8	Flavour	35	117	A3
Ethyl-2-methylbutyrate	7452-79-1	Flavour	2.5	12	B3
3-Ethyl-2-methylpyrazine	15707-23-0	Flavour	15	18	A8
Ethyl myristate	124-06-1	Flavour	5	12	A6
Ethyl nonanoate	123-29-5	Flavour	10	12	A6
Ethyl octanoate	106-32-1	Flavour	7	47	A6, B2
Ethyl palmitate	628-97-7	Flavour	0.05	12	A6
p-Ethylphenol	123-07-9	Flavour	0.05	12	A8
Ethyl phenylacetate	101-97-3	Flavour	30	35	A7
Ethyl propionate	105-37-3	Flavour	30	316	A6
Ethyl salicylate	118-61-6	Flavour	1	12	B2
Ethyl valerate	539-82-2	Flavour	10	12	A6
Ethyl vanillin	121-32-4	Flavour	250	2920	A2
Eucalyptol	470-82-6	Flavour	1	35	A5, B3
Farnesol	4602-84-0	Flavour	2.5	12	A3
Fenugreek extract	84625-40-1	Flavour	175	200	C8
Fenugreek oil	84625-40-1	Flavour	5	12	B3
Fenugreek oleoresin	84625-40-1	Flavour	10	12	B2
Fenugreek tincture	84625-40-1	Flavour	500	585	B3
Fig extract	68916-52-9	Flavour	800	11,700	C8
Fig juice concentrate	90028-74-3	Flavour	800	234	B3
Galbanum extract (resinoid)	9000-24-2	Flavour	1	12	B3

(*continued on next page*)

Table 1 (*continued*)

Ingredient Name	CAS Number[a]	Function	Level (ppm)		Test cigarette
			Max. cig. level[b]	Tested[c]	
Galbanum oil	8023-91-4	Flavour	2	12	A4
Geraniol	106-24-1	Flavour	10	23	A7
Geranium oil	8000-46-2	Flavour	30	35	A4
Geranyl acetate	105-87-3	Flavour	5	12	A7
Geranyl acetone	3796-70-1	Flavour	7.5	12	A7
Geranyl butyrate	106-29-6	Flavour	5	12	A7
Geranyl formate	105-86-2	Flavour	5	12	A7
Ginger oil	8007-08-7	Flavour	57	70	A5
Ginger oleoresin	84696-15-1	Flavour	15	18	B2
Glycerol	56-81-5	Humectant	70,000	70,000	B5, C4
Grapefruit oil, expressed	8016-20-4	Flavour	2.5	12	A3
Grapefruit oil, terpeneless	90045-43-5	Flavour	1	12	B3
Guaiac wood oil	8016-23-7	Flavour	1	12	A5
Guaiacol [*o*-Methoxyphenol]	90-05-1	Flavour	7.5	12	A6
Guar gum	9000-30-0	Binder	2	100	B3
2,4-Heptadienal	4313-03-5	Flavour	1	12	A3
γ-Heptalactone	105-21-5	Flavour	40	47	A2
n-Heptanoic acid	111-14-8	Flavour	1	12	B2
2-Heptanone	110-43-0	Flavour	0.05	12	A3
cis-4-Heptenal	6728-31-0	Flavour	0.05	12	A3
ω-6-Hexadecenlactone	7779-50-2	Flavour	2.5	12	A7
γ-Hexalactone	695-06-7	Flavour	5	12	A2
δ-Hexalactone	823-22-3	Flavour	1	12	A2
Hexanal	66-25-1	Flavour	3	12	A3
Hexanoic acid	142-62-1	Flavour	20	23	A3
Hexen-2-al	505-57-7	Flavour	6	12	A3
3-Hexenoic acid	4219-24-3	Flavour	0.5	12	A3
trans-2-Hexenoic acid	13419-69-7	Flavour	0.5	12	B3
2-Hexenol	2305-21-7	Flavour	0.5	12	A3
cis-3-Hexen-1-ol	928-96-1	Flavour	2.5	12	A3
trans-2-Hexenyl acetate	2497-18-9	Flavour	0.1	12	A3
cis-3-Hexen-1-yl acetate	3681-71-8	Flavour	5	12	A3
cis-3-Hexenyl formate	33467-73-1	Flavour	0.5	12	A3
Hexyl acetate	142-92-7	Flavour	7	12	A3
Hexyl alcohol	111-27-3	Flavour	15	12	A3
Honey	8028-66-8	Flavour	17,000	45,400	C5
Honey absolute	91052-92-5	Flavour	30	35	B3
Hydroxycitronellal	107-75-5	Flavour	5	12	A3
2-Hydroxy-4-methylbenzaldehyde	698-27-1	Flavour	0.5	12	A4
4-(*p*-Hydroxyphenyl)-2-butanone	5471-51-2	Flavour	20	23	A7
Hydroxypropyl cellulose	9004-64-2	Binder	3300	4000	B5
Immortelle, absolute	8023-95-8	Flavour	0.5	47	A4
α-Ionone	127-41-3	Flavour	10	12	B3
β-Ionone	14901-07-6	Flavour	7.5	12	A3
α-Irone	79-69-6	Flavour	1	12	A3
Isoamyl acetate	123-92-2	Flavour	60	70	A3
Isoamyl butyrate	106-27-4	Flavour	20	23	A3
Isoamyl cinnamate	7779-65-9	Flavour	0.5	12	A3
Isoamyl hexanoate	2198-61-0	Flavour	1	12	B3
Isoamyl isovalerate	659-70-1	Flavour	30	35	A3
Isoamyl octanoate	2035-99-6	Flavour	1	18	A3
Isoamyl phenylacetate	102-19-2	Flavour	7.5	12	A8
Isoamyl propionate	105-68-0	Flavour	0.1	12	B2
Isoamyl salicylate	87-20-7	Flavour	0.1	12	A7
Isobornyl acetate	125-12-2	Flavour	0.5	47	A4
Isobutyl acetate	110-19-0	Flavour	10	12	B3
Isobutyl alcohol	78-83-1	Flavour	22	29	A6
Isobutyl butyrate	539-90-2	Flavour	0.05	12	B2
Isobutyl cinnamate	122-67-8	Flavour	0.5	47	A4
Isobutyl phenylacetate	102-13-6	Flavour	5	29	B3
Isobutyraldehyde	78-84-2	Flavour	10	12	B2

(*continued on next page*)

Table 1 (*continued*)

Ingredient Name	CAS Number[a]	Function	Level (ppm)		Test cigarette
			Max. cig. level[b]	Tested[c]	
Isobutyric acid	79-31-2	Flavour	5	35	A8
dl-Isomenthone	491-07-6	Flavour	1	47	A4
Isopropyl myristate	110-27-0	Flavour	2.5	12	A3
Isovaleric acid	503-74-2	Flavour	34	41	A8
Jasmin, absolute	8031-01-4	Flavour	5	47	A4
Jasmin oil	8022-96-6	Flavour	2.5	12	B3
Kola nut extract	68916-19-8	Flavour	40	100	C8
Labdanum, absolute	68917-77-1	Flavour	15	18	B2
Labdanum oil	8016-26-0	Flavour	1	12	B3
Labdanum resinoid/oleoresin	977092-72-0	Flavour	2.5	12	B3
Lactic acid	598-82-3	Flavour	7100	12,400	C8
Lauric acid	143-07-7	Flavour	1	12	A3
Lavender oil	8000-28-0	Flavour	2.5	12	B2
Lemon extract	84929-31-7	Flavour	10	12	B2
Lemon oil, expressed	8020-19-7	Flavour	10	23	A3
Lemon oil, terpeneless	68648-39-5	Flavour	10	12	B3
Lemongrass oil	8007-02-1	Flavour	2.5	29	A3
Licorice extract powder	68916-91-6	Flavour	20,000	20,000	B4, C9
Lime oil, distilled	8008-26-2	Flavour	1	12	A3
Lime oil, expressed	8008-26-2	Flavour	10	12	B3
Linalool	78-70-6	Flavour	10	18	A3
Linalool oxide	60047-17-8	Flavour	5	12	B2
Linalyl acetate	115-95-7	Flavour	7.5	12	A7
Linalyl benzoate	126-64-7	Flavour	1	12	B3
Linalyl isobutyrate	78-35-3	Flavour	5	12	B2
Lovage extract	8016-31-7	Flavour	0.5	12	B2
Lovage oil	8016-31-7	Flavour	7.5	12	A5
Mace oil	8007-12-3	Flavour	10	292	A5, B3
Mace oleoresin	N/A	Flavour	10	23	B2
Malic acid	6915-15-7	Flavour	1700	6500	C4
Malt extract	8002-48-0	Flavour	100	100	B3
Maltol	118-71-8	Flavour	220	257	A6, B2
Mandarin oil	8008-31-9	Flavour	40	47	B2
Maple concentrate syrup	91770-22-8	Flavour	0.5	12	B3
Maple, maple moutain solid extract	91770-23-9	Flavour	20	23	B3
Maple syrup	91770-22-8	Flavour	50	100	C8
Mate extract	68916-96-1	Flavour	5	500	C8
Mate extract, absolute	68916-96-1	Flavour	14	18	B3
p-Mentha-8-thiol-3-one	38462-22-5	Flavour	0.5	12	B2
l-Menthol	89-78-1	Flavour	20,000	23,400	B4
l-Menthone	89-80-5	Flavour	2.5	12	A4
dl-Menthyl acetate	16409-45-3	Flavour	5	12	A7
Menthyl isovalerate	16409-46-4	Flavour	1	12	A7
Methional [(3-Methylthio)-propionaldehyde]	3268-49-3	Flavour	2.5	12	B2
p-Methoxybenzaldehyde	123-11-5	Flavour	200	234	A2
2-Methoxy-4-methylphenol	93-51-6	Flavour	7.5	12	A5
2 or 5 or 6-Methoxy-3-methylpyrazine	68378-13-2	Flavour	7.5	12	A8
4-(*p*-Methoxyphenyl)-2-butanone	104-20-1	Flavour	0.5	12	A3
1-(*p*-Methoxyphenyl)-2-propanone	122-84-9	Flavour	0.5	12	A7
2-Methoxy-4-vinylphenol	7786-61-0	Flavour	10	12	A5
4′-Methylacetophenone	122-00-9	Flavour	10	12	A2
Methyl *p*-anisate	121-98-2	Flavour	30	35	A4
p-Methylanisole	104-93-8	Flavour	4	12	A2
Methylbenzoate	93-58-3	Flavour	25	29	A7
α-Methylbenzyl acetate	93-92-5	Flavour	2.5	12	A7
trans-2-Methyl-2-butenoic Acid	80-59-1	Flavour	0.5	12	B3
2-Methylbutyraldehyde	96-17-3	Flavour	15	18	A8
3-Methylbutyraldehyde	590-86-3	Flavour	40	47	A8
2-Methylbutyric Acid	116-53-0	Flavour	7.5	12	A7
Methyl cinnamate	103-26-4	Flavour	20	23	A5
1-Methyl-2,3-cyclohexadione	3008-43-3	Flavour	0.5	12	A6

(*continued on next page*)

Table 1 (*continued*)

Ingredient Name	CAS Number[a]	Function	Level (ppm)		Test cigarette
			Max. cig. level[b]	Tested[c]	
Methylcyclopentenolone	765-70-8	Flavour	45	53	A6
5*H*-5-Methyl-6,7-dihydrocyclopenta[*b*]pyrazine	23747-48-0	Flavour	5	12	A8
Methyl dihydrojasmonate	24851-98-7	Flavour	10	12	A3
6-Methyl-3,5-heptadien-2-one	1604-28-0	Flavour	5	18	B3
6-Methyl-5-hepten-2-one		1335-09-7	Flavour	7.5	12
Methyl-α-ionone	127-42-4	Flavour	0.5	12	A3
1-Methyl-3-methoxy-4-isopropylbenzene	1076-56-8	Flavour	0.05	12	B3
Methyl-3-(methylthio) propionate	13532-18-8	Flavour	0.5	12	A3
Methyl 2-octynoate	111-12-6	Flavour	0.1	12	A3
3-Methylpentanoic acid	105-43-1	Flavour	3	12	A7
Methyl phenylacetate	101-41-7	Flavour	5	18	A7
2-Methylpyrazine	109-08-0	Flavour	7.5	29	A8
Methyl-2-pyrrolyl ketone	1072-83-9	Flavour	1	12	A8
5-Methylquinoxaline	13708-12-8	Flavour	0.1	12	A8
Methyl salicylate	119-36-8	Flavour	2.5	12	A5
2-Methyltetrahydrofuran-3-one	3188-00-9	Flavour	5	12	A6
4-Methyl-5-thiazole ethanol	137-00-8	Flavour	0.5	12	B2
2-Methylvaleric acid [2-Methylpentanoic acid]	97-61-0	Flavour	5	12	A7
Mimosa, absolute	93685-96-2	Flavour	1	12	A4
Molasses, sugar cane	68476-78-8	Flavour	220	53,500	C8
Myrrh oil	8016-37-3	Flavour	10	12	A5
Nerol	106-52-2	Flavour	2.5	12	A3
Neroli bigarde oil	8016-38-4	Flavour	5	12	B3
Nerolidol	7212-44-4	Flavour	2.5	12	A3
Neryl acetate	141-12-8	Flavour	0.05	12	B2
2,6-Nonadien-1-ol	7786-44-9	Flavour	0.05	12	A4
γ-Nonalactone	104-61-0	Flavour	10	140	A2
δ-Nonalactone	3301-94-8	Flavour	5	12	A2
n-Nonanal	124-19-6	Flavour	5	12	A3
Nonanoic acid	112-05-0	Flavour	10	12	B3
2-Nonanone	821-55-6	Flavour	2.5	12	A7
Nutmeg oil	8008-45-5	Flavour	10	234	A5, B2
Oak chip extract	68917-11-3	Flavour	1	100	C8
Oakmoss, absolute	9000-5-4	Flavour	5	12	A4
γ-Octalactone	104-50-7	Flavour	7.5	12	A2
δ-Octalactone	698-76-0	Flavour	15	18	A2
n-Octanal	124-13-0	Flavour	0.1	12	A3
Octanoic Acid	124-07-2	Flavour	15	18	B3
1-Octanol	111-87-5	Flavour	0.05	12	B2
1-Octen-3-ol	3391-86-4	Flavour	5	12	A3
Olibanum oil	8016-36-2	Flavour	2.5	12	A4
Olibanum resinoid	8050-07-5	Flavour	10	12	B3
Opoponax oil	8021-36-1	Flavour	1	12	B2
Orange flower, absolute	8016-38-4	Flavour	0.05	12	B2
Orange juice essence oil	68606-94-0	Flavour	100	117	B3
Orange oil, bitter	68916-04-1	Flavour	1	12	B3
Orange oil, sweet	8008-57-9	Flavour	100	117	A3
Orange oil, sweet, terpeneless	68606-94-0	Flavour	100	117	B3
Orange sweet, extract	8028-48-6	Flavour	1	12	B3
Orris concrete oil	8002-73-1	Flavour	0.5	12	A4
Orris root extract	8002-73-1	Flavour	2.5	100	C5
Orris root resinoid	8002-73-1	Flavour	0.05	12	B3
Palmarosa oil	8014-19-5	Flavour	2.5	12	A3
Palmitic acid	57-10-3	Flavour	7.5	12	B2
Patchouly oil	8014-09-3	Flavour	10	12	A5
Pectin	9000-69-5	Flavour	80	300	C5
ω-Pentadecalactone	106-02-5	Flavour	5	12	B2
2,3-Pentanedione	600-14-6	Flavour	15	18	A6
2-Pentanone	107-87-9	Flavour	0.5	12	A3
Pent-3-en-4-olide [α-Angelica lactone]	591-12-8	Flavour	1	12	B2
Pepper oil, black	8006-82-4	Flavour	0.5	12	A5

(*continued on next page*)

Table 1 (*continued*)

Ingredient Name	CAS Number[a]	Function	Level (ppm)		Test cigarette
			Max. cig. level[b]	Tested[c]	
Peppermint oil	8006-90-4	Flavour	35	211	A4
Peppermint oil, terpeneless	68606-97-3	Flavour	100	175	B3
Petitgrain oil	8014-17-3	Flavour	2.5	12	A3
α-Phellandrene	99-83-2	Flavour	2.5	12	A5
Phenethyl acetate	103-45-7	Flavour	15	18	A7
Phenethyl alcohol	60-12-8	Flavour	40	47	A7
Phenethyl butyrate	103-52-6	Flavour	0.5	12	B2
Phenethyl cinnamate	103-53-7	Flavour	5	12	A7
Phenethyl isobutyrate	103-48-0	Flavour	30	35	A7
Phenethyl isovalerate	140-26-1	Flavour	5	12	A7
Phenethyl phenylacetate	102-20-5	Flavour	5	12	A7
Phenylacetaldehyde	122-78-1	Flavour	22	29	A7
Phenylacetic acid	103-82-2	Flavour	65	76	A7
2-Phenyl-2-butenal	4411-89-6	Flavour	2.5	12	B3
4-Phenyl-3-buten-2-one	122-57-6	Flavour	10	12	B3
3-Phenyl-1-propanol	122-97-4	Flavour	1	12	A3
3-Phenylpropionaldehyde	104-53-0	Flavour	5	12	B3
3-Phenylpropionic acid	501-52-0	Flavour	5	12	A6
Pineapple extract	97676-27-2	Flavour	10	12	B3
Pineapple juice/concentrate	97676-27-2	Flavour	10	12	B3
α-Pinene	7785-26-4	Flavour	2.5	12	A3
β-Pinene	18172-67-3	Flavour	0.5	12	A3
Pine needle oil	8021-29-2	Flavour	0.5	12	B3
Piperonal	120-57-0	Flavour	200	234	A6, B2
Pipsissewa leaves extract	89997-56-8	Flavour	10	12	B3
Plum extract	90082-87-4	Flavour	40	3500	B3, C5
Plum juice concentrate	90082-87-4	Flavour	40	12	B3
Potassium citrate	866-84-2	Flavour	10	12	B2
Potassium sorbate	24634-61-5	Preservative	500	6000	B3
5-Propenylguaethol	94-86-0	Flavour	5	12	A2
Propionic acid	79-09-4	Flavour	2.5	12	B3
n-Propyl acetate	109-60-4	Flavour	2.5	12	A3
1,2-Propylene glycol	57-55-6	Humectant	47,200	83,300	B2–B4
3-Propylidenephthalide	17369-59-4	Flavour	2.5	12	A5
Prune extract	90082-87-4	Flavour	10,900	17,400	C4
Prune juice concentrate	85594-37-2	Flavour	10,900	20,000	B2
Pyruvic acid	127-17-3	Flavour	25	28	B2
Raisin extract	68915-86-6	Flavour	10,000	11,400	C8
Raisin juice/concentrate	N/A	Flavour	600	600	B4
Raspberry distillate	84929-76-0	Flavour	1	12	B3
Rhodinol	141-25-3	Flavour	2.5	12	A7
Rose, absolute (*R-centifolia*)	8007-01-0	Flavour	0.5	12	B2
Rose oil, red	90106-38-0	Flavour	10	35	A4
Rose oxide	16409-43-1	Flavour	1	12	A4
Rosemary oil	8000-25-7	Flavour	0.5	12	A4
Rum extract (absolute)	90604-30-1	Flavour	10	12	B2
Rum, dark	91450-09-8	Flavour	250	2340	B3
Rum, white	90604-31-1	Flavour	250	5850	A6, B2
Rye extract	91770-60-4	Flavour	8	100	C8
Sage oil (Dalmation)	8022-56-8	Flavour	7.5	12	B2
Sage oil (Spanish)	8022-56-8	Flavour	7.5	12	A4
Sage powder	977002-44-0	Flavour	10	12	B3
Salicylaldehyde	90-02-8	Flavour	20	23	A5
Sandalwood oil, yellow	8006-87-9	Flavour	7.5	18	A4
Sodium acetate	127-09-3	Flavour	10	12	B3
Sodium benzoate	532-32-1	Preservative	5	12	B2, B3
Sodium carbonate	497-19-8	Flavour	10	12	B3
Sodium chloride	7647-14-5	Flavour	0.5	12	B2
Sodium citrate	68-04-2	Flavour	5	12	B3
Sodium ethyl 4-hydroxybenzoate[d]	35285-68-9	Preservative	0[d]	30	B3
Sodium methyl 4-hydroxybenzoate[d]	5026-62-0	Preservative	0[d]	30	B3

(*continued on next page*)

Table 1 (*continued*)

Ingredient Name	CAS Number[a]	Function	Level (ppm)		Test cigarette
			Max. cig. level[b]	Tested[c]	
Sodium propyl 4-hydroxybenzoate[d]	35285-69-9	Preservative	0[d]	60	B3
Sorbic acid	110-44-1	Preservative	0.1	12	A3
Sorbitol	50-70-4	Humectant	24,000	35,300	C5
Spearmint oil	8008-79-5	Flavour	20	936	A4
Starch	9005-25-8	Flavour	19,000	19,000	B5
Styrax extract (resinoid)	8046-19-3	Flavour	56	65	A5
Styrax oil	8024-01-9	Flavour	5	12	B2
Styrax resinoid	8046-19-3	Flavour	30	140	B3
Sugar, brown	N/A	Flavour	62,000	62,000	B2
Sugar, invert	8013-17-0	Flavour	62,000	70,000	C9
Sugar, white	57-50-1	Flavour	62,000	105,000	C3
Tangerine extract	8016-85-1	Flavour	0.5	12	B3
Tangerine oil	8008-31-9	Flavour	1	47	A3
l-Tartaric acid	87-69-4	Flavour	1200	7500	C9
Tea extract, absolute	68916-73-4	Flavour	10	300	A4, C8
Tea extract, distillate	84650-60-2	Flavour	2.5	12	B3
α-Terpineol	98-55-5	Flavour	5	94	A3
Terpinolene	586-62-9	Flavour	0.5	23	A3
Terpinyl acetate	80-26-2	Flavour	1	23	A3
δ-Tetradecalactone	2721-22-4	Flavour	0.05	12	A7
5,6,7,8-Tetrahydroquinoxaline	34413-35-9	Flavour	10	12	A8
Tetramethylpyrazine	1124-11-4	Flavour	20	23	B3
Thyme oil, white	8007-46-3	Flavour	5	12	A4
Thymol	89-83-8	Flavour	0.5	12	B2
Tocopherols (mixed)	52-09-9	Flavour	7.5	12	B3
p-Tolyl acetate	140-39-6	Flavour	5	12	B3
m-Tolyl aldehyde [3-Methylbenzaldehyde]	620-23-5	Flavour	1	12	B2
o-Tolyl aldehyde [2-Methylbenzaldehyde]	529-20-4	Flavour	1	12	B2
p-Tolyl aldehyde [4-Methylbenzaldehyde]	104-87-0	Flavour	5	12	A7
p-Tolyl isobutyrate	103-93-5	Flavour	1	23	A5, B2
Triacetin	102-76-1	Flavour/solvent	300	1400	A3–A8, B2, B3
Triethyl citrate	77-93-0	Flavour	30	819	A3–A8, B2, B3
3,5,5-Trimethyl-1-hexanol	3452-97-9	Flavour	30	35	A5
Trimethylpyrazine	14667-55-1	Flavour	10	12	A8
γ-Undecalactone	104-67-6	Flavour	10	23	A7, B2
δ-Undecalactone	710-04-3	Flavour	7.5	12	A7
2-Undecanone	112-12-9	Flavour	0.5	12	B3
Urea	57-13-6	Flavour	4000	4000	B3
Valeraldehyde	110-62-3	Flavour	0.05	12	B2
Valerian root extract	8057-49-6	Flavour	560	600	C8
Valerian root oil	8008-88-6	Flavour	7.5	117	A4, B2
Valerian tincture	8057-49-6	Flavour	100	234	B3
Valeric acid	109-52-4	Flavour	2	12	A7
γ-Valerolactone	108-29-2	Flavour	25	25	A8
Vanilla, absolute	8024-06-4	Flavour	326	381	A8
Vanilla extract	8024-06-4	Flavour	326	468	B3
Vanilla oleoresin	8023-78-7	Flavour	326	2460	A2
Vanillin	121-33-5	Flavour	864	2550	A8, B2
Veratraldehyde	120-14-9	Flavour	40	47	A8
Verbena oil, European	85116-63-8	Flavour	5	12	B3
Vetiver oil	8016-96-4	Flavour	0.5	12	A4
Violet leaves, absolute	8024-08-6	Flavour	5	12	A4
Water	7732-18-5	Process aid	As needed	76,000	B2-B4
Wine	91082-91-6	Flavour	10	2570	A6
Wine, sherry	8030-68-0	Flavour	40	47	A6
Xanthan gum	11138-66-2	Binder	3800	5000	B5
Ylang ylang oil	8006-81-3	Flavour	5	12	A4

[a] Chemical Abstract Service Registry Number.
[b] Typical maximum use level of the ingredient on British American Tobacco products.
[c] The maximum level of the ingredient tested in an experimental cigarette in this study.
[d] No longer used.

2.4. In vitro bioassay studies

For the Ames in vitro mutation assay, the conditioned cigarettes were smoked on a Borgwaldt RM20CS rotary smoking machine and approximately 300 mg of smoke TPM were collected on 44 mm diameter Cambridge filter pads for each cigarette. A portion of the pad was taken to determine the water and nicotine, and hence NFDPM content. The remainder of the pad was extracted with dimethyl sulphoxide, and the solution was stored in the dark at −78 °C prior to the Ames analyses. They were undertaken within 24 h of generation at the Covance Laboratories in Harrogate, U.K. The assay was performed with five Ames *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537, both in the presence and absence of a metabolic activation system S9, consisting of the post-mitochondrial fraction of the livers from rats treated with Aroclor 1254. The significance of differences between the observed revertants on the plates treated with smoke TPM and solvent-only plates was determined by Dunnet's test (Dunnett, 1955). For those Ames strains yielding statistically significant revertant-NFDPM dose relationships, the slopes of the linear portion of the dose response plots are defined as the specific activity in revertants per μg NFDPM. The Ames protocols used conform to OECD Guideline 471 (1996a), EEC Annex V Test B14 (1967), and the ICH Harmonised Tripartite Guideline (1996, 1997).

For the in vitro micronucleus assay, the smoke TPM collected on the Cambridge filter pad was also extracted with dimethyl sulphoxide, and the solution stored in the dark at −78 °C. The assay was undertaken within 24 h of generating the smoke TPM at the Covance Laboratories in Harrogate, U.K. The testing regime employed in the assay was in accordance with the OECD Guideline 473 (1996b) for chromosome aberration testing and the International Working Group on Genotoxicity Testing (Kirsch-Volders et al., 2003). Briefly, cells in duplicate flasks were exposed to a range of concentrations of smoke TPM. The mixture was subjected to a three-hour pulse treatment followed by a 17 h recovery in the presence and absence of S9 metabolic activation, and a continuous 20 h treatment without S9 metabolic activation. The cell type used for the testing was a V79 Chinese hamster cell line.

In the neutral red uptake cytotoxicity assay, cytotoxicity is expressed as a reduction in the uptake and incorporation of neutral red dye into the lysosomes of viable cells after 48 h of chemical exposure. This energy-dependent process provides an integrated signal of both cell integrity and growth inhibition. All of the assays were performed in the British American Tobacco R&D Centre in Southampton, U.K. Briefly, V79 Chinese hamster cells were seeded onto 96-well plates and maintained in culture for approximately 24 h. These were then exposed to a growth medium containing smoke TPM over a range of eight concentrations varying from 10 to 100 μg NFDPM/ml growth medium. Following 48 h of exposure, the treatment medium was washed off and the cellular uptake of neutral red was measured. The protocol conforms to National Institute of Health (U.S.A.) guidelines (National Institute of Health, 2001).

Neutral red uptake for each of the treatment concentrations was compared to that determined for untreated control cultures. Thus, the % viability for each concentration of the test condensate was calculated. The resulting cell viability data were plotted against the relevant TPM concentrations, and concentration–response curves were fitted by multivariate non-linear regression. These curves were then used to calculate IC_{50} values (the inhibiting concentration that results in a reduction in viability to 50% that of the untreated control cell viability) for each particulate matter sample. A total of four replicate measurements was made for each smoke TPM.

For each in vitro bioassay, the smoke TPM samples from the cigarettes were measured in three or four batches for cigarette series A, B and C. Each bioassay for each test cigarette was always measured in the same batch as its control cigarette.

2.5. In vivo sub-chronic inhalation studies

The three series of test cigarettes (A, B and C) have been evaluated in three sub-chronic inhalation studies, which consisted of exposing groups of rats to smoke for 90 days. A total of 22 groups of animals was exposed to smoke from the various test and control cigarettes, as well as one cage control group and three air control groups (Table 2). In all cases there were 20 animals per group, 10 male and 10 female. Sprague–Dawley CD® (Crl: CD® (SD) IGSBR) rats, *Rattus norvegicus,* were used, approximately 7 weeks of age at the start of the treatment. The target dose levels (chamber concentrations) were 1 mg TPM/l of air, which were selected according to the potential human exposure, existing toxicity data and any limitations imposed by the exposure apparatus and procedure as well as the stability of the experimental atmosphere. The study was undertaken at CTBR (ClinTrials BioResearch) Bio-Research Inc., Senneville, Quebec, Canada and was performed in compliance with the Good Laboratory Practice Regulations as set forth by the U.S. Environmental Protection Agency, Code of Federal Regulations, 40, Part 792 (Code of Federal Regulations, 2001).

The inhalation chamber consisted of a cylindrical aluminium and polycarbonate tube with 20 animal exposure ports arranged radially at four levels. Each level contained five ports of which the conical front section of a polycarbonate tube was inserted. The smoke inlet tube was fitted at the top of the exposure

Table 2
Experimental design of the three groups of animals[a] used in the 90-day sub-chronic inhalation toxcicity studies

Study	Animal groups	Target TPM chamber concentration (mg/l)
1	Cage control (untreated animals)	–
	Air control[b]	–
	Smoke from cigarettes C1–C9[b]	1
	Health screen[d]	–
2	Air control[b]	–
	Smoke from cigarettes A1–A8[c]	1
	Health screen[d]	–
3	Air control[b]	–
	Smoke from cigarettes B1–B5[c]	1
	Health screen[d]	–

[a] Each group consisted of 10 male and 10 female rats.
[b] Animals had air rather than smoke administered to them, same conditions as described in footnote c.
[c] "Nose-only exposure" for 1 h per day, divided as follows: smoke 30 min, air 15 min, smoke 30 min, for 5 days per week.
[d] This group of animals used to test the general health of the rats under study.

chamber and the outlet was through the bottom detachable cap. The tubes, which served to restrain the individual animals, were moulded polycarbonate tubes. One end of the tube was tapered to fit over the shape of the animal's head, and the diameter of the tubes was such that the animal could not turn easily.

Smoke was generated by smoking the cigarettes on a 30-port Baumgartner-Jaeger CSM 2070i rotary smoking machine, manufactured by CH Technologies (U.S.A.) Inc. The cigarettes were smoked to the standard ISO smoking regime of one 35-ml puff of 2-s duration taken every minute. Diluting air was used to attain the chamber concentrations of 1 mg TPM/l of air. The diluted smoke passed from top to bottom through the exposure chamber. Smoke was collected from the animal breathing zone of the chamber at weekly intervals during the studies and TPM, nicotine and carbon monoxide concentrations were determined in a manner similar to that described by Haussmann et al. (1998). All animals were subjected to "nose-only" exposure for one hour per day, divided as follows: smoke 30 min, air 15 min, smoke 30 min, for 5 days per week and a minimum of 13 weeks.

An acclimation period of approximately three weeks was allowed between animal receipt and the start of smoke treatment in order to accustom the animals to the laboratory environment. Approximately one week before initiation of smoke treatment, the animals were weighed and assigned to treatment groups using a randomisation procedure. Randomisation was by stratification using body weight as the parameter. Males and females were randomised separately. Animals in poor health or at the extremes of the body weight range were not assigned to groups. All treated groups of animals were acclimatised to smoke and tube restraint procedures by conducting three days of smoke familiarisation. In addition to the smoke-treated groups of animals, a cage control and air control groups were also used. The cage control group was untreated and the air control group had filtered air instead of smoke administered to them. A further health screen group of animals was used to assess the general health of the rats under study.

Throughout the study the animals were housed at 22 ± 3 °C and $50 \pm 10\%$ relative humidity with a light cycle of 12 h light and 12 h darkness. All animals were examined twice daily for mortality and signs of ill health or reaction to treatment. During the smoke treatment phase, the animals were observed for reaction to treatment during the exposure. Detailed examinations were performed at least once during the pre-treatment period and weekly during the treatment period. Individual body weights were measured weekly commencing the day of randomisation and extending through the treatment period and prior to euthanasia. Six male and six female rats randomly selected from each of the smoked groups were bled once during week 9 of the treatment to determine carboxyhaemoglobin (COHb) concentration (%). Chamber carbon monoxide levels were also measured during this period. At necropsy, all animals were subjected to external and internal macroscopic examinations. Heart, lungs and brain were dissected free of fat and weighed. On completion of gross pathological examination, the majority of tissues were preserved and the larynx/pharynx, nasal cavities and sinuses, mainstream bronchi, trachea, all lobes of the lung and tracheobronchial lymph nodes from all animals were prepared for histopathological examination and histomorphometric assessment. This was done by embedding in paraffin wax, sectioning and staining with hematoxylin and eosin (all tissues) and Alcian blue/Periodic acid Schiff's reagent (left lobe of lung, nasal cavity and trachea only). Tissue sections from all animals were examined microscopically. A veterinary pathologist with experience in cigarette smoke-related changes in the respiratory tract of rodents read the slides in a blind manner. Histopathological findings were scored according to a defined severity scale from 0 (none), 1 (minimal) to 5 (severe). Mean severity scores were calculated based on the male and female rats in each group. Histomorphometric assessment of certain parameters in the respiratory tract was also conducted.

3. Results and discussion

3.1. Pyrolysis studies

Pyrolysis of individual tobacco constituents has been used in many studies since the 1950s to establish

relationships between smoke constituents and their precursors in tobacco (e.g. Chortyk and Schlotzhauer, 1973; Schlotzhauer and Chortyk, 1987; Baker, 1987; Baker and Bishop, 2004). If the pyrolysis conditions are the same as those that occur in the cigarette burning zone during smoking, a series of reaction pathways can be established for the generation of specific smoke constituents from specific tobacco components and ingredients. Unfortunately, because of the large number of processes that occur inside the burning cigarette (combustion, thermal decomposition, distillation etc.), the wide range of temperature and oxygen levels present across the cigarette burning zone, and the very high heating rates that occur during puffing, the experimental conditions used in many published tobacco pyrolysis studies were very different to those that occur inside the burning cigarette (Baker, 1987; Baker and Bishop, 2004). In these circumstances, false predictions can be obtained. In addition, in a burning cigarette products of tobacco-constituent pyrolysis can potentially react further with other tobacco and smoke constituents. This cannot occur during the pyrolysis of individual constituents, as discussed by Rodgman (2002a).

Baker and Bishop (2004) have described work conducted over almost 30 years to determine the precise combustion environment inside the burning cigarette and to develop experimental pyrolysis conditions to mimic, as far as possible, cigarette combustion. They subsequently developed a pyrolysis system based on these conditions, which are summarised in Section 2.1. Baker and Bishop (2004) compared results from this system to those from definitive isotopically-labelled ingredient studies on burning cigarettes. They concluded that the pyrolysis system gave good predictions of the smoke transfer/pyrolytic behaviour of relatively volatile tobacco ingredients. These ingredients are usually added to cigarettes in small amounts (at parts per million levels) and have negligible effect on the major heat producing processes that occur during tobacco combustion. For involatile substances, the pyrolysis system gave over-estimates of the pyrolytic decomposition that occurs in the burning cigarette. It seems that in the experimental pyrolysis system, involatile substances remain in the high temperature region longer than when they are part of the combustion matrix in a burning cigarette, and therefore are subjected to greater decomposition. Nevertheless, the pyrolysis technique is a useful screening tool for indicating which ingredients undergo significant decomposition during the smoking of cigarettes and determining what decomposition products are generated.

Baker and Bishop (2004) pyrolysed 291 single-compound substances, many of which were relatively volatile. Of these, two used as model compounds were naturally-occurring tobacco components (*n*-dotriacontane and nicotine) and one was ammonium hydroxide, for which no products or starting material were detected. In the pyrolysis-mass spectrometer system, water, permanent gases such as carbon monoxide and carbon dioxide, and substances with a molecular weight below about 40 were not detected. Thus, no products were detected from the pyrolysis of ammonium hydroxide, because the experimental system did not allow detection of the potential products, water and ammonia. With this limitation, a summary of the cumulative number of substances transferring out of the pyrolysis zone, at different levels of pyrolytic degradation of the ingredient, is given in Fig. 1. A total of 92 substances (almost a third) transfers with less than 1% pyrolytic degradation, i.e. they transfer at least 99% intact. A total of 184 substances (almost two thirds) transfer with less than 5% pyrolysis (at least 95% intact).

Several of the substances pyrolysed by Baker and Bishop (2004) are likely to have been investigated in other pyrolysis studies. However, the objective of the study was to investigate the behaviour of the substances under pyrolysis conditions that occur inside the burning cigarette. As discussed above, the results from studies that do not simulate these conditions can be irrelevant to a burning cigarette situation. This is illustrated by the pyrolysis of glycerol. Published studies of Stein et al. (1983) have shown that acrolein and acetaldehyde are the principal products from the isothermal pyrolysis of glycerol in steam above 650 °C, and at lower temperatures in the presence of a metal catalyst. However, their pyrolysis conditions were quite different to those that occur inside a burning cigarette. Using pyrolysis conditions more relevant to a burning cigarette, Baker and Bishop (2004) found that 99.8% of the glycerol (boiling point 290 °C) distilled intact out of the pyrolysis system, and neither acrolein nor acetaldehyde was detected. The pyrolysis results of Baker and Bishop are in agreement with unpublished results quoted by the Philip Morris Tobacco Company (2001). These unpublished results were obtained by heating glycerol in air up to 900 °C in 100 s, and two studies on smoking cigarettes in which

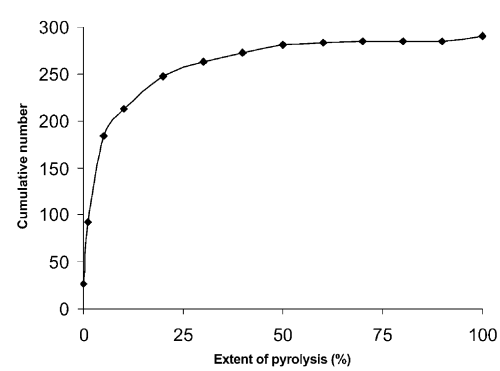


Fig. 1. Cumulative number of ingredients transferring out of the pyrolysis zone as a function of extent of pyrolysis of the ingredient.

radiolabelled glycerol had been added. In all cases, the glycerol distilled intact and the generation of acrolein and acetaldehyde from the glycerol was very small, e.g. less than 0.1% acrolein in the radiolabelled mainstream smoke products. Consequently, published studies with pyrolysis conditions that are not relevant to a burning cigarette are not considered further in the present paper.

For those ingredients that do undergo some degree of pyrolysis, the pyrolysis products detected and their relative amounts were listed by Baker and Bishop (2004). From these relative amounts, an upper level for the maximum amount of each pyrolysis product in mainstream smoke from the ingredient was calculated. This calculation was for an unfiltered (plain) cigarette with the maximum ingredient application level, maximum tobacco weight in the cigarette, maximum proportion of tobacco burnt in puffing and 100% transfer of the ingredient and pyrolysis products to mainstream smoke. It was also assumed in the calculation that all the pyrolysis products were detected. This assumption is not correct for those ingredients that generate water, carbon dioxide, carbon monoxide or involatile ingredients that produce a solid char, for example. The maximum levels of pyrolysis products calculated in mainstream smoke are thus over-estimates in general, and probably large over-estimates for involatile substances.

In considering the contribution of the potential pyrolysis products from ingredients to total smoke, we have chosen to exclude, at this stage, any chemical predicted to be produced from the ingredient at less than 0.03 μg per cigarette. Thus, based on the assumption that a smoker consumes a maximum of 50 cigarettes per day, if a chemical from the ingredient were present in the smoke at 0.03 μg per cigarette, their daily intake of the chemical from the ingredient would be 1.5 μg. It has been reported that the intake of a chemical by ingestion from food at up to 1.5 μg/day is of no toxicological concern (Munro et al., 1999). This is the basis of our cut-off of 0.03 μg per cigarette. It is realised that with smoking the chemical is inhaled and not ingested and so this is a first approximation. However, applying this model does offer a means of prioritising the data produced from pyrolysis studies of this type. This threshold model is applied solely to chemicals in smoke produced from ingredients and is not intended as a means of assessing the significance of smoke per se. We are *not* implying that levels of chemicals in smoke below 0.03 μg per cigarette are 'safe'. Indeed, epidemiology shows that the only way to avoid a smoking-related risk is not to smoke.

Of the approximately 4800 substances in tobacco smoke (Green and Rodgman, 1996), 44 are believed by regulatory authorities in Canada and the U.S.A. to be relevant to smoking-related diseases (Department of Health, Canada, 2000; Massachusetts Tobacco Control Program, 2001). These include, *inter alia*, some volatile carbonyl compounds, tobacco-specific nitrosamines, aromatic amines, phenols, benzo[*a*]pyrene and metals. These substances are sometimes colloquially called "Hoffmann analytes" since similar lists of toxicological substances have been proposed by Hoffmann and co-workers of The American Health Foundation in New York since the mid-1980s. Hoffmann's latest compilation in fact lists 82 substances (Hoffmann and Hoffmann, 1998, 2001; Hoffmann et al., 2001). Substances on any of the three lists that would be in smoke from pyrolysis of the ingredient at or above the threshold limit of 0.03 μg/cigarette, are given in Table 3. Typical smoke levels for an unfiltered cigarette, obtained from Baker (1999) and Hoffmann and Hoffmann (1998), are also included in Table 3 in order to compare the maximum ingredient contribution to smoke yields. Smoke data for unfiltered cigarettes are given in Table 3 in order to compare like with like, since the maximum levels in smoke from pyrolysis of the ingredients were calculated for unfiltered cigarettes. It should be noted that in Table 3 there are some small differences to the maximum levels in smoke, calculated from pyrolysis of the ingredient, from those quoted by Baker and Bishop (2004). This is because, in a few cases, the typical maximum use level of the ingredient on British American Tobacco products has been reduced since the Baker and Bishop paper was written.

In general, the maximum levels of these biologically-active pyrolysis products in smoke from tobacco ingredients are small compared to the typical levels in smoke, although no quantitative smoke data are available for benzofuran yields. From Table 3, the pyrolysis products that could potentially make a measurable contribution to the smoke yield are acetaldehyde and benzene generated from malic acid, which underwent complete decomposition in the experimental pyrolysis study. Malic acid is an involatile solid and, as indicated above, the experimental pyrolysis technique is probably least relevant for such substances. Nevertheless, the data presented in Table 3 have flagged tobacco ingredients that could potentially yield biologically-active pyrolysis products at levels in smoke above the threshold limit. The next step was to smoke cigarettes containing these ingredients to determine their effects on the smoke yields. This has been done for some of the ingredients in Table 3, in which mixtures of ingredients have been added, at or above their maximum levels, to a U.S. blended tobacco and made into filter cigarettes (this is the subject of Section 3.2, described below). The cigarettes were then machine-smoked under the standard ISO conditions of one 35-ml puff of 2-s duration taken every minute. The yields of various constituents in mainstream smoke were determined and the smoke yields of the pyrolysis products listed in Table 3 are given in Table 4, relative to a control cigarette with no

Table 3
Pyrolysis products from tobacco ingredients that are included in the "Hoffmann lists" of smoke components

Ingredient (maximum cigarette level[a], ppm)	Pyrolysis product	Max. level in smoke from ingredient (μg/cig)	Typical smoke levels, unfiltered cigarette (μg/cig)
Anisyl phenylacetate (0.5)	Phenol	0.03	80–160
Benzaldehyde glyceryl acetal (40)	Phenol	0.08	80–160
Benzyl benzoate (500)	Phenol	0.5	80–160
Benzyl cinnamate (80)	Styrene	0.2	10–20
	Phenol	0.04	80–160
	Benzofuran[b]	0.04	Present
n-Butyl alcohol (2.5)	Butyraldehyde	0.04	30–50
Cinnamic acid (2.5)	Styrene	0.07	10–20
Cinnamyl cinnamate (40)	Styrene	0.7	10–20
	Phenol	0.2	80–160
	Benzofuran[b]	0.08	Present
δ-Dodecalactone (140)	Styrene	0.2	10–20
	Benzofuran[b]	0.2	Present
Ethyl cinnamate (15)	Styrene	0.03	10–20
Ethyl salicylate (1)	Phenol	0.1	80–160
Malic acid (1700)	Acetaldehyde	88	500–1400
	Benzene	2	20–70
α-Methylbenzyl acetate (2.5)	Styrene	0.05	10–20
Methyl cinnamate (20)	Styrene	0.03	10–20
Phenethyl alcohol (40)	Styrene	0.1	10–20
Phenethyl isobutyrate (30)	Styrene	0.2	10–20
Phenylacetic acid (65)	Toluene	0.07	100–200
3-Phenylpropionic acid (5)	Styrene	0.04	10–20
Salicylaldehyde (20)	Phenol	0.05	80–160
p-Tolyl acetate (5)	Cresol (*m*-, *o*- or *p*-methylphenol)	0.09	11–37

[a] Typical maximum use level on British American Tobacco products.
[b] Included on Hoffmann's full list (Hoffmann and Hoffmann, 1998) but not on Canadian and Massachusetts lists (Department of Health (Canada), 2000; Massachusetts Tobacco Control Program, 2001).

added ingredients. Differences between the test and control cigarettes that are statistically different are indicated in Table 4.

For a test cigarette (C4) containing 6500 ppm of added malic acid, the smoke yield of acetaldehyde was found to be 69 μg (11%) higher than its control (Table 4). This difference was statistically significant and could be due to the pyrolysis of malic acid. However, this difference was not statistically significant when the long-term variability of the analytical method was taken into account. Also, the acetaldehyde smoke yield was only 69 μg higher than the control, as compared to a maximum of 330 μg predicted from the pyrolysis results for a cigarette containing 6500 ppm of malic acid. This indicates either that the maximum calculated pyrolysis yields in smoke were greatly over-estimated, or that complete pyrolysis of the malic acid does not occur in the burning cigarette, or that the generation of acetaldehyde from malic acid is not too much higher than generation from the tobacco that it replaces in the cigarette, on a per weight basis. The benzene smoke yield from the cigarette containing malic acid (cigarette C4) was found not to be significantly different from the control, again implying that either malic acid does not completely pyrolyse in the burning cigarette, or that generation of benzene from malic acid and tobacco is similar on a per weight basis.

The styrene smoke yields were elevated by 1.3–1.8 μg (11 to 15%) for test cigarettes A6 and A7 relative to their control (Table 4), and the increase with cigarette A7 remained statistically significant when the long-term variability of the method was taken into account. The toluene level in test cigarette A7 was also elevated by 7 μg (7%) although this difference was not significant when the long-term variability of the method was taken into account. These increases are much more than the maximum contributions from the pyrolysis of ingredients (Table 3), suggesting that other factors, such as

analytical variability, may be responsible. On the other hand, the phenol smoke yields for all the test cigarettes listed in Table 4 were lower than their controls, and for cigarettes A5 and B2, the differences were statistically significant. Similarly, the styrene yield from cigarette B2 was also statistically significantly lower than its control cigarette. The other smoke yields listed in Table 4 were not statistically different from their control.

Of course, the list of biologically-active smoke constituents used in this study (Department of Health, Canada, 2000; Massachusetts Tobacco Control Program, 2001; Hoffmann and Hoffmann, 1998) are not definitive. There are possibly other substances with toxicological properties not given on these lists. One of them is furfural, which is on neither the full "Hoffmann list" nor the ones used by Regulatory Authorities in Canada or the U.S.A., and yet it is present in cigarette smoke and furfural is biologically active (Rodgman and Green, 2003). Furfural is generated by the pyrolysis of sorbitol (Baker and Bishop, 2004). Sorbitol is a solid with melting point 98–100 °C and breaks down completely during the pyrolysis experiments. As indicated above, the pyrolysis system is least relevant for such involatile substances. Nevertheless, the pyrolysis study of sorbitol has indicated formation of biologically-active furfural and the next step in the total assessment of the ingredient was to smoke cigarettes containing sorbitol and determine the effect on furfural levels in smoke. This has been done with a test cigarette (code C5) containing 21 mg (3.53% by weight) of sorbitol. This cigarette was then smoked under ISO smoking machine conditions and the level of furfural was

Table 4
Effect of ingredients in test cigarettes on smoke yields for those ingredients giving pyrolysis yields of "Hoffmann analytes" above the 'daily threshold limit'[a]

Ingredients[b] (maximum cigarette level[c], ppm)	Amount (ppm)[d]	Smoke constituent	Smoke yield (S.D.[e]) (μg/cig)	
			Test	Control
Added to test cig. A3				
n-Butyl alcohol (2.5)	12	Butyraldehyde	39.5 (0.9)	36.5 (2.9)
Added to test cig. A5				
Methyl cinnamate (20)	23	Styrene	12.1 (0.5)	11.6 (0.8)
Salicylaldehyde (20)	23	Phenol	12.4 (0.7) [f,g]	13.8 (0.8)
Added to test cig. A6				
3-Phenylpropionic acid (5)	12	Styrene	12.9 (0.7)[f]	11.6 (0.8)
Added to test cig. A7				
Anisyl phenylacetate (0.5)	12	Phenol	13.3 (0.6)	13.8 (0.8)
Benzyl benzoate (500)	585	Styrene	13.4 (0.2) [f,g]	11.6 (0.8)
Benzyl cinnamate (80)	94	Toluene	107 (2)[f]	100 (3)
Cinnamyl cinnamate (40)	47			
δ-Dodecalactone (140)	164			
Ethyl cinnamate (15)	18			
α-Methylbenzyl acetate (2.5)	12			
Phenethyl alcohol (40)	47			
Phenethyl isobutyrate (30)	35			
Phenylacetic acid (65)	76			
Added to test cig. B2				
Cinnamic acid (2.5)	12	Phenol	23.2 (1.7) [f,g]	26.3 (1.8)
Ethyl salicylate (1)	12	Styrene	18.3 (1.0)[f,g]	20.0 (0.5)
Added to test cig. B3				
Benzaldehyde glyceryl acetal (40)	58	Phenol	24.8 (1.4)	26.3 (1.8)
p-Tolyl acetate (5)	12	*m*- + *p*-Cresol	16.1 (1.3)	17.6 (1.1)
		o-Cresol	5.4 (0.2)	5.8 (0.3)
Added to test cig. C4				
Malic acid (1700)	6500	Acetaldehyde	695 (25)[f]	626 (22)
		Benzene	57.4 (1.4)	56.0 (1.0)

[a] 0.03 μg/cigarette, based on smoking 50 cigarettes per day; further details in the text.
[b] Several ingredients were added to each test cigarette. Only those ingredients listed in Table 3 are included here.
[c] Typical maximum use level on British American Tobacco products.
[d] Amount added to test cigarette, parts per million on a dry-weight of tobacco basis.
[e] S.D. is standard deviation, 5 replicate determinations.
[f] Significantly different from control value at 95% confidence level, this data set.
[g] Also significantly different from control value at 95% confidence level when long-term analytical method variability is included.

determined in the smoke and compared to that from a control (no added sorbitol) cigarette (code C1). The furfural was determined in both the particulate and vapour phases of the smoke in separate experiments using a gas chromatography- mass spectrometric technique. Twenty cigarettes were smoked in each experiment and the smoke particulate phase was trapped on a Cambridge pad, which was extracted with methanol prior to analysis. For the smoke vapour phase, the smoke was passed through a Cambridge pad and the vapour phase collected in a gas sampling bag prior to analysis. Two replicate smokings were done for the control and test cigarettes and the furfural levels determined in the mainstream smoke are given in Table 5. For both cigarettes about two thirds of the furfural was in the smoke particulate phase. There was no significant difference in the levels in either phase between the control and test cigarettes. Presumably, during cigarette smoking the sorbitol does not completely decompose, as in the pyrolysis study. Consequently, in this case the pyrolysis result is a false positive. This indicates again that the pyrolysis technique is not suitable for predicting the behaviour of involatile substances in a burning cigarette.

Some pyrolysis products having toxicological significance, for example carbon monoxide, are not detected in the present experimental system. Nevertheless, pyrolysis from tobacco ingredients determined in this study is an important part of the total studies used to assess them.

3.2. Smoke chemistry studies

A total of 482 ingredients has been added at their typical maximum use level in various mixtures to 19 test cigarettes in 3 series of cigarettes (Baker et al., 2004a, b). The ingredients comprised 462 flavours, 1 flavour/solvent, 1 solvent, 7 preservatives, 5 binders, 3 humectants, 1 filler and 2 process aids (one of which was water). A summary of the ingredients used in this study, and which experimental cigarettes they were added to, is given in Table 1. The levels of a variety of smoke constituents in the test cigarettes were compared to those from the relevant control cigarette, with the cigarettes smoked under the standard ISO machine smoking conditions (ISO 3308, 2000). The smoke constituents determined were those 44 analytes colloquially known as the "Hoffmann analytes", outlined in Section 3.1. These are believed by regulatory authorities in Canada and the U.S.A. to be relevant to smoking-related diseases (Department of Health, Canada, 2000, Massachusetts Tobacco Control Program, 2001). The full results of the yields of mainstream smoke constituents from these cigarettes have been given in detailed tables and discussed elsewhere (Baker et al., 2004a, b). Summaries of the results are shown graphically on the "radar" charts of Figs. 2–4 for cigarette series A, B and C, respectively. In these diagrams the yield of each smoke analyte is shown as a percentage of the yield found in the smoke of the appropriate control cigarette. Thus the control cigarettes A1, B1 and C1 are at the 100% level for all the analytes in Figs. 2, 3 and 4, respectively.

From the charts in Figs. 2–4 it is seen that many of the ingredient mixtures in the various test cigarettes had no significant effect on the levels of "Hoffmann analytes" in smoke, typically the levels being 85–115% of the levels of the appropriate control cigarette. These differences are generally not statistically significant, as discussed in detail previously (Baker et al., 2004a, b). It is also seen from the figures that some of the test cigarettes had levels of "Hoffmann analytes" significantly lower than the control [e.g. phenol, *m*- + *p*-cresol and *o*-cresol from cigarettes B4 and B5, Fig. 3(ii)], and some were higher [e.g. formaldehyde from cigarettes B2 and B5 in Fig. 2(ii) and from cigarettes C3, C5, C8 and C9 in Fig. 4(ii)]. The statistical significances of the differences between the test and control cigarettes were determined firstly with a two-tailed Student's *t*-test with unknown and unequal variances, with the standard deviations across the replicate measurements (usually five) undertaken on the specific occasion of the determination. In addition, the statistical significance was determined a second time, again with a Student's *t*-test but based on a standard deviation obtained over many measurements of a reference cigarette in a one-year period, and assuming that the coefficient of variations of the reference, control and test cigarettes were the same (Baker et al., 2004a). The reference cigarette was the University of Kentucky 1R4F cigarette. Where statistically significant differences were found for a particular smoke analyte in a given test cigarette relative to its control, many were not present when the standard deviations over the extended period were used.

The British American Tobacco smoke chemistry study is thus similar to that of the Philip Morris study (Rustemeier et al., 2002). There are, however, two differences. Firstly, in the British American Tobacco study, we have taken into account the long-term variability of the analytical methodology in assessing the significance of differences with the practical approach discussed above. Secondly, in our comparisons of smoke yields from test and control cigarettes, the actual

Table 5
Yield of furfural from cigarette containing sorbitol

Cigarette	Sorbitol	Furfural smoke yield[a], μg/cig.	
	(%)	Particulate phase	Vapour phase
C1 (control)	–	5.2±0.1	2.6±1.0
C5	3.53	6.4±0.5	3.6±0.7

[a] Mean±S.D., $n=2$.

yields of the "Hoffmann analytes" were used rather than the yields normalised to TPM, as compared by Rustemeier et al. (2002). This enabled us to have a direct comparison of yields in the mainstream smoke from the cigarettes. In any case, the maximum increases in TPM in test cigarettes from our study were only 10% relative to the control cigarette, cf. 28% increases in the Philip Morris study (Rustemeier et al., 2002). Consequently, normalisation to TPM in our study would have only a relatively small effect on comparisons.

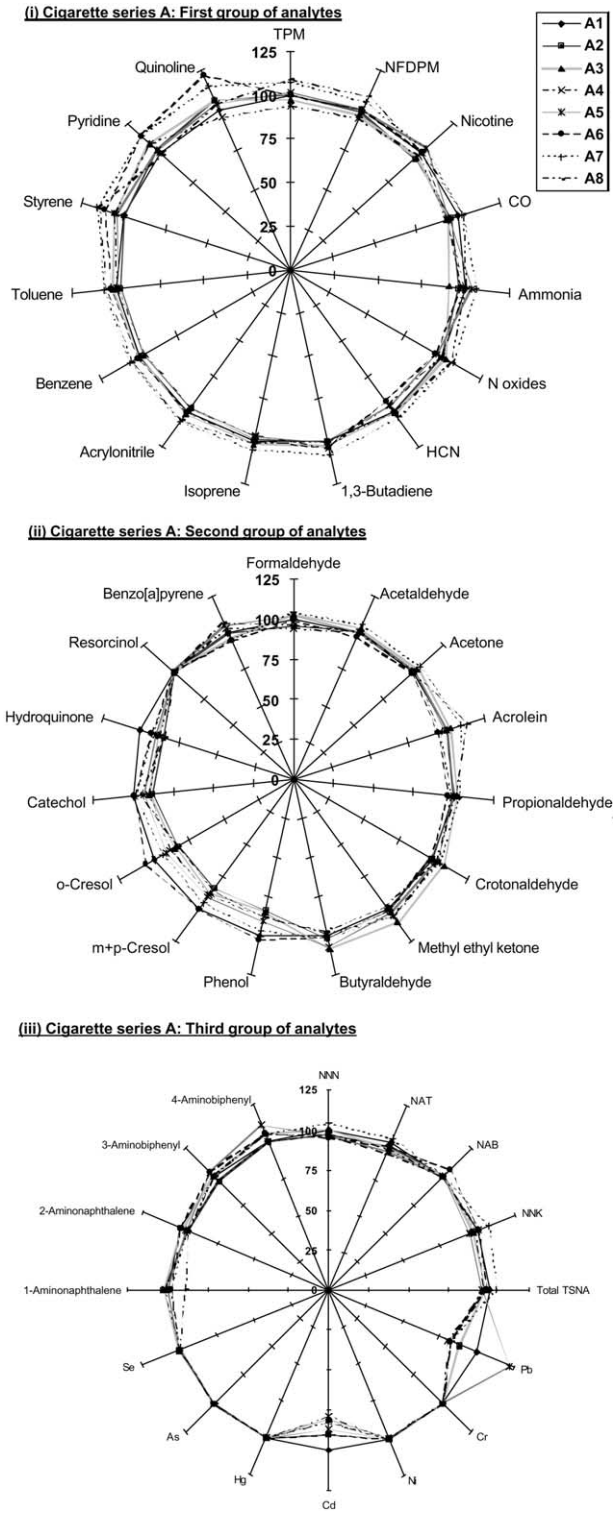


Fig. 2. Yields of smoke components from test cigarettes in series A as percentage of yields from control cigarette A1.

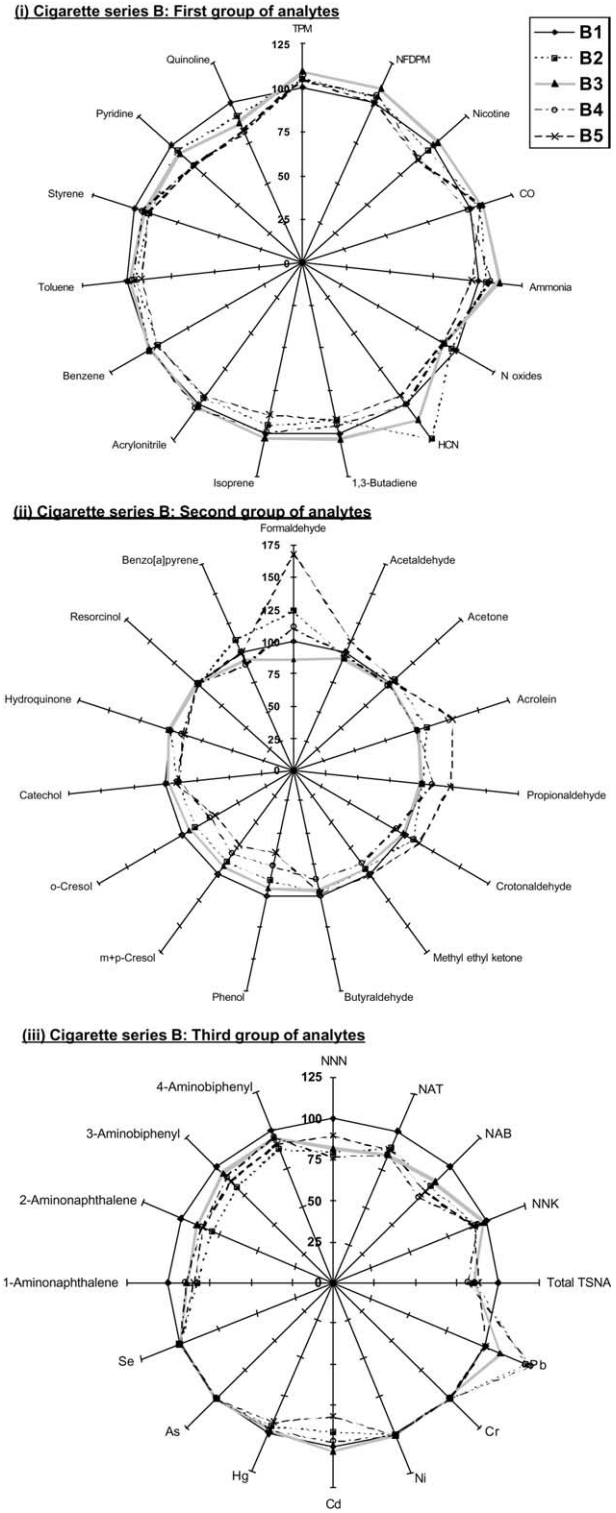


Fig. 3. Yields of smoke components from test cigarettes in series B as percentage of yields from control cigarette B1.

From the 45[1] analytes measured in smoke in each of the 19 test cigarettes, those that are, statistically, significantly higher than those from the relevant control cigarette are listed in Table 6, taking into account the long-term standard deviations. The percentage differences between test and control cigarettes range from 5.5 to 73%, although in some cases the absolute differences are small. Given the high overall long-term variabilities in the results, which of the differences between the control and test cigarettes are 'real', i.e. which would still be present if the analyses were repeated? Over a large sample set, ± 1.96 standard deviations would give a 95% confidence limit of the total variability in a given smoke analyte level (White et al., 1979). If the difference between control and test levels is greater than this total variability, then the difference was considered to be 'real'. The differences concluded to be 'real' are indicated in Table 6; in particular the formaldehyde differences for test cigarettes B2, B5, C3, C8 and C9 are 'real'. In confirmation of this it has been found that the relative differences between the test and control cigarettes were similar when the analyses were repeated on two other occasions, separated by many months (Baker et al., 2004b).

Based on the more detailed discussion of these results given by Baker et al. (2004a,b), the elevated formaldehyde levels in test cigarette B5 were likely to be due to its formation from the pyrolysis of cellulose and other polysaccharide materials that were present in the experimental reconstituted sheet material added to test cigarette B5. The elevated formaldehyde levels in test cigarettes B2, C3, C8 and C9 were likely to be due to its generation from the pyrolysis of the various sugar ingredients included in the casing of this test cigarette.

In the present smoke analysis study, the smoke yields of the 1R4F reference cigarette and two internal British American Tobacco reference cigarettes were also determined. One of these reference cigarettes, designated Ref2, contained 100% flue-cured tobacco and no added tobacco ingredients (Baker et al., 2004a). The formaldehyde smoke levels from this cigarette, determined at the same times as the test cigarettes B2 and B5, and C3, C8 and C9, are compared in Table 7. It is seen that the smoke formaldehyde level of the Ref2 and B5 test cigarettes are very similar. Thus the addition of the cellulose and other polysaccharide ingredients to the all-tobacco U.S. tobacco blend in B1 produced a cigarette (B5) whose smoke had formaldehyde levels similar to a flue-cured cigarette with no added ingredients. Flue-cured tobacco contains relatively high levels of sugars naturally present in the tobacco, produced from tobacco starch during the flue-curing process (Leffingwell, 1999). U.S. blended tobacco (as used in cigarettes B1 and B5) contains relatively low levels of naturally-present sugars. Thus the sugars naturally present in a flue-cured tobacco cigarette (Ref2) produce the same levels of formaldehyde in smoke as the cellulosic and polysaccharide ingredients added to the tobacco-only

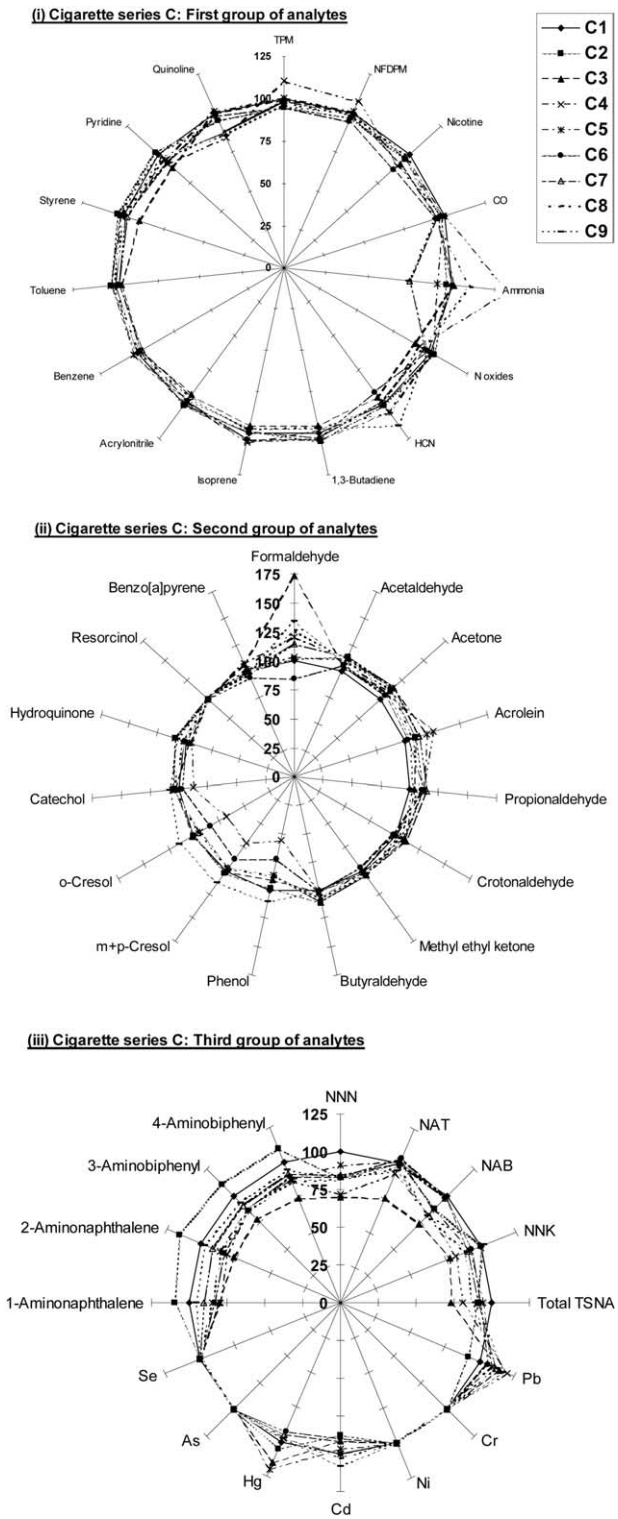


Fig. 4. Yields of smoke components from test cigarettes in series C as percentage of yields from control cigarette C1.

[1] There are 44 "Hoffmann analytes". The total of 45 used here includes both NFDPM and TPM.

Table 6
Increases in levels of "Hoffmann analyte" smoke yields that are statistically significant and long-term analyte variability

Cig. code	Analyte	Unit	Levels in smoke				Variability[a]	Is diff. > variability?
			Control	Test	Diff.	% Diff.		
A7	TPM	mg	17.3	18.6	1.3	7.5	1.97	No
	NFDPM	mg	12.8	13.5	0.7	5.5	1.26	No
A8	TPM	mg	17.3	18.9	1.6	9.2	1.97	No
	NFDPM	mg	12.8	13.9	1.1	8.6	1.26	No
B2	HCN	μg	209	260	51	24	38.0	Yes
	Formaldehyde	μg	23.8	29.5	5.7	24	5.32	Yes
B3	TPM	mg	18.6	20.4	1.8	9.7	2.12	No
	NFDPM	mg	14.2	15.5	1.3	9.2	1.40	No
	CO	mg	14.2	15.2	1.0	7.0	1.75	No
B5	Formaldehyde	μg	23.8	39.9	16.1	68	5.32	Yes
	Acrolein	μg	72.2	93.3	21.1	29	15.8	Yes
	Propionaldehyde	μg	54.1	66.2	12.1	22	10.2	Yes
C3	Formaldehyde	μg	35.6	61.6	26	73	7.96	Yes
C4	TPM	mg	18.6	20.5	1.9	10	2.12	No
	NFDPM	mg	13.3	14.3	1.0	7.5	1.31	No
	NH_3	μg	24.0	31.9	7.9	33	6.25	Yes
	Acrolein	μg	79.9	101	21.1	26	17.4	Yes
C5	Formaldehyde	μg	35.6	43.0	7.4	21	7.96	No
	Acrolein	μg	79.9	95.9	16	20	17.4	No
C8	Formaldehyde	μg	35.6	45.0	9.4	26	7.96	Yes
C9	HCN	μg	238	274	36	15	43.3	No
	o-Cresol	μg	4.14	4.75	0.61	15	0.768	No
	Formaldehyde	μg	35.6	47.7	12.1	34	7.96	Yes

[a] Variability is defined as ±2 S.D.. It was calculated using the S.D. of historical data on the 1R4F reference cigarette for each smoke analyte, determined in the analytical laboratory of the present study, and assuming a constant coefficient of variation between the 1R4F and control cigarettes.

U.S. blended cigarette (B5). Control cigarette C1 gave a higher yield of smoke formaldehyde than control cigarette B1, because the tobacco in C1 contained some cellulose in the reconstituted sheet included in the U.S. tobacco blend (Baker et al., 2004b). The addition of sugar in the casing (test cigarette C3) gave smoke with a substantially higher formaldehyde yield than that from the flue-cured reference cigarette Ref2 (Table 7).

We have dealt in some detail with these smoke chemistry results because there are a small number of statistically significant increases in the presence of some ingredients, in particular formaldehyde levels from sugars and cellulose. These increases are similar to those reported in the Philip Morris study by Rustemeier et al. (2002) and some of the studies included in the review of Paschke et al. (2002). We repeat the statement of Rustemeier et al. (2002) that further research is warranted in order to investigate these differences. However, and again similar to the overall findings in the Philip Morris of Carmines (2002), these chemical differences have not resulted in any differences in the various bioassays undertaken, as indicated in Sections 3.3 and 3.4 below.

3.3. In vitro bioassay studies

Three short term in vitro toxicological tests have been performed on the test and control cigarettes: the Ames test (a bacterial mutation assay), the in vitro micronucleus test (a mammalian cell assay for chromosome structural and numerical damage), and the neutral red

Table 7
Comparison of formaldehyde yields in smoke from test, control and flue-cured reference cigarette

Cigarette	Formaldehyde (μg/cig)
B1 (control)	23.8
B2	29.5
B5	39.9
Ref2[a]	38.6
C1 (control)	35.6
C3	61.6
C8	45.0
C9	47.7
Ref2[a]	42.5

[a] British American Tobacco internal reference cigarette containing 100% flue-cured tobacco and no added tobacco ingredients.

uptake assay (a mammalian cell assay for cytotoxicity). For practical reasons it was not generally possible to perform these bioassays on whole smoke, so solutions of cigarette smoke TPM were used. The bioassays were thus responding to possible changes in the chemical makeup of the particulate phase of the cigarette smoke. The purpose of these tests was to assess whether or not the test cigarettes containing the ingredients had a changed overall specific activity of the smoke TPM relative to the control cigarettes. For all the bioassays, the activity is quoted per μg NFDPM or per μg NFDPM/ml culture medium. Each bioassay was carried out in groups of the experimental cigarette series A, B and C, i.e. the test and relevant control were measured in the same match.

3.3.1. Ames bioassay

TPM from the test and control cigarettes was tested with five Ames bacterial tester strains, TA98, TA100, TA102, TA1535 and TA1537, both in the presence and absence of an S9 metabolic activating fraction. Ames strains TA98 and TA1537 are sensitive to the frame shift mutagens, TA100 and TA1537 are sensitive to base-pair substitution events, and TA102 is sensitive to small deletions (Gatehouse et al., 1990).

In the presence of an S9 metabolic activating fraction with Ames strains TA102 and TA1535, only occasional sporadic increases in revertants were observed with a few smoke TPM dose levels relative to the solvent only. With these strains there was no clear evidence for mutagenic activity. With Ames strains TA98, TA100 and TA1537, all the control and test cigarette smoke TPM samples gave statistically significant positive responses with revertant response-NFDPM dose relationships. A representative data set using strain TA98, in the presence of the S9 metabolic activating fraction, for TPM from the A series of cigarettes is illustrated in Fig. 5. The gradients of the linear portion of these relationships, expressed as revertants per μg NFDPM, are the specific activity and are listed in Table 8. The Tukey least significant differences were also calculated (e.g. Weiss, 1982). These are the least significant differences that can exist between two samples before they are deemed to be statistically significant. For most of the results in Table 8, there was no statistically significant difference between the specific activity of the TPM samples of the test cigarettes and their relevant control cigarette. For six results, the test cigarette had a significantly lower specific activity than its control (cigarettes B5, C3, C4 and C5). Test cigarette A3 with Ames strain TA1537 was on the very edge of statistical significance. The specific activity of test cigarette A3 was 0.21 revertants/μg NFDPM, its control (A1) was 0.16, and the Tukey least significant difference was 0.05. Moreover, examination of the individual revertant/dose response for the A3 and A1 experiments with TA1537 indicated that there was no real difference in the mutagenic activities of the two TPM samples.

In the absence of the S9 metabolic activating fraction, Ames strains TA102 and TA1535 gave occasional sporadic increases in revertants in a few smoke TPM samples, relative to the solvent only. However, there was no clear evidence for mutagenic activity. With the Ames strains TA98, TA100 and TA1537 in the absence of S9, increases in revertant numbers were observed in at least one experimental occasion with the cigarettes. These increases were smaller than those observed in the presence of S9 and were not statistically significant between different dose levels. No assessable differences were observed between smoke TPM samples.

Thus, within the sensitivity and specificity of the Ames test, the specific activity of the cigarette smoke TPM was not increased by the addition of ingredients to the cigarettes.

3.3.2. In vitro mammalian cell micronucleus assay

Within the sensitivity and specificity of the in vitro mammalian cell micronucleus assay, cigarette smoke TPM produced from the test cigarettes did not produce any biologically-relevant increases in the levels of

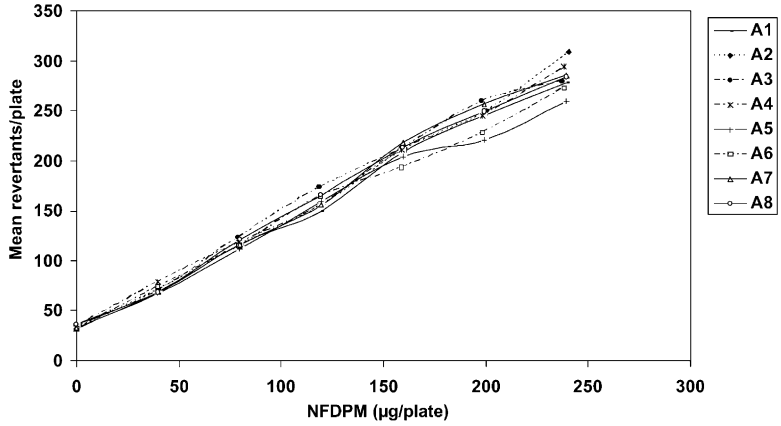


Fig. 5. Ames data using strain TA98 + S9 on smoke particulate matter from control (A1) and test cigarettes A2–A8.

Table 8
Revertants for Ames strains TA98, TA100 and TA1537 in the presence of S9[a]

Particulate matter	Induced revertants/μg NFDPM (Mean±S.D.)[a]			Type of ingredients
	TA98	TA100	TA1537	
A1 (control)	1.00±0.23	0.33±0.13	0.16±0.04	–
A2	1.05±0.27	0.37±0.12	0.18±0.06	Flavourings
A3	1.09±0.23	0.36±0.18	0.21±0.07[c]	Flavourings
A4	1.08±0.19	0.34±0.13	0.17±0.06	Flavourings
A5	0.97±0.16	0.33±0.13	0.16±0.06	Flavourings
A6	1.02±0.24	0.31±0.11	0.17±0.04	Flavourings
A7	1.04±0.22	0.35±0.19	0.19±0.05	Flavourings
A8	1.05±0.21	0.32±0.14	0.17±0.05	Flavourings
Tukey's least significant difference[b]	0.17	0.11	0.05	
B1(Control)	1.30±0.29	0.24±0.14	0.29±0.06	–
B2	1.19±0.30	0.27±0.15	0.24±0.07	Flavourings/casings
B3	1.21±0.39	0.22±0.18	0.27±0.06	Flavourings/casings
B4	1.18±0.26	0.27±0.19	0.23±0.07	Flavourings/casings
B5	1.11±0.27[c]	0.23±0.17	0.20±0.08[c]	Sheet ingredients
Tukey's least significant difference[b]	0.25	0.13	0.06	
C1 (Control)	1.32±0.27	0.48±0.17	0.17±0.09	–
C2	1.31±0.21	0.37±0.22	0.18±0.07	Casings
C3	1.16±0.22	0.31±0.14[c]	0.11±0.09	Casings
C4	1.08±0.33[c]	0.26±0.14[c]	0.14±0.07	Casings
C5	1.25±0.19	0.29±0.22[c]	0.16±0.05	Casings
Tukey's least significant difference[b]	0.22	0.15	0.06	
C1(Control)	1.34±0.25	0.43±0.15	0.14±0.08	–
C6	1.29±0.14	0.27±0.15	0.15±0.09	Casings
C7	1.26±0.17	0.37±0.10	0.13±0.07	Casings
C8	1.31±0.19	0.36±0.14	0.12±0.05	Casings
C9	1.29±0.12	0.40±0.20	0.16±0.06	Casings
Tukey's least significant difference[b]	0.16	0.12	0.06	

[a] Mean±standard deviation (S.D.) of four replicate experiments.
[b] Tukey's least significant difference that can exist between two samples, before they are deemed to be statistically significant.
[c] Significantly different to the control condensate.

micronucleus induction, above those of the relevant control cigarette TPM. A representative plot of data is illustrated in Fig. 6 for some of the cigarettes in the A series. These data are not amenable to description as a single numerical value and so no table of summarised data is given. The tobacco ingredients included in the test cigarettes do not significantly alter the activity of their cigarette smoke TPM samples relative to their control cigarette TPM.

3.3.3. Neutral red uptake cytotoxicity bioassay

A representative data set of the neutral red uptake results is illustrated in Fig. 7 for TPM obtained from the A series of cigarettes The neutral red uptake results obtained with the smoke TPM from the cigarettes in series A, B and C are summarised in Table 9. From both the Tukey pair wise comparison and a two sample *t*-test, no statistically significant differences were found between the IC_{50} values of smoke condensate from any of the test cigarettes and their respective control cigarettes. Consequently, within the sensitivity and specificity of the neutral red uptake cytotoxicity bioassay, none of the test cigarette smoke TPM samples induced biologically-relevant changes in cytotoxicity above those of the control cigarette smoke TPM.

3.4. Sub-chronic inhalation toxicity studies

Three 90-day sub-chronic inhalation studies have been undertaken using smoke generated from the test and control cigarettes in the three cigarette series A, B and C. Other groups of animals were used for cage control, air control and health screen purposes. The experimental design is summarised in Table 2 and the experimental details are described in Section 2.5 above. Analysis of the test atmospheres throughout the inhalation periods indicated that the cigarette mainstream smoke was reproducibly generated and delivered to the exposure chamber to within 6% of the target concentration of 1 mg/l of air. Typical average chamber concentrations during the study using the C series of cigarettes are shown in Table 10.

COHb levels obtained on week 9 were significantly higher in the smoke-exposed animal groups compared to the air control group. COHb levels were similar between all treated groups. There were two deaths that

occurred during the treatment periods in the first and third inhalation studies (for the C and B cigarette series, respectively, Table 2). These deaths were related to tube restraint or bleeding procedures and were not considered to be treatment-related. Mean body weight and/or weight gain of smoke-treated male animals generally decreased from week 3 of exposure onwards. There were no significant changes in body weight or body weight gains of smoke-treated female animals. The weights of lungs from all smoke-treated groups were increased in absolute terms and relative to body and brain weights. The increases were statistically significant relative to body weight. There were no evident differences between animals exposed to smoke from the various different cigarettes.

No treatment associated gross changes were noted at necropsy. The various gross changes that were seen were within the normal range of observations for rats of this age and were considered by the pathologist to be spontaneous in origin.

Treatment-related microscopic changes were observed within the respiratory tracts (nasal cavity/sinuses, larynx

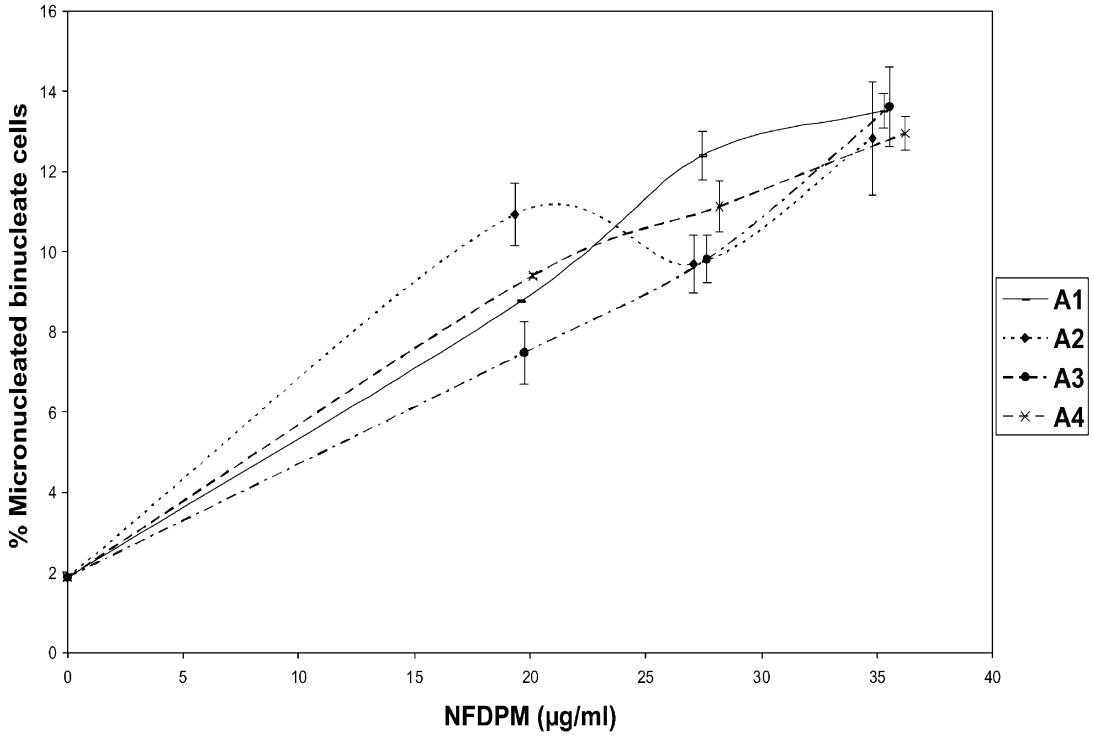


Fig. 6. In vitro micronucleus induction following 3-h treatment in the absence of an S9 metabolic activating fraction, by smoke particulate matter from control (A1) cigarette and test cigarettes, A2, A3 and A4. Mean ± standard error range shown ($n = 2$).

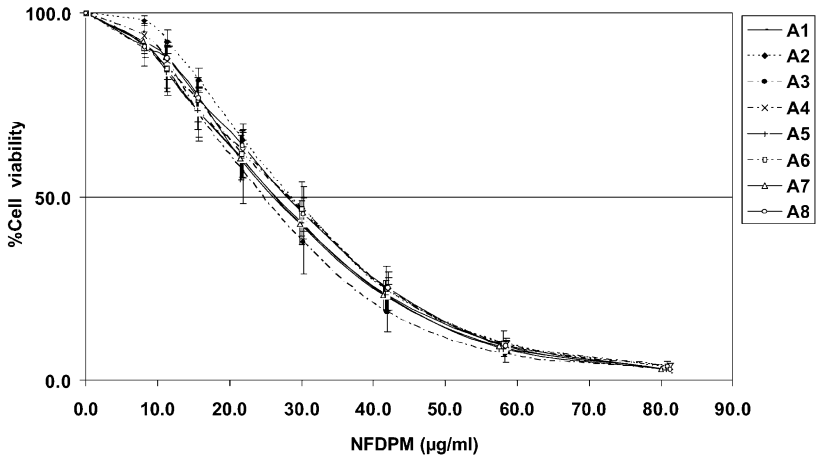


Fig. 7. Cytotoxicity of smoke particulate matter measured by neutral red uptake assay and expressed as % cell viability: particulate matter from control (A1) and test cigarettes A2–A8. Mean ± standard error range shown ($n = 4$).

and lungs) of the animals exposed to cigarette smoke. These changes are typical of those normally seen in animals exposed to cigarette smoke. As all exposed animals were affected, group differentiation could not be made in terms of incidence of lesions. A representative set of the summarised histopathological findings for the male and female groups of rats following 90-day inhalation of air only (control) and smoke from cigarettes in series B is shown in Table 11. The table shows the mean severity score that was assigned to the major treatment-related histopathological changes that were recorded. The scale was 0 (no change), 1 (minimal change) to 5 (severe change).

In the nasal cavities, hyperplasia/degeneration of the transitional epithelium lining the sinuses/turbinates at the first (most cranial) level was observed in all exposed animals. Associated with this change was some degree of inflammation. Occasionally, some hyperplasia of respiratory epithelium and inflammation affecting portions of the central septum were observed, with concomitant loss of goblet cells. Olfactory epithelium was essentially unaffected and changes were confined to this first level.

In the larynx, squamous metaplasia and hyperplasia affected all regions from the epiglottis to the vocal cords. Occasionally, some focal keratinisation and/or erosion were also recorded. In a proportion of animals, necrosis of the U-shaped cartilage adjacent to the ventral pouch was noted. In the lungs some minor hyperplasia and/or goblet cell hypertrophy was noted in a small number of animals. In the lungs of some animals, aggregations of alveolar macrophages, frequently including brown pigment, were recorded. These minor changes probably accounted for the small increases in lung weights of smoke-treated animals, noted above.

In general, there were no clear differences in the tissue response of animals from any single smoke-exposed group of animals. The various other histopathological changes in the tissues examined were typical of those commonly encountered in animals of this species and age and were considered by the pathologist to be spontaneous in origin. There are differences in Table 11 between some of the histopathological parameters for the air control and the smoke-exposed animal groups. There were no statistically significant differences between any of the mean histopathological parameters of the control cigarette (B1) and the test cigarettes B2–B5 in Table 11. This is also the case for the control cigarette A1 and the test cigarettes in the A series, and the control cigarette C1 and test cigarettes in the C series.

Histomorphometric assessment of certain parameters in the respiratory tract was conducted following the routine histological assessment and representative results for the air control and smoke exposure from cigarettes in the B series are given in Table 12. Increases are seen in the density of goblet cells in the respiratory

Table 9
Summary of neutral red cytotoxicity data of smoke total particulate matter from test cigarettes

Particulate matter	Mean IC_{50}±S.D.[a] (μg NFDPM/ml)	Type of ingredients
A1 (Control)	27.7±7.01	–
A2	29.1±5.86	Flavourings
A3	26.0±6.70	Flavourings
A4	27.5±7.07	Flavourings
A5	26.4±4.68	Flavourings
A6	28.7±6.40	Flavourings
A7	26.9±4.97	Flavourings
A8	27.9±3.74	Flavourings
B1 (Control)	21.7±2.57	–
B2	23.3±2.59	Flavourings/casings
B3	24.0±3.12	Flavourings/casings
B4	22.2±3.43	Flavourings/casings
B5	22.1±3.65	Sheet ingredients
C1 (Control)	22.5±1.97	–
C2	21.1±1.75	Casings
C3	23.2±2.13	Casings
C4	29.2±9.48	Casings
C5	22.4±3.30	Casings
C6	23.9±2.49	Casings
C7	23.0±1.56	Casings
C8	23.5±2.69	Casings
C9	22.5±1.97	Casings

[a] The inhibiting concentration that results in a reduction in viability to 50% that of the untreated control cell viability. Data are means± standard deviation (S.D.) of four replicate experiments.

Table 10
Animal groups[a] and average chamber concentrations during the first 90-day inhalation study

Group no.	Cigarette code	Average chamber conc. (μg/l)		
		TPM	Nicotine	CO
1	Cage control[b]	–	–	–
2	Air control[c]	–	–	–
3	C1 (Control)[d]	938	79.6	978
4	C2[d]	977	79.2	929
5	C3[d]	966	76.6	879
6	C4[d]	949	67.9	864
7	C5[d]	971	76.5	858
8	C6[d]	936	67.6	897
9	C7[d]	975	75.5	990
10	C8[d]	967	79.8	860
11	C9[d]	947	75.0	874
12	Health screen[e]	–	–	–

[a] Each group consisted of 10 male and 10 female rats.
[b] This group of animals was untreated.
[c] Animals had air rather than smoke administered to them, same conditions as described in footnote d.
[d] "Nose-only exposure" for 1 h per day, divided as follows: smoke 30 min, air 15 min, smoke 30 min, for 5 days per week.
[e] This group of animals used to test the general health of the rats under study.

Table 11
Summary of histopathological findings[a] in nasal cavity, larynx and lungs of male and female rats following 90-day inhalation of air (air control) and smoke from cigarettes in series B

Cigarette/animal group[b]	Air control	B1 (control)	B2	B3	B4	B5
Nasal cavity/sinuses	*Males*					
Hyperplasia/degeneration:	0.0±0.0	3.2±0.4	3.2±0.4	3.3±0.5	3.2±0.4	3.3±0.5
transitional epithelium	0/10	10/10	10/10	10/10	10/10	10/10
Inflammation: turbinates	0.0±0.0	0.1±0.3	0.0±0.0	0.6±1.0	0.0±0.0	0.2±0.4
	0/10	1/10	0/10	3/10	0/10	2/10
Inflammation: nasal septum	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.7	0.0±0.0	0.0±0.0
	0/10	0/10	0/10	2/10	0/10	0/10
Hyperplasia: respiratory	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.9	0.0±0.0	0.0±0.0
epithelium	0/10	0/10	0/10	1/10	0/10	0/10
Hyperplasia/hypertrophy:	0.0±0.0	0.3±0.7	0.4±0.7	0.0±0.0	0.0±0.0	0.6±1.0
goblet cells	0/10	2/10	3/10	0/10	0/10	3/10
Nasal cavity/sinuses	*Females*					
Hyperplasia/degeneration:	0.0±0.0	3.2±0.4	3.1±0.3	3.3±0.5	3.3±0.5	3.1±0.3
transitional epithelium	0/10	10/10	10/10	10/10	10/10	10/10
Inflammation: turbinates	0.0±0.0	0.0±0.0	0.5±0.9	0.2±0.4	0.0±0.0	0.3±0.7
	0/10	0/10	3/10	2/10	0/10	2/10
Inflammation: nasal septum	0.0±0.0	0.0±0.0	0.1±0.3	0.0±0.0	0.0±0.0	0.1±0.3
	0/10	0/10	1/10	0/10	0/10	1/10
Hyperplasia: respiratory	0.0±0.0	0.0±0.0	0.4±0.8	0.2±0.4	0.0±0.0	0.0±0.0
epithelium	0/10	0/10	2/10	2/10	0/10	0/10
Hyperplasia/hypertrophy:	0.0±0.0	0.0±0.0	0.0±0.0	0.2±0.4	0.2±0.6	0.0±0.0
goblet cells	0/10	0/10	0/10	2/10	1/10	0/10
Larynx	*Males*					
Metaplasia: squamous- epiglottis	0.1±0.3	3.1±0.3	3.0±0.0	3.0±0.0	3.0±0.0	3.1±0.3
	1/10	10/10	10/10	10/10	10/10	10/10
Metaplasia: squamous- arytenoid processes	0.0±0.0	2.4±0.5	2.4±0.5	2.8±0.4	2.3±0.5	2.8±0.4
	0/10	10/10	10/10	10/10	10/10	10/10
Metaplasia: squamous- ventrolateral	0.0±0.0	3.4±0.5	3.6±0.5	3.5±0.5	3.3±0.5	3.6±0.5
	0/10	10/10	10/10	10/10	10/10	10/10
Metaplasia: squamous- vocal cords	0.0±0.0	1.2±0.9	1.7±0.5	2.2±0.4	1.3±0.8	1.7±0.8
	0/10	8/10	10/10	10/10	9/10	10/10
Keratinisation	0.0±0.0	0.0±0.0	0.0±0.0	0.5±0.9	0.1±0.3	0.7±0.8
	0/10	0/10	0/10	1/10	1/10	5/10
Hyperplasia: epithelial	0.0±0.0	2.3±0.5	2.8±0.4	1.9±1.1	2.4±0.5	2.9±0.3
	0/10	10/10	10/10	8/10	10/10	10/10
Erosion	0.0±0.0	0.2±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	0/10	1/10	0/10	0/10	0/10	0/10
Necrosis: cartilage	0.0±0.0	0.0±0.0	0.2±0.6	0.5±1.1	0.2±0.6	0.0±0.0
	0/10	0/10	1/10	2/10	1/10	0/10
Larynx	*Females*					
Metaplasia: squamous- epiglottis	0.0±0.0	3.0±0.0	3.0±0.0	3.1±0.3	3.0±0.0	3.0±0.0
	0/10	10/10	10/10	10/10	10/10	10/10
Metaplasia: squamous- arytenoid processes	0.1±0.3	3.0±0.0	2.8±0.4	3.1±0.3	2.7±0.5	3.2±0.4
	1/10	10/10	10/10	10/10	10/10	10/10
Metaplasia: squamous- ventrolateral	0.0±0.0	3.6±0.5	3.5±0.5	3.5±0.5	3.7±0.5	3.9±0.6
	0/10	10/10	10/10	10/10	10/10	10/10
Metaplasia: squamous- vocal cords	0.0±0.0	2.1±1.1	2.0±0.8	1.6±0.8	1.8±0.8	2.5±1.0
	0/10	9/10	10/10	9/10	10/10	10/10
Keratinisation	0.0±0.0	0.4±0.7	0.2±0.4	0.4±0.5	0.2±0.4	0.5±0.7
	0/10	3/10	2/10	4/10	2/10	4/10
Hyperplasia: epithelial	0.0±0.0	2.9±0.3	2.8±0.4	3.0±0.0	2.5±0.5	3.2±0.4
	0/10	10/10	10/10	10/10	10/10	10/10
Erosion	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	0/10	0/10	0/10	0/10	0/10	0/10
Necrosis: cartilage	0.0±0.0	0.3±0.7	0.5±1.1	1.2±0.9	0.9±0.9	0.5±0.9
	0/10	2/10	2/10	8/10	6/10	3/10
Lung	*Males*					
Hyperplasia/hypertrophy: bronchial/iolar goblet cells	0.0±0.0	0.6±0.7	0.7±0.7	0.3±0.7	0.0±0.0	1.4±1.0
	0/10	5/10	6/10	2/10	0/10	8/10

(*continued on next page*)

Table 11 (*continued*)

Cigarette/animal group[b]	Air control	B1 (control)	B2	B3	B4	B5
Nasal cavity/sinuses	*Males*					
Hyperplasia: BALT[c]	0.0±0.0	0.1±0.3	0.5±0.9	0.3±0.7	0.0±0.0	0.4±0.8
	0/10	1/10	3/10	2/10	0/10	2/10
Macrophage accumulation	0.0±0.0	0.3±0.5	0.7±0.7	0.1±0.3	0.3±0.5	0.1±0.3
	0/10	3/10	6/10	1/10	3/10	1/10
Lung	*Females*					
Hyperplasia/hypertrophy: bronchial/iolar goblet cells	0.0±0.0	0.3±0.5	0.4±0.8	0.4±0.7	1.0±0.9	1.0±1.2
	0/10	3/10	2/10	3/10	6/10	5/10
Hyperplasia: BALT[c]	0.1±0.3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
	1/10	0/10	0/10	0/10	0/10	0/10
Macrophage accumulation	0.0±0.0	0.0±0.0	0.3±0.5	0.4±0.8	0.0±0.0	0.1±0.3
	0/10	0/10	3/10	2/10	0/10	1/10

[a] Mean±standard deviation of histopathology severity index is given for the male or female animals in each group ($n=10$) on a scale 0 (none), 1 (minimal) to 5 (severe). Incidence level is also given in each animal group.
[b] 10 male and 10 female rats in each group.
[c] Bronchus-associated lymphoid tissue.

epithelium lining the trachea, as well as in the bronchioli of the left lung, in all the smoke-exposed groups of animals when compared to the air control group animals. Similarly an increase was observed, in all tobacco smoke exposed groups, in the thickness of the epithelium covering the arytenoids cartilage of the larynx. These smoke-exposed increases were statistically significant relative to the air control group. No meaningful effect was seen with respect to the goblet cell density of the epithelium covering the central and nasal septum between the air control group of animals and the smoke-exposed groups. These observed changes are typical of those observed with smoke treatment.

In general, there were no discernable differences in the various histomorphometric parameters between groups of animals exposed to the various test cigarettes and their respective control cigarette. The data in Table 12 indicate that the mean left lung goblet cell density for the male animal group exposed to smoke from cigarette B4 was significantly lower than that from the B1 (control) cigarette group at the 99% confidence level. On the other hand, the mean left lung goblet cell density for the female animal group exposed to smoke from the same cigarette, B4, was significantly higher than that from the B1 (control) group at the 95% confidence level. When the male and female responses for left lung goblet cell density are combined for cigarette B4, there is no statistically significant difference between the mean (16.2±13.2, $n=20$) and that of the combined male and female response with the control cigarette, B1 (15.7±8.1, $n=20$).

In summary, cigarette smoke inhalation treatment for 13 weeks, 5 days per week at targeted chamber TPM concentrations of 1 mg/l of air, produced evidence of smoke effect on body weights and increased COHb levels. There were no smoke treatment-related clinical

Table 12
Summary of histomorphometry parameters[a] of the respiratory tract tissues of male and female rats following 90-day inhalation of air (air control) and smoke from cigarettes in series B

Cigarette/animal group[b]	Air control	B1 (control)	B2	B3	B4	B5
Male						
Septal goblet cell density (mm^{-1})	287±37	260±37	261±33	277±44	247±31	263±24
Tracheal goblet cell density (mm^{-1})	1.2±0.8	7.8±5.6	4.8±6.2	5.6±2.8	5.9±3.0	6.3±3.8
Left lung goblet cell density (mm^{-1})	3.9±3.3	19±8.1	14±8.3	12±17	7.9±5.5[c]	21±12
Epitheleum thickness (μm)	20±5.0	36±5.7	38±5.1	40±4.3	35±3.9	38±5.4
Female						
Septal goblet cell density (mm^{-1})	270±33	273±40	311±53	296±60	260±27	252±40
Tracheal goblet cell density (mm^{-1})	0.6±0.4	4.9±2.7	7.1±9.5	4.5±2.7	6.2±6.7	5.1±4.7
Left lung goblet cell density (mm^{-1})	1.6±0.8	13±7.6	12±11	13±6.1	24±14[d]	22±16
Epitheleum thickness (μm)	21±6.4	36±5.9	37±4.9	38±4.7	35±5.5	39±8.2

[a] Mean±standard deviation for the male or female animals in each group, $n=10$.
[b] 10 male and 10 female rats in each group.
[c] Significantly lower than B1 (control cigarette) at 99% confidence level.
[d] Significantly higher than B1 (control cigarette) at 95% confidence level.

signs observed during the course of the study. Smoke treatment-related microscopic changes were observed in the nasal cavities, trachea, bronchi, larynx and lungs of all smoke-treated groups. No consistent differentiation in response was observable between animals from the smoke-exposed test groups for the cigarettes in series A, B and C and their respective control cigarettes. Histomorphometry indicated that responses due to tobacco smoke exposure were not distinguishable between the smoke from the test and control cigarettes in the studies for the cigarettes in series A, B and C.

4. Overall summary

1. An overview is given of the effects of 482 tobacco ingredients on various aspects of the chemistry and biological properties of cigarette mainstream smoke. The ingredients comprise: 462 flavours, 1 flavour/solvent, 1 solvent, 7 preservatives, 3 humectants, 5 binders, 1 filler and 2 process aids (one of which is water). The ingredients were added to 19 test cigarettes at or above their typical maximum use levels, in various combinations in three experimental cigarette series. The levels of smoke constituents believed to have biological significance ("Hoffmann analytes"), various in vitro bioassay endpoints, and inhalation toxicity of smoke from the test cigarettes have been compared to those from the appropriate control cigarette. In addition, the behaviour of the single component ingredients during isolated pyrolysis has been measured.
2. A pyrolysis technique has been developed that mimics the combustion conditions inside a burning cigarette. Using this technique on 291 single-substance ingredients, almost a third of the ingredients transferred out of the pyrolysis zone at least 99% intact, and almost two thirds transferred at least 95% intact. For those ingredients that underwent some degree of pyrolysis, the pyrolysis products and their relative amounts were determined. An upper level for the maximum amount of each pyrolysis product in mainstream smoke from the ingredient was calculated, for a plain (unfiltered) cigarette. "Hoffmann analytes" were detected amongst the pyrolysis products of 19 of the 291 substances pyrolysed. The maximum levels in smoke from pyrolysis of the ingredients were predicted to be generally small compared to the levels already typically present in smoke. An exception was acetaldehyde and benzene from the pyrolysis of malic acid. However, subsequent smoke chemistry studies indicated that the maximum predicted pyrolysis levels in smoke are overestimated, or that malic acid does not undergo complete pyrolysis in the burning cigarette and/or generates acetaldehyde and benzene at similar rates to that of tobacco on a per weight basis.
3. When the ingredients were added to tobacco and the resultant cigarettes smoked, the presence of many of the ingredient mixtures had no significant effect on the levels of many of the "Hoffmann analytes" in smoke, while some produced increases or decreases relative to a control cigarette. The study has concentrated on those ingredients that produced increases in "Hoffmann analytes". The long-term variability of the analytical methodology has been determined using historic analyses for the University of Kentucky 1R4F reference cigarette. Many of the differences were found to be not significant when the long-term variability of the analytical methodology was taken into account. However, within this variability, the smoke formaldehyde levels in two of the test cigarettes, B5 and C3, were significantly increased relative to their controls by 16 μg (68%) and 26 μg (73%), respectively. This was likely to be due to the generation of formaldehyde by the pyrolysis of sugars, cellulose and other polysaccharide materials.
4. The specific activity of smoke TPM from the test and control cigarette has been determined with three in vitro bioassays for genotoxicity and cytotoxicity. These were the Ames test, the mammalian cell micronucleus assay, and the neutral red uptake cytotoxicity assay. Within the sensitivity and specificity of these bioassays, the specific activity of the cigarette smoke TPM was not changed by the addition of ingredients to the cigarette.
5. Three 90-day sub-chronic inhalation studies have been undertaken and histopathological and histomorphometric assessments made within the respiratory tracts of animals exposed to smoke from cigarettes containing the various ingredient mixtures and their control cigarettes. The response due to tobacco smoke exposure was not distinguishable between the test and control cigarettes, indicating that the presence of the ingredients had made no discernable differences to the type and severity of the treatment-related changes.

5. General conclusions

This work has contributed to the enormous amount of work that has been conducted over the last fifty years on the effects of tobacco ingredients on the chemical and biological properties of cigarette smoke. This work

was comprehensively reviewed in three major reviews published in 2002. Two of these reviews contained details of many previously unpublished studies from the R.J. Reynolds Tobacco Company. In addition, large studies from Philip Morris and Lorillard Tobacco Company have recently been published on the chemical and biological effects of ingredients used by those companies. The present paper gives an overview of the studies conducted at British American Tobacco. All of these studies have indicated that commonly used tobacco ingredients do not change the toxicity of smoke as measured in specified assays. Also, the ingredients have no effect on the levels of most smoke constituents that may be relevant to smoking-related diseases. The few smoke constituents where increases have been observed in the presence of some tobacco ingredients, in particular formaldehyde, are highlighted in this paper.

Acknowledgements

It is a pleasure to acknowledge Alan Rodgman, who has made significant contributions to this paper, both by reviewing it and also sharing his considerable knowledge on the subject with us.

References

Baker, R.R., 1975. Temperature variation within a cigarette combustion coal during the smoking cycle. High Temp. Sci. 7, 236–247.

Baker, R.R., 1977. Combustion and thermal decomposition regions inside a burning cigarette. Combust. Flame 30, 21–32.

Baker, R.R., 1981a. Product formation mechanisms inside a burning cigarette. Prog. Energy Combust. Sci. 7, 135–153.

Baker, R.R., 1981b. Variation of the gas formation regions within a cigarette combustion coal during the smoking cycle. Beitr. Tabakforsch. Int 11, 1–17.

Baker, R.R., 1987. A review of pyrolysis studies to unravel reaction steps in burning tobacco. J. Anal. Appl. Pyrol. 11, 555–573.

Baker, R.R., 1999. Smoke chemistry. In: Davis, D.L., Nielsen, M.T. (Eds.), Tobacco-production, Chemistry and Technology. Blackwell Science, Oxford, UK, pp. 398–439. Chapter 12.

Baker, R.R., Bishop, L.J., 2004. The pyrolysis of tobacco ingredients. J. Anal. Appl. Pyrol. doi:10.1016/S0165-2370(03)00090-1.

Baker, R.R., Pereira da Silva, J.R., Smith, G., 2004a. The effect of tobacco ingredients on smoke chemistry. Part 1: Flavourings and additives. Food and Chem. Toxicol. doi:10.1016/S0278-6915(03)00189-3, in this issue.

Baker, R.R., Pereira da Silva, J.R., Smith, G., 2004b. The effect of ingredients on smoke chemistry. Part II: Casing ingredients. Food and Chem. Toxicol. doi:10.1016/j.fct.2003.08.009, in this issue.

Bombick, D.W., 2002. Assessment of tobacco smoke by in vitro cell toxicity assays. Recent Adv. Tob. Sci. 28, 104–136.

Carmines, E.L., 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 1: Cigarette design, testing approach, and review of results. Food and Chem. Toxicol. 40, 77–91.

Chortyk, O.T., Schlotzhauer, W.S., 1973. Studies on the pyrogenesis of tobacco smoke constituents (a review). Beitr. Tabak 7, 165–178.

Code of Federal Regulations (U.S.A.), revised 2001. Title 40, Protection of the environment, Part 792, Good laboratory practice standards. U.S. Government Printing Office; Internet website: http://www.access.gpo.gov/nara/cfr/waisidx_01/140cfrv28_01.htlm.

Department of Health, Canada, 2000. Proposed Tobacco Reporting Regulations, Schedule 2, Official methods for the collection of emission data on mainstream smoke. Canada Gazette Part 11, 133 (15), 19 July 2000, 1781–1784.

Dunnett, C.W., 1955. A multiple comparison procedure for comparing treatments with a control. J. Am. Stat. Assoc. 50, 1096–1121.

EEC (European Economic Commission) Directive 67/548, 1967. Toxicology and chemical substances. Testing methods of Annex V. Test B14.

Gatehouse, D.G., Wilcox, P., Forster, R., Rowland, I.R., Callander, R.D., 1990. Bacterial mutation assays. In: Kirkland, D.J. (Ed.), Basic Mutagenicity Tests: UKEMS Recommended Procedures. Cambridge University Press, Cambridge, UK, pp. 13–61.

Gaworski, C.L., Dozier, M.M., Gerhart, J.M., Rajendran, N., Brennecke, L.H., Aranyi, C., Heck, J.D., 1997. 13-week inhalation toxicity study of menthol cigarette smoke. Food Chem. Toxicol. 35, 683–692.

Gaworski, C.L., Dozier, M.M., Heck, J.D., Gerhart, J.M., Rajendran, N., David, R.M., Brennecke, L.H., Morrisey, R., 1998. Toxicological evaluation of flavor ingredients added to cigarette tobacco: 13-week inhalation exposure in rats. Inhal. Toxicol. 10, 357–381.

Gaworski, C.L., Heck, J.D., Bennett, M.B., Wenk, M.L., 1999. Toxicological evaluation of flavor ingredients added to cigarette tobacco: skin painting bioassay of cigarette smoke condensate in SENCAR mice. Toxicology 139, 1–17.

Green, C.R., Rodgman, A., 1996. The Tobacco Chemists' Research Conference: a half century forum for advances in analytical methodology of tobacco and its products. Recent Adv. Tob. Sci. 22, 131–304.

Haussmann, H.J., Anskeit, E., Becker, D., Kuhl, P., Stinn, W., Teredesai, A., Voncken, P., Walk, R.A., 1998. Comparison of fresh and room-aged cigarette sidestream smoke in a subchronic inhalation study on rats. Toxicological Sciences 41, 100–116.

Heck, J.D., Gaworski, C.L., Rajendran, N., Morrisey, R.L., 2002. Toxicological evaluation of humectants added to cigarette tobacco: 13-week smoke inhalation study of glycerine and propylene glycol in Fischer 344 rats. Inhal. Toxicol 14, 1135–1152.

Hoffmann, D., Hoffmann, I., 1997. The changing cigarette, 1950–1995. J. Toxicol. Environ. Health 50, 307–364.

Hoffmann, D., Hoffmann, I., 1998. Tobacco smoke components. Betir. Tabak. Int. 18, 49–52.

Hoffmann, D., Hoffmann, I., 2001. The changing cigarette: chemical studies and bioassays. In National Cancer Institute (NCI) Monograph 13, Risks associated with smoking cigarettes with low machine-measured yields of tar and nicotine. U.S. Department of Health and Human Services, Public Health Service, National Institute of Health, National Cancer Institute, Chapter 5, pp. 159–191.

Hoffmann, D., Hoffmann, I., El-Bayoumy, K., 2001. The less harmful cigarette: a controversial issue. A tribute to Ernst L. Wynder. Chem. Res. Toxicol. 14, 767–790.

ICH (International Committee on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use) Tripartite Harmonised Guideline, Step 4, 1996. Genotoxicity: guidance on specific aspects of regulatory tests for pharmaceuticals. Federal Register 61, 24 April 1996, 18199.

ICH (International Committee on Harmonisation of Technical Requirements for the Registration of Pharmaceuticals for Human Use) Tripartite Harmonised Guideline, Step 4, 1997. Genotoxicity: a standard battery for genotoxicity testing for pharmaceuticals. Federal Register, 21 November 1997.

International Organisation for Standardisation: International Standard ISO 8454, 1995. Cigarettes. Determination of carbon monoxide in the vapour phase of cigarette smoke- NDIR method.

International Organisation for Standardisation: International

Standard ISO 10362-1, 1999. Cigarettes. Determination of water in smoke condensates- gas chromatographic method.

International Organisation for Standardisation: International Standard ISO 3308, 2000. Routine analytical cigarette-smoking machine. Definitions and standard conditions, 4th Edition.

International Organisation for Standardisation: International Standard ISO 4387, 2000. Cigarettes. Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine, 3rd Edition.

International Organisation for Standardisation: International Standard ISO 10315, 2000. Cigarettes. Determination of nicotine in smoke condensates-gas chromatographic method, 2nd Edition.

Kirsch-Volders, M., Sofuni, T., Aardema, M., Albertini, S., Eastmond, D., Fenech, M., Ishidate Jr., M., Kirchner, S., Lorge, E., Morita, T., Norppa, H., Surrallés, J., Vanhauwaert, A., Wakata, A., 2003. Report from the *in vitro* micronucleus assay working group. Mutation Research 540, 153–163.

LaVoie, E.J., Hecht, S.S., Hoffmann, D., Wynder, E.L., 1980. The less harmful cigarette and tobacco smoke flavours. In: Gori, G.B., Bock, F.G. (Eds.), Banbury Report 3, A safe cigarette? Cold Spring Harbor Laboratory, New York, USA, pp. 251–260.

Leffingwell, J.C., 1999. Basic chemical constituents of tobacco leaf and differences among tobacco types. In: Davis, D.L., Nielsen, M.T. (Eds.), Tobacco-production, chemistry and technology. Blackwell Science, Oxford, UK, pp. 265–284. Chapter 8a.

Massachusetts Tobacco Control Program, 2001. 665-200 Testing for mainstream smoke constituents; Internet website: http://www.state.ma.us/dph/mtcp/report/smoktox.htm.

Massey, E.D., Kalirai, K.K., Smith, G., 2002. *In vitro* genotoxicity of smoke from cigarettes containing tobacco ingredients. Paper presented at (a) U.K. Environmental Mutagen Society Meeting, Plymouth, U.K. July 2002, Mutagenesis 2003, 17, 566–571, (b) CORESTA Congress, New Orleans, U.S.A., September 2002, Programme and Abstract Booklet, no. ST6.

Munro, I.C., Kennepohl, E., Kroes, R., 1999. A procedure for the safety evaluation of flavouring substances. Food Chem. Tox. 37, 207–232.

NIH (National Institute of Health), 2001, Guidance document on using *in vitro* data to estimate *in vivo* starting doses for acute toxicity, Publication No. 01-4500.

OECD (Organisation for Economic Co-operation and Development), 1996a. Environmental Directorate. Guidelines for the testing of chemicals. Guideline 471: Bacterial reverse mutation test.

OECD (Organisation for Economic Co-operation and Development), 1996b. Environmental Directorate. Guidelines for the testing of chemicals. Guideline 473: *In vitro* mammalian chromosome aberration test.

Paschke, T., Scherer, G., Heller, W.-D., 2002. Effects of ingredients on cigarette smoke composition and biological activity: a literature overview. Beitr. Tabak. Int. 20, 107–247.

Philip Morris USA, 2001. Evaluation of glycerol for use as a cigarette ingredient. Submission to the U.K. Department of Health. Unpublished report – can be obtained from the corresponding author upon request.

Report of the Scientific Committee on Tobacco and Health, Chairman Poswillo, D., 1998. Guidelines for testing and use of new additives in tobacco products in the U.K., The Stationery Office, London, Annex K, Appendix 1, 126–128.

Rodgman, A., 2002a. Some studies of the effects of additives on cigarette mainstream smoke properties. I. Flavorants. Beitr. Tabak. Int. 20, 83–103.

Rodgman, A., 2002b. Some studies of the effects of additives on cigarette mainstream smoke properties. II. Casing materials. Beitr. Tabak. Int. 20, 279–299.

Rodgman, A., Green, C.R., 2003. Toxic chemicals in cigarette mainstream smoke-hazard and hoopla. Beitr. Tabak. Int. 20, 481–545.

Roemer, E., Tewes, F.J., Mesigen, T.J., Veltel, D.J., Carmines, E.L., 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 3: *In vitro* genotoxicity and cytotoxicity. Food and Chem. Toxicol. 40, 105–111.

Rustemeier, K., Stabbert, R., Haussmann, H.-J., Roemer, E., Carmines, E.L., 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 2: Chemical composition of mainstream smoke. Food and Chem. Toxicol. 40, 93–104.

Schlotzhauer, W.S., Chortyk, O.T., 1987. Recent advances in studies on the pyrosynthesis of cigarette smoke constituents. J. Anal. Appl. Pyrol 12, 193–222.

Seeman, J.I., Dixon, M., Haussmann, H.-J., 2002. Acetaldehyde in mainstream tobacco smoke: formation and occurrence in smoke and bioavailability in the smoker. Chem. Res. Toxic 15, 1331–1350.

Stein, Y.S., Antal Jr., M.J., Jones Jr., M., 1983. A study of the gas-phase pyrolysis of glycerol. J. Anal. Appl. Pyrol. 4, 283–296.

United States Public Health Service, 1979. Smoking and health. A report of the Surgeon General. DHEW Publication No. PHS 79-50066, pp. 63–64.

United States Public Health Service, 1981. The health consequences of smoking. The changing cigarette. A report of the Surgeon General. DHHS Publication No. PHS 81-50156, pp. 51–52.

Vanscheeuwijck, P.M., Teredesai, A., Terpstra, P.M., Verbeeck, J., Kuhl, P., Gerstenberg, B., Gebel, S., Carmines, E.L., 2002. Evaluation of the potential effects of ingredients added to cigarettes. Part 4: Subchronic inhalation toxicity. Food Chem. Toxicol. 40, 113–131.

Weiss, N.A., 1982. Introductory Statistics, 4th Edition, TSBN 0-201-60046-3. Tukey's multiple-comparison method, pp. 889–894.

White, R., Yeats, A., Skipworth, G., 1979 Tables for statisticians. Stanley Thornes (Publishers) Ltd., Cheltenham, U.K., Third edition. pp 13–14.

World Health Organization (WHO) Monograph., 2001. Advancing knowledge on regulating tobacco products. WHO, Geneva, Switzerland, pp 40–46.

Wynder, E.L., Hoffmann, D., 1967. Tobacco and Tobacco Smoke. Studies in Experimental Carcinogenesis. Academic Press, New York and London. pp. 526–528.

Available online at www.sciencedirect.com
SCIENCE DIRECT®
J. Anal. Appl. Pyrolysis 71 (2004) 223–311
JOURNAL of ANALYTICAL and APPLIED PYROLYSIS
www.elsevier.com/locate/jaap

The pyrolysis of tobacco ingredients

Richard R. Baker*, Louise J. Bishop

Research and Development Centre, British American Tobacco, Regent's Park Road, Southampton SO15 8TL, UK

Received 8 January 2003; accepted 5 May 2003

Abstract

Relationships between tobacco components and smoke products are complex and often difficult to unravel. Pyrolysis experiments have commonly been used to establish such relationships. However, unless they are performed under dynamic conditions that are relevant to those that occur during tobacco burning, results can be obtained which have little resemblance to those obtained during cigarette smoking. The relevance of pyrolysis experiments to the behaviour of tobacco ingredients in a burning cigarette is considered. Based on the temperature, heating rate, oxygen levels and gas flow conditions that occur inside the burning zone of a cigarette, together with a review of relevant pyrolysis and smoking experiments, a set of pyrolysis conditions has been developed that approximates those occurring in the pyrolysis region of the burning cigarette. The conditions include heating the sample at 30 °C s^{-1} from 300 to 900 °C under a flow of 9% oxygen in nitrogen. Experiments on the pyrolytic behaviour of eleven relatively volatile substances under these conditions give results that are in good agreement with results from thirteen published studies in which cigarettes incorporating labelled versions of the substances were smoked. Subsequently, 291 single-compound tobacco ingredients have been pyrolysed under this set of conditions, most of which are relatively volatile. This enables the behaviour of these ingredients in a burning cigarette to be estimated in terms of intact transfer to mainstream smoke versus pyrolytic decomposition. It is predicted that almost a third of the substances would transfer to mainstream smoke at least 99% intact, and almost two-thirds would transfer 95% intact. Where pyrolytic decomposition does occur, the products are listed together with an estimate of the levels in smoke that would arise from the ingredient.
© 2003 Elsevier B.V. All rights reserved.

Keywords: Burning; Cigarette; Combustion; Ingredient; Pyrolysis; Smoke; Tobacco

* Corresponding author. Tel.: +44-23-8079-3739; fax: +44-23-8079-3076.
E-mail address: richard_baker@bat.com (R.R. Baker).

0165-2370/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0165-2370(03)00090-1

1. Introduction

Commercial cigarettes are made by blending various types of tobacco and processed tobacco. During the blending and processing, humectants such as glycerol and propylene glycol may be added to increase the moisture-holding capacity of tobacco and aid processing. In addition, in some markets such as the USA, flavour ingredients are used to complement the subjective characteristics of the smoke. When the cigarette is smoked these ingredients either distil out of the tobacco and transfer intact to smoke, or undergo pyrolytic reactions and their products transfer to smoke, or the pyrolysis products can react with other smoke constituents. Consequently, when assessing the influence of tobacco ingredients on the overall chemistry of smoke it is necessary to know whether or not they are pyrolysed during smoking, and what pyrolysis products are generated.

In the present study the behaviour of 291 single-substance ingredients have been investigated in pyrolysis experiments. The work is aimed at assessing the degree of generation of pyrolysis products from the tobacco ingredients, particularly those that may be biologically active. The pyrolysis conditions were carefully chosen in order to simulate the conditions that occur inside the burning zone of the cigarette during smoking. In order to set this study in context, the combustion and smoke formation processes occurring inside the cigarette during smoking are reviewed briefly. Within this general framework, the relevance of isolated pyrolysis studies to smoking mechanisms is considered.

2. Smoke generation and tobacco combustion

Throughout this paper, the term ‘tobacco constituent’ is defined as a substance naturally present in tobacco. The term ‘tobacco ingredient’ is defined as a substance, generally a flavour material, that is added to tobacco during the cigarette manufacturing process.

Tobacco smoke is generated when tobacco is burnt incompletely during the smoking of cigarettes, cigars or pipes. Tobacco is a plant material and consists of some 3800 constituents, ranging from small organic and inorganic molecules to biopolymers [1]. The small molecules belong to numerous classes of compounds such as hydrocarbons, terpenes, alcohols, phenols, acids, aldehydes, ketones, quinones, esters, nitriles, sulphur compounds, carbohydrates, amino acids, alkaloids, sterols, isoprenoids, Amadori compounds etc. The biopolymers consist of cellulose, hemicellulose, pectin, lignin, proteins and peptides, nucleic acids etc. During smoking, these are all subjected to temperatures up to 950 °C in the presence of varying concentrations of oxygen in the burning zone of the cigarette or other tobacco product [2]. About 4800 individual substances have been identified in tobacco smoke. Most of these are at trace levels and current limits of detection for smoke components are of the order of about 100 pg (i.e. 100×10^{-12} g) [3]. Many of the smoke components arise from a variety of different routes including distillation from tobacco, combustion, pyrolysis and pyrosynthetic reactions. Approximately 2800 constituents are found in tobacco smoke but not tobacco, indicating the importance of pyrolysis and pyrosynthetic formation mechanisms.

Many studies have been undertaken to determine the conditions inside the burning zone of the cigarette that influence product formation mechanisms. This work has been sum-

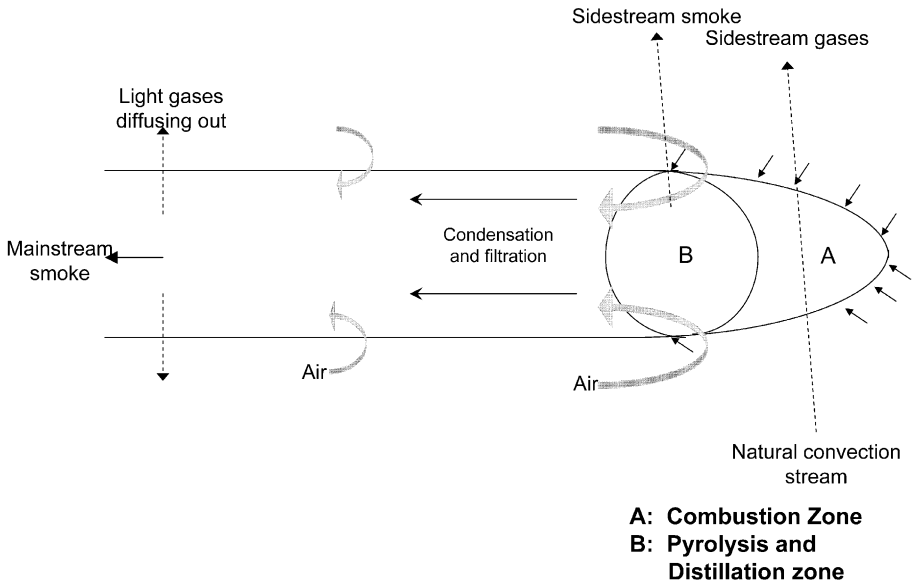


Fig. 1. The burning cigarette.

marised in Ref. [2] and, from it, a pictorial representation of the major smoke formation mechanisms occurring inside the cigarette can be obtained (Fig. 1). Fig. 2 shows the approximate relationships of the major combustion processes involved in smoke generation [4]. The interior of the burning zone is oxygen-deficient and hydrogen-rich and can be effectively divided into two regions: an exothermic combustion zone and an endothermic pyrolysis/distillation zone [2]. As air is drawn into the cigarette during the puff, oxygen is consumed by combustion with carbonised tobacco and the simple combustion products carbon monoxide, carbon dioxide and water are formed, together with the release of heat which sustains the whole burning process. Temperatures in this region of between approximately 700 and 950 °C are generated and heating rates as high as 500 °C s^{-1} are achieved. Immediately downstream of the combustion region is the pyrolysis/distillation zone, where the temperatures are between approximately 200 and 600 °C and which is still low in oxygen levels. The majority of smoke products are generated in this region by a variety of mechanisms which are essentially endothermic. A highly concentrated, probably supersaturated, vapour is generated and, during a puff, is drawn down the tobacco rod to form the mainstream smoke. Its residence time in the formation region is only a few milliseconds.

As the generated vapour is drawn out of the pyrolysis/distillation region during the puff, it cools very rapidly in the presence of diluting air entering at the paper burn line. This brings the vapours of the less volatile compounds quickly to their saturation point and condensation occurs as the vapour cools below about 350 °C. A dense aerosol consisting of growing droplet particles is formed.

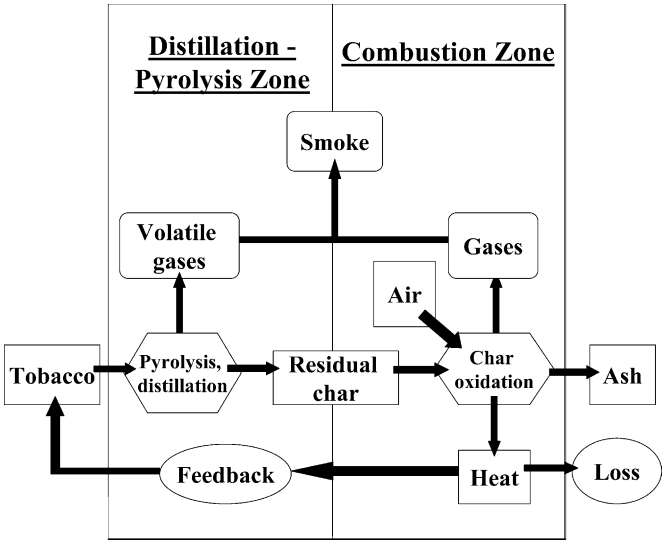


Fig. 2. Schematic diagram of the major processes occurring in cigarette combustion (after Muramatsu [4]).

3. The relevance of isolated pyrolysis studies to smoking mechanisms

The goal of many studies has been to establish relationships between tobacco constituents and smoke products. However, the complexity of the tobacco burning process makes this very difficult. As described above, tobacco itself consists of thousands of chemical constituents and each can undergo a variety of chemical reactions when the tobacco is smoked. Many of the thousands of combustion products are formed from many reactions of many tobacco constituents occurring in parallel. Thus it is virtually impossible to add an isolated tobacco constituent or ingredient to tobacco, burn the tobacco and unambiguously identify any new reaction product.

To address this complexity, two specialised techniques have been commonly used by tobacco scientists to establish relationships between tobacco constituents and smoke products. The first technique is the analysis of radioactive products in smoke from cigarettes loaded with isotopically labelled substances, reviewed by Jenkins et al. [5,6] for radioactively labelled compounds. Such studies are definitive for calculating the amount of transfer of tobacco constituents and ingredients to smoke during smoking, and the amount of thermal decomposition and oxidation that occurs during transfer. The second technique is the isolated pyrolysis of different constituents, reviewed in Refs. [7–14].

Literally hundreds of papers on pyrolysis of tobacco and tobacco constituents have been published or presented at conferences. If the pyrolysis experiments are performed authentically, a series of discrete reaction steps can be deduced for the generation of specific products in the burning cigarette. Unfortunately, because of the large number of processes occurring inside the cigarette (combustion, thermal decomposition, etc.), the wide range of temperatures and oxygen levels present at different positions inside the burning zone,

and the very high heating rates that occur during puffing, the conditions employed in isolated pyrolysis experiments can be very different than those that occur inside the burning cigarette. These conditions can make large differences in the results obtained.

This is amply illustrated by the results of Schmeltz et al. [15] who compared the products of nicotine pyrolysis to those obtained from nicotine in the burning cigarette. (Nicotine is a constituent alkaloid of tobacco.) They adsorbed radioactively labelled nicotine onto tobacco or silica gel, isothermally pyrolysed the mixture under nitrogen in combustion tubes at various temperatures between 600 and 900 °C, and analysed the labelled products (which must come from the nicotine) in the particulate phase. Under the pyrolysis conditions the nicotine underwent degradation to pyridines, which involves simple bond cleavages, and more extensive degradation and re-arrangement to quinolines, arylnitriles and aromatic hydrocarbons. Schmeltz et al. [15] also smoked the cigarette containing the labelled nicotine on a smoking machine. In this case, it was found that a substantial portion (approximately 42%) of the nicotine distilled unchanged into the mainstream and sidestream smoke, up to 11% was converted by simple degradation to pyridines, but no significant amounts of quinolines, arylnitriles or aromatic hydrocarbons could be found. Furthermore, in a cigarette 12.5% of the nicotine was oxidised to carbon dioxide. Clearly, in this example much more extensive thermal degradation had occurred in the pyrolysis experiment than in the burning cigarette.

The importance of the results of Schmeltz et al. [15] was not only their reporting of the differences between pyrolysis of an individual compound versus smoking it in a lit cigarette but also their reporting of such data that contradicted the repeated assertion by Wynder and Hoffmann [16,17] since the mid 1950s that the fate of a compound during pyrolysis was equivalent to its fate in tobacco during smoking. Schmeltz et al. [15] also reported that the difference found might also occur with other tobacco compounds.

As a further example, Schmeltz and Schlotzhauer pyrolysed menthol at 600 and 860 °C and found 22 and 84% of the menthol, respectively was pyrolysed [18]. They found, inter alia, phenol and benzo[*a*]pyrene in the pyrolysate at 860 °C. However, two other studies [19,20] have independently shown that when a cigarette containing radiolabelled menthol is smoked on a smoking machine, 99% of the menthol mainstream smoke transfers intact to. During puffing only 0.5% of the menthol underwent pyrolysis to menthane and menthone, and 0.1% underwent oxidation to the carbon oxides. Clearly, the behaviour of the menthol in the pyrolysis experiments at 600 and 860 °C was very different to the behaviour in a cigarette being smoked.

As a final example *n*-dotriacontane, a C_{32} straight-chain alkane and one of the waxes found in tobacco leaves, has been widely studied. Two independent studies [21,22] have reported that its pyrolysis in nitrogen at temperatures of 700 and 860 °C produces a variety of products including aromatic hydrocarbons such as benzene, naphthalene and polynuclear aromatic hydrocarbons. However, other studies [23–26] in which radiolabelled *n*-dotriacontane was added to cigarettes that were subsequently smoked have shown that 95% of the *n*-dotriacontane that transfers to mainstream cigarette smoke is intact. The rest of the *n*-dotriacontane undergoes pyrolysis and oxidation, essentially to C_1–C_{10} compounds and oxides of carbon [24].

When substances are subjected to slow heating, or kept at a constant temperature, they can thermally decompose at different temperatures, and/or yield different products than when they are heated very rapidly [27]. Consequently, in order for pyrolysis studies to produce

results that are relevant to cigarette smoking, the conditions of the pyrolysis experiment must simulate the conditions that occur in the burning zone of the cigarette when it is smoked. These conditions are a dynamic heating rate of 2 °C s^{-1} during smoulder and up to hundreds of °C s^{-1} in the puff [28], over the range room temperature to above 900 °C; realistic oxygen levels and gas flow conditions over the pyrolysing material; realistic size of the sample being pyrolysed and residence time of the products in the high temperature region.

In recent years, this has been appreciated and several pyrolysis techniques have been developed to simulate cigarette burning conditions. Thus, Tiller and Gentry [29] have measured kinetic parameters for the overall decomposition of reconstituted tobacco sheet at heating rates up to 500 °C s^{-1}, which are known to occur inside the burning zone during a puff. The effect of heating rate on the bulk decomposition of tobacco has also been investigated by Muramatsu et al. [30]. Using a mathematical model of pyrolysis, Baker [27] has extrapolated the pyrolytic profiles for carbon monoxide and carbon dioxide formation from tobacco at a heating rate of 1.3 °C s^{-1} to the high heating rates occurring inside a burning cigarette. The mathematical model results indicate that the formation peaks in the carbon monoxide and dioxide pyrolysis profiles move towards higher temperatures and become less resolved as the tobacco heating rate is systematically increased. Inside the cigarette burning zone during a puff the majority of the tobacco is below 900 °C [28]. Thus, during a puff sufficiently high temperatures are not reached for all the decomposition carbon monoxide to be completely evolved. In practice, in a burning cigarette the tobacco constituents that can potentially form the carbon oxides by pyrolysis at high temperature, will undergo lower temperature combustion reactions, also to generate the carbon oxides [31].

Cullis et al. [32] impregnated cigarette paper (cellulose containing 29% calcium carbonate filler) with various inorganic salts and investigated the effect of heating rate on the amount of carbon monoxide formed during non-isothermal pyrolysis in air. The results indicate that most of the salts decrease carbon monoxide at the lower heating rate of 0.2 °C s^{-1}. However, at the higher heating rate of 300 °C s^{-1} the salts tend to increase the amount of carbon monoxide generated. The effect of the salts on the mechanism of cellulose combustion apparently differs at the two heating rates. Since the burning of the cigarette paper at the edge of the combustion zone of the cigarette can have a large effect on the air entering the combustion zone and hence the burning conditions, this study again illustrates the importance of matching pyrolysis conditions to those that occur in the cigarette. However, the heating rate of cigarette paper during the puff of a cigarette is of the order of 50–100 °C s^{-1}, so that neither of the rates actually used in the particular study quoted [32] was applicable to those relevant during puffing.

Attempts have been made to simulate the puff/smoulder burning of a cigarette in pyrolysis experiments. Smith et al. [33] described a ‘pulsed’ pyrolysis apparatus in which the tobacco was placed in a quartz tube, and then a heating block surrounding the tube was moved along the tube linearly, thereby simulating the burning of a cigarette. Measurements of the amount of hydrogen cyanide produced showed that there was no difference between the pulsed and non-pulsed techniques. The levels of phenols produced by pyrolysis of tobacco were shown to be increased with the pulsed technique [34]. Higman et al. [35] developed a pyrolysis apparatus similar to that of Smith et al. Their study of polynuclear aromatic hydrocarbons, neutral constituents and phenols in the pyrolysate indicate that the pulsed technique more nearly simulates cigarette smoking than the unpulsed technique.

4. Development of pyrolysis conditions relevant to a burning cigarette

During the 1970s and early 1980s the precise conditions inside the burning zone of the cigarette throughout the smoking cycle were mapped, summarised in Refs. [2,36]. This has enabled pyrolysis conditions to be developed that mimic the cigarette's burning zone environment. Furthermore, the reliability and accuracy of results obtained using these pyrolysis conditions can be assessed by comparison to results from the definitive labelled smoking studies, discussed above.

In the first of two studies [37,38] on the pyrolytic behaviour of tobacco ingredients, Stotesbury et al. [37] based their experimental pyrolysis conditions on the known conditions inside the burning zone of a cigarette. They examined the detailed oxygen levels and temperature conditions inside various positions of the cigarette burning zone at various times during a puff, published by Baker [39]. Stotesbury et al. [37] subsequently analysed the frequency at which the various oxygen levels and temperatures occurred throughout the cigarette burning zone puffing history. Based on this analysis they pyrolysed individually six tobacco ingredients isothermally at seven different temperature conditions between 100 and 900 °C, and oxygen levels of 2 and 10%, making 14 pyrolysis conditions in all. From these pyrolysis experiments they were able to deduce the amount of intact (i.e. without thermal decomposition) transfer of volatile ingredients from tobacco to smoke, together with an estimate of any decomposition products from the ingredient as it transfers to smoke. Using this set of pyrolysis conditions, Stotesbury et al. [37] demonstrated that the tobacco ingredients anisole, isoamyl alcohol and vanillin could transfer intact to smoke at 200 °C, *p*-methoxybenzaldehyde (*p*-anisaldehyde) and methyl cinnamate would transfer to smoke at about 400 °C with 3 and 1% thermal decomposition, respectively, and that benzaldehyde would transfer to smoke at 200 °C but about 26% would oxidise to benzoic acid. With the exception of benzaldehyde, these results are in agreement with direct transfer results published by Green et al. [40]. They added radiolabelled versions of these six ingredients to cigarettes and showed that for five of them, including benzaldehyde, the transfer to mainstream smoke was 100% intact. With *p*-methoxybenzaldehyde, there was some thermal decomposition (8.6%) during transfer to the cigarette mainstream smoke.

In a second study, Stotesbury et al. [38] examined the pyrolysis of *p*-methoxybenzaldehyde and vanillin labelled with ^{18}O and ^{13}C. For this study, they used just three isothermal pyrolysis conditions: 200 °C/2%; 500 °C/10%; and 800 °C/2% oxygen. The pyrolysis results were consistent with their initial study using 14 isothermal pyrolysis conditions [37]. Furthermore, their pyrolysis results were also consistent with studies in which they added the labelled ingredients to cigarettes and measured the direct transfer of the ingredient to mainstream smoke during standard machine smoking (see Section 6.1 below).

Following the work of Stotesbury et al., a sub-group of the UK. Tobacco Manufacturers' Association Additives Working Party considered the pyrolysis of tobacco ingredients during 1999 and 2000. This sub-group consisted of relevant tobacco scientists from the research centres of cigarette manufacturers operating in the UK: British American Tobacco, Gallaher Ltd., Imperial Tobacco Ltd., Japan Tobacco International and Philip Morris. Its objective was to establish a set of pyrolysis conditions that could be used to predict the pyrolytic behaviour of tobacco ingredients as they transfer from tobacco to smoke during smoking. Following collaborative studies, the sub-group concluded that pyrolysis is a useful

Table 1
Pyrolysis conditions and rationale for their use

Condition	Rationale
Atmosphere 9% O_2 in N_2	Approximate mean oxygen level throughout the pyrolysis/distillation zone inside the burning cigarette during a puff (see Fig. 1)
Pyroprobe initial temperature of 300 °C held for 5 s	Simulation of tobacco ingredient heated in smoulder period prior to puff
Pyroprobe ramp: 30 °C s^{-1} from 300 to 900 °C	Approximate mean heating rate throughout the pyrolysis/distillation zone during a puff. Approximate temperature range to which the cigarette burning zone is subjected
Pyroprobe final temperature of 900 °C held for 5 s	Maximum duration of high burning zone temperature during puff under extreme human smoking conditions
Sample size: 200 μg in 2 μl ethanol solution on quartz wool inside pyroprobe	Sample size similar order of magnitude for many tobacco ingredients. Quartz wool simulates tobacco strand structure
Total pyrolysis gas flow: 4.6 ml s^{-1}	Sweeps products out of the pyrolysis zone, as in a real burning cigarette
Pyrolysis/GC interface temperature: 250 °C	Prevention of condensation of pyrolysate in the interface

qualitative technique for predicting the behaviour of relatively volatile ingredients in a burning cigarette. The sub-group considered that the following general non-isothermal pyrolysis conditions were most suitable: pyrolysis atmosphere between 2 and 20% oxygen to mimic smoking; pyrolysis carried out over a ramped temperature range of preferably 300–900 °C. The sub-group found that the level of oxygen in the pyrolysis atmosphere (range 2–20%), and the ramped heating rate in non-isothermal pyrolysis (range 10–50 °C s^{-1}) did not alter significantly the distribution of pyrolysis products obtained.

Based on these recommendations, and further considerations of the variation of the environment inside the cigarette burning zone as given in Refs. [28,39], the pyrolysis conditions in the current experimental study were as given in Table 1.

5. Experimental procedure

A CDS Pyrolyser 2000 was coupled to a Hewlett Packard 6890 gas chromatograph (GC) and Hewlett Packard 5973 mass spectrometer (MS). The GC and MS were both controlled by computer. The pyrolyser was operated by a programmable, handheld control unit. A Gerstel CIS injector was used to interface the pyrolyser to the GC column. During an experiment this interface was maintained at 250 °C to prevent condensation of pyrolysis products in the interface. The injector was held at −60 °C, which initially collected all the pyrolysis products prior to GC analysis. An HP5-MS (5% diphenyl–95% dimethylsiloxane) GC column was used to separate the pyrolysis products. This non-polar column provided robust separation over a wide range of compounds with broad peak shape for acids and carbonyl compounds.

The substances pyrolysed were mostly obtained from Aldrich (UK). 'Food grade' chemicals were pyrolysed, similar to the grade of the ingredient used commercially when added to tobacco as an ingredient.

The substance to be pyrolysed was made up in ethanol solution to a concentration of 10 mg ml^{-1}. *n*-Dotriacontane, a model compound pyrolysed in the study, was made up in *n*-hexane solution; ammonium hydroxide was in aqueous solution. Solid ingredients ascorbic acid, malic acid, sorbitol and *l*-tartaric acid were pyrolysed whole. Two microlitres of the solution was microsyringed onto a piece of quartz wool (1 mg, approximately 2 mm long) contained in a quartz tube (2 mm internal diameter, 25 mm long). The quartz tube was placed in the heating unit of the pyroprobe, which was then inserted into the pyrolysis unit and sealed. The flow of the 9% O_2 in N_2 gas mixture through the pyrolysis unit was set, and the pyrolysis initiated using the heating sequence and conditions given in Table 1. The complete pyrolysis lasted 30 s during which time the products were collected in the cold inlet. After a further minute the pyrolyser valve was closed, the inlet was heated at 12 °C s^{-1} to 260 °C and the products eluted to the GC column. Details of the GC and MS conditions are given in Table 2.

Each pyrolysis was carried out at least in duplicate. Before each set of replicates, the quartz tube and wool were analysed as a blank to ensure that there was no carryover between samples.

The percentages of substances in the pyrolysate were calculated assuming all pyrolysis products had the same peak area/quantity relationships as the original material being pyrolysed. Compounds were identified by their mass spectra with the Wiley library of mass spectra. Contamination in GC peaks due to ethanol solvent and column bleed was identified and removed from quoted products.

6. Results

6.1. Comparison of pyrolysis results to labelled smoking results

In the published scientific literature thirteen studies are reported in which labelled substances have been added to cigarettes and their transfer to mainstream smoke determined during smoking. Eleven of these studies used radioactive studies labelled with ^{14}C, one study [38] used the stable isotopic labels ^{13}C and ^{18}O, and one study [45] used deuterium as the label. The results from these studies should be definitive of the behaviour of the substances in a burning cigarette in terms of intact transfer to smoke versus pyrolytic decomposition. The 11 substances used in these studies have been pyrolysed in the present study. The present results are compared to the labelled smoking results in Table 3.

For nine of the 11 substances, there is a high degree of agreement between the two types of study. Those substances that are found to transfer largely intact (>95%) to the pyrolysate in the present study are also found to transfer largely intact to smoke in the radiolabelled studies. The two exceptions are phenylacetic acid and *n*-dotriacontane. In the present study, only 52% of phenylacetic acid transfers intact to the pyrolysate, and radiolabelled studies show that 80% transfers intact to mainstream smoke [41]. Similarly, in the present pyrolysis studies only 54% of the *n*-dotriacontane transfers intact to the pyrolysate, compared to 94–98.5

Table 2
GC and MS operating conditions

Instrument/procedure	Details
GC	
Instrument	HP 6890
Column	HP5-MS
Column dimensions	30 m×0.25 mm id×0.25 μm film thickness
Injection mode	Split
Split flow	100 ml min^{-1}
Carrier gas	Helium at 1ml min^{-1} flow
Column injector conditions	−60 °C for 1 min. Ramped 12 °C s^{-1} to 260 °C and then to 350 °C where it was held for 5 min
Oven conditions	40 °C for 3 min. Ramped at 10 °C min^{-1} to 240 °C. Ramped at 20 °C min^{-1} to 280 °C and then held for 5 min
MS	
Instrument	HP 5973 MSD
Resolution	Nominal
Mass range	29–400 amu
Threshold	100
Solvent delay	5.2 min
Transfer line	280 °C
MS source temperature	230 °C
MS quad temperature	150 °C
Integration parameters	
Integrator	RTE with smoothing on
Sampling	1
Start threshold	0.05
Stop threshold	0.01
Filtering	5
Minimum area	0.1% of largest peak
Maximum number of peaks	250
Peak location	Top
If leading or trailing	<100
Prefer	Baseline drop, else tangent
Peak separation	5

in the smoking studies. In these cases the pyrolysis studies have over-predicted the amount of pyrolytic decomposition. In contrast, the present study indicates that 99.8% of glycerol transfers intact to the pyrolsate whereas labelled studies [42,43] indicate 83–85% intact transfer to mainstream smoke. It is also seen in Table 3 that there is some disagreement amongst labelled studies for the amount of vanillin transfer to mainstream smoke: Green et al. [40] and Stotesbury et al. [38] show 100% transfer whereas Eble [41] gives 69% transfer.

The first six substances listed in Table 3 were also pyrolysed using fourteen isothermal conditions by Stotesbury et al. [37]. The levels of intact transfer in the study of Stotesbury are in good agreement with the results from the non-isothermal pyrolysis conditions used in the present study, with the exception of benzaldehyde. Stotesbury et al. [37] found only

Table 3
Comparison of intact transfer to mainstream smoke predicted from pyrolysis (this study) to transfer indicated by labelled smoking

Compound	Intact transfer (%) (pyrolysis)	Intact transfer to mainstream[b] (%) (smoking studies)	Smoking study reference	Boiling or melting point (°C)
Anisole	98.7	100	40	bp 155
Benzaldehyde	94.9	100	40	bp 178
Isoamyl isovalerate	99.8	100	40	bp 192
Methyl cinnamate	98.4	100	40	bp 263
p-Methoxybenzaldehyde	99.3	91.4	40	bp 248
(*p*-Anisaldehyde)		99.1	38	
Vanillin	99.6	100[c]	40	mp 44.5
		69.3	41	bp 80
		99.9	38	
Menthol	99.0	98.9	20	mp 42.5
		97.9	41	bp 216
Glycerol	99.8	85.3	42	mp 17.8
		83.0[a]	43	bp 290
Phenylacetic acid	51.6	79.7	41	bp 265.5
Nicotine	97.7	83–93	15	bp 246
		76.4	44	
		85.3	42	
		88	25	
		91.4	26	
		100[c]	42	
n-Dotriacontane	53.8	98.5	23	mp 74–75
		93.7	24	bp 245 at
		97.4	25	1.5 mm Hg
		97.1	26	

Most studies used ^{14}C as the label except Ref. [38], which used ^{13}C and ^{18}O as the label, and Ref. [45], which used deuterium.

[a] First three puffs only.

[b] Either measured directly [15,20,24,38,40,44,45] or from distribution of ^{14}C between mainstream particulate and vapour phases [23,25,26,41–43].

[c] Some decomposition products detected in sidestream smoke.

74% intact transfer for benzaldehyde, the rest pyrolysed to benzoic acid at temperatures below 200 °C. This has not been found in the present study.

Where they are formed, the pyrolytic decomposition products from all the substances pyrolysed are listed in the present study. Pyrolysis products have been determined in six of the labelled smoking studies. The products from the two types of study are compared in Table 4. The minor decomposition products are essentially similar in the two types of study, although not identical.

With the exception of *n*-dotriacontane, the small proportion of pyrolysis products essentially involve formation via minor molecular re-arrangements. On the other hand, *n*-dotriacontane (a C_{32} alkane) has undergone extensive degradation to over 40 C_4–C_{15} aldehydes, ketones and oxygen-heterocyclic products in the present study. The products in the radiolabelled smoking study of Jenkins et al. [24] were also similarly extensively

Table 4
Comparison of decomposition products from additive

Compound	Composition of products from additive (%)		Reference
	Pyrolysis (this study)	Labelled smoking study[a]	
Menthol	99.0 Menthol 0.9 Menthone 0.1 Menthene	98.9 Menthol 0.4 Menthone 0.2 Menthane 0.1 CO_2 0.4 Unknown	[20]
Nicotine	97.7 Nicotine 1.4 Nicotyrine 0.6 Myosmine 0.3 Cotinene	83.0–93.3 Nicotine 0.7–0.9 Pyridine 0.7–5.2 Monomethylpyridines 0.4–1.3 Dimethylpyridines 0.7–4.8 Ethenylpyridines 2.7–5.7 Myosmine 0.7 Bipyridyl $\leq$12.5 CO_2	[15]
		78.9 Nicotine[b] 4.1 Myosmine[b] 3.2 3-Ethenylpyridine[b] 2.2 β-Nicotyrine[b] 0.8 Methylpyridine[b] 0.8 Cotinine[b] 0.7 Pyridine[b] 0.5 3-Ethylpyridine[b] 0.5 3-Allylpyridine[b] 0.5 3-Pyridinecarbonitrile[b]	[45]
n-Dotriacontane ($C_{32}H_{66}$)	53.8 *n*-$C_{32}H_{66}$ 2.1 $CH_3(CH_2)_9CHO$ 1.9 $CH_3(CH_2)_{10}CHO$ 1.9 $CH_3(CH_2)_5CHO$ 40.3 38 C_4–C_{15} aldehydes, Ketones and *O*-heterocyclics	95.1 C_{31}–C_{33} compounds 4.2 C_1–C_{10} compounds 0.7 CO+CO_2	[24]
Vanillin	99.6 0.2 2-Methoxyphenol? 0.1 Vanillin methyl ether 0.1 Piperonal?	99.9 Vanillin 0.1 2-Methoxyphenol	[38]
p-Methoxy benzaldehyde	99.3 *p*-Methoxybenzaldehyde 0.3 Methoxy benzyl alcohol 0.2 Methoxyphenol 0.2 Anisole	99.1 *p*-Methoxybenzaldehyde 0.3 Anisole 0.6 Phenol	[38]

[a] Products determined in mainstream smoke, except Ref. [45].

[b] These results refer to total products detected in mainstream and sidestream smoke. The decomposition products were found in sidestream smoke only.

degraded, although a much lower proportion of the original *n*-dotriacontane was pyrolysed in their smoking study. The formation of a variety of carbonyl products can be anticipated from studies on combustion of hydrocarbons [46,47]. Furthermore, it is not surprising that in the pyrolysis experiment of the present study almost half of the involatile *n*-dotriacontane is pyrolysed before it can distil out of the tube in the pyroprobe. It is, in fact, surprising that in the smoking experiment 95% of the *n*-dotriacontane transfers to mainstream smoke intact. One possible explanation is that in the burning cigarette elution is contributing to its intact transfer to smoke. Elution is a mechanism that has previously been postulated [48], in which a material is distilled from tobacco and trapped in the smoke by aerosol particles. In the pyrolysis experiment, in the absence of smoke aerosol particles, such a mechanism would not operate. Further work would be needed to test this hypothesis further.

It would appear that the present pyrolysis system gives good predictions of the smoke transfer pyrolytic behaviour of relatively volatile tobacco ingredients. For involatile substances it gives an over-estimation of the amount of pyrolysis that occurs relative to the situation in a burning cigarette.

6.2. Data from pyrolytic experiments

The detailed results of the 291 substances pyrolysed in the present study are given in the Appendix A. The composition of the pyrolysate is the mean of two pyrolyses for each ingredient. Generally, the variation between the replicates was less than 5%. Either all pyrolysis products are shown, or the 10 major products are listed. In this latter case, where an abbreviated list of products is given, any pyrolysis product that is part of the 'Hoffmann list' of toxicological substances in smoke (see Section 7 below) is also included in the Appendix A. The listed products were identified from the Wiley library of mass spectra. Some mass spectra were only identified tentatively and these are listed with a question mark after the name. Some products are also listed as a combination of substances, which may be either a genuine mixture or an indecision on identification. Many of these ingredients are listed by their names as commonly used by flavour chemists rather than their systematic chemical names. In order to avoid any ambiguity, the Chemical Abstract Service (CAS) Registry Number for each ingredient is also listed, together with the chemical formula or structure. The boiling and/or melting points of the ingredients, listed in the Appendix A, were obtained from standard reference texts of flavour and organic chemicals [49,50].

The purity of the sample of the ingredient pyrolysed is also listed in the Appendix A. It is not possible to state categorically that all the substances listed in the 'Composition of pyrolysate' column are actually pyrolysis products; some could be impurities in the original sample. For example, the pyrolysate of *m*-tolylaldehyde apparently contains traces of chloroxylene and dichloroxylene. These must be impurities in the original *m*-tolylaldehyde. The materials pyrolysed in this study were similar 'food grade' chemicals as those that are used commercially when added to tobacco as ingredients. Thus, from a consumer smoker's point of view this will not actually matter—the substances listed in the 'Composition of pyrolysate' column will reach the consumer whether they are pyrolysis products or impurities in the tobacco ingredient.

In the present system, water and permanent gases such as carbon monoxide and carbon dioxide are not detected. Where pyrolysis in the presence of oxygen does occur it is likely

that these will also be products. Furthermore, since the mass range scanned was from 29 to 400 amu (Table 2), products with a molecular weight below about 40 will not be quantitatively detected.

The maximum level of each pyrolysis product in mainstream smoke ($\text{Product}_{\text{max}}$) obtained from the ingredient is also listed in the Appendix A, calculated as follows:

$$\begin{aligned}\text{Product}_{\text{max}}\ (\mu\text{g}) &= \text{Weight of ingredient in cigarette}\ (\mu\text{g}) \\ &\quad \times \text{Proportion of product in pyrolysate} \\ &\quad \times \text{Proportion of tobacco burnt in puffing} \qquad (1)\end{aligned}$$

Cigarettes typically contain 0.7–0.8 g of tobacco. If a maximum weight of 1.0 g tobacco is used, the maximum weight of ingredient in the cigarette (μg) is equal to the maximum ingredient application level (ppm). The proportion of tobacco burnt in puffing is between 30 and 50% for typical cigarette designs [51]. With the maximum of 50%, Eq. (1) becomes:

$$\begin{aligned}\text{Product}_{\text{max}}\ (\mu\text{g}) &= 0.5 \times \text{Maximum ingredient application level (ppm)} \\ &\quad \times \text{Proportion of product in pyrolysate} \qquad (2)\end{aligned}$$

The use of Eq. (2) assumes that during a puff, 100% of the ingredient and its pyrolysis products transfer to mainstream smoke, i.e. none transfers to sidestream or is filtered on the tobacco rod. Both these processes are known to occur in practice and would reduce the level of ingredient and its products in mainstream smoke. As examples of this, the radiolabelled studies of Newell et al. [19] and Jenkins et al. [20] have both determined the distribution of menthol added to the tobacco in the mainstream and sidestream smoke as well as the cigarette butt, following smoking. The results in Table 5 indicate that only 29–43% of the menthol transfers to mainstream smoke, while 40–44% transfers to sidestream smoke and 19–27% transfers to the butt.

Eq. (2) also gives the mainstream yield for an unfiltered, plain cigarette. Filters on cigarettes typically have filtration efficiencies of 30–60%. Such filters would reduce the level of particulate-phase smoke components by approximately 30–60%, respectively. Thus, Eq. (2) incorporates the maximum ingredient application level, the maximum tobacco

Table 5
Distribution of radiolabelled menthol added to a cigarette following smoking

	Radioactivity (%)	
	Newell et al. [19]	Jenkins et al. [20]
Mainstream TPM[a]	41.9	27.9
Mainstream vapour phase	1.06	1.0
Sidestream TPM[a]	33.0	23.1
Sidestream vapour phase	4.93	21.2
Butt	19.1	26.9
Total	100.0	100.1

[a] Total particulate matter.

weight in a cigarette, the maximum proportion of tobacco burnt in puffing, the maximum possible transfer to mainstream smoke, and assumes that the smoke is unfiltered. Consequently, the calculated levels in smoke from the ingredients, listed in the Appendix A, are very much upper limits.

It is beyond the scope of this paper to consider in detail the significance of these pyrolysis results in terms of smoke toxicity. However, a threshold of toxicological concern of 1.5 μg per day has been suggested for the ingestion of untested chemicals in food [52]. This has been stated as meaning that with less than 1.5 μg of the substance per day, the cancer risk from the chemical is less than 10^{-6} [52]. With the assumption that a smoker consumes a maximum of 50 cigarettes per day, this daily threshold level equates to 0.03 μg per cigarette.

6.3. *Results of tobacco ingredient pyrolyses*

A total of 291 substances is listed in Appendix A, of which two were used as model compounds and are naturally present in tobacco (nicotine and *n*-dotriacontane) and one was inorganic (ammonium hydroxide) and neither the substance itself nor any products were detected. Of the 290 substances that yielded pyrolysis products, 233 were monofunctional compounds, 49 were bifunctional and 8 were trifunctional. The number of substances showing particular levels of intact transfer out of the pyrolysis zone, predictably into smoke, is given in Table 6. Almost a third of the substances transfer at least 99% intact, i.e. with less than 1% pyrolysis. Almost two-thirds of the substances transfer with less than 5% pyrolysis (95% intact).

Intact transfer for ingredients in various monofunctional organic compound classes are shown in Table 7, and containing two and three organic functional groups in Tables 8 and 9, respectively. A few trends are apparent. Fifteen pyrazines or (pyrazine or pyrrole + carbonyl) compounds have been pyrolysed and all of them transfer at least 99% intact. All of the

Table 6
Number of ingredients transferring intact out of pyrolysis zone

% Intact transfer	Number of ingredients
100	26
≥99	92
≥95	184
≥90	213
≥80	248
≥70	263
≥60	273
≥50	281
≥40	283
≥30	284
≥20	284
≥10	284
Total	290

Table 7
Intact transfer of substances in various monofunctional compound classes

Ingredient class	Number of ingredients with % intact transfer							
	≥99	≥95	≥90	≥80	≥70	≥50	≥30	Total
Hydrocarbons	0	0	2	3	4	6	6	6
Carboxylic acids	5	13	15	23	23	25	25	26
Alcohols	6	13	18	25	25	25	26	27
Aldehydes	2	11	17	18	22	25	25	25
Ketones	9	15	18	22	23	24	25	25
Esters	27	56	66	72	76	81	81	81
Internal esters (lactones)	9	16	17	19	19	19	19	19
Ethers	4	5	5	5	5	5	5	5
Internal ethers (furans etc.)	3	3	3	3	3	3	3	3
Phenols	1	3	3	3	3	3	3	3
Pyrazines	12	12	12	12	12	12	12	12
Amide	0	0	0	0	0	0	0	1
Total (monofunctional ingredients)								233

monofunctional ethers, internal ethers (furans etc.) and phenols transfer at least 95% intact, and mostly at least 99% intact. All the hydrocarbons studied transfer with some pyrolysis. For most of the substances, the functional group appeared not to have much effect on the proportion of the substances that underwent significant pyrolysis.

Six of the tobacco ingredients studied break down completely: ascorbic acid, malic acid, sorbitol, *l*-tartaric acid, 2,5-dimethyldihydrofuranolone [systematic chemical name

Table 8
Intact transfer of tobacco ingredients containing two organic functional groups

	Number of ingredients with % intact transfer							
	≥99	≥95	≥90	≥80	≥70	≥50	≥30	Total
Ester + alcohol	0	0	0	0	1	1	1	2
Ester + carbonyl	1	2	2	2	2	2	2	2
Ester + ether	1	1	2	3	4	5	5	5
Alcohol + acid	1	1	1	1	2	2	3	4
Alcohol + carbonyl	0	2	2	2	2	3	3	3
Alcohol + ether	0	1	1	3	3	3	3	3
Ether + carbonyl	4	6	7	7	7	7	7	7
Acid + carbonyl	0	0	0	0	0	1	1	1
Phenol + ether/carbonyl/ester	1	9	11	12	12	12	12	12
Thiazole + alcohol/carbonyl	1	2	2	2	2	2	2	2
Thiether + ester	1	1	1	2	2	2	2	2
Thioalcohol + ester	1	1	1	2	2	2	2	2
Pyrrole/pyrazine + ether/carbonyl	3	3	3	3	3	3	3	3
Two heterocyclic N rings	0	1	1	1	1	1	1	1
Total (bifunctional ingredients)								49

Table 9
Intact transfer of tobacco ingredients containing three organic functional groups

	Number of ingredients with % intact transfer							
	≥99	≥95	≥90	≥80	≥70	≥50	≥30	Total
Alcohol + ether + acid	0	0	0	0	0	0	0	1
Alcohol + ether + carbonyl	1	1	2	2	3	3	3	5
Phenol + ether + carbonyl	2	2	2	2	2	2	2	2
Total (trifunctional ingredients)								8

4-hydroxy-2,5-dimethyl-3(2*H*)-furanone] and 4,5-dimethyl-3-hydroxy-2,5-dihydrofuran-2-one. The first five of these are involatile solids with melting points in the range 70–190 °C. Clearly, they decompose before they can distil out of the pyrolysis zone. The 4,5-dimethyl-3-hydroxy-2,5-dihydrofuran-2-one is highly volatile and probably decomposes as it boils.

Urea is also a solid with a melting point of 133 °C. Only 6.6% of the pyrolysate is urea, the rest is isocyanic acid, possibly with other products also present (the mass spectrum of the isocyanic acid GC peak in the pyrolysate had possible contributions from other substances). Other pyrolysis studies [53,54] have shown that isocyanic acid is a major product from the pyrolysis of urea, originally Wöhler in 1829 [55]:

$$NH_2CONH_2 \rightarrow HNCO + NH_3$$

This primary reaction occurs just above urea's melting point of 133 °C. At 150 °C secondary reaction occurs to generate biuret [54] although none was specifically observed in the present study:

$$NH_2CONH_2 + HNCO \rightarrow NH_2CONHCONH_2$$

At temperatures above 175 °C further reactions occur to produce the heterocyclic compounds cyanuric acid and ammelide [54], although none were observed in the present study.

Several of the substances pyrolysed in the present study are likely to have been investigated in other pyrolysis studies. However, the objective of the present study was to investigate the behaviour of the substances under pyrolysis conditions that simulate those that occur inside the burning cigarette. As discussed in Section 3 above, 'The relevance of isolated pyrolysis studies to smoking mechanisms', the results from studies that do not simulate these conditions are irrelevant to a burning cigarette situation. Thus, the pyrolytic results for menthol, nicotine and *n*-dotriacontane, obtained under harsher pyrolytic conditions, are not in agreement with the results of the present study. The present results are, however, in agreement with labelled smoking studies, discussed in Section 6.1 above. This is further illustrated by the pyrolysis of glycerol. Published studies of Stein and Antal [56] have shown that acrolein and acetaldehyde are the principal products from the isothermal pyrolysis of glycerol in steam above 650 °C, and at lower temperatures in the presence of a

metal catalyst. However, in the present study 99.8% of the glycerol (boiling point 290 °C) is found intact in the pyrolysate, and neither acrolein nor acetaldehyde is detected. The pyrolysis results of the present study are in agreement with unpublished results quoted by the Philip Morris tobacco company [57]. These unpublished results were obtained by heating glycerol in air up to 900 °C in 100 s, and two studies on smoking cigarettes in which radiolabelled glycerol had been added. In all cases the glycerol distilled intact and the presence of acrolein and acetaldehyde from the glycerol was very small, e.g. less than 0.1% acrolein in the radiolabelled mainstream smoke products. Consequently, published studies in which harsh pyrolysis conditions that are not relevant to a burning cigarette are not considered further in the present paper.

7. Significance of the results

Based on the arguments developed in Sections 3 and 4 above, and comparison of the current pyrolytic results to labelled smoking data discussed in Section 6.1, we believe that the pyrolysis results in the present study are a reasonable approximation to the behaviour of the tobacco ingredients in a burning cigarette. This is the case for the relatively volatile ingredients that distil largely unchanged out of the pyrolysis zone. If anything, the results of the present study tend to over-estimate the amount of pyrolysis that occurs during transfer of the ingredient to smoke. This is especially likely to be the case for involatile ingredients. Where results in the present study indicate significant pyrolysis of the ingredient, further investigation should be undertaken. In particular, the ingredient should be added to a cigarette and the mainstream smoke analysed to assess the effect of the ingredient on the level of products in question. The present results may thus be regarded as a first screening of the impact of the ingredient on smoke chemistry.

In fact, the present study is part of a much wider investigation. In other aspects of the investigation, mixtures of the tobacco ingredients have been added to cigarettes and the resultant smoke has been assessed for any changes in the levels of biologically-active chemical constituents and in the response to in vitro bioassays [58–60]. Discussion of the results from those investigations is beyond the scope of the present study. Suffice it to say that in most cases the ingredients produced no statistically significant increase in yields of the biologically-active smoke constituents, or on the activity of the smoke condensate in various bioassays. These results are in agreement with those published in other studies on the mainstream smoke from cigarettes containing several different multi-ingredient mixtures [61–67].

It is also beyond the scope of the present report to comment on the toxicological implications of the pyrolysis products listed in the Appendix A. However, some preliminary comments are worth making. Of the approximately 4800 substances in tobacco smoke [3], 44 are believed by Regulatory Authorities in Canada and the USA to be relevant to smoking-related diseases [68,69]. These include, inter alia, some volatile carbonyl compounds, tobacco-specific *N*-nitrosamines, aromatic amines, phenols, volatile alkenes, benzo[*a*]pyrene and metals. These substances are sometimes colloquially called ‘Hoffmann analytes’ since similar lists of toxicological substances have been proposed by Dietrich Hoffmann et al. of the American Health Foundation in New York since the mid 1980s. The latest

Table 10
Pyrolysis products from tobacco ingredients that are included in the 'Hoffmann lists' of smoke components [68–72]

Ingredient	Pyrolysis product	Max. level in smoke from ingredient (μg per cigarette)	Typical smoke levels, unfiltered cigarette [2,70] (μg per cigarette)
Anisyl phenylacetate	Phenol	0.03	80–160
Benzaldehyde glyceryl acetal	Phenol	0.08	80–160
Benzyl benzoate	Phenol	0.5	80–160
Benzyl cinnamate	Styrene	0.2	10–20
	Phenol	0.04	80–160
	Benzofuran[a]	0.04	Present
n-Butyl alcohol	Butanal	0.04	30–50
Cinnamic acid	Styrene	0.07	10–20
Cinnamyl cinnamate	Styrene	0.7	10–20
	Phenol	0.2	80–160
	Benzofuran[a]	0.08	Present
δ-Dodecalactone	Styrene	0.2	10–20
	Benzofuran[a]	0.2	Present
Ethyl cinnamate	Styrene	0.03	10–20
Ethyl salicylate	Phenol	0.1	80–160
Malic acid	Acetaldehyde	330	500–1400
	Benzene	7	20–70
α-Methylbenzyl acetate	Styrene	0.1	10–20
Methyl cinnamate	Styrene	0.03	10–20
Phenethyl alcohol	Styrene	0.1	10–20
Phenethyl isobutyrate	Styrene	0.2	10–20
Phenylacetic acid	Toluene	0.07	100–200
3-Phenylpropionic acid	Styrene	0.04	10–20
Salicylaldehyde	Phenol	0.05	80–160
p-Tolyl acetate	Cresol (*m*-, *o*- or *p*-methylphenol)	0.09	11–37

[a] Included on Hoffmann's full list [70–72] but not on Canadian and Massachusetts lists [68,69].

compilation by Hoffmann et al. in fact lists 82 substances [70–72]. Substances on any of the three lists [68–72] that are predicted to be in smoke from pyrolysis of the ingredient, at or above the threshold limit of 0.03 μg per cigarette, detailed in the Appendix A, are listed in Table 10. Typical smoke levels reported for an unfiltered cigarette are also included in Table 10 in order to compare the maximum ingredient contribution to smoke yields. Smoke data for unfiltered cigarettes are given in Table 10 in order to compare like with like—see discussion on Eq. (2) in Section 6.2 above.

In general, the maximum levels of these biologically-active pyrolysis products in smoke from tobacco ingredients are small compared to the typical levels in smoke, although no quantitative smoke data are available for benzofuran yields. From Table 10, the pyrolysis products that could potentially make a significant contribution to the smoke yield are acetaldehyde and benzene generated from malic acid, which undergoes complete pyrolysis in the present study. As discussed in Section 6.3 above, malic acid is an involatile solid and the pyrolysis technique is probably least relevant for such substances. Nevertheless, the data presented in Table 10 have flagged tobacco ingredients that could potentially yield

biologically-active pyrolysis products at levels in smoke above the threshold limit. As outlined above, the next step is to smoke cigarettes containing these ingredients to determine their effects on the smoke yields. This has been done for the ingredients in Table 10, in which mixtures of ingredients have been added at or above their maximum levels (given in the Appendix A), to an American blended tobacco and made into filter cigarettes [58,59]. The cigarettes were then machine-smoked under the standard ISO conditions of a one 35 ml puff of 2 s duration taken every minute. The yields of various constituents in mainstream smoke were determined and the smoke yields of the 'Hoffmann analytes' listed in Table 10 are given in Table 11, relative to a control cigarette with no added ingredients. Differences between the test and control cigarettes that are statistically significantly different are indicated in the Table. In the smoke chemistry study [59] the analytical results obtained with a reference cigarette were found to vary over several months due to the variability of the analytical methodology for the very low levels of smoke analytes being determined. This long-term variability for these smoke analytes has been reported in another study by Rickert and Wright [73]. When this long-term analytical variability was taken into account, some of the comparisons between the test and control cigarettes were found to no longer have statistically significant differences and this is also indicated in Table 11. (The nomenclature for the test cigarettes in Table 11, A3, A5 etc. is consistent with that used in the paper describing the smoke chemistry study in more detail [59]).

The smoke yield of acetaldehyde is 69 μg higher with the cigarette containing added malic acid (cigarette C4) relative to the control cigarette, which is statistically different and this could be due to the pyrolysis of malic acid. However, this difference is not statistically significant when the long-term variability of the analytical method is taken into account [59]. Also, the 69 μg increased acetaldehyde yield is lower than the maximum of 330 μg predicted from the pyrolysis results (Table 10). This implies that either: (a) the maximum calculated pyrolysis yields in smoke are greatly over-estimated; or (b) complete pyrolysis of the malic acid does not occur in the burning cigarette; or (c) the generation of acetaldehyde from malic acid is not too much higher than generation from the tobacco that it replaces in the cigarette, on a per weight basis. Over-estimation of the maximum calculated yields is very likely given the assumptions made and discussed in Section 6.2 above. The benzene smoke yield from the cigarette containing malic acid is not significantly different from the control, again implying either over-estimation of the maximum pyrolysis yield, or that malic acid does not completely pyrolyse in the burning cigarette, or that generation of benzene from malic acid and tobacco are similar on a per weight basis.

From the smoke chemistry results in Table 11, the yields of 'Hoffmann analytes' have statistically significant differences between the test and control cigarettes for test cigarette A5 (for phenol), A7 (for styrene) and B2 (for styrene and phenol), even when the long-term variability of the smoke constituent analytical methodology is taken into account. The significant differences between the test and control cigarettes are compared to those predicted by pyrolysis of the ingredients in Table 12. The maximum predicted increase in smoke styrene in test cigarette A7 is 1.53 μg relative to its control (no ingredient) cigarette, compared to an observed increase of 1.8 μg. Given the assumptions made in calculating the maximum level from pyrolysis (Section 6.2 above) and the variability in the smoke

Table 11
Effect of ingredients on smoke yields for those ingredients giving yields of 'Hoffmann analytes' above the daily threshold limit

Ingredients[a]	Smoke constituent	Smoke yield (s.d.) (μg per cigarette)	
		Test	Control
Added to test cig. A3			
n-Butyl alcohol	Butanal	39.5 (0.9)	36.5 (2.9)
Added to test cig. A5			
Methyl cinnamate	Styrene	12.1 (0.5)	11.6 (0.8)
Salicylaldehyde	Phenol	12.4 (0.7)[b,c]	13.8 (0.8)
Added to test cig. A6			
3-Phenylpropionic acid	Styrene	12.9 (0.7)[b]	11.6 (0.8)
Added to test cig. A7			
Anisyl phenylacetate	Phenol	13.3 (0.6)	13.8 (0.8)
Benzyl benzoate	Styrene	13.4 (0.2)[b,c]	11.6 (0.8)
Benzyl cinnamate	Toluene	107 (2)[b]	100 (3)
Cinnamyl cinnamate			
δ-Dodecalactone			
Ethyl cinnamate			
α-Methylbenzyl acetate			
Phenethyl alcohol			
Phenethyl isobutyrate			
Phenylacetic acid			
Added to test cig. B2			
Cinnamic acid	Phenol	23.2 (1.7)[b c]	26.3 (1.8)
Ethyl salicylate	Styrene	18.3 (1.0)[b c]	20.0 (0.5)
Added to test cig. B3			
Benzaldehyde glyceryl acetal	Phenol	24.8 (1.4)	26.3 (1.8)
p-Tolyl acetate	*m*- + *p*-Cresol	16.1 (1.3)	17.6 (1.1)
	o-Cresol	5.4 (0.2)	5.8 (0.3)
Added to test cig. C4			
Malic acid	Acetaldehyde	695 (25)[b]	626 (22)
	Benzene	57.4 (1.4)	56.0 (1.0)

s.d. is standard deviation, five replicate determinations. Significant differences were determined using a two-tailed Student's *t*-test.

[a] Several ingredients were added to each test cigarette [58,59]. Only those ingredients listed in Table 10 are included here.

[b] Significantly different from control value at 95% confidence level—this data set.

[c] Also significantly different from control value at 95% confidence level when long-term analytical method variability is included.

Table 12
Comparison of statistically significant differences in smoke yields and those predicted by pyrolysis of ingredients

Test cigarette	Ingredients[a]	Smoke constituent	Max. level in smoke (μg)[b]	(Test-control) cigarette yield (μg)
A5	Salicylaldehyde	Phenol	0.05	−1.4
A7	Benzyl cinnamate	Styrene	0.2	1.8
	Cinnamyl cinnamate	Styrene	0.7	
	δ-Dodecalactone	Styrene	0.2	
	Ethyl cinnamate	Styrene	0.03	
	α-Methylbenzyl acetate	Styrene	0.1	
	Phenethyl alcohol	Styrene	0.1	
	Phenethyl isobutyrate	Styrene	0.2	
		Total styrene	1.53	
B2	Cinnamic acid	Phenol	0.003	−3.1
	Ethyl salicylate	Phenol	0.1	
		Total phenol	0.10	
B2	Cinnamic acid	Styrene	0.07	−1.7

Significantly different from control value at 95% confidence level when long-term analytical method variability is included.

[a] Several ingredients were added to each test cigarette [58,59]. Only those ingredients listed in Table 10 and producing the analyte during pyrolysis (Appendix A) are included here.

[b] Maximum level predicted in cigarette smoke from pyrolysis of the ingredient.

analytical methodology [59], this agreement is good. For test cigarettes A5 and B2 (Table 12), the levels of phenol and styrene in the smoke of the test cigarettes are lower than in their respective control cigarettes. For the relevant ingredients, salicylaldehyde, cinnamic acid and ethyl salicylate, the maximum predicted levels of phenol and styrene in smoke generated from their pyrolysis is small ($\leq$0.1 μg). For these ingredients and cigarettes, generation of 'Hoffmann analytes' by pyrolysis of ingredients is negligible.

Of course, the lists of biologically-active smoke constituents [68–72] used in this study are not definitive. There are possibly other substances with toxicological properties not given on these lists. One of them is furfural which is not on either the full "Hoffmann list" or the ones used by Regulatory Authorities in Canada or the U.S.A. and yet it is present in cigarette smoke and furfural is bilolgically active. Furfural is generated by the pyrolysis os sorbitol in the present study. Sorbitol is a solid material and breaks down conpletely in the present study (see Appendix). As indicated above, the pyrolysis system is least relevant for such involatile substances. Nevertheless, the pyrolysis of sorbitol has indicated formation

of biologically-active furfural and the next step in the total assessment of the ingredient was to smoke cigarettes containing sorbitol and determin the effect on furfural levels in smoke. This has been done with a test cigarette containing 21 mg (3.5% by weight) of sorbitol. When smoked the furfural level determined in the mainstream snoke (6.4 μg per cigarette) was found to be not significantly different from the control (no sorbitol) cigarette. Presumably, during cigarette smoking the sorbitol does not completely deconpose, as in the pyrolysis study. Consequently, in this case the pyrolysis result is a false positive. This indicates again that the pyrolysis technique is noe suitable for predicting the behaviour of involatile substances in a burning cigarette.

Some pyrolysis products having toxicologicat significance, for example carbon monoxide, are not detected in the presents experemental system. Nevertheless, pyrolysis from tobacco ingredients determined in this study is an important part of the total studies used to assess them.

The work described in this paper has been aimed at using a pyrolysis technique to assess the behaviour of tobacco ingredients inside the burning zone of a cigarette. Potentially, ingredients can undertake one or more of the following processes during tobacco combustion in the smoking process:

1) Distil directly into smoke;
2) Pyrolyse or oxidise and their products enter smoke;
3) Pyrolyse or oxidise and the reaction products react with tobacco and smoke constituents and affect their yields or generate other smoke products.

The present study has shown that for almost two thirds of the 291 ingredients assessed, process (1) occurs predominantly, with the tobacco ingredients distilling into smoke at least 95% intact. Of course, for those ingredients that do undergo significant pyrolysis, process (3) can potentially occur in the burning zone of the cigarette but not in the pyrolysis system, where the pyrolysing ingredient is not in contact with tobacco or smoke components. This inherent difference between pyrolysis in isolation, and pyrolysis within a burning cigarette, has been discussed recently by Rodgman et al. [74,75]. Rodgman et al. [74] described the differences as follows:

> During the smoking process, the reaction system is much more complex than in the pyrolysis. The compound itself, whether added to or inherent in the tobacco, as well as fragments produced from it have the opportunity not only to react with volatilized tobacco components [over 3800 tobacco components have been identified and only a few are non-volatile, e.g. biopolymers (celluloses, pectins, starch, lignin), proteins] but also to react with the decomposition fragments produced not only from each of them but also from those produced from the many other unidentified tobacco components.

The present study is not aimed at being a definitive mechanistic study on smoke formation. Identifying the potential reactions that could occur in process (3) above is not the subject of this study. Rather, it is aimed at providing a practical approach to assessing the degree of generation of constituents from tobacco ingredients, in particular biologically-active ones. The results are compatible with parallel studies in which the ingredients are added to tobacco and the effects on cigarette smoke constituents are measured. In general, the

number of 'Hoffmann analytes' detected amongst the pyrolysis products of the ingredients, and their levels, are low.

8. Conclusions

i) In order to produce results that are relevant to a burning cigarette, tobacco ingredients must be pyrolysed under experimental conditions that mimic the combustion conditions inside the cigarette burning zone.
ii) The pyrolysis system developed gives good predictions of the smoke transfer/pyrolytic behaviour of relatively volatile tobacco ingredients. For involatile substances it gives an over-estimation of the amount of pyrolytic decomposition that occurs in the burning cigarette.
iii) Of the 291 tobacco ingredients pyrolysed, almost a third transfer out of the pyrolysis zone at least 99% intact, and almost two thirds transfer at least 95% intact.
iv) Pyrolysis products from the ingredients are listed, and these have been converted to maximum levels that would be obtained in mainstream cigarette smoke, based on maximum levels used commercially by British American Tobacco.
v) Particular attention has been paid to assessing the generation of 'Hoffmann analytes', i.e. biologically-active analytes in smoke, from the pyrolysis of the ingredients. In general, the number of 'Hoffmann analytes' detected amongst the pyrolysis products of the ingredients, and their levels, are low.
vi) The present pyrolysis technique is a part of the total assessment methodology of tobacco ingredients, and is a useful screening tool for indicating which ingredients undergo significant decomposition during smoking of cigarettes.
vii) Where necessary, results from such pyrolysis studies should be confirmed in the burning cigarette.

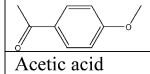
Acknowledgements

We would like to thank Simon Price and Dorethée Nicodéme for technical assistance with the pyrolysis experiments, André Bishop for assistance with the computing of the results, and Fran Lake for collating and word processing the data in the Appendix A.

Appendix A. Pyrolytic behaviour of individual tobacco ingredients

CAS No. is Chemical Abstract Service Registry Number; Mol. wt. (M) is molecular weight; bp is boiling point; mp is melting point; Max. cig. appln. level (ppm) is the maximum recommended level of this ingredient applied commercially to a cigarette; Composition of pyrolysate refers to those pyrolysis products detected. Water, CO, CO_2 and products with a molecular weight below about 40 are not detected; Max. level in smoke (μg) is the maximum level in cigarette smoke obtained from the ingredient, calculated as indicated in the text; Levels *in italics* are at or below the daily threshold level of 0.03 μg for one cigarette.

Appendix A. Pyrolytic behaviour of individual tobacco ingredients

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M) bp or mp (°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
Acetanisole CAS 100-06-1 	Ketone Aryl ether	M=150 bp 153 at 26 mm Hg	42	98	Acetanisole Anisaldehyde Acetic acid *t*-Butyltoluene Acetophenone	99.6 0.15 0.14 0.07 0.06	21 *0.03* *0.03* *0.01* *0.01*
Acetic acid CAS 64-19-7 CH_3CO_2H	Acid	M=60 bp 118	4,500	99.5	Acetic acid Acetic acid anhydride Ethanol	95.9 4.0 0.1	2160 90 2
Acetoin CAS 513-86-0 	Alcohol Ketone	M=88 bp 148	140	96	Acetoin Acetic acid Acetic anhydride Tetramethyl trioxobicyclooctane Butandiol diacetate Ethyl acetate Diacetyl Methylhexanone ? Tridecane 5 Unidentified compounds	33.5 27.8 17.4 11.4 2.6 2.0 1.4 0.5 0.1 3.3	23 19 12 8 2 1 1 0.4 0.07 2

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)	Max. level in smoke (µg)
Acetophenone CAS 98-86-2	Aryl ketone	M=202 bp 120	45	98	Acetophenone 99.8 Benzoic acid 0.2	22 0.04
2-Acetylpyrazine CAS 22047-25-2	Pyrazine Ketone	M=122 bp 79-80	15	99	2-Acetylpyrazine 100	7.5
2-Acetylthiazole CAS 24295-03-2	Ketone Thiazole	M=127 bp 89 at 12 mm Hg	1	99	2-Acetylthiazole 100	0.5
Ambroxide CAS 3738-00-9	Furan	M=236 mp 74-76	5	99	Ambroxide 99.6 Aromadendrene 0.1 Butylhydroxy toluene 0.1 2 Unidentified compounds 0.2	2.5 *0.002* *0.002* *0.005*
Ammonium hydroxide CAS 1336-21-6 NH_4OH	Inorganic	M=35	6,400	Aqueous solution	No product detected. -	-
Amyl butyrate CAS 540-18-1	Ester	M=158 bp 185-186	15	98	Amyl butyrate 67.4 Isoamyl butyrate 32.2 Di-*n*-amyl ether 0.3 Ethyl butyrate 0.1	5 2 *0.02* *0.008*

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
α-Amylcinnamaldehyde CAS 122-40-7	Unsaturated aldehyde	M=202 bp 284-287	1	97	α-Amylcinnamaldehyde Methylphenylhexanone + Pentyl nonenal Benzaldehyde Naphthalene	97.8 1.3 0.7 0.2	0.5 *0.007* *0.004* *0.001*
Amyl hexanoate CAS 540-07-8	Ester	M=186 bp 222-227	0.5	98	Amyl hexanoate Isoamyl hexanoate Ethyl caproate Hexanoate?	97.7 1.6 0.4 0.3	0.2 *0.004* *0.001* *0.001*
Anisole (Methoxybenzene) CAS 100-66-3	Aryl ether	M=108 mp 37-38 bp 155	1	99	Anisole Methylanisole Benzaldehyde	98.7 1.1 0.2	0.5 *0.006* *0.001*
Anisyl acetate CAS 104-21-2	Ester Aryl ether	M=180 bp 137-139	20	97	Anisyl acetate Anisaldehyde Anisyl formate Anise alcohol Methylanisole Anisole	94.1 3.6 1.3 0.6 0.2 0.2	9 0.4 0.1 0.06 *0.02* *0.02*
Anisyl alcohol CAS 105-13-5	Alcohol Aryl ether	M=138 bp 136	10	98	Anisyl alcohol Anisaldehyde Methoxybenzeneacetic acid Anisole Methyl anisate	85.9 13.1 0.6 0.3 0.1	4 0.7 *0.03* *0.02* *0.005*

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
Anisyl formate CAS 122-91-8	Ester Aryl ether	M=166 bp 100	5	90	Anisyl formate	60.2	1.5
					Anise alcohol	20.7	0.5
					Anisaldehyde	17.3	0.4
					Anisyl acetate	0.5	*0.01*
					Methyl anisole	0.4	*0.01*
					2 Unidentified compounds	0.9	*0.02*
Anisyl phenylacetate CAS 102-17-0	Ester Aryl ether	M=256 bp 370	0.5	97	Anisyl phenylacetate	71.2	0.2
					Anisaldehyde	7.5	*0.02*
					Benzaldehyde	7.2	*0.02*
					Anisyl alcohol	4.1	*0.01*
					Anisyl formate	3.7	*0.01*
					Phenol	1.0	*0.03*
					Ethyl phenylacetate	0.9	*0.002*
					Anisyl acetate	0.3	*0.0008*
					Benzyl phenylacetate	0.3	*0.0008*
					Cresol	0.1	*0.0003*
					Ethyl phenol	0.1	*0.0003*
					10 Minor compounds	3.6	*0.01*
Anisyl propionate CAS 7549-33-9	Ester Aryl ether	M=194 bp 277	1	98	Anisyl propionate	86.9	0.4
					Anisaldehyde	4.3	*0.02*
					Anisyl formate	3.8	*0.02*
					Methylanisole	2.4	*0.01*
					Anisyl alcohol	1.5	*0.008*
					Phenol	0.3	*0.002*
					Anisyl isobutyrate	0.2	*0.001*
					Anisole	0.2	*0.001*
					Cresol	0.2	*0.001*
					Anisyl acetate	0.1	*0.0005*
					1 Unidentified compound	0.1	*0.0005*

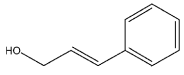
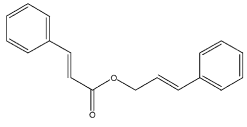
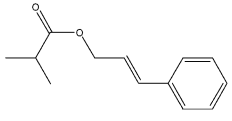
Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
Ascorbic acid CAS 50-81-7	Alcohol Unsaturated γ-lactone	M=176 mp 190	0.05	99	Furfural	51.0	*0.01*
					Acetic acid	7.4	*0.002*
					Pyrone	6.5	*0.002*
					Butenal	6.0	*0.002*
					Heptanone?	3.6	*0.001*
					Butyrolactone + Furanone?	1.8	*0.0005*
					Methylfuranone?	1.8	*0.0005*
					Acetylfuran	1.4	*0.0004*
					Acetol	0.9	*0.0002*
					Phenol	0.5	*0.0001*
					Cresol	0.2	*0.00005*
					Benzene	0.2	*0.00005*
					13 Minor compounds,10 unidentified	18.7	*0.005*
Benzaldehyde CAS 100-52-7	Aryl aldehyde	M=106 bp 178	300	98	Benzaldehyde	94.9	140
					Benzoic acid	0.2	0.3
					Ethyl benzoate	0.1	0.2
					1 Unidentified compound	4.8	7
Benzaldehyde glyceryl acetal CAS 1319-88-6	Cyclic ether Alcohol	M=180 mp 64 (*cis*), 83 (*trans*) bp 280	40	95 (Mixture of isomers)	Benzaldehyde glyceryl acetal	82.7	17
					Benzaldehyde glyceryl acetal isomer	5.8	1
					Benzaldehyde	9.2	2
					Phenol	0.4	0.08
					Ethyl benzoate	0.2	0.04
					7 Unidentified compounds	1.7	0.3
Benzoic acid CAS 65-85-0	Aryl acid	M=122 mp 121-123 bp 249	140	99.5	Benzoic acid	97.2	68
					Isopropyl benzoate	1.6	1
					Phenylacetic acid	1.1	0.8
					Phenyl benzoate	0.1	0.07

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
Benzophenone CAS 119-61-9	Aryl ketone	M=182 mp 48.5 bp 305	30	99	Benzophenone Fluorenone	99.7 0.3	15 0.05
Benzyl alcohol CAS 100-51-6	Aryl alcohol	M=108 bp 206	1,700	99	Benzyl alcohol Benzaldehyde	95.2 4.8	800 40
Benzyl benzoate CAS 120-51-4	Aryl ester	M=212 bp 323	500	99	Benzyl benzoate Benzaldehyde Benzyl alcohol Ethyl benzoate Phenol	98.5 0.7 0.4 0.2 0.2	250 2 1 0.5 0.5
Benzyl butyrate CAS 103-37-7	Aryl ester	M=178 bp 239	20	98	Benzyl butyrate Benzaldehyde Ethyl butyrate	98.4 1.2 0.4	10 0.1 0.04
Benzyl cinnamate CAS 103-41-3	Unsaturated ester	M=238 bp 195 at 5 mm Hg	80	98	Benzyl cinnamate Benzaldehyde Benzyl alcohol + Phenylbutenol Styrene Ethyl cinnamate Benzyl alcohol Benzofuran Phenol Acetophenone 3 Minor compounds	95.1 1.9 0.8 0.6 0.4 0.4 0.1 0.1 0.1 0.5	38 0.8 0.3 0.2 0.2 0.2 0.04 0.04 0.04 0.2

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
Benzyl formate CAS 104-57-4	Aryl ester	M=136 bp 202-203	0.5	95	Benzyl formate Benzyl alcohol Benzaldehyde Benzyl acetate 1 Unidentified compound	75.5 17.3 6.6 0.4 0.2	0.2 0.04 *0.02* *0.001* *0.0005*
Benzyl isobutyrate CAS 103-28-6	Aryl ester	M=178 bp 229	10	97	Benzyl isobutyrate Benzyl alcohol Benzaldehyde Benzyl butyrate	98.2 1.0 0.6 0.2	5 0.05 *0.03* *0.01*
Benzyl isovalerate CAS 103-38-8	Aryl ester	M=192 bp 246	1	98	Benzyl isovalerate Benzaldehyde Benzyl alcohol	98.3 1.2 0.5	0.5 *0.006* *0.003*
n-Butyl acetate CAS 123-86-4 $CH_3COOCH_2CH_2CH_2CH_3$	Ester	M=116 bp 126	15	99	Butyl acetate	100	7.5
n-Butyl alcohol CAS 71-36-3 $CH_3CH_2CH_2CH_2OH$	Alcohol	M=74 bp 118	2.5	99.5	*n*-Butyl alcohol *iso*-Butyl alcohol? Butanal 1 Unidentified compound	90.7 6.3 2.8 0.2	1 0.08 0.04 *0.003*
n-Butyl butyrate CAS 109-21-7	Ester	M=144 bp 165	110	98	*n*-Butyl butyrate Hexyl butyrate Ethyl butyrate	99.7 0.2 0.1	55 0.1 0.06
Butyl isovalerate CAS 109-19-3	Ester	M=158 bp 175	10	98	Butyl isovalerate	100	5

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
n-Butyl valerate CAS 591-68-4	Ester	M=158 bp 186.5	0.5	98	*n*-Butyl valerate	98.7	0.2
					Butylmethyl butyrate	0.6	*0.002*
					Ethyl valerate	0.2	*0.0005*
					Isopentyl valerate	0.2	*0.0005*
					1 Unidentified product	0.3	*0.0008*
n-Butyric acid CAS 107-92-6 $CH_3CH_2CH_2CO_2H$	Acid	M=88 bp 164	110	99	*n*-Butyric acid	97.5	54
					iso-Butyric acid?	1.3	0.7
					1 Unidentified compound	1.2	0.7
Carvacrol CAS 499-75-2	Phenol	M=150 bp 238	10	98	Carvacrol	99.7	5
					Carvacryl acetate	0.2	*0.01*
					Carvacrol methyl ether	0.1	*0.005*
4-Carvomenthenol CAS 562-74-3	Unsaturated cyclic alcohol	M=154 bp 88 at 6 mm Hg	1	92	4-Carvomenthenol	96.0	0.5
					Methylisopropylbenzenes	1.7	*0.009*
					Terpinenes	0.5	*0.003*
					Cuminaldehyde?	0.3	*0.002*
					Carvacrol	0.2	*0.001*
					Benzyl isovalerate	0.2	*0.001*
					6 Minor compounds (3 unidentified)	1.1	*0.006*
d-Carvone CAS 2244-16-8	Unsaturated cyclic ketone	M=150 bp 230	5	96	*d*-Carvone	95.7	2
					Dihydrocarveol	1.6	0.04
					Isolimonene	0.5	*0.01*
					Carveol	0.5	*0.01*
					Isodihydrocarveol	0.2	*0.005*
					Menthadienal	0.2	*0.005*
					α-Terpinolene	0.1	*0.002*
					2 Unidentified compounds	1.2	*0.03*
l-Carvone CAS 6485-40-1	Unsaturated cyclic ketone	M=150 bp 231	1	97	*l*-Carvone	99.3	0.5
					Dimethylcyclohexadiene	0.3	*0.002*
					Dimethylethenylcyclohexene	0.2	*0.001*
					Methoxypropenylbenzene	0.2	*0.001*

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %) Composition		Max. level in smoke (µg)
β-Caryophyllene CAS 87-44-5	Cyclic alkene	M=204 bp 254-257	250	80	ß-Caryophyllene	81.1	100
					Caryophylladienol?	12.8	16
					Caryophyllene oxide	1.4	2
					Other caryophyllene isomers	0.7	0.9
					α-Humulene	0.4	0.5
					Benzoic acid	0.3	0.4
					Dimethyldivinylcyclohexane?	0.3	0.4
					Farnesene	0.2	0.3
					2 Unidentified compounds	2.8	4
Caryophyllene oxide CAS 1139-30-6	Cyclic alcohol	M=220 mp 60-62	15	95	Caryophyllene oxide	96.1	7
					Heptyltetrahydropyranone	0.2	*0.02*
					3 Unidentified compounds	3.7	0.3
Cinnamic acid CAS 621-82-9	Unsaturated aryl acid	M=148 bp 300	2.5	99	Cinnamic acid	85.9	1
					Styrene	5.5	0.07
					Benzaldehyde	4.7	0.06
					Ethyl cinnamate	0.8	*0.01*
					Acetophenone	0.6	*0.008*
					Hyacinthin	0.6	*0.008*
					Phenylacetylene	0.3	*0.004*
					Phenylnaphthalene	0.2	*0.003*
					Phenol	0.2	*0.003*
					Benzofuran	0.2	*0.003*
					4 Minor compounds	1.0	*0.01*
Cinnamyl acetate CAS 103-54-8	Unsaturated ester	M=176 bp 262-265	10	98	Cinnamyl acetate	95.7	5
					Cinnamaldehyde	1.5	*0.008*
					Benzaldehyde	0.5	*0.003*
					Cinnamyl formate	0.2	*0.001*
					Phenylpropene	0.2	*0.001*
					5 Unidentified compounds	1.9	0.1

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
Cinnamyl alcohol CAS 104-54-1 	Unsaturated alcohol	M=134 bp 258	10	98	Cinnamyl alcohol	81.6	4
					Cinnamaldehyde	13.7	0.7
					Benzaldehyde	2.3	0.1
					Phenylpropanol	0.9	0.05
					Cinnamyl formate	0.7	0.04
					Phenyl vinyl ketone	0.2	*0.01*
					Styrene	0.2	*0.01*
					Phenol	0.1	*0.005*
					4 Minor compounds	0.3	*0.02*
Cinnamyl cinnamate CAS 122-69-0 	Unsaturated ester	M=264 mp 42 bp 370	40	95	Cinnamyl cinnamate	53.6	11
					Benzaldehyde	7.0	1
					Diphenylhexadiene	5.1	1
					Styrene	3.6	0.7
					Diphenylpentadiene	2.8	0.6
					Cinnamaldehyde	2.2	0.4
					Phenol	1.1	0.2
					Propynylbenzene	1.0	0.2
					Methylstyrene	0.6	0.1
					Benzofuran	0.4	0.08
					26 Minor compounds (9 unidentified)	22.6	5
Cinnamyl isobutyrate CAS 103-59-3 	Unsaturated ester	M=204 bp 254	2.5	97	Cinnamyl isobutyrate	94.0	2
					Cinnamaldehyde	2.1	*0.03*
					Cinnamyl alcohol	1.0	*0.01*
					Phenylpropyl acetate	0.7	*0.009*
					Benzaldehyde + Propenyl benzene	0.7	*0.009*
					Propenylbenzene	0.4	*0.005*
					Phenyl vinyl ketone	0.3	*0.004*
					1 Unidentified compound	0.8	*0.01*

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
Citral CAS 5392-40-5	Unsaturated aldehyde	M=152 bp 103-104 at 12 mm Hg	20	96	E-Citral	59.8	6
					Z-Citral	33.9	3
					Thujone?	1.1	0.1
					Dill ether	0.8	0.08
					Verbenyl ethyl ether	0.7	0.07
					Isopropylidenebicyclooctane?	0.7	0.07
					Menthadienol	0.5	0.05
					Methylcyclohexenone	0.1	*0.01*
					Trimethyl hexadiene	0.1	*0.01*
					5 Minor products	2.3	0.2
Citric Acid CAS 77-92-9	Hydroxy tricarboxylic acid	M=192 bp 142-143	11,360	99.8	Citric acid	100	5,700
Citronellal CAS 106-23-0	Unsaturated aldehyde	M=154 bp 207	1	80	Citronellal	78.7	0.4
					Dihydrocitronellal + photocitral	6.1	*0.03*
					Isopulegol	6.0	*0.03*
					Carveol?	1.1	*0.006*
					Citral	0.8	*0.004*
					Methone	0.6	*0.003*
					Dimethylheptenal	0.4	*0.002*
					ß-Citronellol	0.4	*0.002*
					14 Minor compounds (7 unidentified)	5.9	*0.03*
Citronellol CAS 106-22-9	Unsaturated alcohol	M=156 bp 225	10	99	Citronellol	92.8	5
					Citronellyl acetate	2.4	0.1
					Citronella	2.0	0.1
					Isogeraniol	1.5	0.08
					ß-Pinene	0.4	*0.02*
					Rose oxide?	0.2	*0.01*
					Lauryl ethoxylate	0.2	*0.01*
					Citronellyl propionate	0.1	*0.005*
					2 Unidentified compounds	0.4	*0.02*

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
Citronellyl acetate CAS 150-84-5	Unsaturated ester	M=198 bp 229	13	80	Citronellyl acetate	92.0	6
					Decene	0.8	0.05
					Dimethyloctadiene	0.7	0.05
					α-Fenchene	0.6	0.04
					ß-Citronellol	0.4	*0.03*
					ß-Pinene	0.4	*0.03*
					10 Minor compounds	5.1	0.3
Cuminaldehyde CAS 122-03-2	Aryl aldehyde	M=148 bp 232	30	98	Cuminaldehyde	98.0	15
					Cumene	0.6	0.09
					Cuminic acid	0.5	0.08
					Diisopropylbenzene	0.2	*0.03*
					1 Unidentified compound	0.7	0.1
Cyclamen aldehyde CAS 103-95-7	Aryl aldehyde	M=190 bp 270	15	90	Cyclamen aldehyde	77.7	6
					Butyltrimethylbenzene	11.2	0.8
					Cuminaldehyde	4.7	0.4
					Butylstyrene	0.9	0.07
					Dimethylbutylstyrene	0.6	0.05
					Dimethyldiethylbenzene	0.2	*0.02*
					13 Minor compounds (8 unidentified)	4.7	0.4
β-Damascenone CAS 23696-85-7	Unsaturated ketone	M=190 bp 116 at 13 mm Hg	10	95	ß-Damascenone	94.2	5
					ß-Farnesene?	0.7	0.04
					Dimethylcrotonophenone	0.6	*0.03*
					α-Phellandrene	0.3	*0.03*
					Dihydromethyl(methylpropenyl)benzofuran	0.2	*0.01*
					Ketoisophorone	0.1	*0.005*
					Cresol	0.1	*0.005*
					11 Minor compounds (9 unidentified)	3.8	0.2

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
β-Damascone CAS 23770-92-3 or 35044-68-9	Unsaturated ketone	M=192 bp 97 at 3 mm Hg	15	90	ß-Damascone (α+δ+γ)-Damascone Isopropylidenemethylcyclopentanecarboxaldehyde Hydroxydimethylethylphenylpropanone ? 5 Minor compounds	88.6 9.2 0.08 0.5 0.9	7 0.7 0.06 0.04 0.07
δ-Decalactone CAS 705-86-2	δ-Lactone	M=170 bp 117-120 at 0.02 mm Hg	5	98	δ-Decalactone δ-Tetradecalactone? Heptanal Methoxyethyl acetate? δ-Octalactone γ-Undecalactone Octanal	97.5 0.8 0.5 0.4 0.4 0.2 0.2	2 *0.02* *0.01* *0.01* *0.01* *0.005* *0.005*
γ-Decalactone CAS 706-14-9	γ-Lactone	M=170 bp 281	15	98	γ-Decalactone δ-Undecalactone 2 Unidentified compounds	98.2 0.8 1.0	7 0.06 0.08
Decanal CAS 112-31-2	Aldehyde	M=156 bp 209	1	95	Decanal Nonanal Decanol Undecanone Nonene Epoxyoctane 6 Minor compounds	94.8 2.0 0.6 0.4 0.3 0.2 1.7	0.5 *0.01* *0.003* *0.002* *0.002* *0.001* *0.009*

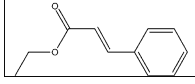
Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp (°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
Decanoic acid CAS 334-48-5	Acid	M=172 bp 268	15	98	Decanoic acid	84.0	6
					Nonanal	6.0	0.5
					Ethyl decanoate	4.1	0.3
					Octanal	1.3	0.1
					Nonene	0.9	0.07
					Hexanol	0.7	0.05
					γ-Decalactone	0.6	0.05
					Heptanal	0.6	0.05
					Nonanone	0.3	*0.02*
					Butyltetrahydrofuran	0.2	*0.02*
					2 Unidentified compounds	1.3	0.1
Diacetyl CAS 431-03-8	Dicarbonyl ketone	M=86 bp 87-88	7.5	95	Diacetyl	95.7	4
					Tetramethyltrioxabicyclooctane	0.7	*0.03*
					2 Unidentified compounds	1.3	0.1
Diethyl malonate CAS 105-53-3	Di ester	M=160 bp 199-200	10	98	Diethyl malonate	100	5
2,3-Diethylpyrazine CAS 15707-24-1	Pyrazine	M=136 bp 180-182	1	98	2,3-Diethylpyrazine	99.6	0.5
					Methylisopropylpyrazine	0.4	*0.002*
m-Dimethoxybenzene CAS 151-10-0	Aryl di ether	M=138 bp 216	7.5	98	*m*-Dimethoxybenzene	99.7	4
					Dimethoxytoluene	0.3	*0.01*

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
p-Dimethoxybenzene CAS 150-78-7	Aryl di ether	M=138 mp 56 bp 109 at 20 mm Hg	25	99	*p*-Dimethoxybenzene Dimethoxytoluene	99.8 0.2	12 *0.03*
2,6-Dimethoxyphenol CAS 91-10-1	Phenol Ether	M=154 mp 56-57 bp 262	7.5	98	2,6-Dimethoxyphenol Other Dimethoxyphenol Trimethoxybenzene Vanillin Trimethylnaphthalene Methyloctalactone 1 Unidentified compound	84.9 13.9 0.3 0.1 0.1 0.1 0.6	3 0.5 *0.01* *0.003* *0.003* *0.003* *0.02*
3,4-Dimethyl-1,2- cyclopentadione CAS 13494-06-9	Cyclic dicarbonyl ketone	M=126 mp 68-72	15	97	3,4-Dimethyl-1,2-cyclopentadione Dimethylcyclopentanone Methylcyclopentenone Dimethylfurandione 1 Unidentified compound 4 Unidentified minor compounds	78.6 0.2 0.2 0.2 19.3 1.5	6 *0.02* *0.02* *0.01* 1 0.1
2,5-Dimethyldihydrofuranolone [2,5-dimethyl-4-hydroxy-3(2*H*)-furanone] CAS 3658-77-3	Unsaturated cyclic ketone Cyclic ether Alcohol	M=128 mp 70	20	98	Acetic acid Acetic anhydride Ethyl pyruvate Acetol acetate Benzaldehyde	70.1 25.4 2.3 1.8 0.4	7 2.5 0.2 0.2 0.04

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
4,5-Dimethyl-3-hydroxy-2,5-dihydrofuran-2-one CAS 28664-35-9	Unsaturated cyclic ketone Cyclic ether Alcohol	M=128 bp 81	7.5	95	Acetic acid Ethyl acetate Acetic anhydride Propylene glycol	39.7 28.7 28.2 3.4	1.5 1 1 0.1
3,7-Dimethyl-6-octenoic acid CAS 502-47-6	Unsaturated acid	M=170 bp 121 at 1mm Hg	0.05	94	3,7-Dimethyl-6-octenoic acid 4 Unidentified compounds	97.2 2.8	0.02 *0.0007*
2,3-Dimethylpyrazine CAS 5910-89-4	Pyrazine	M=108 bp 156	15	95	2,3-Dimethylpyrazine Trimethylpyrazine Ethyldimethylpyrazine	99.7 0.2 0.1	7 *0.02* *0.01*
2,5-Dimethylpyrazine CAS 123-32-0	Pyrazine	M=108 bp 155	15	98	2,5-Dimethylpyrazine Methylpyrrole	99.7 0.3	7 *0.02*
2,6-Dimethylpyrazine CAS 108-50-9	Pyrazine	M=108 mp 47-48 bp 156	1	98	2,6-Dimethylpyrazine Aminodimethylpyridine? Dimethylethylpyrazine	99.5 0.3 0.2	0.5 *0.002* *0.001*

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
δ-Dodecalactone CAS 713-95-1	δ-Lactone	M=198 bp 140-141 at 1 mm Hg	140	98	δ-Dodecalactone	87.9	62
					Heptanal	1.8	1
					Tetradecalactone	1.6	1
					Decenal	1.1	0.8
					Octanal	1.0	0.7
					2 Undecalactones	1.0	0.7
					Pentylbenzene	0.8	0.06
					Undecene?	0.7	0.5
					Hexanal	0.7	0.5
					Styrene	0.3	0.2
					Benzofuran	0.3	0.2
					6 Minor compounds, 4 unidentified	2.8	2
γ-Dodecalactone CAS 2305-05-7	γ-Lactone	M=198 bp 131	10	97	γ-Dodecalactone	98.4	5
					δ-Dodecalactone	0.8	0.04
					2 Unidentified compounds	0.8	0.04
n-Dotriacontane CAS 544-85-4 $CH_3(CH_2)_{30}CH_3$	Alkane	M=450 mp 74-5 bp 245 at 1.5 mm Hg	0 Naturally present in tobacco. Model compound.	97	*n*-Dotriacontane	53.8	*
					Undecanal, $CH_3(CH_2)_9CH0$	2.1	*
					Dodecanal, $CH_3(CH_2)_{10}CHO$	1.9	*
					Heptanal, $CH_3(CH_2)_5CHO$	1.9	*
					Tetradecanal, $CH_3(CH_3)_{12}CHO$	1.8	*
					Nonanal, CH_3 $(CH_2)_7CHO$	1.8	*
					Octanal, $CH_3(CH_2)_6CHO$	1.8	*
					Hexanal, $CH_3(CH_2)_4CHO$	1.8	*
					Hexadecanal, $CH_3(CH_2)_{14}CHO$	1.4	*
					Hexanone, $CH_3(CH_2)_3COCH_3$	1.3	*
					32 $C_4 – C_5$ Aldehydes, ketones and *O*-heterocyclics	30.4	*
							*Not relevant
p-Ethoxybenzaldehyde CAS 10031-82-0	Aryl aldehyde Aryl ether	M=150 mp 14 bp 140 at 20 mm Hg	0.05	99	*p*-Ethoxybenzaldehyde	99.7	*0.02*
					Ethoxybenzene	0.3	*0.0001*

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp** **(°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
Ethyl acetate CAS 141-78-6 $CH_3COOCH_2CH_3$	Ester	M=88 bp 77	550	99	Ethyl acetate	100	275
Ethyl acetoacetate CAS 141-97-9	Ester Ketone	M=130 bp 181	7.5	99	Ethyl acetoacetate Acetol acetate 1 Unidentified compound	99.2 0.3 0.5	4 *0.01* *0.02*
Ethyl alcohol CAS 64-17-5 CH_3CH_2OH	Alcohol	M=46 bp 78	20,000	99	Ethanol Acetic acid Acetic anhydride 2 Unidentified compounds	99.1 0.3 0.3 0.3	990 30 30 30
4-Ethylbenzaldehyde CAS 4748-78-1	Aryl aldehyde	M=134 bp 221	5	97	4-Ethylbenzaldehyde 2 or 3-Ethylbenzaldehyde Ethylbenzene Furylcyclopentene? Cinnamaldehyde Ethylphenol Dimethylbenzenedicarboxaldehyde 4 Unidentified compounds	95.9 0.8 0.4 0.2 0.2 0.2 0.1 2.2	2 *0.02* *0.01* *0.005* *0.005* *0.005* *0.03* 0.06
Ethyl benzoate CAS 93-89-0	Aryl ester	M=150 bp 213	10	99	Ethyl benzoate	100	5
Ethyl *trans*-2-butenoate CAS 10544-63-5	Unsaturated ester	M= 114 bp 136	10	96	Ethyl *trans*-2-butenoate Diethyl oxalate Crotonic acid? Acetaldehyde? 3 Unidentified compounds	97.5 0.6 0.4 0.1 1.4	5 *0.03* *0.02* *0.005* 0.07
Ethyl butyrate CAS 105-54-4	Ester	M=116 bp 121	250	98	Ethyl butyrate	100	125

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp** **(°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
Ethyl cinnamate CAS 103-36-6 	Unsaturated ester	M=176 bp 271-272	15	98	Ethyl cinnamate Benzaldehyde Styrene Methyl cinnamate	98.7 0.7 0.4 0.2	7 0.05 *0.03* *0.02*

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
Ethyl decanoate CAS 110-38-3	Ester	M=200 bp 243	40	98	Ethyl decanoate Long chain hydrocarbon	98.0 2.0	20 0.4
2-Ethyl-3,(5 or 6)-dimethylpyrazine CAS 13360-65-1; 13925-07-0 (2-Ethyl-3,5…) (2-Ethyl-3,6….)	Pyrazine	M=136 bp 180-181	15	95	2-Ethyl-3,(5 or 6)-dimethylpyrazine	100	8
4-Ethylguaiacol CAS 2785-89-9	Phenol Aryl ether	M=152 bp 235	2.5	98	4-Ethylguaiacol Vinylmethoxyphenol Hydroxymethoxyacetophenone Other ethyl methoxy phenols Ethylcyclohexenone + ethylphenol Methylguaiacol 3 Unidentified compounds	97.5 0.6 0.5 0.5 0.2 0.2 0.5	1 *0.008* *0.006* *0.006* *0.003* *0.003* *0.006*
Ethyl heptanoate CAS 106-30-9	Ester	M=158 bp 189	55	98	Ethyl heptanoate Other esters	99.6 0.4	27 0.1
Ethyl hexanoate CAS 123-66-0	Ester	M=144 bp 166	55	98	Ethyl hexanoate	100	28
3-Ethyl-2-hydroxy-2-cyclopenten-1-one CAS 21835-01-8	Unsaturated cyclic ketone, alcohol	M=126 mp 36-43 bp 78-80	0.5	97	3-Ethyl-2-hydroxy-2-cyclopenten-1-one Methylcyclopentene? Ethylhydroxycyclopentenone? 2 Unidentified compounds	98.9 0.4 0.2 0.5	0.2 *0.001* *0.0005* *0.001*

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
5-Ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone CAS 698-10-2	Unsaturated cyclic ketone Cyclic ether Alcohol	M=142 mp 31-35 bp 83-86 at 0.5 mm Hg	2.5	97	5-Ethyl-3-hydroxy-4-methyl-2(5*H*)-furanone Methylpentenal Methylhexenone Isopropylfuran Ethyldimethylcyclopentenone 7 Unidentified compounds	54.2 15..5 2.9 1.8 0.8 24.8	0.7 0.2 0.04 *0.02* *0.01* 0.3
2-Ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone CAS 27538-10-9	Unsaturated cyclic ketone Cyclic ether Alcohol	M=142 bp 103 at 15 mm Hg	1	97	2-Ethyl-4-hydroxy-5-methyl-3(2*H*)-furanone Acetoxybutenone Methylpentanone? 9 Unidentified compounds	79.3 4.3 3.2 13.2	0.4 *0.02* *0.02* 0.07
Ethyl isobutyrate CAS 97-62-1	Ester	M=116 bp 112-113	120	98	Ethyl isobutyrate Hydrazoic acid?	98.8 1.2	59 0.7
Ethyl isovalerate CAS 108-64-5	Ester	M=130 bp 135	15	98	Ethyl isovalerate	100	8
Ethyl lactate CAS 97-64-3	Ester Alcohol	M=118 bp 154	5	98	Ethyl lactate Acetol acetate? Acetic acid? 4 Unidentified compounds	73.4 22.2 0.3 4.1	2 0.6 *0.008* 0.1

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp** **(°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
Ethyl laurate CAS 106-33-2	Ester	M=228 bp 269	160	98	Ethyl laurate Ethyl myristate	99.9 0.1	80 0.08
Ethylmaltol CAS 4940-11-8	Hydroxy δ-pyrone (Unsaturated cyclic ketone, ether, alcohol)	M=140 mp 89-93	100	99	Ethylmaltol Ethylmaltol isomer? 2 Unidentified compounds	92.8 6.2 4.1	46 3 0.1
Ethyl 2-methylbutyrate CAS 7452-79-1	Ester	M=130 bp 133	5	98	Ethyl 2-methylbutyrate Dimethyl butanoate Ethyl dimethylbutyrate 1 Unidentified compound	99.4 0.3 0.2 0.1	2 *0.008* *0.005* *0.003*
3-Ethyl-2-methylpyrazine CAS 15707-23-0	Pyrazine	M=122 bp 57 at 10 mm Hg	15	98	3-Ethyl-2-methylpyrazine Isopropenylpyrazine	99.8 0.2	7 *0.002*
Ethyl myristate CAS 124-06-1	Ester	M=256 bp 178 at 12 mm Hg	10	98	Ethyl myristate	100	5
Ethyl nonanoate CAS 123-29-5	Ester	M=186 bp 229	10	98	Ethyl nonanoate	100	5

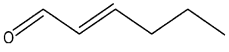
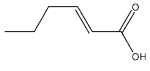
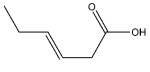
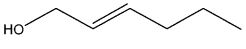
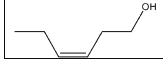
Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
Ethyl octanoate CAS 106-32-1	Ester	M=172 bp 209	40	98	Ethyl octanoate Ethyl decanoate Methoxyethyl acetate? Ethyl benzoate Ethyl nonanoate + undecanone?	99.5 0.2 0.1 0.1 0.1	20 0.04 *0.02* *0.02* *0.02*
Ethyl palmitate CAS 628-97-7	Ester	M=284 bp 303	0.1	95	Ethyl palmitate Ethyl myristate Tetradecane Ethyl stearate	98.4 0.9 0.4 0.3	0.05 *0.0005* *0.0002* *0.0002*
p-Ethylphenol CAS 123-07-9	Phenol	M=122 mp 47-48 bp 218-219	0.05	98	*p*-Ethylphenol Vinylphenol Cresol Isopropylphenol 3 Other phenols	98.1 0.9 0.4 0.3 0.3	0.02 *0.0002* *0.0001* *0.0001* *0.0001*
Ethyl phenylacetate CAS 101-97-3	Aryl ester	M=164 bp 229	30	98	Ethyl phenylacetate Ethylbenzoyl formate Benzaldehyde	98.5 0.8 0.7	15 0.1 0.1
Ethyl propionate CAS 105-37-3	Ester	M=102 bp 99	270	97	Ethyl propionate	100	135
Ethyl salicylate CAS 118-61-6	Phenol Ester	M=166 mp 2-3 bp 234	1	99	Ethyl salicylate Phenol	97.1 2.9	0.5 0.1
Ethyl valerate CAS 539-82-2	Ester	M=130 bp 145	10	98	Ethyl valerate Ethyl valerate isomer? Limonene Ethyl methylbutyrate	85.3 14.2 0.3 0.2	4 0.7 *0.02* *0.01*

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp** **(°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
Ethylvanillin CAS 121-32-4	Phenol Aldehyde Ether	M=166 bp 285	250	98	Ethylvanillin	99.2	120
					Benzofurancarboxaldehyde	0.4	0.5
					Diethoxybenzaldehyde	0.1	0.1
					Pyranone	0.1	0.1
					1 Unidentified compound	0.2	0.1
Eucalyptol CAS 470-82-6	Cyclic ether	M=154 bp 176	1	99	Eucalyptol	99.9	0.5
					Menthone	0.1	*0.0005*
Farnesol CAS 4602-84-0	Unsaturated alcohol	M=222 bp 263	2.5	95	Farnesol	88.4	1
					ß-Farnesene	5.1	0.06
					Farnesal	3.6	0.05
					α-Farnesene	2.9	0.04
Geraniol CAS 106-24-1	Unsaturated alcohol	M=154 bp 130	2,000	97	Geraniol	85.6	860
					Citral	4.6	46
					ß-Myrcene	3.0	30
					Ocimene	1.8	18
					Neryl acetate	1.3	13
					Alloöcimene	0.7	7
					Menthatriene	0.5	5
					Limonene	0.4	4
					20 Minor compounds	2.1	21

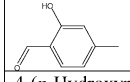
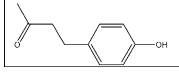
Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
Geranyl acetate CAS 105-87-3	Unsaturated ester	M=196 bp 245	5	98	Geranyl acetate	85.3	2
					ß-Pinene	6.3	0.2
					ß-Myrcene	3.7	0.09
					ß-Ocimene	1.4	0.04
					Limonene	0.4	*0.01*
					α-Ocimene	0.4	*0.01*
					Alloöcimene	0.3	*0.008*
					E-Citral	0.3	*0.008*
					Camphene	0.2	*0.005*
					6 Minor compounds (3 unidentified)	1.7	0.04
Geranylacetone CAS 3796-70-1	Unsaturated ketone	M=194 bp 247	7.5	97	Geranylacetone	97.1	4
					Butanone	0.2	*0.008*
					Methylheptenone	0.2	*0.008*
					Methyldihydrofuran	0.2	*0.008*
					9 Unidentified compounds	2.3	0.09
Geranyl butyrate CAS 106-29-6	Unsaturated ester	M=224 bp 253	5	96	Geranyl butyrate	58.8	1.5
					Citronellyl butyrate	16.2	0.4
					Geranyl butyrate isomer?	14.0	0.4
					ß-Myrcene	4.7	0.1
					ß-Ocimene	1.9	0.05
					Limonene	0.9	*0.02*
					12 Minor compounds	3.5	0.09
Geranyl formate CAS 105-86-2	Unsaturated ester	M=182 bp 216	5	99	Geranyl formate	75.9	2
					Nerol or δ-3-Carene	7.9	0.2
					β-Myrcene	3.7	0.09
					Citronellyl formate	2.9	0.07
					Citral	2.0	0.05
					Ocimene	2.0	0.05
					13 Minor compounds	5.6	0.1
Glycerol CAS 56-81-5	Trihydric alcohol	M=92 mp 18 bp 290	70,000	99.5	Glycerol	99.8	35,000
					2 Unidentified products	0.2	70

Ingredient CAS Number Formula or structure	Chemical Class	Mol. Wt. (M) bp or mp (°C)	Max. cig. appln. Level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
Guaiacol (*o*-Methoxy phenol) CAS 90-05-1	Phenol Ether	M=124 mp 28 bp 203-206	7.5	98	Guaiacol Guaiacol acetate Indanone Dimethoxybenzene Cinnoline	92.5 6.3 0.7 0.3 0.2	3 0.2 *0.03* *0.01* *0.008*
2,4-Heptadienal CAS 4313-03-5	Unsaturated aldehyde	M=110 bp 84	1	88	2,4-Heptadienal Benzaldehyde 8 Minor compounds, (4 unidentified)	98.3 0.4 1.3	0.5 *0.002* *0.007*
γ-Heptalactone CAS 105-21-5	γ-Lactone	M=128 bp 61-62 at 2 mm Hg	40	98	γ-Heptalactone Isopropylmethylbutyrolactone? γ-Octalactone Dimethylcyclobutanone Methoxyethyl acetate?	99.2 0.3 0.2 0.2 0.1	20 0.06 0.04 0.04 *0.02*
n-Heptanoic acid CAS 111-14-8	Acid	M=130 bp 223	1	98	*n*-Heptanoic acid Propyl heptanoate Heptalactone 4 Minor compounds	98.9 0.6 0.4 0.1	0.5 *0.003* *0.002* *0.005*
2-Heptanone CAS 110-43-0	Ketone	M=114 bp 151	5	98	2-Heptanone Other Heptanone	87.5 12.5	2 0.3
cis-4-Heptenal CAS 6728-31-0	Unsaturated aldehyde	M=112 bp 153	0.05	99	*cis*-4-Heptenal *trans*-4-Heptenal 4 Minor compounds	94.2 4.3 1.5	*0.02* *0.001* *0.0004*

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
ω-*6*-Hexadecenlactone CAS 7779-50-2	Unsaturated ω lactone	M=252 bp 185 at 16 mm Hg	2.5	98	ω-6-Hexadecenlactone 2 Unidentified compounds	99.8 0.2	1 *0.003*
δ-Hexalactone CAS 823-22-3	δ-Lactone	M=114 bp 110-112 at 15 mm Hg	1	98	δ-Hexalactone 3 Unidentified compounds	99.5 0.5	0.5 *0.003*
γ-Hexalactone CAS 695-06-7	γ-Lactone	M=114 bp 100-102 at 18 mm Hg	10	98	γ-Hexalactone Methylethylperhydrofuranone? Caprolactone?	99.6 0.2 0.2	5 *0.01* *0.01*
Hexanal CAS 66-25-1	Aldehyde	M=100 bp 131	3	97	Hexanal Hexanoic acid Butyloctenal Cyclododecene 15 Minor compounds, 8 unidentified	66.0 8.8 3.9 0.7 20.6	1 0.1 0.06 *0.01* 0.3
Hexanoic Acid CAS 142-62-1	Acid	M=116 bp 223	20	98	Hexanoic acid 2 Hexanoic acid isomers Undecanone Methylpentanoic anhydride?	89.6 6.9 2.8 0.7	9 0.7 0.3 0.07

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M) bp or mp (°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
trans-2-Hexenal CAS 6728-26-3 	Unsaturated aldehyde	M=98 bp 47 at 17 mm Hg	10	95	*trans*-2-Hexenal Hexenoic acid *cis*-2-Hexenal Ethylhydroxycyclopentenone Cyclohexene oxide 4 Unidentified compounds	74.9 8.9 6.5 3.0 0.8 5.9	4 0.4 0.3 0.2 0.04 0.3
trans-2-Hexenoic acid CAS 13419-69-7 	Unsaturated acid	M=114 bp 217	0.5	98	*trans*-2-Hexenoic acid Pentenal	99.1 0.9	0.2 *0.002*
3-Hexenoic acid CAS 4219-24-3 	Unsaturated acid	M=114 bp 208	0.5	98	3-Hexenoic acid Ethylfuranone?	99.7 0.3	0.2 *0.0008*
trans-2-Hexen-1-ol CAS 928-95-0 	Unsaturated alcohol	M=100 bp 158	0.5	96	*trans*-2-Hexen-1-ol *cis*-2-Hexen-1-ol Hexenal Hexenyl formate Hexenyl butyrate 4 Unidentified compounds	71.5 11.8 6.9 4.8 1.1	0.2 *0.03* *0.02* *0.01* *0.003*
cis-3-Hexen-1-ol CAS 928-96-1 	Unsaturated alcohol	M=100 bp 156	2.5	98	*cis*-3-Hexen-1-ol *trans*-3-Hexen-1-ol Methylbutenylbenzene	95.3 4.6 0.1	1 0.06 *0.001*

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
cis-3-Hexen-1-yl acetate CAS 3681-71-8	Unsaturated ester	M=142 bp 198	5	98	*cis*-3-Hexen-1-yl acetate *trans*-3-Hexen-1-yl acetate Ethylfuran Hexenol 10 Minor compounds, 4 unidentified	79.9 15.7 0.9 0.8 2.7	2 0.4 *0.02* *0.02* 0.07
trans-2-Hexenyl acetate CAS 2497-18-9	Unsaturated ester	M=142 bp 166	0.5	98	*trans*-2-Hexenyl acetate *cis*-2-Hexenyl acetate Hexyl acetate Hexenol Hexenal 5 Unidentified compounds	88.7 4.2 3.7 0.6 0.5 2.3	0.2 *0.01* *0.009* *0.002* *0.001* *0.006*
cis-3-Hexenyl formate CAS 33467-73-1	Unsaturated ester	M=128 bp 155	0.5	94	*cis*-3-Hexenyl formate Hexenol *trans*-3-Hexenyl formate 4 Unidentified compounds	90.4 8.0 0.8 0.8	0.2 *0.02* *0.002* *0.002*
Hexyl acetate CAS 142-92-7	Ester	M=144 bp 168-170	7	98	Hexyl acetate Hexanol Dimethylacetal? Pentyl acetate 1 Unidentified compound	97.3 1.9 0.5 0.2 0.1	3 0.07 *0.02* *0.007* *0.004*
Hexyl alcohol CAS 111-27-3	Alcohol	M=102 bp 157	15	98	Hexyl alcohol Hexanal Ethyl hexanoate Undecane	96.3 3.5 0.1 0.1	7 0.3 *0.008* *0.008*
Hydroxycitronellal CAS 107-75-5	Hydroxy aldehyde	M=172 bp 241	5	95	Hydroxycitronellal Dimethyloctadiene Citronellal Dimethylheptanol 4 Undentified compounds	96.6 1.4 0.4 0.2 1.4	2 0.04 *0.01* *0.005* 0.04

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp** **(°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
2-Hydroxy-4-methylbenzaldehyde CAS 698-27-1 	Phenol Aldehyde	M=136 bp 58-60	0.5	98	2-Hydroxy-4-methylbenzaldehyde 2-Hydroxy-4-methylbenzaldehyde isomer Cresol	99.0 0.8 0.2	0.2 *0.002* *0.0005*
4-(*p*-Hydroxyphenyl)-2-butanone CAS 5471-51-2 	Phenol Ketone	M=164 mp 82-84	20	98	4-(*p*-Hydroxyphenyl)-2-butanone 4-(*p*-Hydroxyphenyl)-2-butanone isomer Dihydrobenzofuran 1 Unidentified compound	96.8 3.0 0.1 0.1	10 0.3 *0.01* *0.01*

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp (°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
α-Ionone CAS 127-41-3	Unsaturated ketone	M=192 bp 237	10	90	α-Ionone Trimethyldihydronaphthalene +? β-Ionone γ-Ionone Trimethyltetrahydronapthalene Ethylidenemethylcyclopentene? 6 Minor products	91.8 3.4 1.3 1.2 0.8 0.4 1.1	5 0.2 0.07 0.06 0.04 *0.02* *0.06*
β-Ionone CAS 14901-07-6	Unsaturated ketone	M=192 bp 239	7.5	97	β-Ionone α-Ionone α-Ionene 6 Minor products, 3 unidentified	95.4 1.1 0.8 2.7	4 0.04 *0.03* *0.01*
α-Irone CAS 79-69-6	Unsaturated ketone	M=206 bp 110-112	5.5	90	α-Irone Menthofuran Methylionone + propylidenephthalide 22 Minor products, 11 unidentitifed, others partially identified.	62.8 4.9 2.5 29.8	2 0.1 0.07 0.8
Isoamyl acetate CAS 123-92-2	Ester	M=130 bp 145	60	97	Isoamyl acetate Dimethylpropanol acetate Ethyl hexanol	99.6 0.2 0.2	30 0.06 0.06
Isoamyl butyrate CAS 106-27-4	Ester	M=158 bp 179	20	98	Isoamyl butyrate Ethyl butyrate Amyl butyrate Amyl isobutyrate	99.6 0.2 0.1 0.1	10 *0.02* *0.01* *0.01*

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp** **(°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
Isoamyl cinnamate CAS 7779-65-9	Unsaturated ester	M=218 bp 310	1	97	Isoamyl cinnamate Benzaldehyde Styrene + ethylbenzene Ethyl cinnamate Acetophenone Propenylbenzene 10 Minor products	93.2 2.4 0.9 0.7 0.6 0.5 1.7	0.5 *0.01* *0.005* *0.004* *0.003* *0.003* *0.009*
Isoamyl hexanoate CAS 2198-61-0	Ester	M=186 bp 222	1	98	Isoamyl hexanoate Ethyl caproate Amyl hexanoate	99.6 0.3 0.1	0.5 *0.002* *0.0005*
Isoamyl isovalerate CAS 659-70-1	Ester	M=172 bp 192	30	98	Isoamyl isovalerate Ethyl isovalerate Isobutyl isovalerate	99.8 0.1 0.1	15 *0.02* *0.02*
Isoamyl octanoate CAS 2035-99-6	Ester	M=214 bp 267-268	15	98	Isoamyl octanoate Ethyl octanoate Isobutyl caprylate + Isoamyl heptanoate Amyl caprylate	99.5 0.3 0.2 0.1	7 *0.02* *0.02* *0.008*
Isoamyl phenylacetate CAS 102-19-2	Ester	M=206 bp 268	7.5	98	Isoamyl phenylacetate Benzaldehyde Ethyl phenylacetate Methylbutanol Phenyl benzoate	94.7 3.8 0.9 0.4 0.2	4 0.1 *0.03* *0.02* *0.008*

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp (°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
Isoamyl propionate CAS 105-68-0	Ester	M=144 bp 156	0.1	98	Isoamyl propionate Propyl propionate Di(methylbutyl) ether Isoamyl acetate 1 Unidentified compound	99.1 0.3 0.3 0.1 0.2	0.05 *0.0002* *0.0002* *0.00005* *0.0001*
Isoamyl salicylate CAS 87-20-7	Phenol Ester	M=208 bp 277	0.1	98	Isoamyl salicylate Ethyl salicylate Phenol Pentyl salicylate Other salicylates	96.6 1.4 0.8 0.7 0.5	0.05 *0.0007* *0.0004* *0.0004* *0.0003*
Isobornyl acetate CAS 125-12-2	Bridged cyclohexane Ester	M=196 bp 227	0.5	90	Isobornyl acetate Camphene α-Fenchene Trimethyltricycloheptane α-Terpinene β-Fenchene 3 Unidentified compounds	91.0 4.7 0.4 0.4 0.1 0.1 3.3	0.2 *0.01* *0.001* *0.001* *0.0003* *0.0003* *0.008*
Isobutyl acetate CAS 110-19-0	Ester	M=116 bp 116	10	98	Isobutyl acetate *n*-Butyl acetate 1 Unidentified compound	99.8 0.1 0.1	5 *0.005* *0.005*
Isobutyl alcohol CAS 78-83-1 $(CH_3)_2CHCH_2OH$	Alcohol	M=74 bp 108	25	99	Isobutyl alcohol	100	12.5
Isobutyl butyrate CAS 539-90-2	Ester	M=144 bp 157	0.05	98	Isobutyl butyrate Butyl butyrate	99.9 0.1	*0.03* *0.00003*

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M) bp or mp (°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
Isobutyl cinnamate CAS 122-67-8	Unsaturated ester	M=204 bp 271	0.5	98	Isobutyl cinnamate Ethyl cinnamate Benzaldehyde Acetophenone Butyl cinnamate	96.9 2.0 0.8 0.2 0.1	0.2 *0.005* *0.002* *0.0005* *0.0003*
Isobutyl phenylacetate CAS 102-13-6	Ester	M=192 bp 247	25	98	Isobutyl phenylacetate Benzaldehyde Ethyl phenylacetate Butyl phenyl ketone?	98.2 1.1 0.5 0.2	12 0.1 0.06 *0.03*
Isobutyraldehyde CAS 78-84-2 $(CH_3)_2CHCHO$	Aldehyde	M=72 bp 63	10	98	Isobutyraldehyde Other aldehydes	95.6 4.4	15 0.2
Isobutyric acid $(CH_3)_2CHCO_2H$ CAS 79-31-2	Acid	M=88 bp 155	30	99	Isobutyric acid *n*-Butyric acid Acetic acid 2 Unidentified compounds	60.5 35.5 1.2 2.8	9 5 0.2 0.4
dl-Isomenthone CAS 491-07-6	Cyclic ketone	M=154 bp 210	1	98	*dl*-Isomenthone Menthenone Citronellol? Carvomenthone 2 Unidentified compounds	98.9 0.7 0.2 0.1 0.1	0.5 *0.004* *0.001* *0.0005* *0.0005*
Isopropyl myristate CAS 110-27-0	Ester	M=270 bp 192 at 22 mm Hg	2.5	98	Isopropyl myristate Isopropyl palmitate Ethyl myristate Tridecanal Isopropyl decanoate	98.8 0.5 0.3 0.3 0.1	1 *0.006* *0.004* *0.004* *0.001*

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp** **(°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
Isovaleric acid CAS 503-74-2	Acid	M=102 bp 175	35	99	Isovaleric acid	90.1	16
					Epoxyhexanone?	5.1	0.9
					Other valeric acid	4.0	0.7
					Isoamyl isovalerate	0.3	0.05
					1 Unidentified compound	0.5	0.09
Lactic acid CAS 598-82-3; 50-21-5 $CH_3CH(OH)CO_2H$	Acid Alcohol	M=90 bp 122 at 15 mm Hg	12,400	98	Lactic acid	83.2	5,200
					Methylmaleic anhydride	3.7	230
					2 Lactides	13.1	810
Lauric acid (*n*-Dodecanoic acid) CAS 143-07-7	Acid	M=200 bp 225 at 100 mm Hg	1	98	Lauric acid	85.4	0.5
					Undecanal	4.4	*0.02*
					Ethyl laurate	2.7	*0.01*
					Undecene	1.2	*0.006*
					Undecalactone	0.8	*0.004*
					8 Minor products	4.3	*0.02*
Linalool CAS 78-70-6	Unsaturated alcohol	M=154 bp 198	15	97	Linalool	97.9	7
					Dihydrolinalool	1.6	0.1
					Methylheptenone	0.3	*0.02*
					β-Myrcene	0.2	*0.02*
Linalool oxide CAS 1365-19-1; 5983-33-3	Alcohol Cyclic ether	M=170 bp 188	5	97	Linalool oxide	95.1	2
					Methylvinylbutyrolactone	2.3	0.06
					Methylcaprolactone?	0.8	*0.02*
					Diethylmethoxycyclopropane?	0.5	*0.01*
					Methylheptenone	0.3	*0.008*
					Pentylacetyl propanoate	0.1	*0.003*
					3 Unidentified compounds	0.9	*0.02*

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp** **(°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
Linalyl acetate CAS 115-95-7	Unsaturated ester	M=196 bp 220	7.5	97	Linalyl acetate	78.6	3
					β-Myrcene	6.5	0.2
					δ-3-Carene	3.6	0.1
					β-Ocimene	3.1	0.1
					Dihydrolinalool	2.0	0.08
					α-Ocimene	1.1	0.04
					Limonene	1.1	0.04
					11 Minor products, 3 unidentified	4.0	0.2
Linalyl benzoate CAS 126-64-7	Unsaturated ester	M=258 bp 263	1	98	Linalyl benzoate	52.9	0.3
					Linalyl-related benzoate	16.9	0.08
					β-Ocimene	7.3	0.04
					β-Myrcene	5.8	*0.03*
					β-Pinene + Phenol	4.4	*0.02*
					Limonene	1.6	*0.008*
					Alloöcimene	1.3	*0.007*
					25 Minor products, 1 unidentified	9.8	0.05
Linalyl isobutyrate CAS 78-35-3	Unsaturated ester	M=224 bp 230	5	97	Linalyl isobutyrate	80.7	2
					β-Myrcene	4.5	0.1
					Isopulegone	3.1	0.08
					β-Ocimene	2.7	0.07
					Dihydrolinalool?	1.6	0.04
					Geraniol	1.4	0.04
					Menthatriene	0.9	*0.02*
					δ-3-Carene	0.9	*0.02*
					Isobutyric acid	0.9	*0.02*
					11 Minor products, 3 unidentified	3.3	0.08

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp** **(°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
Malic acid CAS 6915-15-7	Dicarboxylic acid	M=134 mp 131-135	6,500	99	Maleic anhydride	84.1	2,730
					Acetaldehyde	10.3	330
					Acrylic acid	1.5	150
					Acetic acid	1.0	33
					Pyruvic acid	0.9	30
					Maleic anhydride-related	0.8	30
					Methylmaleic anhydride	0.4	10
					Dimethylmaleic anhydride	0.3	10
					Benzene	0.2	7
					Methylcyclobutane	0.2	7
					2 Unidentified compounds	0.3	10
Maltol CAS 118-71-8	Hydroxy γ-pyrone	M=126 mp 160-164	220	99	Maltol	99.8	110
					Acetoxymethyl pyranone	0.2	0.2
p-Mentha-8-thiol-3-one CAS 38462-22-5	Cyclic ketone Thio alcohol	M=186 bp 120 at 10 mm Hg	0.5	94 (Mixture of *cis* and *trans* isomers)	*p*-Mentha-8-thiol-3-one	55.7	0.2
					Pulegone	28.8	0.07
					Isopulegone	12.5	*0.03*
					Menthofuran	0.7	*0.002*
					Methylcyclohexanone	0.7	*0.002*
					Thiothymol	0.3	*0.0008*
					Sulphur dioxide	0.3	*0.0008*
					3 Unidentified compounds	1.0	*0.003*
l-Menthol CAS 89-78-1; 2216-51-5	Cyclic alcohol	M=156 mp 42-43 bp 212	20,000	99	Menthol	99.0	10,000
					Menthone	0.9	90
					Menthene	0.1	10

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp** **(°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
l-Menthone CAS 89-80-5	Cyclic ketone	M=154 bp 207	2.5	96	Menthone Menthenone Isopulegol + Menthene Limonene	99.0 0.6 0.3 0.1	1 *0.008* *0.004* *0.001*
dl-Menthyl acetate CAS 16409-45-3	Ester	M=198 bp 228-229	5.5	97	Menthyl acetate Menthene Menthol Cymene Menthone	96.9 2.3 0.5 0.2 0.1	3 0.06 *0.01* *0.006* *0.003*
Menthyl isovalerate CAS 16409-46-4	Ester	M=240 bp 260-262	1	98	Menthyl isovalerate Menthene Menthol Cymene	94.9 4.5 0.3 0.3	0.5 *0.02* *0.002* *0.002*
Methional [(3-Methylthio)-propionaldehyde] CAS 3268-49-3	Thio ether Ester	M=104 bp 165-166	2.5	98	Methional Methional isomer Dimethyl disulphide Dithiapentane 3 Unidentified compounds	88.1 10.0 0.2 0.2 1.5	1 0.1 *0.003* *0.003* *0.02*
p-Methoxybenzaldehyde CAS 123-11-5	Aldehyde Aryl ether	M=136 bp 243-244	200	97.5	*p*-Methoxybenzaldehyde Methoxybenzyl alcohol Methoxyphenol Anisole	99.3 0.3 0.2 0.2	100 0.3 0.2 0.2

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M) bp or mp (°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
2-Methoxy-4-methylphenol CAS 93-51-6	Phenol Aryl ether	M=138 mp 5.5 bp 220-221	7.5	98	2-Methoxy-4-methylphenol 2-Methoxy-4-methylphenol isomer Methylbenzal acetone Dimethoxytoluene 1 Unidentified compound	90.9 7.7 0.6 0.5 0.3	3 0.3 *0.02* *0.02* *0.01*
2 or 5 or 6-Methoxy-3-methylpyrazine CAS 68378-13-2	Pyrazine Ether	M=124	7.5	99 (Mixture of Isomers)	2-Methoxy-3-methylpyrazine 2-Methoxy-5-methylpyrazine Ethoxymethylpyrazine Methylpyrazine 1 Unidentified compound	79.2 20.2 0.2 0.2 0.2	3 0.8 *0.008* *0.008* *0.008*
4-(*p*-Methoxyphenyl)-2-butanone CAS 104-20-1	Ketone Aryl ether	M=178 bp 277	0.5	98	4-(*p*-Methoxyphenol)-2-butanone Vinylanisole Anisaldehyde Methoxyphenylbutenone Methylenedioxyphenylbutanone? 1 Unidentified compound	96.2 1.4 1.1 0.8 0.3 0.2	0.2 *0.004* *0.003* *0.002* *0.008* *0.0005*
1-(*p*-Methoxyphenyl)-2-propanone CAS 122-84-9	Ketone Aryl ether	M=164 bp 264-265	0.5	97	1-(*p*-Methoxyphenyl)-2-propanone Anisaldehyde Methoxyacetophenone 2 Unidentified compounds	91.2 6.0 0.1 2.7	0.2 *0.02* *0.003* *0.007*
2-Methoxy-4-vinylphenol CAS 7786-61-0	Phenol Aryl ether	M=150 bp 224	10	98	2-Methoxy-4-vinylphenol Vanillin 2-Methoxy-4-vinylphenol isomer 1 Unidentified compound	98.8 0.6 0.5 0.1	5 *0.03* *0.03* *0.005*

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp** **(°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
4'-Methylacetophenone CAS 122-00-9	Aryl ketone	M=134 mp 22-24 bp 226	10	95	4'-Methylacetophenone 3'-Methylacetophenone 2'-Methylacetophenone	95.6 3.7 0.7	5 0.2 0.04
Methyl *p*-anisate CAS 121-98-2	Aryl ester Aryl ether	M=166 mp 48-52 bp 244-245	30	99	Methyl *p*-anisate Ethyl anisate Anisole	99.3 0.5 0.2	15 0.08 *0.03*
p-Methylanisole CAS 104-93-8	Aryl ether	M=122 bp 174	5	99	*p*-Methylanisole	100	2.5
Methyl benzoate CAS 93-58-3	Aryl ester	M=136 bp 198	25	98	Methyl benzoate Ethyl benzoate	99.6 0.4	12 0.05
α-Methylbenzyl acetate CAS 93-92-5	Aryl ester	M=164 bp 94-95 at 12 mm Hg	5.5	98	α-Methylbenzyl acetate Styrene Acetophenone Phenylethanol Ethylphenylethyl ether Benzaldehyde Isopropylcyclohexanol 1 Unidentified compound	93.1 4.0 1.2 1.0 0.2 0.2 0.2 0.1	3 0.1 *0.03* *0.03* *0.006* *0.006* *0.006* *0.003*

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp** **(°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
trans-2-Methyl-2-butenoic acid CAS 80-59-1	α,β Unsaturated acid	M=100 mp 61-67 bp 199	0.5	99	*trans*-2-Methyl-2-butenoic acid 1 Unidentified compound	99.8 0.2	0.2 *0.0005*
2-Methylbutyraldehyde CAS 96-17-3	Aldehyde	M=86 bp 90-92	15	90	2-Methylbutyraldehyde Methylbutyric acid + ethylmethyl butyrate Valeraldehyde diethyl acetal 1 Unidentified compound	63.6 22.8 10.4 3.2	5 2 0.8 0.2
3-Methylbutyraldehyde CAS 590-86-3	Aldehyde	M=86 bp 93	40	97	3-Methylbutyraldehyde Acetic acid Acetic anhydride Methylbutyric acid Diethoxymethylbutane Dimethylheptanone or isobutyl ketone 2 Unidentified compounds	75.7 11.5 7.3 3.0 0.3 0.2 2.0	15 2 1.0 0.6 0.06 0.04 0.4
2-Methylbutyric acid CAS 116-53-0	Acid	M=102 bp 176	7.5	98	2-Methylbutyric acid Propanoic acid Methyl pentanoate? Dimethyl acetal? Amyl isovalerate 2 Unidentified compounds	89.6 1.4 0.8 0.7 0.3 0.4	3 0.05 *0.03* *0.03* *0.01* 0.08
Methyl cinnamate CAS 103-26-4	α,β Unsaturated ester	M=162 mp 34-38 bp 263	20	98	Methyl cinnamate Ethyl cinnamate Benzaldehyde Styrene	98.4 0.7 0.6 0.3	10 0.07 0.06 *0.03*

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M) bp or mp (°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
1-Methyl-2,3-cyclohexadione CAS 3008-43-3	Dicarbonyl cyclic ketone	M=126 mp 61-63 bp 69-72 at 1 mm Hg	0.5	98	3-Methyl-1,2-cyclohexadione 3-Methyl-1,2-cyclohexadione isomer Dimethylpyranone?	99.1 0.8 0.1	0.2 *0.02* *0.0003*
Methylcyclopentenolone CAS 765-70-8	Alcohol Unsaturated γ-lactone	M=112 mp 105-108	45	99	Methylcyclopentenolone Methylcyclohexenone? 3 Unidentified compounds	85.3 0.3 14.4	19 0.07 3
5*H*-5-Methyl-6,7-dihydrocyclopenta(*b*)pyrazine CAS 23747-48-0	Pyrazine	M=134 Mp-121-123 bp 96-99 at at 20 mm Hg	5	97	5*H*-5-Methyl-6,7-dihydrocyclopenta[*b*]pyrazine Dihydrocyclopentapyrazine Trimethyldihydrocyclopentapyrazine 3 Unidentified products	99.0 0.6 0.2 0.2	2 *0.02* *0.005* *0.005*
Methyl dihydrojasmonate CAS 24851-98-7	Ester Cyclic ketone	M=226 bp >300	10	96	Methyl dihydrojasmonate Pentylcyclopentenone? Methyl jasmonate 2 Unidentified compounds	96.4 2.1 0.5 1.0	5 0.1 *0.03* 0.05
6-Methyl-3,5-heptadien-2-one CAS 1604-28-0	Unsaturated ketone	M=124 bp 190	15	98	6-Methyl-3,5-heptadien-2-one	100	7.5

Ingredient CAS Number Formula or structure	Chemical Class	Mol. Wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
6-Methyl-5-hepten-2-one CAS 110-93-0	Unsaturated ketone	M=126 bp 73 at 18 mm Hg	10	98	6-Methyl-5-hepten-2-one Methylheptadienone 5 Unidentified compounds	97.8 1.2 1.0	5 0.06 0.05
Methyl-α-ionone CAS 127-42-4	Unsaturated ketone	M=206 bp 238	0.5	90	Methyl-α-ionone Methyl-γ-ionone Methyl-β-ionone *Iso*-Methyl-β-ionone Dimethylcyclohexenylmethyl ketone Toluene 16 Minor products, 10 unidentified	40.4 36.2 11.6 2.8 0.3 0.1 8.6	0.1 0.09 0.09 0.03 *0.0008* *0.0003* *0.02*
1-Methyl-3-methoxy-4-isopropylbenzene CAS 1076-56-8	Aryl ether	M=164 Bp 215	0.05	97	1-Methyl-3-methoxy-4- isopropylbenzene	100	*0.03*
Methyl 3-(methylthio) propionate CAS 13532-18-8	Ester Thio ether	M=134 bp 74-78 at 13 mm Hg	0.5	98	Methyl 3-(methylthio)propionate Sulphur dioxide Ethyl thioisobutyrate Dimethyl disulphide	99.4 0.3 0.2 0.1	0.2 *0.0008* *0.0005* *0.0003*
Methyl 2-octynoate CAS 111-12-6	Unsaturated ester	M=154 bp 217	1	99	Methyl 2-octynoate Methyl 2-octynoate isomer Octatriene + Methylnaphthalene? 2 Unidentified compounds	92.8 4.7 0.2 2.3	0.5 *0.02* *0.001* *0.01*

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp** **(°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
3-Methylpentanoic acid CAS 105-43-1	Acid	M=116 bp 197-198	3	98	3-Methylpentanoic acid Dimethylcyclobutanone? 1 Unidentified compound	99.2 0.3 0.5	1.5 *0.005* *0.008*
Methyl phenylacetate CAS 101-41-7	Ester	M=150 bp 215	15	98	Methyl phenylacetate Ethyl phenylacetate Phenethyl alcohol	98.4 0.9 0.7	7 0.07 0.05
2-Methylpyrazine CAS 109-08-0	Pyrazine	M=94 bp 137	25	99	2-Methylpyrazine	100	12.5
Methyl-2-pyrrolyl ketone CAS 1072-83-9	Pyrrole Ketone	M=109 bp 220	1	98	Methyl-2-pyrrolyl ketone Acetylpyrrole 1 Unidentified compound	99.3 0.4 0.3	0.5 *0.002* *0.002*
5-Methylquinoxaline CAS 13708-12-8	Pyrazine	M=144 mp 20-21 bp 246	0.1	99	5-Methylquinoxaline Quinoxaline	99.9 0.1	0.05 *0.00005*
Methyl salicylate CAS 119-36-8	Phenol Ester	M=152 bp 222	2.5	98	Methyl salicylate Ethyl salicylate Phenol	98.6 1.1 0.3	1 0.01 *0.004*

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp** **(°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
2-Methyltetrahydrofuran-3-one CAS 3188-00-9	Cyclic ether Cyclic ketone	M=100 bp 139	5	97	2-Methyltetrahydrofuran-3-one Other tetrahydrofuranone? Ethyl ethoxy propionate 4 Unidentified compounds	69.2 8.1 3.4 19.3	2 0.2 0.09 0.5
4-Methyl-5-thiazole ethanol CAS 137-00-8	Thiazole Alcohol	M=143 bp 135 at 7 mm Hg	0.5	98	4-Methyl-5-thiazole ethanol Methylvinylthiazole Trimethylthiazole? Choroethylmethylthiazole Acetylmethylthiazole Methylthiazoleacetaldehyde 1 Unidentified compound	95.6 1.3 2.0 0.3 0.2 0.2 0.4	0.2 *0.003* *0.005* *0.0008* *0.0005* *0.0005* *0.001*
2-Methylvaleric acid CAS 97-61-0	Acid	M=116 bp 196-197	5	98	Methylvaleric acid Epoxyethylbutanone? Styrene 1 Unidentified compound	95.9 3.5 0.4 0.2	2 0.09 *0.01* *0.005*

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (^{o}C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
Nerol CAS 106-25-2	Unsaturated alcohol	M=154 bp 227	2.5	97	Nerol	86.0	1
					Z-Citral	8.2	0.1
					E-Citral	1.8	*0.03*
					δ-3-Carene	0.6	*0.008*
					Menthadienol	0.4	*0.005*
					Citronella	0.4	*0.005*
					Limonene	0.3	*0.004*
					Isogeraniol?	0.3	*0.004*
					γ-Terpinene	0.3	*0.004*
					α-Terpinolene	0.3	*0.004*
					10 Minor products, 1 unidentified	1.4	*0.02*
Nerolidol CAS 7212-44-4	Unsaturated alcohol	M=222 bp 276	2.5	97 Mixture of *cis* and *trans* isomers	Nerolidol	82.5	1
					Naphthalene	10.1	0.1
					β-Farnesene +Biphenylene	4.6	0.06
					α-Farnesene	1.7	*0.02*
					Limonene	0.8	*0.01*
					Styrene	0.3	*0.004*
Neryl acetate CAS 141-12-8	Ester	M=196 bp 134 at 25 mm Hg	2	98	Neryl acetate	82.0	0.8
					Pulegone	4.9	0.05
					Limonene	2.8	*0.03*
					β-Myrcene	1.9	*0.02*
					β-Ocimene	1.6	*0.02*
					β-Pinene	1.1	*0.01*
					α-Terpinolene	0.9	*0.009*
					δ-3-Carene	0.8	*0.008*
					16 Minor products, 2 unidentified	3.7	0.04
Nicotine CAS 54-11-5	Pyridine Pyrrolidine	M=162 bp 246	0 Naturally present in tobacco. Model compound.	99	Nicotine	97.7	*
					Nicotyrine	1.4	*
					Myosmine	0.6	*
					Cotinine	0.3	*
							* Not relevant

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (^{o}C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
2,6-Nonadien-1-ol CAS 7786-44-9	Unsaturated alcohol	M=140 bp 196	0.05	95	2,6-Nonadien-1-ol	98.1	*0.02*
					α-Farnesene	0.5	*0.0001*
					Decanol	0.4	*0.0001*
					4 Minor products, partically identified	1.0	*0.0003*
δ-Nonalactone CAS 3301-94-8	δ-Lactone	M=156 bp 115 at 2 mm Hg	5	98	δ-Nonalactone	98.6	2
					Tetradecalactone?	0.8	*0.02*
					Undecalactone?	0.5	*0.01*
					Octalactone	0.1	*0.003*
γ-Nonalactone CAS 104-61-0	γ-Lactone	M=156 bp 121 at 6 mm Hg	20	98	γ-Nonalactone	99.7	10
					Dihydromethylpentylfuranone?	0.2	*0.02*
					1 Unidentified product	0.1	*0.01*
n-Nonanal CAS 124-19-6	Aldehyde	M=142 bp 93 at 23 mm Hg	5	95	*n*-Nonanal	97.8	2
					Decahydromethylbenzocycloheptenone?	0.8	*0.02*
					Octanal	0.7	*0.02*
					Methylundecanal?	0.4	*0.01*
					Nonanol isomer	0.2	*0.005*
					Methylanisole	0.1	*0.003*
Nonanoic acid CAS 112-05-0	Acid	M=158 mp 31-32 bp 268-269	10	96	Nonanoic acid	85.5	4
					Octanal	5.4	0.3
					Ethyl nonanoate	2.8	0.1
					Heptanal	1.9	0.1
					Pentylbutanolide	1.0	0.05
					Octanone	0.9	0.05
					Octene	0.6	*0.03*
					Methylheptanoic acid	0.6	*0.03*
					Hexanal	0.6	*0.03*
					Nonalactone	0.4	*0.02*
					Butylpentanolide	0.3	*0.02*

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
2-Nonanone CAS 821-55-6	Ketone	M=142 bp 195	2.5	99	2-Nonanone Undecanone Decanone	99.8 0.1 0.1	1 *0.001* *0.001*
δ-Octalactone CAS 698-76-0	δ-Lactone	M=142 bp 115-117 at 12 mm Hg	15	98	δ-Octalactone Tetradecalactone? Octalactone isomer	98.7 1.0 0.3	7 0.08 *0.02*
γ-Octalactone CAS 104-50-7	γ-Lactone	M=142 bp 234	7.5	97	γ-Octalactone Dimethylhydroxyheptanoic acid lactone?	99.6 0.4	4 *0.02*
n-Octanal CAS 124-13-0	Aldehyde	M=128 bp 171	0.5	92	*n*-Octanal Octanol Heptanal Nonanone Ethyl octanoate 1 Unidentified compound	95.3 1.0 0.5 0.2 0.1 2.9	0.2 *0.003* *0.001* *0.0005* *0.0003* *0.007*
Octanoic acid CAS 124-07-2	Acid	M=144 bp 237	15	98	Octanoic acid Heptanal Dihydropentylfuranone Undecanone Methyl laurate Octalactone	98.8 0.4 0.2 0.2 0.2 0.2	7 *0.03* *0.02* *0.02* *0.02* *0.02*
1-Octanol CAS 111-87-5	Alcohol	M=130 bp 195	0.06	98	1-Octanol Octanal Decanol?	95.7 4.1 0.2	*0.03* *0.001* *0.00006*
1-Octen-3-ol CAS 3391-86-4	Unsaturated alcohol	M=128 bp 175	5	98	1-Octen-3-ol	100	2.5

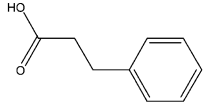
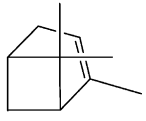
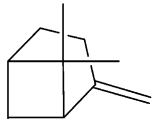
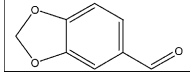
Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (^{o}C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
Palmitic acid CAS 57-10-3	Acid	M=256 mp 55 bp ›200	7.5	98	Palmitic acid	84.8	3
					Palmitic aldehyde	3.7	0.1
					Ethyl palmitate	2.6	0.1
					Tetradecanal	1.0	0.04
					Pentadecene	1.0	0.04
					Tetradecanal	1.0	0.04
					Pentadecene	1.0	0.04
					Palmitolactone	0.9	*0.03*
					Dodecanal	0.6	*0.02*
					10 Minor products, 1 unidentified	3.4	0.1
ω-Pentadecalactone CAS 106-02-05	ω-Lactone	M=240 mp 32 bp 137 at 2 mm Hg	5	98	ω-Pentadecalactone	99.6	2.5
					2 Unidentified compounds	0.4	*0.01*
2,3-Pentanedione CAS 600-14-6	Dione	M=100 bp 108	15	96	2,3-Pentanedione	89.5	7
					Dimethylbutanone?	3.6	0.3
					Acetic acid	1.4	0.1
					Dimethylpentanone?	1.4	0.1
					Butanedione	1.3	0.1
					Ethanol	0.9	0.07
					Acetic anhydride	0.4	*0.03*
					Methanol	0.2	*0.02*
					Propionic anhydride	0.2	*0.02*
					5 Minor products, 1 unidentified	1.1	0.08

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (^{o}C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
2-Pentanone CAS 107-87-9	Ketone	M=86 bp 102	0.5	98	2-Pentanone	100	0.3
Pent-3-en-4-olide (α-Angelica lactone, or 5-Methyl-2(3*H*)-furanone) CAS 591-12-8	Unsaturated γ-Lactone	M=98 mp 18 bp 167	1	98	Pent-3-en-4-olide δ-Angelica lactone Protoanemonine Ethyl levulinate Acetic acid? 2 Unidentified compounds	86.6 5.1 3.9 1.7 0.7 2.0	0.4 *0.03* *0.02* *0.009* *0.004* *0.01*
α-Phellandrene CAS 99-83-2	Hydrocarbon	M=136 bp 175-176	5	95	α-Phellandrene Cymene β-Myrcene α-Terpinene α-Thujene δ-4-Carene + Isopropenylmethylbenzene Sabinol? γ-Terpinene β-Ocimene 7 Minor products, 4 unidentified	64.2 18.9 3.7 3.4 3.4 0.6 0.5 0.3 0.2 4.8	2 0.5 0.09 0.09 0.09 *0.02* *0.01* *0.008* *0.005* 0.1
Phenethyl acetate CAS 103-45-7	Ester	M=164 bp 232	15	99	Phenethyl acetate Phenethyl alcohol Styrene	98.7 1.1 0.2	7 0.08 *0.02*
Phenethyl alcohol CAS 60-12-8	Alcohol	M=122 bp 219	40	99	Phenethyl alcohol Benzaldehyde Hyacinthin Styrene	96.8 1.7 0.9 0.6	19 0.3 0.2 0.1

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
Phenethyl butyrate CAS 103-52-6	Ester	M=192 bp 238	5.5	98	Phenethyl butyrate	94.1	3
					Phenethyl alcohol	3.4	0.09
					Benzaldehyde	1.0	*0.03*
					Styrene	0.7	*0.02*
					Acetophenone ?	0.4	*0.01*
					Hyacinthin	0.2	*0.006*
					Phenethyl isobutyrate	0.2	*0.006*
Phenethyl cinnamate CAS 103-53-7	Ester	M=252 mp 54	5	96	Phenethyl cinnamate	97.5	2
					Phenylethyl alcohol	0.9	*0.02*
					Benzaldehyde	0.6	*0.02*
					Styrene	0.3	*0.008*
					3 Long chain hydrocarbons	0.7	*0.02*
Phenethyl isobutyrate CAS 103-48-0	Ester	M=192 bp 263	30	98	Phenethyl isobutyrate	97.7	15
					Styrene	1.1	0.2
					Benzaldehyde	0.4	0.06
					Ethyl isobutyrate?	0.2	*0.03*
					Phenethyl alcohol	0.2	*0.03*
					Phenethyl acetate	0.2	*0.03*
					Phenethyl butyrate	0.2	*0.03*
Phenethyl isovalerate CAS 140-26-1	Ester	M=206 bp 264	5	98	Phenethyl isovalerate	99.5	2.5
					Phenethyl alcohol	0.4	*0.01*
					Ethenylmethylbenzene?	0.1	*0.003*

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
Phenethyl phenylacetate CAS 102-20-5	Ester	M=240 bp 325	5	98	Phenethyl phenylacetate	82.1	2
					Phenethyl alcohol	2.9	0.07
					Benzaldehyde	4.8	0.1
					Acetophenone ?	3.5	0.09
					Ethyl phenylacetate	2.0	0.05
					Styrene	1.2	*0.03*
					Phenol	0.5	*0.01*
					Hyacinthin	0.5	*0.01*
					Dibenzyl + Benzofuran	0.2	*0.0005*
					Toluene	0.1	*0.0003*
					8 Minor products	2.2	0.06
Phenylacetaldehyde CAS 122-78-1	Aldehyde	M=120 bp 195	25	90	Phenylacetaldehyde	84.0	11
					Benzaldehyde	11.8	1
					Phenylpropanedione	1.5	0.2
					Methylphenyl cinnoline	0.9	0.1
					Phenylacetaldehyde isomer?	0.7	0.09
					Ethyl benzoate +?	0.3	0.04
					Benzyl formate	0.3	0.04
					Isobutyrophenone	0.3	0.04
					Octyl acetate?	0.2	*0.03*
Phenylacetic acid CAS 103-82-2	Acid	M=136 bp 265	65	99	Phenylacetic acid	51.6	11
					Benzaldehyde	38.5	13
					Ethylphenyl acetate	6.0	2
					Diphenylethanone	1.1	0.4
					Benzoic acid	1.0	0.3
					Dibenzyl	0.3	0.1
					Phenylstyrene or stilbene	0.2	0.07
					Benzyl phenylacetate	0.2	0.07
					Benzophenone	0.2	0.07
					Toluene	0.2	0.07
					Acetophenone	0.1	*0.03*
					4 Minor products, 1 unidentified	0.8	0.3

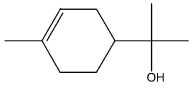
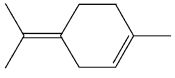
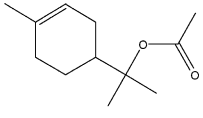
Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
2-Phenyl-2-butenal CAS 4411-89-6	Aldehyde	M=146 bp 177 at 15 mm Hg	2.5	97	2-Phenyl-2-butenal	96.8	1
					Phenylpropanone	1.4	*0.02*
					Biphenyl	0.7	*0.009*
					Phenylfuran	0.6	*0.008*
					1 Unidentified compound	0.5	*0.006*
4-Phenyl-3-buten-2-one CAS 122-57-6	Unsaturated ketone	M=146 bp 131 at 12 mm Hg	10	99	4-Phenyl-3-buten-2-one	97.7	5
					Benzaldehyde	1.3	0.07
					4-Phenyl-3-buten-2-one isomer?	0.5	0.03
					Styrene	0.3	*0.02*
					1 Unidentified compound	0.2	*0.01*
3-Phenyl-1-propanol CAS 122-97-4	Alcohol	M=136 bp 236	1	98	3-Phenyl-1-propanol	90.4	0.5
					Phenylpropanal	3.8	*0.02*
					Benzaldehyde	2.5	*0.01*
					Styrene	1.5	*0.008*
					Phenylpropenal	1.0	*0.005*
					Phenyl vinyl ketone	0.4	*0.002*
					Acetophenone	0.2	*0.001*
					Methylstyrene or phenylpropene	0.1	*0.0005*
					1 Unidentified compound	0.1	*0.0005*
3-Phenylpropionaldehyde CAS 104-53-0	Aldehyde	M=134 bp 104-105 at 13 mm Hg	5	90	3-Phenylpropionaldehyde	93.1	2
					Phenylpropanol	1.1	*0.03*
					Benzaldehyde	0.8	*0.02*
					Cinnamaldehyde	0.7	*0.02*
					Phenylpentanal?	0.5	*0.01*
					Acetophenone + Toluene?	0.4	*0.01*
					Methylnaphthol	0.3	*0.008*
					Hyacinthin	0.3	*0.008*
					Ethyl phenylpropionate	0.3	*0.008*
					1 Unidentified compound	2.5	*0.006*

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
3-Phenylpropionic acid CAS 501-52-0 	Acid	M=150 bp 280	5	99	3-Phenylpropionic acid	85.0	2
					Benzaldehyde	4.4	0.1
					Acetophenone	3.1	0.08
					Styrene	1.5	0.04
					Hyacinthin	1.4	0.04
					Dihydrocoumarin + Biphenyl?	1.1	*0.03*
					Diethylbiphenylene?	0.4	*0.01*
					Phenol	0.3	*0.008*
					Stilbene	0.2	*0.005*
					19 Minor products, 1 unidentified	2.6	0.07
α-Pinene CAS 80-56-8 	Hydrocarbon	M=136 bp 155	3.5	98	α-Pinene	94.8	2
					Pinene oxide	1.8	*0.03*
					Verbenene?	1.2	*0.02*
					Verbenene isomer	1.2	*0.02*
					Pinenone	0.4	*0.007*
					β-Pinene	0.3	*0.005*
					Myrtenal +?	0.3	0.005
β-Pinene CAS 127-91-3 	Hydrocarbon	M=136 bp 165	0.5	97	β-Pinene	91.8	0.2
					Nopinone?	2.6	*0.007*
					Myrtenol	1.5	*0.004*
					Myrtenal	1.1	*0.003*
					β-Myrcene	0.8	*0.002*
					Pinocarvone	0.6	*0.002*
					α-Pinene	0.4	*0.001*
					Menthatriene?	0.3	*0.0008*
					Camphene	0.2	*0.0005*
					5 Minor products, 2 unidentified	0.7	*0.002*
Piperonal CAS 120-57-0 	Aldehyde	M=150 bp 264	200	99	Piperonal	99.8	100
					Methoxybenzoic acid?	0.2	0.2

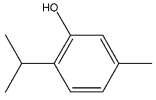
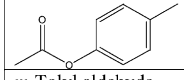
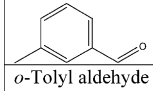
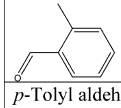
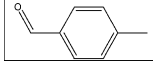
Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (^{o}C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
5-Propenylguaethol CAS 94-86-0	Phenol Ether	M=178 mp 85-88	5	99	5-Propenylguaethol	95.4	2
					Methylnaphthol	0.6	*0.02*
					Propylbenzene	0.2	*0.005*
					Ethylvanillin	0.2	*0.005*
					Ethylmethylphenol or propylphenol	0.2	*0.005*
					Methoxymethylindanone	0.1	*0.003*
					Hexahydroindacene	0.1	*0.003*
					Dimethylindanone	0.1	*0.003*
					4 Unidentified compounds	3.1	*0.08*
Propionic acid CAS 79-09-4 $CH_3CH_2CO_2H$	Acid	M=74 bp 141	5	99.5	Propionic acid	97.6	1
					Ethanol	1.2	*0.03*
					Propionic anhydride?	1.1	*0.03*
					Acetic acid	0.1	*0.003*
n-Propyl acetate CAS 109-60-4	Ester	M=102 bp 102	2.5	98	*n*-Propyl acetate	100	1
1,2-Propylene glycol CAS 57-55-6	Diol	M=76 bp 187	50,000	99.5	1,2-Propylene glycol	86.3	22,000
					1,3-Propylene glycol?	6.2	1,600
					Acetol or acetic anhydride	4.7	1,200
					Pyruvaldehyde	2.8	700
3-Propylidenephthalide CAS 17369-59-4	γ-Lactone	M=174 mp 5 bp 170 at 12 mm Hg	2.5	96	3-Propylidenephthalide	93.6	1
					Phthalic anhydride	1.8	*0.02*
					Ethyl phthalate	0.9	*0.01*
					Phenol	0.4	*0.005*
					Methylnaphthoquinone + Dibenzofuran	0.2	*0.003*
					Naphthoquinone	0.2	*0.003*
					Propyl phenyl ketone	0.2	*0.003*
					Ethyl benzoate	0.2	*0.003*
					Indenedione	0.1	*0.001*
					12 Minor products, 9 unidentified	2.4	*0.03*

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (^{o}C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
Pyruvic acid CAS 127-17-3	Acid Ketone	M=88 mp 12-14 bp 165 with decomposition	25	97	Pyruvic acid Cyclobutanone Methylsuccinic anhydride Methylmaleic anhydride Methylmaltol Protoanemonine Methylbutadiene? Cyclobutanone Hexanedione 19 Minor products, 15 unidentified	61.9 10.4 7.5 1.7 1.1 1.0 0.4 0.3 0.3 15.4	8 1 0.9 0.2 0.1 0.1 0.05 0.04 0.04 2
Rhodinol CAS 141-25-3; 6812-78-8	Unsaturated alcohol	M=156 bp 68-70 at 1.8 mm Hg	2.5	82	Rhodinal δ-3-Carene Guaiadiene E-Citral Caryophyllene Ledene + ? β-Bourbonene Hexahydrodimethylmethylethylnaphthalene +Tetrahydrodimethylmethylethylnaphthalene Aromadendrene	49.2 32.2 7.8 3.1 2.4 1.6 1.6 1.3 0.8	0.6 0.4 0.1 0.04 *0.03* *0.02* *0.02* *0.02* *0.01*
Rose Oxide CAS 16409-43-1	Tetrahydro-pyran	M=154 bp 70 at 12mm Hg	1	98 Mixture of *cis* & *trans* isomers	Rose oxide 2 Unidentified compounds	99.8 0.2	0.5 *0.001*
Salicylaldehyde CAS 90-02-8	Phenol Aldehyde	M=122 bp 197	20	98	Salicylaldehyde Phenol 1 Unidentified compound	97.4 0.5 2.1	10 0.05 0.2

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (°C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
Sorbic acid CAS 110-44-1	Unsaturated acid	M=112 mp 133 bp 228	0.1	99	Sorbic acid	99.4	0.05
					Phenol	0.5	*0.0003*
					Decanal	0.1	*0.00005*
Sorbitol CAS 50-70-4	Polyalcohol	M=182 mp 98-100	35,245	98	Furfural	31.4	5,500
					Propylfuran	9.7	1,700
					Acetylfuran	7.7	1,400
					Furanone	6.4	1,100
					Methoxycyclopentenone + ?	5.2	920
					Pyruvaldehyde	3.7	650
					Dimethylformylfuran	3.4	600
					Methylfuran	3.1	550
					Methylfurfural	2.6	460
					Methylfuranone	2.4	420
					Cyclopentenediol	2.4	420
					Benzenediol	2.3	410
					Furfuryl alcohol	2.3	410
					Butanediol	2.0	350
					Acetol	1.9	340
					Cyclopentenedione	1.6	280
					6 Minor products	4.3	760
					1 Unidentified product	7.6	1,340
l-Tartaric acid CAS 87-69-4	Dicarboxylic acid Alcohol	M=150 mp 170	7,475	99.5	Acetol	60.2	22.50
					Methylfurandione	18.0	670
					Pyruvaldehyde	8.1	300
					Furanone	6.2	230
					Cyclobutanone	5.0	190
					Furandione	1.8	67
					Methyl acetate	0.7	26

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (^{o}C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
α-Terpineol CAS 10482-56-1; 98-55-5 	Alcohol	M=154 bp 217	40	96	α-Terpineol	81.7	16
					Isoterpinolene	12.4	2
					Terpineol (unspecified isomer)	1.6	0.3
					Isolimonene	0.8	0.2
					Borneol	0.8	0.2
					β-Terpineol	0.7	0.1
					Limonene	0.4	0.08
					Allyltoluene or Cymenyl?	0.3	0.06
					Toluene	0.1	*0.02*
					6 Minor products, 3 unidentified	1.2	0.3
Terpinolene CAS 586-62-9 	Unsaturated cyclic hydrocarbon	M=136 bp 183-185	0.5	90	Terpinolene	70.8	0.2
					Limonene	6.9	*0.02*
					Isocineole + α-Terpinene	5.9	*0.01*
					Cineole	4.0	*0.01*
					Cymene	3.7	*0.009*
					α-Pinene	2.2	*0.006*
					γ-Terpinene	1.6	*0.004*
					Allytoluene + Methylacetophenone	1.2	*0.003*
					14 Minor products, 1 unidentified	3.7	*0.009*
Terpinyl acetate CAS 80-26-2  (α isomer)	Ester	M=196 bp 270	5.5	95 (Mixture of isomers)	Terpinyl acetate	69.6	2
					Limonene	10.7	0.3
					Menthadiene	3.9	0.1
					α-Terpinolene	3.9	0.1
					α-Terpinene	3.6	0.1
					Borneol acetate	0.9	*0.02*
					β-Terpinene	0.6	*0.02*
					γ-Terpinene	0.6	*0.02*
					14 Minor products, 6 unidentified	6.2	0.2

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp** **(°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
δ-Tetradecalactone CAS 2721-22-4	δ-Lactone	M=226 bp 290	0.05	98	δ-Tetradecalactone γ-Undecalactone Nonanal Decanal Octalactone? δ-Undecalactone	98.8 0.4 0.2 0.2 0.2 0.2	*0.02* *0.0001* *0.00005* *0.00005* *0.00005* *0.00005*
5,6,7,8-Tetrahydroquinoxaline CAS 34413-35-9	Pyrazine	M=134 bp 108-111	10	97	5,6,7,8-Tetrahydroquinoxaline	100	5
Tetramethylpyrazine CAS 1124-11-4	Pyrazine	M=136 mp 85-90 bp 190	20	98	Tetramethylpyrazine	100	10

Ingredient CAS Number Formula or structure	**Chemical Class**	**Mol. wt. (M) bp or mp (°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
Thymol CAS 89-83-8 	Phenol	M = 150 bp 232	0.5	99	Thymol Thymol acetate Methylcinnamic acid? Cinnamaldehyde or methylbenzofuran Butylphenol Acetic acid Thymoquinone Cumenol 1 Unidentified compound	95.6 1.6 1.5 0.3 0.2 0.2 0.1 0.1 0.4	*0.2* *0.004* *0.004* *0.0008* *0.0005* *0.0005* *0.0003* *0.0003* *0.001*
p-Tolyl acetate CAS 140-39-6 	Ester	M=150 bp 208-212	5	98	*p*-Tolyl acetate Cresol Methylbenzyl acetate	96.3 3.5 0.2	2 0.09 *0.005*
m-Tolyl aldehyde (3-Methylbenzaldehyde) CAS 620-23-5 	Aldehyde	M=120 bp 201	1	97	*m*-Tolyl aldehyde Dichloroxylene (impurity) Chloroxylene (impurity)	99.4 0.4 0.2	0.5 *0.002* *0.001*
o-Tolyl aldehyde (2-Methylbenzaldehyde) CAS 529-20-4 	Aldehyde	M=120 bp 197	1	97	*o*-Tolyl aldehyde Chloroxylene (impurity) Methylbenzyl alcohol Isobenzofuranone Chloromethylethylbenzene (impurity)	97.9 1.5 0.3 0.2 0.1	0.5 *0.008* *0.002* *0.001* *0.0005*
p-Tolyl aldehyde (4-Methylbenzaldehyde) CAS 104-87-0 	Aldehyde	M=120 bp 204-205	5	97	*p*-Tolyl aldehyde Cresol Methyl toluate 1 Unidentified compound	90.0 0.4 0.3 9.3	2 *0.01* *0.008* *0.2*

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (^{o}C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
p-Tolyl isobutyrate CAS 103-93-5	Ester	M=178 bp 237	1	97	*p*-Tolyl isobutyrate Cresol Tolyl acetate Ethyl isobutyrate Isopropenylmethylbenzene 1 Unidentified compound	98.6 0.5 0.3 0.3 0.1 0.2	0.5 *0.003* *0.002* *0.002* *0.0005* *0.001*
Triacetin CAS 102-76-1	Tri ester	M=218 mp 32 bp 131 at 7 mm Hg	1,190	99	Triacetin Diacetin 1 Unidentified compound	98.5 1.4 0.1	590 8 0.6
Triethyl citrate CAS 77-93-0	Tri ester	M=276 bp 127 at 1 mm Hg	700	99	Triethyl citrate Diethyl malonate Ester? Triethyl acetylcitrate 1 Unidentified compound	96.2 1.3 0.4 0.2 1.9	340 5 1 0.7 7
3,5,5-Trimethyl-1-hexanol CAS 3452-97-9	Alcohol	M=144 bp 142	30	85	3,5,5-Trimethyl-1-hexanol 3,5,5-Trimethylhexanal Trimethylhexanol (unspecified isomer) Methylcyclohexane Diisoamylene or trimethylhexene Dimethyl pentanone Nonenol?	91.4 4.6 3.0 0.4 0.3 0.2 0.1	14 0.7 0.5 0.06 0.05 *0.03* *0.02*

Ingredient CAS Number Formula or structure	Chemical Class	Mol. wt. (M) bp or mp (^{o}C)	Max. cig. appln. level (ppm)	Purity of sample pyrolysed (%)	Composition of pyrolysate (Compound, %)		Max. level in smoke (µg)
Trimethylpyrazine CAS 14667-55-1	Pyrazine	M=122 bp 171	10	99	Trimethylpyrazine Ethyldimethylpyrazine Tetramethylpyrazine	99.8 0.1 0.1	5 *0.005* *0.005*
δ-Undecalactone CAS 710-04-3	δ-Lactone	M=184 bp 152-155 at 11 mm Hg	7.5	97	δ-Undecalactone δ-Tetradecalactone Hexanal γ-Decalactone Heptanal Butylbenzene Nonenal 2 Unidentified compounds	96.8 1.0 0.7 0.5 0.4 0.2 0.2 0.2	4 0.04 *0.03* *0.02* *0.02* *0.008* *0.008* *0.008*
γ-Undecalactone CAS 104-67-6	γ-Lactone	M=184 bp 297	20	98	γ-Undecalactone Dimethylhydroxyheptanoic acid? Butyl octanoate Heptanal	99.2 0.4 0.3 0.1	10 0.04 *0.03* *0.01*
2-Undecanone CAS 112-12-9	Ketone	M=170 bp 231-232	0.5	98	2-Undecanone Nonanal Octanal Methylundecene 1 Unidentified compound	99.3 0.3 0.2 0.1 0.1	0.2 *0.0008* *0.0005* *0.0003* *0.0003*
Urea CAS 57-13-6 NH_2CONH_2	Amide	M=60 mp 133	4,000	Aqueous solution	Isocyanic acid +? Urea	93.4 6.6	1,900 130
Valeraldehyde CAS 110-62-3	Aldehyde	M=86 bp 103	0.05	97	Valeraldehyde 2 Unidentified compounds	93.5 6.5	*0.02* *0.002*
Valeric acid CAS 109-52-4	Acid	M=102 bp 186	5.5	99	Valeric acid Butenyl hexyl ether? Butyric acid? Butyl valerate Amyl isovalerate 1 Unidentified compound	95.4 2.4 1.0 0.4 0.4 0.4	3 0.07 *0.03* *0.01* *0.01* *0.01*

Ingredient **CAS Number** **Formula or structure**	**Chemical Class**	**Mol. wt. (M)** **bp or mp** **(°C)**	**Max. cig. appln. level (ppm)**	**Purity of sample pyrolysed (%)**	**Composition of pyrolysate (Compound, %)**		**Max. level in smoke (µg)**
γ-Valerolactone CAS 108-29-2	γ-Lactone	M=100 bp 207	25	98	γ-Valerolactone Dimethylbutyrolactone	99.0 1.0	12 0.1
Vanillin CAS 121-33-5	Aldehyde Phenol Ether	M=152 mp 81-83 bp 170 at 15 mm Hg	2,180	97	Vanillin 2-methoxyphenol? Piperonal Vanillin methyl ether	99.6 0.2 0.1 0.1	1,100 2 1 1
Veratraldehyde CAS 120-14-9	Aldehyde Ether	M=166 mp 43-45 bp 281	40	98	Veratraldehyde Veratrole Methyl veratrate Dimethyl oxalate	99.6 0.2 0.1 0.1	20 0.04 *0.02* *0.02*

References

[1] J.C. Leffingwell, in: D.L. Davis, M.T. Nielsen (Eds.), Tobacco Production, Chemistry and Technology, Blackwell Science, Oxford, UK, 1999, pp. 265–284 Chapter 8A.
[2] R.R. Baker, in: D.L. Davis, M.T. Nielsen (Eds.), Tobacco Production, Chemistry and Technology, Blackwell Science, Oxford, UK, 1999, pp. 398–439 Chapter 12.
[3] C.R. Green, A. Rodgman, Rec. Adv. Tob. Sci. 22 (1996) 131–304.
[4] M. Muramatsu, Sci. Papers Cent. Res. Inst., Japan Tobacco & Salt Monop. Corp. 123 (1981) 9–77.
[5] R.W. Jenkins Jr., R.A. Comes, R.T. Bass, Rec. Adv. Tob. Sci. 1 (1975) 1–30.
[6] R.W. Jenkins Jr., Beitr. Tabakforsch. Int. 14 (1990) 353–378.
[7] O.T. Chortyk, W.S. Schlotzhauer, Beitr. Tabakforsch. 7 (1973) 165–178.
[8] C.R. Green, in: Proc. 173rd Am. Chem. Soc. Symp., Recent advances in the chemical composition of tobacco and tobacco smoke, 1977, pp. 426–470.
[9] S.S. Hecht, I. Schmeltz, D. Hoffmann, Rec. Adv. Tob. Sci. 3 (1977) 59–93.
[10] K.D. Brunnemann, D. Hoffmann, Rec. Adv. Tob. Sci. 8 (1982) 103–140.
[11] R.R. Baker, J. Anal. Appl. Pyrolysis 11 (1987) 555–573.
[12] W.S. Schlotzhauer, O.T. Chortyk, J. Anal. Appl. Pyrolysis 12 (1987) 193–222.
[13] R.A. Fenner, Rec. Adv. Tob. Sci. 14 (1988) 82–113.
[14] R.W. Jenkins Jr., D.D. McRae, Rec. Adv. Tob. Sci. 22 (1996) 337–392.
[15] I. Schmeltz, A. Wenger, D. Hoffmann, T.C. Tso, J. Agric. Food Chem. 27 (1979) 602–608.
[16] E.L. Wynder, D. Hoffmann, Adv. Cancer Res. 8 (1964) 249–453.
[17] E.L. Wynder, D. Hoffmann, Tobacco and Tobacco Smoke: Studies in Experimental Carcinogenesis, Academic Press, New York, USA, 1967.
[18] I. Schmeltz, W.S. Schlotzhauer, Nature 219 (1968) 370–371.
[19] M.P. Newell, P.H. Latimer, L.R. Haefele, 22nd Tobacco Chemists' Research Conference, Programme Booklet and Abstracts, vol. 22, Paper 24, 1968, p. 18.
[20] R.W. Jenkins Jr., R.H. Newman, M.K. Chavis, Beitr. Tabakforsch. 5 (1970) 299–301.
[21] G.M. Badger, J.K. Donnelly, T.M. Spotswood, Aust. J. Chem. 18 (1965) 1249–1266.
[22] W.S. Schlotzhauer, I. Schmeltz, Beitr. Tabakforsch. 5 (1969) 5–8.
[23] B.R. Davis, T.H. Houseman, H.R. Roderick, Beitr. Tabakforsch. 7 (1973) 148–153.
[24] R.W. Jenkins Jr., R.H. Newman, R.D. Carpenter, T.S. Osdene, Beitr. Tabakforsch. 5 (1970) 295–298.
[25] R.W. Jenkins Jr., M.K. Chavis, R.H. Newman, F.A. Morrell, Int. J. Appl. Radiat. Isot. 22 (1971) 691–697.
[26] R.W. Jenkins Jr., R.T. Bass, R.H. Newman, M.K. Chavis, Beitr. Tabakforsch. Int. 9 (1977) 126–130.
[27] R.R. Baker, Thermochim. Acta 28 (1979) 45–57.
[28] R.R. Baker, High Temp. Sci. 7 (1975) 236–247.
[29] C.O. Tiller, E.M. Gentry, Beitr. Tabakforsch. Int. 9 (1977) 7–12.
[30] M. Muramatsu, S. Umemura, K. Ohta, T. Okado, Netsusokutei 6 (1979) 137–145.
[31] R.R. Baker, J. Anal. Appl. Pyrolysis 4 (1983) 297–334.
[32] C.F. Cullis, D. Goring, M.M. Hirschler, in: J.F. Kennedy (Ed.), Cellulose and its Derivatives, Ellis Horwood/Wiley, Chichester, UK, 1985, pp. 401–410.
[33] W.T. Smith Jr., S.P. Chen, J.M. Patterson, Tob. Sci. 19 (1975) 53–55.
[34] J.M. Patterson, N.F. Haidar, S.P. Chen, W.T. Smith Jr., Tob. Sci. 20 (1976) 108–110.
[35] E.B. Higman, R.F. Severson, R.F. Arrendale, O.T. Chortyk, J. Agric. Food Chem. 25 (1977) 1201–1207.
[36] R.R. Baker, Prog. Energy Combust. Sci. 7 (1981) 135–153.
[37] S.J. Stotesbury, H. Digard, L.J. Willoughby, A. Couch, Beitr. Tabakforsch. Int. 18 (1999) 147–163.
[38] S.J. Stotesbury, L.J. Willoughby, A. Couch, Beitr. Tabakforsch. Int. 19 (2000) 55–64.
[39] R.R. Baker, Beitr. Tabakforsch. Int. 11 (1981) 1–17.
[40] J.D. Green, J. Chalmers, P.J. Kinnard, Beitr. Tabakforsch. Int. 14 (1989) 283–288.
[41] S.A. Eble, in: Proceedings of the International Conference on the Physical and Chemical Processes Occurring in a Burning Cigarette, R.J. Reynolds Tobacco Co., Winston-Salem, NC, USA, 1987, pp. 261–279.
[42] P.S. Larson, E.S. Harlow, Radioiso. Sci. Res. 3 (1958) 62–78.
[43] F.W. Best, in: Proceedings of the International Conference on the Physical and Chemical Processes Occurring in a Burning Cigarette, R.J. Reynolds Tobacco Co., Winston-Salem, NC, USA, 1987, pp. 244–260.

[44] T.H. Houseman, Beitr. Tabakforsch. 7 (1973) 142–147.
[45] N.A. Stevens, M.F. Borgerding, Anal. Chem. 71 (1999) 2179–2185.
[46] R.R. Baker, D.A. Yorke, J. Chem. Educ. 49 (1972) 351–355.
[47] R.W. Walker, C. Morley, in: R.G. Compton, G. Hancock (Eds.), Chemical Kinetics, vol. 35, Low-temperature Combustion and Autoignition (Ed. By M.J. Pilling), Elsevier, 1997, Chapter 1, pp. 1–124.
[48] J.G. Curran Jr., E.G. Miller Jr., Beitr. Tabakforsch. 5 (1969) 64–70.
[49] G.A. Burdock (Ed.), Fenaroli's Handbook of Flavor Ingredients, CRC Press, 2002.
[50] J. Buckingham, S.M. Donaghy (Eds.), Dictionary of Organic Compounds, Chapman and Hall, New York and London, 5th ed., 1982.
[51] P.D. Case, in: Papers Presented at the Meeting of the Smoke and Technology Groups, CORESTA Congress, Brighton, UK, 1998, pp. 145–154.
[52] I.C. Munro, E. Kennepohl, R. Kroes, Food Chem. Toxicol. 37 (1999) 207–232.
[53] M. Koebel, M. Elsener, J. Chromatogr., A 689 (1995) 164–169.
[54] P.M. Schaber, J. Colson, S. Higgins, E. Dietz, D. Thielen, B. Anspach, J. Brauer, Am. Lab. (1999) 13–21. August.
[55] F. Wöhler, Ann. Phys. Chem. 15 (1829) 619–630.
[56] Y.S. Stein, M.J. Antal Jr., J. Anal. Appl. Pyrolysis 4 (1983) 283–296.
[57] Philip Morris USA, Evalution of glycerol for use as a cigarette ingredient, Submission to the UK Department of Health, October 2001.
[58] R.R. Baker, G. Smith, Paper presented at CORESTA Congress, New Orleans, USA, September, 2002, Programme and Abstracts Booklet, no. ST25.
[59] R.R. Baker, J.R. Pereira da Silva, G. Smith, Papers submitted to Food Chem. Toxicol.
[60] E.D. Massey, K.K. Kalirai, G. Smith, Paper presented at: (a) UK Environmental Mutagen Society Meeting, Plymouth, UK, July 2002, Mutagenesis, 17 (2003) 566–567; (b) CORESTA Congress, New Orleans, USA, September 2002, Programme and Abstracts Booklet, no. ST6.
[61] E.L. Carmines, Food Chem. Toxicol. 40 (2002) 77–91.
[62] K. Rustemeier, R. Stabbert, H.-J. Haussmann, E. Roemer, E.L. Carmines, Food Chem. Toxicol. 40 (2002) 93–104.
[63] E. Roemer, F.J. Tewes, T.J. Mesigen, D.J. Veltel, E.L. Carmines, Food Chem. Toxicol. 40 (2002) 105–111.
[64] P.M. Vanscheeuwijck, A. Teredesai, P.M. Terpstra, J. Verbeeck, P. Kuhl, B. Gerstenberg, S. Gebel, E.L. Carmines, Food Chem. Toxicol. 40 (2002) 113–131.
[65] C.L. Gaworski, M.M. Dozier, J.M. Gerhart, N. Rajendran, L.H. Brennecke, C. Aranyi, J.D. Heck, Food Chem. Toxicol. 35 (1997) 683–692.
[66] C.L. Gaworski, M.M. Dozier, J.D. Heck, J.M. Gerhart, N. Rajendran, R.M. David, L.H. Brennecke, R. Morrisey, Inhal. Toxicol. 10 (1998) 357–381.
[67] C.L. Gaworski, J.D. Heck, M.B. Bennett, M.L. Wenk, Toxicology 139 (1999) 1–17.
[68] Massachusetts Tobacco Control Program: 665.200 Testing for mainstream smoke constituents, 2001. Internet website: http://www.state.ma.us/dph/mtcp/report/smoktox.htm.
[69] Department of Health (Canada): Proposed tobacco reporting regulations, schedule 2, Official methods for the collection of emission data on mainstream smoke; Canada Gazette Part II, 133 (15) 19 July, 2000, pp. 1781–1784.
[70] D. Hoffmann, I. Hoffmann, Beitr. Tabakforsch. Int. 18 (1998) 49–52.
[71] D. Hoffmann, I. Hoffmann, in: D.M. Burns, N.L. Benowitz (Eds.), Risks Associated with Cigarettes with Low Machine—Measured Yields of Tar and Nicotine, NCI (National Cancer Institute) Smoking and Control, Monograph 13, US Department of Health and Human Services, Bethesda, MD, 2001, Chapter 5, pp. 159–191.
[72] D. Hoffmann, I. Hoffmann, K. El-Bayoumy, Chem. Res. Toxicol. 14 (2001) 767–790.
[73] W.S. Rickert, W. Wright, Paper presented at the CORESTA Congress, New Orleans, USA, 2002, Programme and Abstracts Booklet, no. ST26.
[74] A. Rodgman, C.J. Smith, T.A. Perfetti, Hum. Exp. Toxicol. 19 (2000) 773–795.
[75] A. Rodgman, Beitr. Tabakforsch. Int. 20 (2002) 83–103.

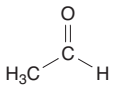
Acetaldehyde

CAS No. 75-07-0

Reasonably anticipated to be a human carcinogen

First listed in the *Sixth Annual Report on Carcinogens* (1991)

Also known as ethanal



Carcinogenicity

Acetaldehyde is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity from studies in experimental animals.

Cancer Studies in Experimental Animals

Exposure to acetaldehyde by inhalation caused tumors in two rodent species and at two different tissue sites. In rats of both sexes, it caused cancer of the nasal mucosa (squamous-cell carcinoma and adenocarcinoma), and in hamsters of both sexes, it caused cancer of the larynx (carcinoma) (IARC 1985, 1987). Inhalation of acetaldehyde also promoted the induction of respiratory-tract tumors by intratracheal instillation of the known carcinogen benzo[*a*]pyrene in hamsters of both sexes.

Since acetaldehyde was listed in the *Sixth Annual Report on Carcinogens,* an additional study in rats has been identified. Administration of acetaldehyde in drinking water increased the incidences of hemolymphoreticular cancer (leukemia and lymphoma combined), benign tumors of the pancreas (islet-cell adenoma), and cancer of the bone (osteosarcoma) and nasal cavity (carcinoma) in males and benign mammary-gland tumors (fibroma or fibroadenoma) in females (Soffritti *et al.* 2002). Increased incidences of tumors observed at other sites occurred only at one of the lower doses tested.

Cancer Studies in Humans

The data available from epidemiological studies are inadequate to evaluate the relationship between human cancer and exposure specifically to acetaldehyde. A survey of workers producing acetaldehyde and other aldehydes in Germany reported 9 cases of cancer, including 5 of lung cancer and 2 of oral-cavity cancer, among an unspecified number of workers; these incidences reportedly were higher than expected, but the observations were confounded by the fact that all cases of cancer occurred in tobacco smokers (IARC 1985, 1987).

Since acetaldehyde was listed in the *Sixth Annual Report on Carcinogens,* additional epidemiological studies have been identified, primarily case-control studies of populations exposed to acetaldehyde (the main initial metabolite of alcohol) following consumption of alcoholic beverages. Alcoholic beverage consumption is listed in the Report on Carcinogens as *known to be a human carcinogen*. In its 1999 review, the International Agency for Research on Cancer noted that three small case-control studies found increased risks of alcohol-related cancer (of the oral cavity, pharynx, larynx, and esophagus) among individuals with genetic variations (polymorphisms) that result in increased levels of acetaldehyde after alcohol consumption. However, IARC concluded that the data available were inadequate to evaluate the carcinogenicity of acetaldehyde (IARC 1999). Since then, a number of review articles and meta-analyses have summarized the results of subsequent studies that found dose-response relationships between alcohol consumption and cancer of the oral cavity, pharynx, larynx, and esophagus, and possibly the stomach and colorectum, among individuals with genetic polymorphisms that increase blood or salivary levels of acetaldehyde (Bagnardi *et al.* 2001, Zeka *et al.* 2003, Boffetta and Hashibe 2006, Baan *et al.* 2007, Boccia *et al.* 2009, Salaspuro 2009). In 2009, IARC concluded that acetaldehyde associated with alcohol consumption was carcinogenic to humans (Secretan *et al.* 2009). Few studies have been conducted on the association of these polymorphisms with cancer at other tissue sites, and the role of acetaldehyde in pancreatic, liver, bladder, or breast cancer is not clear (van Dijk *et al.* 2001, Terry *et al.* 2006, Seitz and Becker 2007, Visvanathan *et al.* 2007, Druesne-Pecollo *et al.* 2009).

Studies on Mechanisms of Carcinogenesis

Alcohol is metabolized to acetaldehyde by alcohol dehydrogenases (ADH), and acetaldehyde is metabolized to acetic acid by aldehyde dehydrogenases (ALDH). In some individuals, genetic polymorphisms in these enzymes can result in either higher rates of acetaldehyde production from alcohol or lower rates of acetaldehyde metabolism to acetic acid, resulting in higher blood acetaldehyde levels after a given level of alcohol intake than in individuals without these polymorphisms. Five ADH genes have been identified in humans, two of which have been shown to be polymorphic. The variant allele of the *ALDH2* gene, which is prevalent in Asians, encodes an enzyme that has almost no ability to detoxify acetaldehyde (IARC 1999).

Properties

Acetaldehyde is an aliphatic aldehyde that exists at room temperature as a colorless gas with a fruity, pungent odor. It is miscible with water, ether, benzene, gasoline, solvent naphtha, toluene, xylene, turpentine, and acetone. It is very flammable and is unstable in air (Akron 2009, HSDB 2009). Physical and chemical properties of acetaldehyde are listed in the following table.

Property	Information
Molecular weight	44.0[a]
Specific gravity	0.79 at 16°C/4°C[a]
Melting point	–124°C[a]
Boiling point	21°C[a]
Log K_{ow}	–0.34[b]
Water solubility	1,000 g/L at 25°C[a]
Vapor pressure	902 mm Hg at 25°C[a]
Vapor density relative to air	1.5[a]
Dissociation constant (pK_a)	13.6 at 25°C[a]

Sources: [a]HSDB 2009, [b]ChemIDplus 2009.

Use

Acetaldehyde is used primarily as a chemical intermediate in the production of acetic acid, pyridine and pyridine bases, peracetic acid, pentaerythritol, butylene glycol, and chloral. It is also used in the synthesis of crotonaldehyde, flavor and fragrance acetals, acetaldehyde 1,1-dimethylhydrazone, acetaldehyde cyanohydrin, acetaldehyde oxime, various acetic acid esters, paraldehyde, metaldehyde (a molluscicide widely used to kill slugs and snails), polymers, and various halogenated derivatives (IARC 1985, 1999). Acetaldehyde has been used in the manufacture of aniline dyes, plastics, and synthetic rubber, to silver mirrors, and to harden gelatin fibers. It has also been used in the production of polyvinyl acetal resins, in fuel compositions, to inhibit mold growth on leather, and in the manufacture of disinfectants, pesticides, drugs, explosives, lacquers and varnishes, photographic chemicals, phenolic and urea resins, and rubber accelerators and antioxidants (EPA 1994).

Acetaldehyde is considered by the U.S. Food and Drug Administration to be generally recognized as safe for use as a flavoring agent and adjuvant (Furia and Bellanca 1975, HSDB 2009). It is an important component of food flavorings and is added to milk products, baked

goods, fruit juices, candy, desserts, and soft drinks; it is especially useful for imparting orange, apple, and butter flavors. The concentration of acetaldehyde in food generally is up to 0.047%. In 1976, about 8,600 kg (19,000 lb) of acetaldehyde was used as food additives. Acetaldehyde is also used in the manufacture of vinegar and as a fruit and fish preservative. It is approved for use in phenolic resins in molded containers for contact with non-acidic foods. Acetaldehyde is no longer registered as an active ingredient in any pesticide. When it was used as a fumigant for storage of apples and strawberries, it was exempted from a residue tolerance (IARC 1985, EPA 1994, HSDB 2009).

Production

Acetaldehyde was first produced commercially in 1916, and its U.S. production peaked at 1.65 billion pounds in 1969 (IARC 1985). In 2015, combined U.S. production and imports were in the range of 250 million to 500 million pounds (EPA 2016), similar to the range of 100 million to 500 million pounds reported from 1994 to 2002 (EPA 2004). Data on U.S. imports and exports of acetaldehyde indicated that although exports have decreased substantially from the 42.6 million pounds reported in 1989 (USITC 2009), they have continued to greatly exceed imports (as shown in the table below). In 2009, acetaldehyde was available from 49 suppliers, including 21 U.S. suppliers (ChemSources 2009).

Category	Year	Quantity (lb)
Production + imports[a]	2015	250 million to 500 million
U.S. imports[b]	2017	177,000
U.S. exports[b]	2017	4.5 million

Sources: [a]EPA 2016. [b]USITC 2018.

Exposure

There is high potential for exposure of the general population to acetaldehyde through ingestion, inhalation, and dermal contact and of workers through inhalation and dermal contact. The main source of exposure of the general population is through consumption of alcoholic beverages and the subsequent metabolism of alcohol to form acetaldehyde (HSDB 2009). Because acetaldehyde may form in wine and other alcoholic beverages after exposure to air (Hagemeyer 2002), alcoholic beverages (including wines, beer, and spirits) also frequently contain acetaldehyde as a volatile component (HSDB 2009).

Acetaldehyde is a product of most hydrocarbon oxidation reactions and is a normal intermediate in the respiration of most higher plants. It is found in trace amounts in many plant products, including apples, broccoli, coffee, grapefruit, grapes, lemons, mushrooms, onions, oranges, peaches, nectarines, pears, pineapples, raspberries, strawberries, cranberries, sour cherries, and mango. It has been detected in the essential oils of alfalfa, rosemary, balm, clary sage, daffodil, bitter orange, camphor, angelica, fennel, mustard, peppermint, and lychee, and in oak and tobacco leaves and cotton leaves and blossoms (IARC 1985, Burdon *et al.* 1996, Gorny *et al.* 1999, Gunes *et al.* 2002, Bonerz *et al.* 2007, Mahattanatawee *et al.* 2007). Acetaldehyde has also been detected in breast milk. Consumers may be exposed to acetaldehyde in many milk products, including all types of cheese, yogurt, and milk of varying fat content (Mistry and Hassan 1992, Barbieri *et al.* 1994, Jandal 1996, Beshkova *et al.* 1998, Van Aardt *et al.* 2001, Kondyli *et al.* 2002, Boscaini *et al.* 2003, Di Cagno *et al.* 2004, Fernandez-Garcia *et al.* 2004, Blagden and Gilliland 2005, Gadaga *et al.* 2007, Kaminarides *et al.* 2007). Acetaldehyde has also been detected in cooked beef, chicken, and fish (HSDB 2009, Yasuhara and Shibamoto 1995) and is used as a synthetic flavoring ingredient in processed foods, especially margarine (HSDB 2009).

According to EPA's Toxics Release Inventory, environmental releases of acetaldehyde have increased slightly since 1988, when 9.5 million pounds was released, 73% to air, 23% to underground injection wells, and the remainder to surface water and landfills. Since then, releases to underground injection wells have decreased, and releases to surface water have increased. In 2007, 11.4 million pounds of acetaldehyde was released from 336 facilities that processed, produced, or used the chemical; 29 facilities each released more than 100,000 lb. Of the total amount, 94% was released to air, 3.1% to underground injection wells, and 2.8% to water (TRI 2009). Acetaldehyde will volatilize rapidly from water or land, and it will leach into the ground, where it will biodegrade (HSDB 2009). Acetaldehyde is also degraded readily in soil, sewage, and natural waters by microorganisms (EPA 1987).

Acetaldehyde is a natural product of photooxidation of hydrocarbons commonly found in the atmosphere and occurs naturally as emissions from forest fires, volcanoes, and animal wastes. In the 1990s, annual emissions of acetaldehyde from all sources in the United States were estimated at 12.1 million kilograms (27 million pounds) (IPCS 1995). Burning wood produces acetaldehyde at approximately 0.7 g/kg of wood, and fireplace emissions range from 0.083 to 0.20 g/kg of wood burned (HSDB 2009). In the 1990s, annual emissions from residential burning in the United States were estimated at 5,000 metric tons (11 million pounds) (IPCS 1995). Acetaldehyde is also a combustion product of some plastics (e.g., polycarbonate) and some hard and soft polyurethane foams. It also occurs in gasoline exhaust (1.4 to 8.8 mg/m^3) and diesel exhaust (0.05 to 6.4 mg/m^3); however, very little is emitted from small engines such as lawn mowers or leaf blowers (IARC 1985, Baldauf *et al.* 2006).

Many individuals are exposed to acetaldehyde by inhalation. The highest ambient-air concentrations of acetaldehyde were reported for urban or suburban areas or near sources of combustion (HSDB 2009). In ambient air, concentrations of acetaldehyde generally averaged 5 μg/m^3. Indoor air concentrations were higher than ambient concentrations in all locations where acetaldehyde air concentrations were measured, both in the United States and in other countries (Miguel *et al.* 1995, Mukund *et al.* 1996, Brickus *et al.* 1998, MacIntosh *et al.* 2000, Possanzini *et al.* 2002, Baez *et al.* 2003, Hellen *et al.* 2004, Hodgson *et al.* 2004, Park and Ikeda 2004, Saijo *et al.* 2004, Sax *et al.* 2004, Shendell *et al.* 2004, Gilbert *et al.* 2005, Cavalcante *et al.* 2006, Ohura *et al.* 2006, Pang and Mu 2006, Sax *et al.* 2006, Hodgson *et al.* 2007, Possanzini *et al.* 2007). Acetaldehyde is also found in tobacco and marijuana cigarette smoke (1,220 μg per cigarette) and tobacco cigarettes (980 to 1,370 μg per cigarette).

In 1988–89, acetaldehyde was detected in 4 of 10 surveyed water supplies (EPA 1987). In surface water, concentrations generally are less than 0.1 μg/L, and the contribution from drinking water to human exposure is considered negligible (IPCS 1995).

The National Occupational Exposure Survey (conducted from 1981 to 1983) estimated that 216,533 workers, including 97,770 women, potentially were exposed to acetaldehyde (NIOSH 1990). Workers potentially exposed include those involved in the manufacture or use of industrial organic chemicals, dyes, fabricated rubber, plastics, urea-formaldehyde foam insulation, fuels, drugs, explosives, varnishes, pesticides, food additives, leather goods, and mirrors (IARC 1985, EPA 1994).

Regulations

Coast Guard, Department of Homeland Security

Minimum requirements have been established for safe transport of acetaldehyde on ships and barges.

Department of Transportation (DOT)

Acetaldehyde is considered a hazardous material, and special requirements have been set for marking, labeling, and transporting this material.

Environmental Protection Agency (EPA)

Clean Air Act

Mobile Source Air Toxics: Listed as a mobile source air toxic for which regulations are to be developed.
National Emission Standards for Hazardous Air Pollutants: Listed as a hazardous air pollutant.
New Source Performance Standards: Manufacture of acetaldehyde is subject to certain provisions for the control of volatile organic compound emissions.
Prevention of Accidental Release: Threshold quantity (TQ) = 10,000 lb.
Urban Air Toxics Strategy: Identified as one of 33 hazardous air pollutants that present the greatest threat to public health in urban areas.

Clean Water Act

Designated a hazardous substance.

Comprehensive Environmental Response, Compensation, and Liability Act

Reportable quantity (RQ) = 1,000 lb.

Emergency Planning and Community Right-To-Know Act

Toxics Release Inventory: Listed substance subject to reporting requirements.

Resource Conservation and Recovery Act

Listed Hazardous Waste: Waste code for which the listing is based wholly or partly on the presence of acetaldehyde = U001.

Occupational Safety and Health Administration (OSHA, Dept. of Labor)

While this section accurately identifies OSHA's legally enforceable PELs for this substance in 2018, specific PELs may not reflect the more current studies and may not adequately protect workers.
Permissible exposure limit (PEL) = 200 ppm (360 mg/m^3).
Considered a highly hazardous chemical: Threshold quantity (TQ) = 2,500 lb.

Guidelines

American Conference of Governmental Industrial Hygienists (ACGIH)

Threshold limit value – ceiling (TLV-C) = 25 ppm.

National Institute for Occupational Safety and Health (NIOSH, CDC, HHS)

Immediately dangerous to life and health (IDLH) limit = 2,000 ppm.
Listed as a potential occupational carcinogen.

References

Akron. 2009. *The Chemical Database.* The Department of Chemistry at the University of Akron. http://ull.chemistry.uakron.edu/erd and search on CAS number. Last accessed: 7/2/09.

Baan R, Straif K, Grosse Y, Secretan B, El Ghissassi F, Bouvard V, Altieri A, Cogliano V. 2007. Carcinogenicity of alcoholic beverages. *Lancet Oncol* 8(4): 292-293.

Baez A, Padilla H, Garcia R, Torres MD, Rosas I, Belmont R. 2003. Carbonyl levels in indoor and outdoor air in Mexico City and Xalapa, Mexico. *Sci Total Environ* 302(1-3): 211-226.

Bagnardi V, Blangiardo M, La Vecchia C, Corrao G. 2001. A meta-analysis of alcohol drinking and cancer risk. *Br J Cancer* 85(11): 1700-1705.

Baldauf R, Fortune C, Weinstein J, Wheeler M, Blanchard F. 2006. Air contaminant exposures during the operation of lawn and garden equipment. *J Expo Sci Environ Epidemiol* 16(4): 362-370.

Barbieri G, Bolzoni L, Careri M, Mangia A, Parolari G, Spagnoli S, Virgili R. 1994. Study of the volatile fraction of Parmesan cheese. *J Agric Food Chem* 42(5): 1170-1176.

Beshkova D, Simova E, Frengova G, Simov Z. 1998. Production of flavour compounds by yogurt starter cultures. *J Ind Microbiol Biotechnol* 20(3-4): 180-186.

Blagden TD, Gilliland SE. 2005. Reduction of levels volatile components with the "beany" flavor in soymilk by lactobacilli and streptococci. *J Food Sci* 70(3): M186-M189.

Boccia S, Hashibe M, Galli P, De Feo E, Asakage T, Hashimoto T, *et al.* 2009. Aldehyde dehydrogenase 2 and head and neck cancer: A meta-analysis implementing a Mendelian randomization approach. *Cancer Epidemiol Biomarkers Prev* 18(1): 248-254.

Boffetta P, Hashibe M. 2006. Alcohol and cancer. *Lancet Oncol* 7(2): 149-156.

Bonerz D, Würth K, Dietrich H, Will F. 2007. Analytical characterization and the impact of ageing on anthocyanin composition and degradation in juices from five sour cherry cultivars. *Eur Food Res Technol* 224(3): 355-364.

Boscaini E, Van Ruth S, Biasioli F, Gasperi F, Märk TD. 2003. Gas chromatography-olfactometry (GC-O) and proton transfer reaction-mass spectrometry (PTR-MS) analysis of the flavor profile of Grana Padano, Parmigiano Reggiano, and Grana Trentino cheeses. *J Agric Food Chem* 51(7): 1782-1790.

Brickus LSR, Cardoso JN, Neto FRD. 1998. Distributions of indoor and outdoor air pollutants in Rio de Janeiro, Brazil: Implications to indoor air quality in bayside offices. *Environ Sci Technol* 32(22): 3485-3490.

Burdon J, Dori S, Marinansky R, Pesis E. 1996. Acetaldehyde inhibition of ethylene biosynthesis in mango fruit. *Postharvest Biol Technol* 8(2): 153-161.

Cavalcante RM, Campelo CS, Barbosa MJ, Silveira ER, Carvalho TV, Nascimento RF. 2006. Determination of carbonyl compounds in air and cancer risk assessment in an academic institute in Fortaleza, Brazil. *Atmos Environ* 40(29): 5701-5711.

ChemIDplus. 2009. *ChemIDplus Advanced*. National Library of Medicine. http://chem.sis.nlm.nih.gov/chemidplus/chemidheavy.jsp and select Registry Number and search on CAS number. Last accessed: 7/2/09.

ChemSources. 2009. *Chem Sources - Chemical Search*. Chemical Sources International. http://www.chemsources.com/chemonline.html and search on acetaldehyde. Last accessed: 7/2/09.

Di Cagno R, Tamborrino A, Gallo G, Leone C, De Angelis M, Faccia M, Amirante P, Gobbetti M. 2004. Uses of mares' milk in manufacture of fermented milks. *Int Dairy J* 14(9): 767-775.

Druesne-Pecollo N, Tehard B, Mallet Y, Gerber M, Norat T, Hercberg S, Latino-Martel P. 2009. Alcohol and genetic polymorphisms: Effect on risk of alcohol-related cancer. *Lancet Oncol* 10(2): 173-180.

EPA. 1987. *Health Assessment Document for Acetaldehyde*. EPA/600/8-86-015A. Research Triangle Park, NC: U.S. Environmental Protection Agency.

EPA. 1994. *Chemical Summary for Acetaldehyde.* U.S. Environmental Protection Agency. http://www.epa.gov/chemfact/s_acetal.txt.

EPA. 2004. *Non-confidential IUR Production Volume Information*. U.S. Environmental Protection Agency. http://www.epa.gov/oppt/iur/tools/data/2002-vol.html and search on CAS number.

EPA. 2016. *Chemical Data Reporting Summary: Acetaldehyde.* U.S. Environmental Protection Agency. https://chemview.epa.gov/chemview and search on CAS number or substance name and select Manufacturing, Processing, Use, and Release Data Maintained by EPA and select Chemical Data Reporting Details.

Fernandez-Garcia E, Carbonell M, Gaya P, Nuñez M. 2004. Evolution of the volatile components of ewes raw milk Zamorano cheese. Seasonal variation. *Int Dairy J* 14(8): 701-711.

Furia TE, Bellanca N. 1975. *Fenaroli's Handbook of Flavour Ingredients*, vol. 2, Cleveland, OH: CRC Press.

Gadaga TH, Viljoen BC, Narvhus JA. 2007. Volatile organic compounds in naturally fermented milk and milk fermented using yeasts, lactic acid bacteria and their combinations as starter cultures. *Food Technol Biotechnol* 45(2): 195-200.

Gilbert NL, Guay M, Miller JD, Judek S, Chan CC, Dales RE. 2005. Levels and determinants of formaldehyde, acetaldehyde, and acrolein in residential indoor air in Prince Edward Island, Canada. *Environ Res* 99(1): 11-17.

Gorny JR, Hess-Pierce B, Kader AA. 1999. Quality changes in fresh-cut peach and nectarine slices as affected by cultivar, storage atmosphere and chemical treatments. *J Food Sci* 64(3): 429-432.

Gunes G, Liu RH, Watkins CB. 2002. Controlled-atmosphere effects on postharvest quality and antioxidant activity of cranberry fruits. *J Agric Food Chem* 50(21): 5932-5938.

Hagemeyer HJ. 2002. Acetaldehyde. In *Kirk-Othmer Encyclopedia of Chemical Technology,* vol. 1. Online edition. New York: John Wiley & Sons. pp. 99-114.

Hellen H, Hakola H, Reissell A, Ruuskanen TM. 2004. Carbonyl compounds in boreal coniferous forest air in Hyytiala, Southern Finland. *Atmos Chem Phys* 4: 1771-1780.

Hodgson AT, Shendell DG, Fisk WJ, Apte MG. 2004. Comparison of predicted and derived measures of volatile organic compounds inside four new relocatable classrooms. *Indoor Air* 14(s8): 135-144.

Hodgson AT, Destaillats H, Sullivan DP, Fisk WJ. 2007. Performance of ultraviolet photocatalytic oxidation for indoor air cleaning applications. *Indoor Air* 17(4): 305-316.

HSDB. 2009. *Hazardous Substances Data Bank.* National Library of Medicine. http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB and search on CAS number. Last accessed: 7/2/09.

IARC. 1985. Acetaldehyde. In *Allyl Compounds, Aldehydes, Epoxides, and Peroxides*. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 36. Lyon, France: International Agency for Research on Cancer. pp. 101-132.

IARC. 1987. Acetaldehyde. In *Overall Evaluations of Carcinogenicity*. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, suppl. 7. Lyon, France: International Agency for Research on Cancer. pp. 77-78.

IARC. 1999. Acetaldehyde. In *Re-evaluation of Some Organic Chemicals, Hydrazine, and Hydrogen Peroxide*. IARC Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans, vol. 71. Lyon, France: International Agency for Research on Cancer. pp. 319-335.

IPCS. 1995. *Environmental Health Criteria No. 167. Acetaldehyde*. International Programme on Chemical Safety. http://www.inchem.org/documents/ehc/ehc/ehc167.htm.

Jandal JM. 1996. Studies on dried fermented dairy products prepared from sheep milk. *Small Rumin Res* 21(3): 217-220.

Kaminarides S, Stamou P, Massouras T. 2007. Comparison of the characteristics of set type yoghurt made from ovine milk of different fat content. *Int J Food Sci Tech* 42(9): 1019-1028.

Kondyli E, Katsiari MC, Masouras T, Voutsinas LP. 2002. Free fatty acids and volatile compounds of low-fat Feta-type cheese made with a commercial adjunct culture. *Food Chem* 79(2): 199-205.

MacIntosh DL, Zimmer-Dauphinee SA, Manning RO, Williams PL. 2000. Aldehyde concentrations in ambient air of coastal Georgia, USA. *Environ Monit Assess* 63(3): 409-429.

Mahattanatawee K, Perez-Cacho PR, Davenport T, Rouseff R. 2007. Comparison of three lychee cultivar odor profiles using gas chromatography—Olfactometry and gas chromatography—Sulfur detection. *J Agric Food Chem* 55(5): 1939-1944.

Miguel AH, Neto FBD, Cardoso JN, Vasconcellos PD, Pereira AS, Marquez KSG. 1995. Characterization of indoor air-quality in the cities of Sao-Paulo and Rio-de-Janeiro, Brazil. *Environ Sci Technol* 29(2): 338-345.

Mistry VV, Hassan HN. 1992. Manufacture of nonfat yogurt from a high milk protein powder. *J Dairy Sci* 75(4): 947-957.

Mukund R, Kelly TJ, Spicer CW. 1996. Source attribution of ambient air toxic and other VOCs in Columbus, Ohio. *Atmos Environ* 30(20): 3457-3470.

NIOSH. 1990. *National Occupational Exposure Survey (1981-83)*. National Institute for Occupational Safety and Health. Last updated: 7/1/90. http://www.cdc.gov/noes/noes1/01038sic.html.

Ohura T, Amagai T, Senga Y, Fusaya M. 2006. Organic air pollutants inside and outside residences in Shimizu, Japan: Levels, sources and risks. *Sci Total Environ* 366(2-3): 485-499.

Pang XB, Mu YJ. 2006. Seasonal and diurnal variations of carbonyl compounds in Beijing ambient air. *Atmos Environ* 40(33): 6313-6320.

Park JS, Ikeda K. 2004. Exposure to the mixtures of organic compounds in homes in Japan. *Indoor Air* 14(6): 413-420.

Possanzini M, Di Palo V, Cecinato A. 2002. Sources and photodecomposition of formaldehyde and acetaldehyde in Rome ambient air. *Atmos Environ* 36(19): 3195-3201.

Possanzini M, Tagliacozzo G, Cecinato A. 2007. Ambient levels and sources of lower carbonyls at Montelibretti, Rome (Italy). *Water Air Soil Pollut* 183(1-4): 447-454.

Saijo Y, Kishi R, Sata F, Katakura Y, Urashima Y, Hatakeyama A, *et al.* 2004. Symptoms in relation to chemicals and dampness in newly built dwellings. *Int Arch Occup Environ Health* 77(7): 461-470.

Salaspuro M. 2009. Acetaldehyde as a common denominator and cumulative carcinogen in digestive tract cancers. *Scand J Gastroenterol* 44(8): 912-925.

Sax SN, Bennett DH, Chillrud SN, Kinney PL, Spengler JD. 2004. Differences in source emission rates of volatile organic compounds in inner-city residences of New York City and Los Angeles. *J Expo Anal Environ Epidemiol* 14(suppl. 1): S95-S109.

Sax SN, Bennett DH, Chillrud SN, Ross J, Kinney PL, Spengler JD. 2006. A cancer risk assessment of inner-city teenagers living in New York City and Los Angeles. *Environ Health Perspect* 114(10): 1558-1566.

Secretan B, Straif K, Baan R, Grosse Y, El Ghissassi F, Bouvard V, *et al.* 2009. A review of human carcinogens—Part E: tobacco, areca nut, alcohol, coal smoke, and salted fish. *Lancet Oncol* 10(11): 1033-1034.

Seitz HK, Becker P. 2007. Alcohol metabolism and cancer risk. *Alcohol Res Health* 30(1): 38-47.

Shendell DG, Winer AM, Stock TH, Zhang L, Zhang J, Maberti S, Colome SD. 2004. Air concentrations of VOCs in portable and traditional classrooms: Results of a pilot study in Los Angeles County. *J Expo Anal Environ Epidemiol* 14(1): 44-59.

Soffritti M, Belpoggi F, Lambertini L, Lauriola M, Padovani M, Maltoni C. 2002. Results of long-term experimental studies on the carcinogenicity of formaldehyde and acetaldehyde in rats. *Ann N Y Acad Sci* 982: 87-105.

Terry MB, Gammon MD, Zhang FF, Knight JA, Wang Q, Britton JA, Teitelbaum SL, Neugut AI, Santella RM. 2006. ADH3 genotype, alcohol intake and breast cancer risk. *Carcinogenesis* 27(4): 840-847.

TRI. 2009. *TRI Explorer Chemical Report*. U.S. Environmental Protection Agency. Last updated: 6/07. http://www.epa.gov/triexplorer and select Acetaldehyde. Last accessed: 7/2/09.

USITC. 2009. *USITC Interactive Tariff and Trade DataWeb.* United States International Trade Commission. http://dataweb.usitc.gov/scripts/user_set.asp and search on HTS no. 2912120000.

USITC. 2018. *USITC Interactive Tariff and Trade DataWeb.* United States International Trade Commission. http://dataweb.usitc.gov/scripts/user_set.asp and search on HTS no. 2912120000.

Van Aardt M, Duncan SE, Bourne D, Marcy JE, Long TE, Hackney CR, Heisey C. 2001. Flavor threshold for acetaldehyde in milk, chocolate milk, and spring water using solid phase microextraction gas chromatography for quantification. *J Agric Food Chem* 49(3): 1377-1381.

Van Dijk BAC, Van Houwelingen KP, Witjes JA, Schalken JA, Kiemeney LALM. 2001. Alcohol dehydrogenase type 3 (ADH3) and the risk of bladder cancer. *Eur Urol* 40(5): 509-514.

Visvanathan K, Crum RM, Strickland PT, You X, Ruczinski I, Berndt SI, *et al.* 2007. Alcohol dehydrogenase genetic polymorphisms, low-to-moderate alcohol consumption, and risk of breast cancer. *Alcohol Clin Exp Res* 31(3): 467-476.

Yasuhara A, Shibamoto T. 1995. Quantitative analysis of volatile aldehydes formed from various kinds of fish flesh during heat treatment. *J Agric Food Chem* 43(1): 94-97.

Zeka A, Gore R, Kriebel D. 2003. Effects of alcohol and tobacco on aerodigestive cancer risks: A meta-regression analysis. *Cancer Causes Control* 14(9): 897-906.

Acetaldehyde

75-07-0

Hazard Summary

Acetaldehyde is mainly used as an intermediate in the synthesis of other chemicals. It is ubiquitous in the environment and may be formed in the body from the breakdown of ethanol. Acute (short-term) exposure to acetaldehyde results in effects including irritation of the eyes, skin, and respiratory tract. Symptoms of chronic (long-term) intoxication of acetaldehyde resemble those of alcoholism. Acetaldehyde is considered a probable human carcinogen (Group B2) based on inadequate human cancer studies and animal studies that have shown nasal tumors in rats and laryngeal tumors in hamsters.

Please Note: The main sources of information for this fact sheet are EPA's Health Assessment Document for Acetaldehyde (1) and the Integrated Risk Information System (IRIS) (4), which contains information on inhalation chronic toxicity of acetaldehyde and the RfC. Other secondary sources include the International Agency for Research on Cancer (IARC) Monographs on Chemicals Carcinogenic to Humans. (6)

Uses

- The predominant use of acetaldehyde is as an intermediate in the synthesis of other chemicals. (1)
- Acetaldehyde is used in the production of perfumes, polyester resins, and basic dyes. Acetaldehyde is also used as a fruit and fish preservative, as a flavoring agent, and as a denaturant for alcohol, in fuel compositions, for hardening gelatin, and as a solvent in the rubber, tanning, and paper industries. (1,2)

Sources and Potential Exposure

- Acetaldehyde is ubiquitous in the ambient environment. It is an intermediate product of higher plant respiration and formed as a product of incomplete wood combustion in fireplaces and woodstoves, coffee roasting, burning of tobacco, vehicle exhaust fumes, and coal refining and waste processing. Hence, many individuals are exposed to acetaldehyde by breathing ambient air. It should be noted that residential fireplaces and woodstoves are the two highest sources of emissions, followed by various industrial emissions. (1)
- In Los Angeles, California, levels of acetaldehyde up to 32 parts per billion (ppb) have been measured in the ambient environment. (1)
- Exposure may also occur in individuals occupationally exposed to acetaldehyde during its manufacture and use. (1,2)
- In addition, acetaldehyde is formed in the body from the breakdown of ethanol; this would be a source of acetaldehyde among those who consume alcoholic beverages. (1)

Assessing Personal Exposure

- Acetaldehyde can be detected in the blood and breath to determine whether or not exposure has occurred. (12)

Health Hazard Information

Acute Effects:

- The primary acute effect of inhalation exposure to acetaldehyde is irritation of the eyes, skin, and

respiratory tract in humans. At higher exposure levels, erythema, coughing, pulmonary edema, and necrosis may also occur. (1)

- Acute inhalation of acetaldehyde resulted in a depressed respiratory rate and elevated blood pressure in experimental animals. (1)
- Tests involving acute exposure of rats, rabbits, and hamsters have demonstrated acetaldehyde to have low acute toxicity from inhalation and moderate acute toxicity from oral or dermal exposure. (3)

Chronic Effects (Noncancer):

- Symptoms of chronic intoxication of acetaldehyde in humans resemble those of alcoholism. (5)
- In hamsters, chronic inhalation exposure to acetaldehyde has produced changes in the nasal mucosa and trachea, growth retardation, slight anemia, and increased kidney weight. (1,4)
- The Reference Concentration (RfC) for acetaldehyde is 0.009 milligrams per cubic meter (mg/m^3) based on degeneration of olfactory epithelium in rats. The RfC is an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups), that is likely to be without appreciable risk of deleterious noncancer effects during a lifetime. It is not a direct esimator of risk but rather a reference point to gauge the potential effects. At exposures increasingly greater than the RfC, the potential for adverse health effects increases. Lifetime exposure above the RfC does not imply that an adverse health effect would necessarily occur. (4)
- EPA has medium confidence in the principal studies because appropriate histopathology was performed on an adequate number of animals and a no-observed-adverse-effect level (NOAEL) and a lowest-observed-adverse-effect level (LOAEL) were identified, but the duration was short and only one species was tested; low confidence in the database due to the lack of chronic data establishing NOAELs and due to the lack of reproductive and developmental toxicity data; and, consequently, low confidence in the RfC. (4)
- EPA has not established a Reference Dose (RfD) for acetaldehyde. (4)

Reproductive/Developmental Effects:

- No information is available on the reproductive or developmental effects of acetaldehyde in humans.
- Acetaldehyde has been shown, in animals, to cross the placenta to the fetus. (1,4)
- Data from animal studies suggest that acetaldehyde may be a potential developmental toxin. In one study, a high incidence of embryonic resorptions was observed in mice injected with acetaldehyde. In rats exposed to acetaldehyde by injection, skeletal malformations, reduced birth weight, and increased postnatal mortality have been reported. (1,6)

Cancer Risk:

- Human data regarding the carcinogenic effects of acetaldehyde are inadequate. Only one epidemiology study is available that has several limitations including short duration, small number of subjects, and concurrent exposure to other chemicals and cigarettes. (1,4,6)
- An increased incidence of nasal tumors in rats and laryngeal tumors in hamsters has been observed following inhalation exposure to acetaldehyde. (1,4,6)
- EPA has classified acetaldehyde as a Group B2, probable human carcinogen. (1,4)
- EPA uses mathematical models, based on human and animal studies, to estimate the probability of a person developing cancer from breathing air containing a specified concentration of a chemical. EPA calculated an inhalation unit risk of 2.2×10^{-6} $(\mu g/m^3)^{-1}$. EPA estimates that, if an individual were to continuously breathe air containing acetaldehyde at an average of 0.5 $\mu g/m^3$ (5×10^{-4} mg/m^3) over his or her entire lifetime, that person would theoretically have no more than a one-in-a-million increased chance of developing cancer as a direct result of breathing air containing this chemical. Similarly, EPA estimates that breathing air containing 5.0 $\mu g/m^3$ (5×10^{-3} mg/m^3) would result in not greater than a one-in-a-hundred thousand increased chance of developing cancer, and air containing 50.0 $\mu g/m^3$ (5×10^{-2} mg/m^3) would result in not greater than a one-in-ten thousand increased chance of developing cancer. For a detailed discussion of confidence in the potency estimates, please see IRIS. (4)

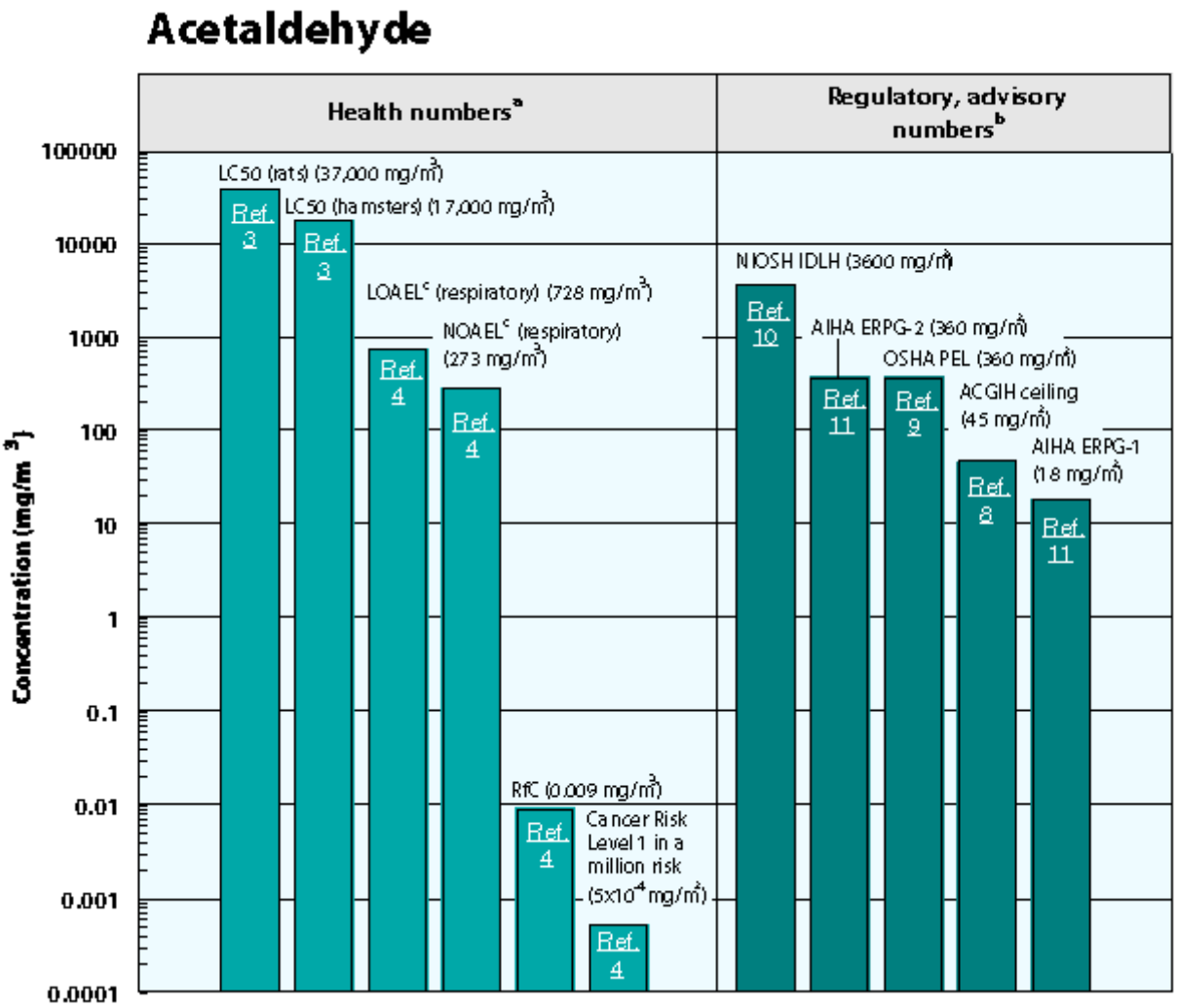
Physical Properties

- The chemical formula for acetaldehyde is CH_3CHO, and it has a molecular weight of 44.06 g/mol. (1)
- Acetaldehyde is a colorless mobile liquid that is flammable and miscible with water. (1,6)
- Acetaldehyde has a pungent suffocating odor, but at dilute concentrations it has a fruity and pleasant odor. The odor threshold of acetaldehyde is 0.05 parts per million (ppm) (0.09 mg/m^3). (1,7)
- The vapor pressure for acetaldehyde is 740 mm Hg at 20 °C, and it has a log octanol/water partition coefficient (log K_{ow}) of 0.43. (1)

Conversion Factors (only for the gaseous form):

To convert concentrations in air (at 25°C) from ppm to mg/m^3: mg/m^3 = (ppm) × (molecular weight of the compound)/(24.45). For acetaldehyde: 1 ppm = 1.8 mg/m^3. To convert concentrations in air from $\mu g/m^3$ to mg/m^3: mg/m^3 = ($\mu g/m^3$) × (1 mg/1,000 µg).

Health Data from Inhalation Exposure



AIHA ERPG--American Industrial Hygiene Association's emergency response planning guidelines. ERPG 1 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed up to one hour without experiencing other than mild transient adverse health effects or perceiving a clearly defined objectionable odor; ERPG 2 is the maximum airborne concentration below which it is believed nearly all individuals could be exposed up to one hour without experiencing or developing irreversible or other serious health effects that could impair their abilities to take protective action.

ACGIH ceiling-- American Conference of Governmental and Industrial Hygienists' threshold limit value ceiling; the concentration of a substance that should not be exceeded during any part of the working exposure.
LC_{50} (Lethal Concentration $_{50}$)--A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.
NIOSH IDLH --National Institute of Occupational Safety and Health's immediately dangerous to life or health limit; NIOSH recommended exposure limit to ensure that a worker can escape from an exposure condition that is likely to cause death or immediate or delayed permanent adverse health effects or prevent escape from the environment.
OSHA PEL --Occupational Safety and Health Administration's permissible exposure limit expressed as a time-weighted average; the concentration of a substance to which most workers can be exposed without adverse effect averaged over a normal 8-h workday or a 40-h workweek.

The health and regulatory values cited in this factsheet were obtained in December 1999.
[a] Health Numbers are toxicological numbers from animal testing or risk assessment values developed by EPA.
[b] Regulatory numbers are values that have been incorporated in Government regulations, while advisory numbers are nonregulatory values provided by the Government or other groups as advice. OSHA numbers are regulatory, whereas NIOSH, ACGIH, and AIHA numbers are advisory.
[c] The LOAEL and NOAEL are from the critical study used as the basis for the EPA RfC.

Summary created in April 1992, updated in January 2000

References

1. U.S. Environmental Protection Agency. Health Assessment Document for Acetaldehyde. EPA/600/8-86-015A. Environmental Criteria and Assessment Office, Office of Health and Environmental Assessment, Office of Research and Development, Research Triangle Park, NC. 1987.
2. M. Sittig. Handbook of Toxic and Hazardous Chemicals and Carcinogens. 2nd ed. Noyes Publications, Park Ridge, NJ. 1985.
3. U.S. Department of Health and Human Services. Registry of Toxic Effects of Chemical Substances (RTECS, online database). National Toxicology Information Program, National Library of Medicine, Bethesda, MD. 1993.
4. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS) on Acetaldehyde. National Center for Environmental Assessment, Office of Research and Development, Washington, D.C. 1999.
5. The Merck Index. An Encyclopedia of Chemicals, Drugs, and Biologicals. 11th ed. Ed. S. Budavari. Merck and Co. Inc., Rahway, NJ. 1989.
6. International Agency for Research on Cancer (IARC). IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans: Allyl Compounds, Aldehydes, Epoxides and Peroxides. Volume 36. World Health Organization, Lyon. 1985.
7. J.E. Amoore and E. Hautala. Odor as an aid to chemical safety: Odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. Journal of Applied Toxicology, 3(6):272-290. 1983.
8. American Conference of Governmental Industrial Hygienists (ACGIH). 1999 TLVs and BEIs. Threshold Limit Values for Chemical Substances and Physical Agents, Biological Exposure Indices. Cincinnati, OH. 1999.
9. Occupational Safety and Health Administration (OSHA). Occupational Safety and Health Standards, Toxic and Hazardous Substances. Code of Federal Regulations. 29 CFR 1910.1000. 1998.
10. National Institute for Occupational Safety and Health (NIOSH). Pocket Guide to Chemical Hazards. U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention. Cincinnati, OH. 1997.
11. American Industrial Hygiene Association (AIHA). The AIHA 1998 Emergency Response Planning Guidelines and Workplace Environmental Exposure Level Guides Handbook. 1998.
12. U.S. Department of Health and Human Services. Hazardous Substances Data Bank (HSDB, online database). National Toxicology Information Program, National Library of Medicine, Bethesda, MD. 1993.

TOBACCO SMOKING

Tobacco smoking was considered by previous IARC Working Groups in 1986, 1987 and 2002 (IARC, 1986, 1987, 2004a). Since that time, new data have become available, these have been incorporated into the *Monograph*, and taken into consideration in the present evaluation.

1. Exposure Data

1.1 Smoked tobacco products

Smoked forms of tobacco include various kinds of cigarettes (manufactured, hand-rolled, filtered, un-filtered and flavoured), cigars and pipes. While cigarette smoking, particularly manufactured cigarettes, is by far the main form of tobacco smoked globally, in some countries other forms of smoked tobacco are dominant (IARC, 2004a). In India, for example, bidis (made of coarse and uncured tobacco) account for about 60% of smoked tobacco products whereas cigarettes account for 20% (Ray & Gupta, 2009; IIPS, 2010). Water pipes, another form of smoked tobacco known by other various names such as gaza, hookah, narghile, shisha, hubble-bubble, are commonly smoked in the Eastern Mediterranean region, in some parts of Asia including India, and in North Africa (Asma *et al.*, 2009).

1.2 Chemical composition of tobacco smoke

1.2.1 Smoke from cigarettes

One cubic cm of fresh, un-aged cigarette mainstream smoke [the smoke emerging from the mouth end of a cigarette during smoking] has about 4×10^9 particles with a mean diameter of about 0.2 µm (Borgerding & Klus, 2005). The size of the particles increases as the smoke ages. Temperatures in the burning cone of the cigarette are about 800 °C during the smoulder period between puffs and increase to 910–920 °C at the periphery of the cone during puffing (Borgerding & Klus, 2005). Hydrogen is generated in the glowing cone, resulting in an oxygen deficient reducing atmosphere (Borgerding & Klus, 2005). The approximate composition of mainstream smoke of a plain cigarette is summarized in Table 1.1 (Borgerding & Klus, 2005). The total particulate matter, after subtraction of the amounts of nicotine and water, is referred to as 'tar'.

Over 5300 compounds have been identified in tobacco smoke (Rodgman & Perfetti, 2009). Classes of compounds include but are not limited to neutral gases, carbon and nitrogen oxides, amides, imides, lactams, carboxylic acids, lactones, esters, aldehydes, ketones,

Table 1.1 Approximate chemical composition of mainstream smoke generated by a plain cigarette

Compound or class of components	Relative amount w/w (%)
Nitrogen	58
Oxygen	12
Carbon dioxide	13
Carbon monoxide	3.5
Hydrogen, argon	0.5
Water	1
Volatile organic substances	5
Particulate phase	8

From Borgerding & Klus (2005)

alcohols, phenols, amines, *N*-nitrosamines, *N*-heterocyclics, aliphatic hydrocarbons, monocyclic and polycyclic aromatic hydrocarbons (PAHs), nitriles, anhydrides, carbohydrates, ethers, nitro compounds and metals (Rodgman & Perfetti, 2009).

The addictive properties of tobacco smoke are attributed to nicotine, the principal tobacco alkaloid in smoke (Hukkanen *et al.*, 2005). Minor tobacco alkaloids include nornicotine, anatabine and anabasine (Hukkanen *et al.*, 2005). The tobacco alkaloids are not generally considered carcinogenic, but are accompanied by carcinogens in each puff of smoke.

There are over 70 carcinogens in tobacco smoke that have been evaluated by the *IARC Monographs* programme as having sufficient evidence for carcinogenicity in either laboratory animals or humans (IARC, 2004a). The different chemical classes of carcinogens and representatives of each are presented in Table 1.2 (IARC, 2004a). Sixteen of these – benzo[*a*]pyrene (BaP), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and *N*′-nitrosonornicotine (NNN), 2-naphthylamine, 4-aminobiphenyl, formaldehyde, 1,3-butadiene, benzene, vinyl chloride, ethylene oxide, arsenic, beryllium, nickel compounds, chromium VI, cadmium, and polonium-210 – are classified as carcinogenic to humans (Group 1). Structures of some representative carcinogens in cigarette smoke are shown in Fig. 1.1. There are other likely carcinogens in cigarette smoke that have not been evaluated by the *IARC Monographs* programme. These include, for example, PAHs with incompletely characterized occurrence levels and carcinogenic activities; over 500 PAHs have been identified (Rodgman & Perfetti, 2006).

PAHs, tobacco-specific *N*-nitrosamines, aromatic amines, aldehydes and certain volatile organics likely contribute significantly to the carcinogenic activity of tobacco smoke (Hecht, 2003).

In the early part of the 20th century, PAHs were identified as carcinogenic constituents of coal tar (Phillips, 1983). They are products of incomplete combustion of all organic matter and occur, always as complex mixtures, in tars, soots, broiled foods, vehicle engine exhaust and tobacco smoke. PAHs are generally locally acting carcinogens, and some, such as the prototypic compound BaP, have strong carcinogenic activity on mouse skin and in rodent lung. Heterocyclic analogues of PAHs also occur in cigarette smoke. Concentrations of individual PAHs in mainstream cigarette smoke are generally in the range of 1–50 ng per cigarette (IARC, 2004a).

Among the carcinogenic *N*-nitrosamines in tobacco smoke are tobacco-specific *N*-nitrosamines, which are derived from, and structurally related to, the tobacco alkaloids. Two of the most important of these are NNK and NNN (Hecht & Hoffmann, 1988). Levels of NNK and NNN in cigarette smoke vary depending on tobacco type and other factors, but are frequently in the range of 50–200 ng per cigarette (IARC, 2004a).

Aromatic amines were first identified as human carcinogens from industrial exposures in the dye industry in the early part of the 20th century. They include the well known human bladder carcinogens 2-naphthylamine and 4-aminobiphenyl which, along with other

Table 1.2 Tobacco smoke carcinogens evaluated in the *IARC Monographs*

Chemical Class	Number of Carcinogens	Representative Carcinogens
Polycyclic aromatic hydrocarbons (PAHs) and their heterocyclic analogues	15	Benzo[*a*]pyrene (BaP) Dibenz[*a*,*h*]anthracene
N-Nitrosamines	8	4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) *N*′-Nitrosonornicotine (NNN)
Aromatic amines	12	4-Aminobiphenyl 2-Naphthylamine
Aldehydes	2	Formaldehyde Acetaldehyde
Phenols	2	Catechol Caffeic acid
Volatile hydrocarbons	3	Benzene 1,3-Butadiene Isoprene
Other organics	12	Ethylene oxide Acrylonitrile
Inorganic compounds	8	Cadmium Polonium-210

There are many other carcinogens in cigarette smoke that have not been evaluated in an *IARC Monograph*.
From IARC (2004a)

isomers, are found in cigarette smoke, but their levels are generally quite low (1–20 ng per cigarette) (IARC, 2004a).

Aldehydes such as formaldehyde and acetaldehyde occur widely in the human environment and are also found in human blood. Concentrations of acetaldehyde and formaldehyde in cigarette smoke are far higher than those of PAHs, *N*-nitrosamines or aromatic amines but their carcinogenic activities are weak (Hecht, 2003). Cigarette mainstream smoke typically contains 10–30 µg formaldehyde/cigarette and 800–900 µg acetaldehyde/cigarette (IARC, 2004a).

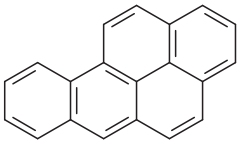
Volatile hydrocarbons in cigarette smoke include 1,3-butadiene, a powerful multi-organ carcinogen in the mouse, and benzene, a known human leukaemogen. 1,3-Butadiene (20–40 µg/cigarette) and benzene (12–50 µg/cigarette) are two of the most prevalent strong carcinogens in cigarette smoke (IARC, 2004a).

In summary, cigarette smoke is an exceedingly complex mixture which contains over 5300 compounds including multiple toxicants and carcinogens.

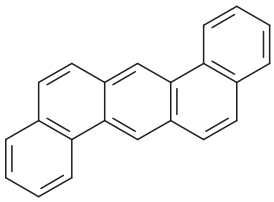
1.2.2 Smoke from other tobacco products

Some constituents have been measured in roll-your-own cigarettes, and their levels are comparable to or higher than those in commercial brands. Carcinogen and toxicant levels expressed per unit are higher in cigars than in cigarettes because of their larger size, and in some instances are also higher per litre of smoke. Levels of nicotine and tobacco-specific nitrosamines were comparable in bidis and commercial Indian cigarettes; bidis also contain high levels of eugenol, as do kreteks. Levels of NNK and NNN in chuttas were considerably higher than in standard cigarettes (IARC, 2004a).

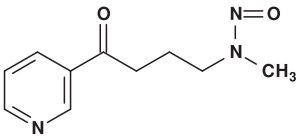
Fig. 1.1 Structures of some representative tobacco smoke carcinogens



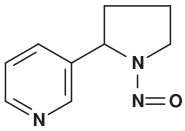
benzo[*a*]pyrene (BaP)



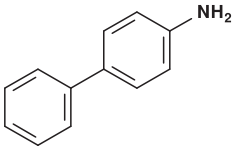
dibenz[*a,h*]anthracene



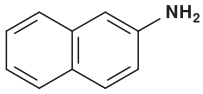
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)



N'-nitrosonornicotine (NNN)

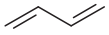


4-aminobiphenyl



2-naphtylamine

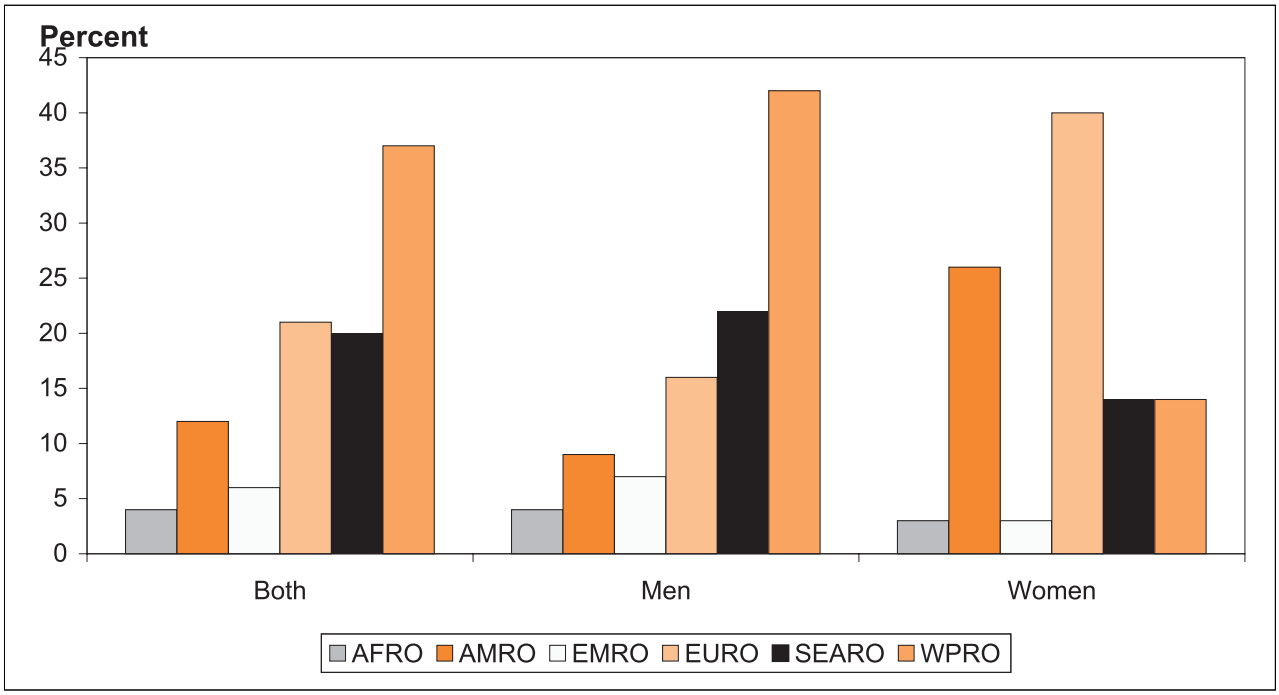
benzene



1,3-butadiene

ethylene oxide

Fig. 1.2 Proportion of adult smokers by WHO region in 2009



From WHO (2011)

1.3 Prevalence of tobacco smoking

1.3.1 Data collection and methods

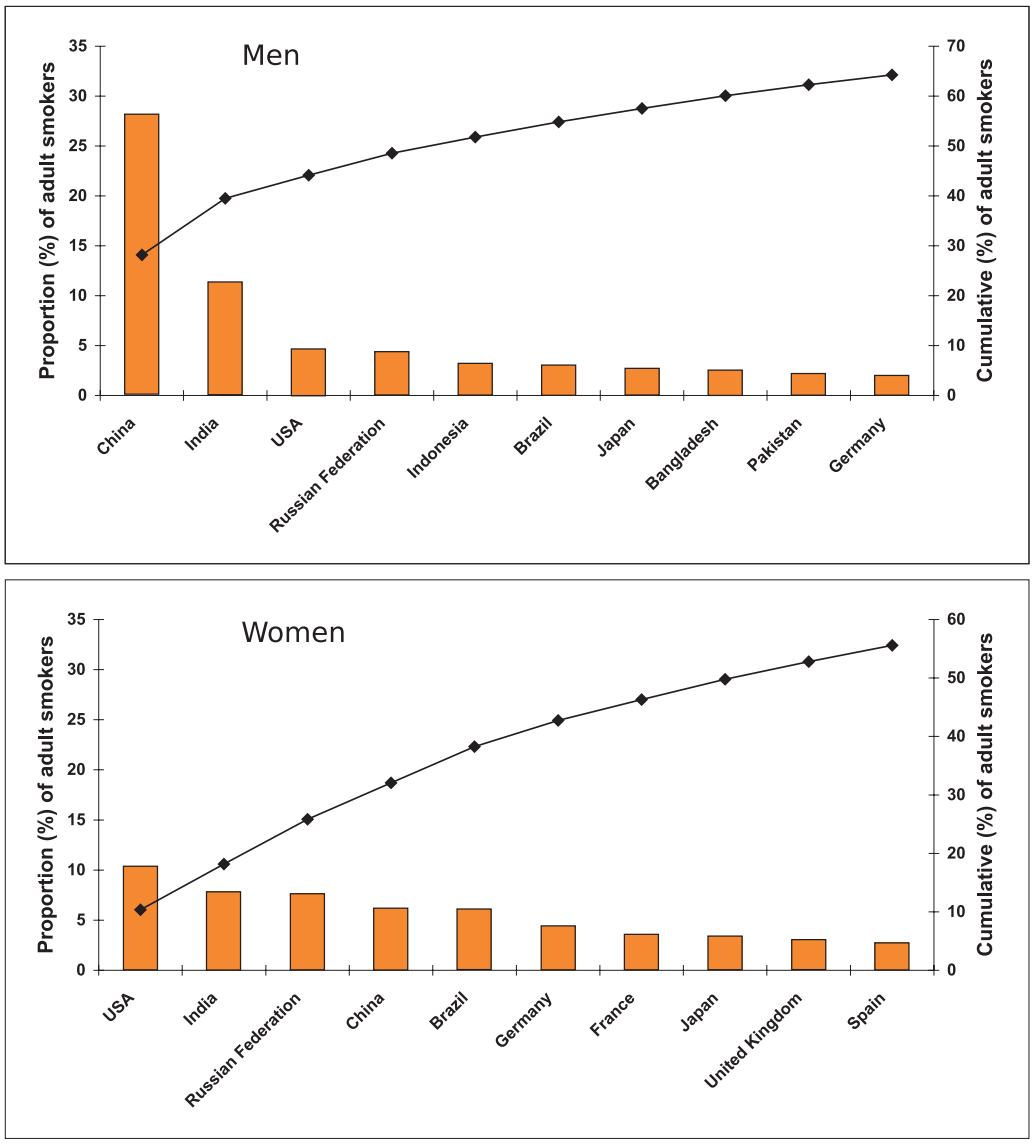
Data on smoking tobacco are available from WHO's Global Infobase (www.who.int/infobase) and the WHO Global Health Observatory (www.who.int/gho/en) – repositories of information on tobacco use and other risk factors in young people (13–15 years old) and adults (aged 15 years and over). The data span several years and are acquired from government reports, journals and unpublished sources. WHO has in the recent past used and modelled these data to produce estimates of tobacco smoking prevalence, published in the WHO Reports on the Global Tobacco Epidemic. For a complete explanation of methods used, the reader is referred to the Technical Note on Prevalence in the 3rd WHO Report on the Global Tobacco Epidemic (WHO, 2011). The six WHO regions are: EMRO, Eastern Mediterranean Region; EURO, European Region; AFRO, African Region; WPRO, Western Pacific Region; SEARO, South East Asian Region; AMRO, Region of the Americas. A listing of the countries in each region can be viewed at http://www.who.int/about/structure/en/index.html.

1.3.2 Distribution of smokers by WHO region and country

WHO estimates that in 2009, there was about 1.1 billion adult smokers worldwide, representing nearly a quarter (22%) of the global adult population (WHO, 2011). A disaggregation by the six WHO regions (Fig. 1.2) shows that over a third of smokers worldwide live in WPRO (highly influenced by the People's Republic of China), followed by SEARO, which has around a fifth of the world's smokers (influenced by India and Indonesia).

The number of smokers in any country is a function of both the prevalence of smoking and the size of the population. A further

Fig. 1.3 Proportion and cumulative percentage of smokers in high-burden countries, in men (A) and women (B) in 2009



From WHO (2011)

disaggregation of the regions by country shows that a few countries account for a large proportion of tobacco smokers. Ranked in descending order of the number of smokers, the five countries of China, India, United States of America (USA), Russian Federation and Indonesia account for about 52% of adult smokers in the world, with China and India alone accounting for 40% (Fig. 1.3). Furthermore, nearly two-thirds of the world's smokers live in only ten countries of the world.

1.3.3 Distribution of smokers by sex

With a global average smoking prevalence of 36%, men account for just over 80% of all smokers. The male adult prevalence is 4–5 times that for women, at 8%. This difference varies across WHO

regions. Smoking among men, concentrated in the five countries of China, India, Indonesia, Russian Federation and USA (Fig. 1.3), accounts for about 56% of global smoking among men. Women smokers are mostly concentrated in EURO and AMRO. These two regions account for 40% and 26% of all women smokers globally, respectively. The prevalences for women in these two regions are about half of those in men, whereas the difference is substantially greater in the other regions. Just as men smoke more than women everywhere, so too among young people, boys generally smoke more than girls. There is an increasing concern, however, that the gap may diminish, not because of a reduction in boys prevalence but because of an increase in the proportion of girls who are taking up smoking (Warren *et al.*, 2006).

1.3.4 The four stage smoking model

(a) The four stages of tobacco use

Lopez *et al.* (1994) used trend data on smoking prevalence and tobacco attributable mortality to show the evolution of tobacco use in a country. Four stages of smoking and attributable mortality have been identified to represent the growth and eventual decline of smoking among men and women (Fig. 1.4).

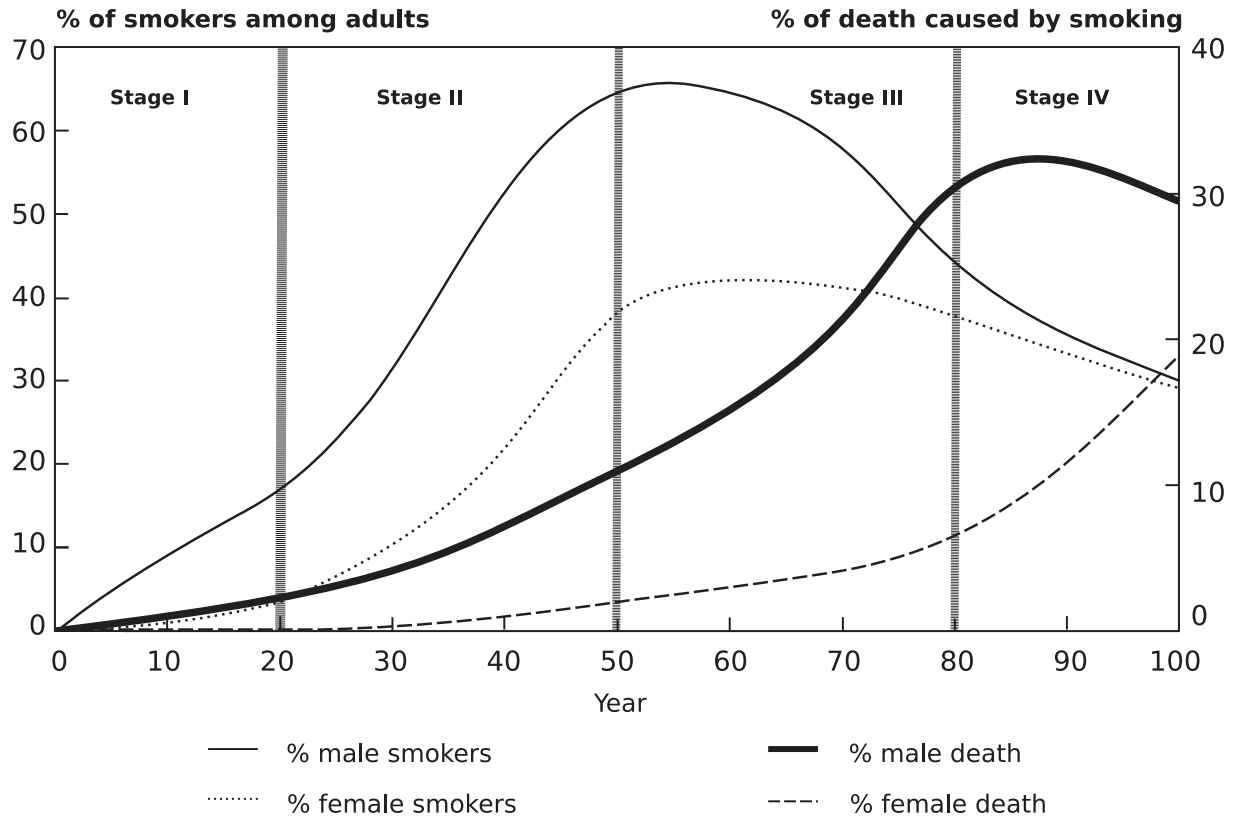
Stage 1 is characterized by low smoking prevalence in men (less than 15%) and very low in women (less than 10%). Death and disease from smoking are not apparent in this phase, as nearly all health effects from smoking are related to past smoking habits and their cumulative effects rather than current smoking. In Stage 2, smoking prevalence in men rapidly increases while it increases more slowly in women. Towards the end of this stage, smoking prevalence in men typically peaks to lie at 50–60%, with 10% of deaths in men attributable to smoking; deaths in women are comparatively fewer. After a protracted period of high smoking prevalence, Stage 3 shows a decline in smoking prevalence in men to around 40%. Smoking prevalence in women peaks and then begins to decline; towards the end of this stage the gap between men's and women's prevalence starts to narrow. However, smoking attributable deaths in men increase from around 10% to 25–30% within a span of three decades; in women the deaths are increasing but are still quite low. In the final Stage 4, smoking prevalences in both men and women continue to decline albeit relatively slowly in comparison with Stage 3, with the gap substantially narrowing to lie at around five percentage points, and as little as one percentage point in some countries. In Stage 4, smoking mortality in men peaks to between 30–35% and then declines to below 30% at the end of this period. In women, the health effects from past smoking persist, with increasing mortality, but remain lower than in men, and recently have begun to decline in some countries.

(b) Smoking prevalence worldwide

Using prevalence data for men and women collected in 2006 for 140 countries, WHO determined at which stage of the tobacco epidemic countries are in the model of Lopez *et al.* (1994). In Fig. 1.5, countries have been ranked by smoking prevalence in men in ascending order for Stages 1 and 2, and then in descending order for Stages 3 and 4. (Smoking prevalence in men is almost always higher than in women, with a few exceptions observed in the fourth stage.) While most countries fit the classification, there are a few exceptions, most of which in the last stage. Prevalence between Stage 3 and Stage 4 is discontinuous in both sexes. This is due to the classification followed, which puts countries with a relatively narrow difference in prevalence between men and women in Stage 4 even though their prevalence is largely comparable with those in Stage 3.

Most African countries fall in the first stage of the smoking model, characterized by low smoking prevalence in men and very low prevalence in women. Three of the five high burden

Fig. 1.4 The four stages of the tobacco epidemic



From Lopez *et al.* (1994)

countries fall in stage 2 (India, Indonesia and China), with the rest comprising a combination of countries from Africa, South East Asia, eastern Europe and the Middle East. At this stage smoking prevalences in women continue to remain very low, most countries having a prevalence in adult women of less than 10%.

Stage 3 includes the fourth high burden country (Russian Federation), along with countries in eastern Europe, South America and western Europe, which fall at the end of Stage 3. Stage 4 is populated entirely by the developed countries of western Europe, North America and Oceania. The USA, the fifth high burden country, fall in the last stage as a result of the relatively small difference in the smoking prevalence between men and women compared to the other intermediate stages. As mentioned before, Stage 4 includes countries where the smoking prevalence is higher in women than in men, with a small (< 8%) difference.

(c) Age-specific prevalence

Age-specific prevalence for men and women aged 15 years or older is presented for six representative countries for current smoking (Fig. 1.6). There are wide variations in age-specific prevalence between these countries. In men, prevalence varies from less than 10% to 75% in the 15–19 years age range to lie between 10% and 55% in the oldest age range. Prevalence among women varies from less than 1% to as high as 45% in young adults (15–19 years). Unlike men, prevalence in women tends to converge after age

Fig. 1.5 Prevalence of smoking in 140 countries in 2009, staged according to the model by Lopez

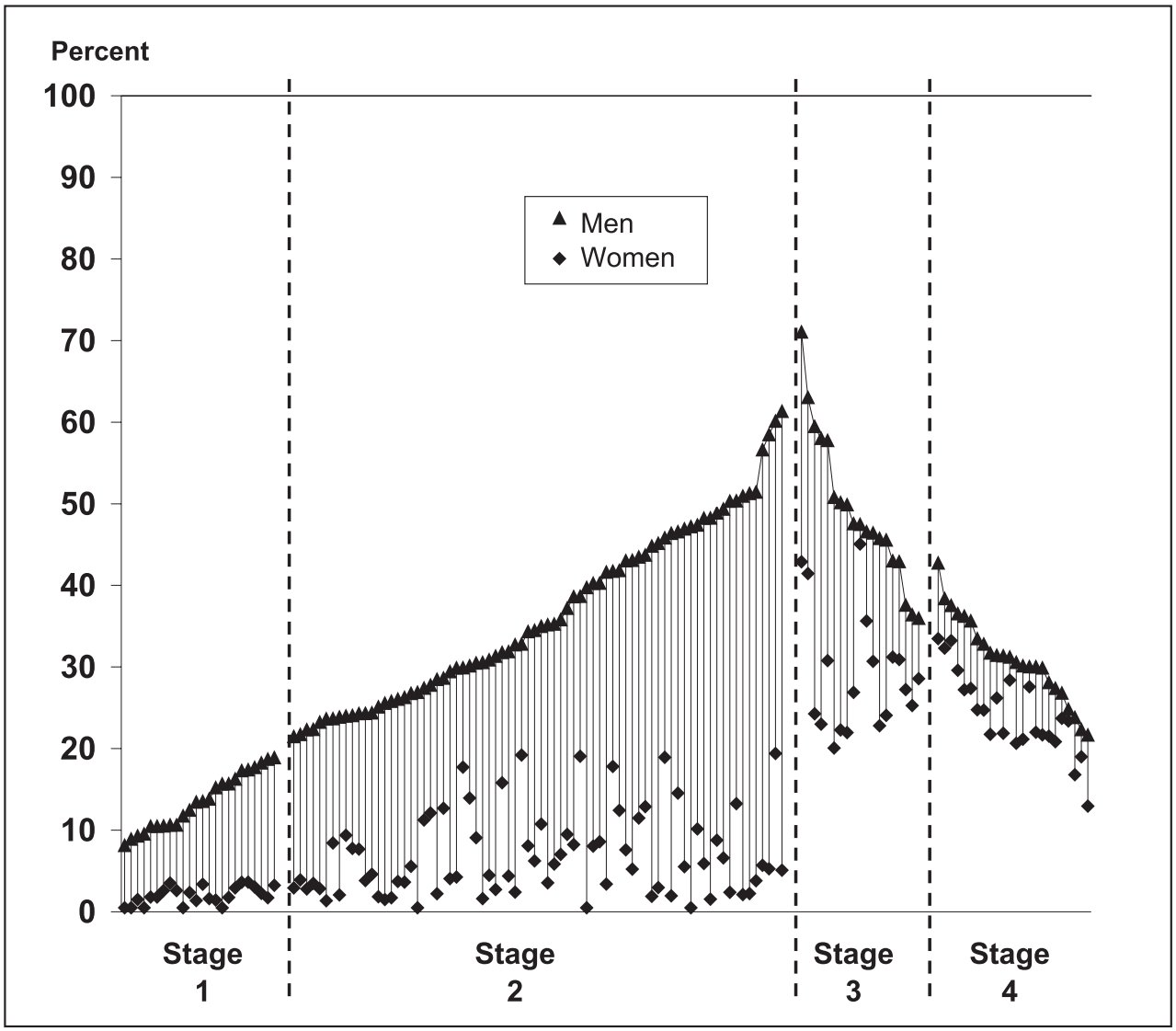
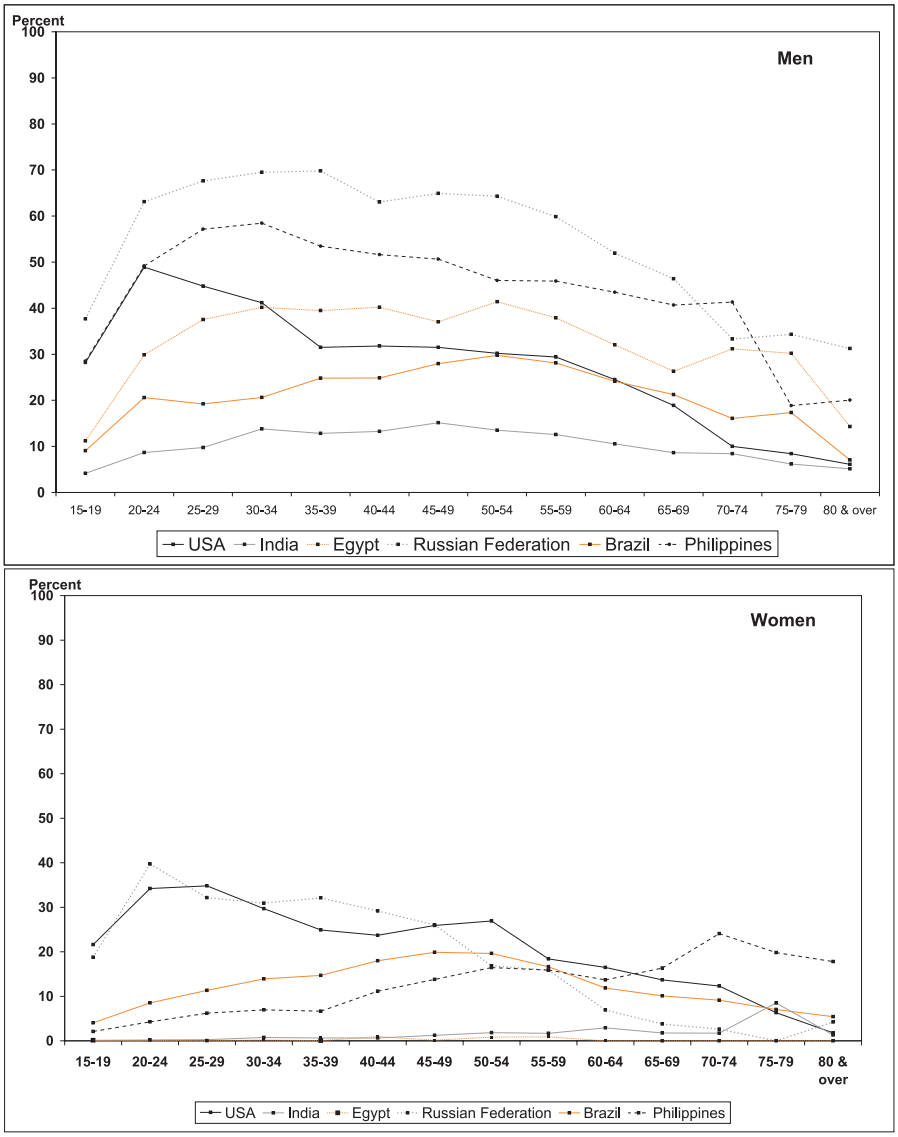


Fig. 1.6 Age-specific rates of smoking prevalence, in men and women in 2009



50, lying within 15 percentage points. Prevalence in women is almost always lower than in men in all age groups.

Initiation of smoking is shifting, and is taking place at earlier ages in both developed and developing countries. In developed countries, quitting smoking is also shifting to occur at a younger age, whereas in developing countries there is no such evidence.

(d) Smoking in youth

Information on smoking habits in youth are collected from a variety of youth surveys that include the Global Youth Tobacco Survey (GYTS), Global school-based Student Health Survey (GSHS) and Health Behaviour in School Aged Children (HBSC). Some countries have their own youth surveys, or have them as part of a general health or household survey, such as the Student Survey in Argentina, the Youth Smoking Survey in Canada, and New Zealand's Tobacco Survey.

The GYTS is a school-based survey designed to monitor tobacco use among youths aged 13 to 15 years. The GYTS uses a standard set of questions and sampling methods in over 160 countries. The survey has core questions that span seven thematic areas pertinent to tobacco. In addition to these, countries can include country-specific questions that allow assessment of tobacco use unique to the country. To assess prevalence of smoking, students are asked to report their smoking habits for both cigarettes and other tobacco products that they may have consumed over the past 30 days. Since its inception in 1999, the GYTS has covered over 2 million students. Although most GYTS are national surveys, in some countries they are limited to subnational locations. Further, countries conduct the GYTS in different years, rendering comparison for the same year difficult.

Prevalence of current tobacco use [including smokeless tobacco] in youth in 2004–09 for fourteen high burden low and middle income countries is shown in Fig. 1.7. The Russian Federation has the highest prevalence of current tobacco use among the high burden countries for which national data are available. Further, in the Americas and Europe the difference in prevalence between boys and girls is smaller than in other regions. In contrast, in Egypt, India and Thailand, prevalences in boys are significantly higher than in girls.

Fig. 1.8 shows the range of current tobacco use by WHO region for boys and for girls and for both sexes combined. There are wide variations in current tobacco use within each region. The largest variations are observed in EMRO and SEARO irrespective of sex, reflecting potentially disparate initiation rates in countries within the region. In AFRO, the range of current tobacco use between boys and girls is virtually the same. In some countries (e.g. Argentina, Peru, Sierra Leone, Bulgaria, Croatia, Cook Islands, New Zealand), tobacco use in girls exceeds that in boys; but overall boys and girls show remarkably similar propensity to take up tobacco use.

Warren *et al.* (2006) present global estimates and regional averages for current tobacco smoking in youth using GYTS data spanning 1999–2005. Their estimates show that one in five boys and one in seven girls currently smoke tobacco. Prevalence of current smoking for both boys and girls combined was highest in AMRO (22.2%) and lowest in WPRO (11.4%). AMRO have the highest average for current tobacco smoking for boys (24%) and for girls (20.4%) whereas the lowest prevalence was in WPRO among boys (15%) and in SEARO among girls (7.1%).

1.4 Regulations and policies: the WHO Framework Convention on Tobacco Control

The WHO Framework Convention on Tobacco Control (WHO FCTC) – the first multilateral evidence-based treaty on tobacco control

Fig. 1.7 Prevalence of current tobacco use in youth for selected countries, 2005–2009

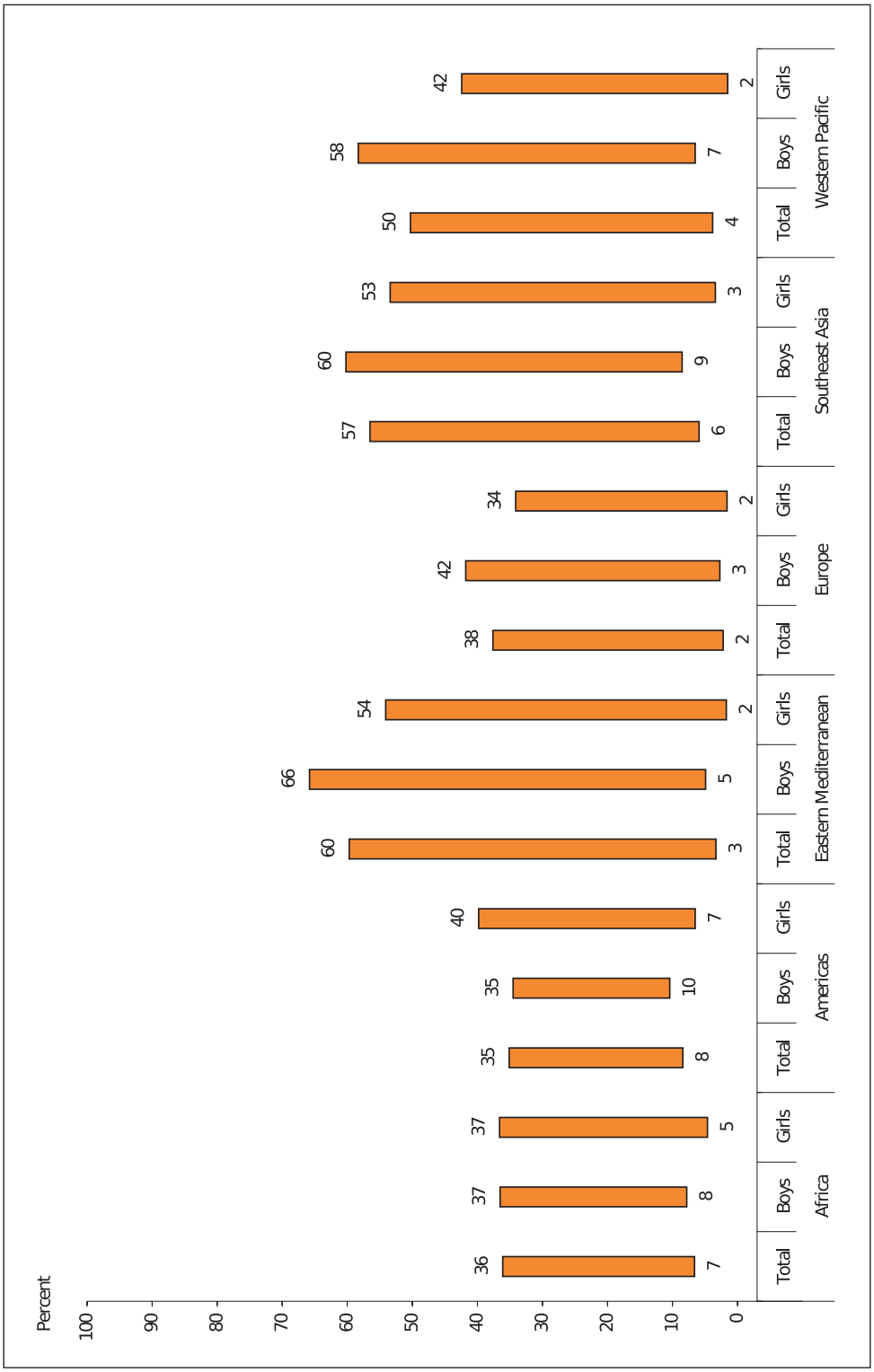
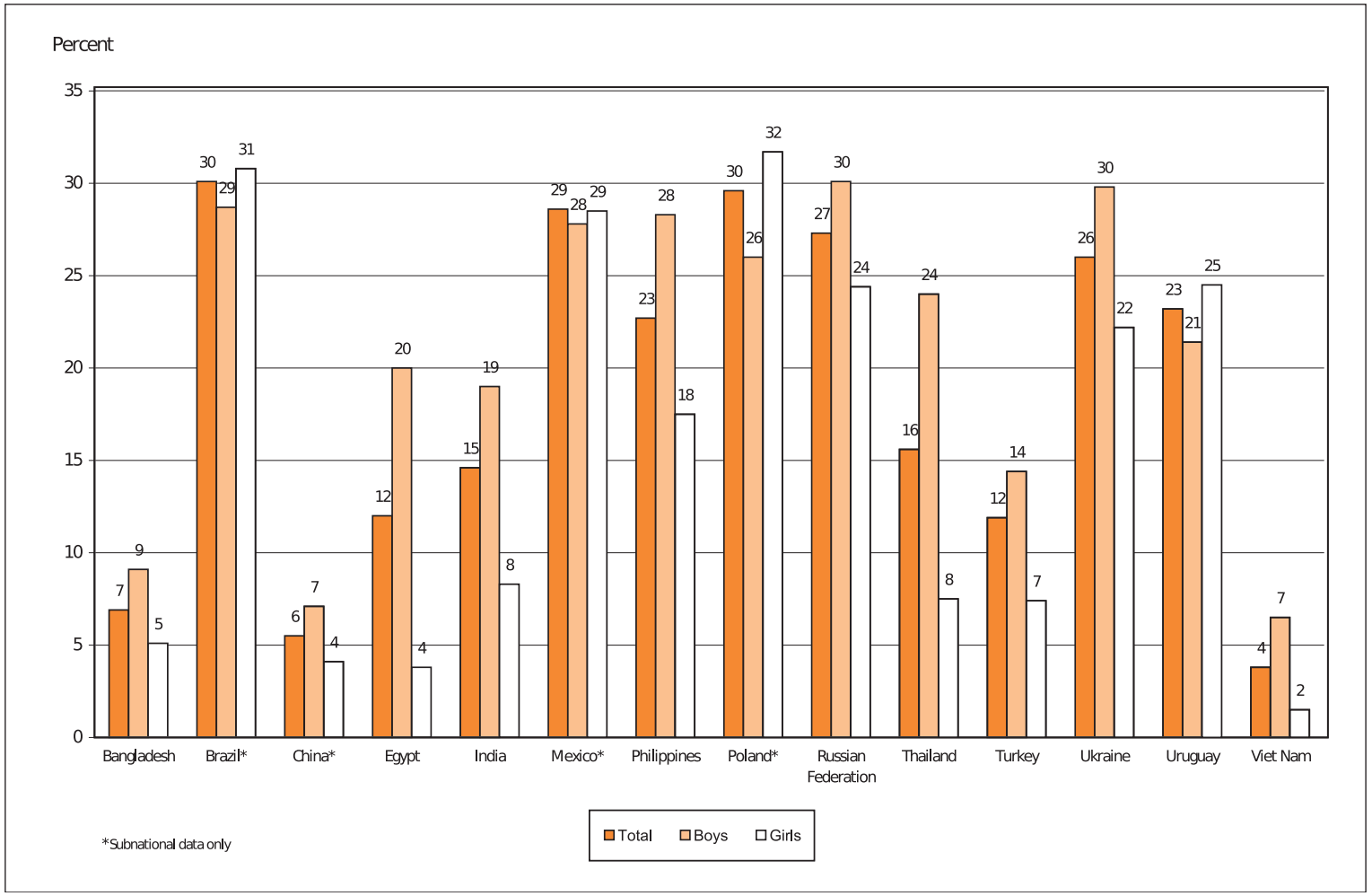


Fig. 1.8 Range of prevalence of current tobacco use in youth, 2005–2009, by WHO region



Figures have been rounded off and show prevalences in countries with national and subnational jurisdiction.

– articulates tobacco control measures available to countries to counter the growing tobacco epidemic. This treaty, which entered into force in 2005, represents one of the most universal treaties in the United Nations history. In 2008, the WHO launched MPOWER, a technical assistance package comprised of six strategies that reflects one or more of the WHO FCTC measures and helps countries meet their commitments to the WHO FCTC.

2. Cancer in Humans

2.1 Introduction

The available knowledge on the relationship between tobacco smoking and a variety of human cancers is based primarily on epidemiological evidence. An immense amount of such evidence has been obtained, and only a small proportion can be referred to here. The cancers considered to be causally related to tobacco smoking in the previous *IARC Monograph* on tobacco smoking (IARC, 2004a) included lung, oral cavity, nasal cavity and paranasal sinuses, nasopharynx, oropharynx, hypopharynx, larynx, oesophagus (adenocarcinoma and squamous cell carcinoma), upper aerodigestive tract combined, stomach, pancreas, liver, kidney (body and pelvis), ureter, urinary bladder, cervix and myeloid leukaemia. In addition, it was concluded that there was evidence suggesting lack of carcinogenicity for cancers of the breast and of the endometrium.

Since 2002, there have been additional cohort and case–control studies on the relationship of tobacco smoking in different forms to these and other cancers in many countries. A large body of evidence has been obtained from cohort studies with respect to different cancer sites and types of tobacco product. These cohort studies are described briefly in Table 2.1 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.1.pdf), listed by country. Case–control studies are described in the sections pertaining to cancer sites. More studies are now available from countries and populations that are still at an early stage of the tobacco epidemic. These studies are prone to underestimate the true strengths of the association between tobacco smoking and any specific cancer as the full effect of duration of smoking cannot be evaluated.

2.2 Cancer of the lung

2.2.1 Overview of studies

The main cause of lung cancer in humans is tobacco smoking and most information establishing this fact comes from epidemiological studies in which the assessment of exposure was based on self-reported information on personal smoking habits via self-administered questionnaire or in-person interviews. Since the previous *IARC Monograph* (IARC, 2004a), numerous studies have been published on the issues of tobacco smoking and sex and racial/ethnic susceptibility, 'tar' yields as measured by machine smoking, the relationship between histological changes and the design of cigarettes, dose–response association, genetic susceptibilities and interactions.

2.2.2 Factors affecting risk

Recent epidemiological studies incorporating measures of smoking metabolites in serum or urine are helping to refine our understanding of exposure-response relationships with tobacco smoke. Dose–response evidence has been obtained from three cohort studies (Flanders *et al.*, 2003; Boffetta *et al.*, 2006; Yuan *et al.*, 2009; Table 2.2 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.2.pdf) and four pooled analyses (Lubin & Caporaso, 2006; Lubin *et al.*, 2007a, b, 2008; Table 2.3 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.3.pdf)

since the previous *IARC Monograph* (IARC, 2004a).

The US American Cancer Society Cancer Prevention Study-II (CPS-II) is the largest cohort study on smoking and lung cancer risk using questionnaire assessment of exposure (Flanders *et al.*, 2003). In this study cigarette smoking duration is a much stronger predictor of lung cancer mortality than is cigarette smoking intensity, regardless of age in both men and women. These results are qualitatively similar to those reported by Doll & Peto (1978) and are consistent with IARC (2004a).

In a questionnaire-based assessment of the association of tobacco smoking with lung cancer risk, smokers at higher smoking intensities seem to experience a "reduced potency" per pack such that for equal total exposure, the excess odds ratio per pack–year decreases with intensity (Lubin *et al.* 2008). Below 15–20 cigarettes/day, the excess odds ratio/pack–year increases with intensity (Lubin & Caporaso, 2006; Lubin *et al.*, 2007a) while above 20 cigarettes/day, there is an 'inverse-exposure-rate' effect (Lubin *et al.*, 2007a) suggesting a greater risk for total exposure delivered at lower intensity (or a longer duration) than the equivalent exposure delivered at a higher intensity. The intensity effects were also statistically homogeneous across diverse cancer types, indicating that after accounting for risk from total pack–years, intensity patterns were comparable for cancer of the lung, bladder, oral cavity, pancreas and oesophagus. These analyses suggest that the risk of lung cancer increases with increasing tobacco exposure at all dose levels, but there is some levelling-off effect at the highest intensity of tobacco smoking.

However, when serum cotinine was used as a measure of exposure to tobacco smoking, rather than questionnaire-based data, the odds ratio of lung cancer increased linearly over the full range of exposure from ≤ 5 ng/mL through ≥ 378 ng/mL, with an odds ratio of 55.1 (95% confidence interval (CI): 35.7–85.0) in the highest exposure group. These results suggest that the decreased rate of lung cancer risk at high intensity of tobacco smoke previously described is a statistical artefact. Such an effect may be due to an inaccurate assessment of total tobacco smoke exposure from questionnaire-based studies at high exposure levels (Boffetta *et al.*, 2006). Somewhat similar results were obtained when both 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) and total cotinine in urine were measured in subjects of two large cohort studies from Shanghai men and Singapore men and women (Yuan *et al.*, 2009). Among smokers with comparable smoking histories (as noted in questionnaire data) there is a 9-fold variation in subsequent risk of lung cancer between those with high and those with low levels of total urinary NNAL and cotinine. Thus measurements of urinary cotinine and total NNAL at a single point in time in a smoker could substantially improve the predictive power of a lung cancer assessment model based solely on self-reported smoking history (number of cigarettes/day, number of years of regular smoking). A positive NNAL-lung cancer association of comparable magnitude was observed in both Shanghai and Singapore subjects despite differences in the NNK content of tobacco smoked. The independent association between total urinary cotinine and lung cancer risk, after adjustment for total urinary NNAL and smoking history, suggests that tobacco smoke compounds other than NNK play a role in lung cancer development in smokers. Further, a single measurement of urinary NNAL may closely predict the average level of NNAL measured over a much longer period of time.

2.2.3 Types of tobacco or of cigarette

(a) Tar levels

In a previous *IARC Monograph* (IARC, 1986), it was concluded on the basis of the case–control, cohort studies and ecological evaluations

available at the time that prolonged use of 'high-tar' and unfiltered cigarettes is associated with greater risks than prolonged use of filter-tipped and 'low-tar' cigarettes. More recently (IARC, 2004a), it has been recognized that the actual quantitative impact of reduced 'tar' and filter-tipped cigarettes is difficult to assess because of, respectively, the concomitant increase in tobacco-specific nitrosamines that accompanies the greater use of blend tobacco and the compensatory changes in smoking behaviour by smokers attempting to maintain their accustomed level of nicotine intake. Nevertheless, it was concluded that changes in cigarette types since the 1950s have probably tended to reduce the risk for lung cancer associated with tobacco smoking.

Additional refinement in assessing the health effects associated with smoking cigarettes of various tar content has been possible since the publication of the earlier reports. Compared with smokers of medium tar (15–21 mg) filtered cigarettes risk was higher among men and women who smoked high tar (≥ 22 mg) non-filtered brands but there was no difference in risk among men and women who smoked 'very low tar' or 'low tar' brands compared with those who smoked 'medium tar' brands (Harris *et al.*, 2004). Regardless of tar content of their cigarettes, all current smokers had a far greater risk for lung cancer than people who had stopped smoking or had never smoked (Harris *et al.*, 2004).

(b) Mentholated cigarettes

In the previous *IARC Monograph* (IARC, 2004a) the conclusion was drawn that there is no additional risk associated with smoking mentholated cigarettes when total consumption (pack–years) was controlled versus non-mentholated ones. Recent evidence supports that conclusion.

Mentholated cigarettes first appeared in the 1920s, but were not widely used until the mid-1950s (Bogen, 1929; Federal Trade Commission, 2001). Since the early 1970s, menthol varieties have accounted for 25–60% of all cigarettes sold in the USA (Federal Trade Commission, 2001). There are strong ethnic differences in the use of menthol cigarettes; more than 60% of Black smokers of both sexes use menthol brands compared to fewer than 25% of White smokers (Royce *et al.*, 1993; Hymowitz *et al.*, 1995). Studies have generally not demonstrated an increased risk of lung cancer for mentholated cigarettes versus non-mentholated cigarettes (Kabat & Hebert, 1994; Carpenter *et al.*, 1999; Brooks *et al.*, 2003; Stellman *et al.*, 2003). Recent evidence also suggests that users of mentholated cigarettes smoke fewer pack–years than those of non-mentholated cigarettes.

The higher incidence of lung cancer among Blacks is an important public health concern but the causes remain unclear. Mentholated cigarette use does not appear to explain the racial disparity observed in lung cancer risk among those having the same total tobacco consumption.

2.2.4 Histology

Compiled databases from IARC and other sources indicated that squamous cell carcinoma rates [per 100000 person-years] among men declined by 30% or more in North America and some European countries between 1980–82 and 1995–97, while changing less dramatically in other areas; small cell carcinoma rates decreased less rapidly. In contrast, the proportion of adenocarcinoma cases rose among men and women in virtually all areas, with the increases among men exceeding 50% in many areas of Europe (Devesa *et al.*, 2005).

Based on a comparison of two large cohort studies initiated by the American Cancer Society (ACS) (CPS-I and CPS-II) in 1960 and 1980, respectively, a stronger association between smoking and adenocarcinoma was observed in recent compared to earlier follow-up periods (Thun & Heath, 1997). Additionally, an association between cigarette smoking and bronchioloalveolar carcinoma was also found in several

studies (Falk *et al.*, 1992; Morabia & Wynder, 1992).

A meta-analysis of 8 cohort and 14 case–control studies conducted in Japan among active smokers indicated significant excess lung cancer risks among men for both squamous cell carcinoma (relative risk (RR), 11.7) and adenocarcinoma (RR, 2.30). Among women the risks were 11.3 for squamous cell carcinoma and 1.37 for adenocarcinoma (Wakai *et al.*, 2006).

2.2.5 Population characteristics

(a) Sex

Meta-analyses on sex-specific susceptibility to lung cancer associated with tobacco smoking are presented in Table 2.4 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.4.pdf) and cohort studies in Table 2.5 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.5.pdf).

In the 1990s, two case–control studies indicated that relative risks for lung cancer associated with specific amounts and duration of cigarette smoking may be higher among women than among men (Risch *et al.*, 1993; Zang & Wynder, 1996).

In the large NIH-AARP [National Institutes of Health-American Association of Retired People] cohort (Freedman *et al.*, 2008), smoking was associated with lung cancer risk in both men and women. Age-standardized incidence rates for lung cancer tended to be higher in men than in women with comparable smoking histories (for current smokers and for quitters of less than 10 years), and in cases with squamous cell tumours. However, lung cancer risk was generally similar between men and women.

In a joint analysis, results from the Nurses' Health Study of women and the Health Professionals Follow-up Study in men (Bain *et al.*, 2004) suggest little difference in lung cancer susceptibility between men and women given equal smoking exposure. The hazard ratio in women ever smokers compared with men was 1.11 (95%CI: 0.95–1.31).

Serum cotinine levels were analysed in lung cancer cases and controls (Boffetta *et al.*, 2006). The lung cancer odds ratios (ORs) estimated for men and women were very similar for those with comparable serum cotinine levels. Other studies that have carefully quantified tobacco exposure via self-administered questionnaire or interview provide additional evidence of a comparable increase in lung cancer risk in the two sexes (Kreuzer *et al.*, 2000; Flanders *et al.*, 2003; Bain *et al.*, 2004).

In a meta-analysis of observational studies on cigarette smoking and cancer from 1961–2003 (conducted on 177 case–control studies, 75 cohort studies and two nested case–control studies), dose–response estimates were available in 44 studies: 19 with estimates for men only, 11 with estimates for women only and 14 with separate estimates for men and women (Gandini *et al.*, 2008). Overall, the risk of lung cancer for men and women increased by 7% for each additional cigarette smoked per day (RR, 1.07; 95%CI: 1.06–1.08). The increased risk appears to be slightly higher in women (RR, 1.08; 95%CI: 1.07–1.10) than in men (RR, 1.07; 95%CI: 1.05–1.08) ($P < 0.001$; adjusting for study type).

(b) Ethnicity

It has been postulated that susceptibility to lung cancer from tobacco smoking may differ by race and ethnicity (Schwartz & Swanson, 1997; Peto *et al.*, 1999; Stellman *et al.*, 2001; Kiyohara *et al.*, 2004, 2005, 2006; Pinsky, 2006; Wakai *et al.*, 2006; Vineis *et al.*, 2007; Takahashi *et al.*, 2008; Table 2.6 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.6.pdf). Lung cancer incidence rates vary considerable across racial/ethnic groups in the USA and elsewhere. Black men have higher rates than white men, while Hispanics, Asians and American Indians of both sexes have lower rates than whites (Stellman *et al.*, 2003; SEER, 2004).

Nutritional habits, smoking patterns, type of tobacco smoked and genetic factors may play a role in such differences between racial and ethnic groups.

The association of tobacco smoking and lung cancer does not appear to be as strong among Japanese as among populations of North America or Europe (Wakai *et al.*, 2006). In a meta-analysis of 8 cohort studies and 14 case–control studies conducted in Japan, the excess lung cancer risks observed for both men (RR, 4.39; 95%CI: 3.92–4.92) and women (RR, 2.79; 95%CI: 2.44–3.20) in both case–control and cohort studies were lower than would have been expected from studies in North America and Europe. The lower lifetime consumption of cigarettes in Japanese, due in part to a later initiation of smoking and a lower consumption per day has been suggested to explain this. Other differences that may have etiological significance include tobacco ingredients, different filters on cigarettes, lifestyle factors including diet, and possibly differences in genetic susceptibility. [The Working Group noted that North American or European populations were not directly included in any of these studies.]

Data from the Asian Pacific Cohort Studies Collaboration, 31 studies involving 480125 persons, evaluated the risk of death from lung cancer associated with smoking habits in Australia, New Zealand and Asia (Huxley *et al.*, 2007b). Among Asian men the hazard ratio was 2.48 versus 9.87 in men in Australia and New Zealand. Among women, the corresponding estimates were 2.35 and 19.33, respectively. [In these studies, Asian populations smoked fewer cigarettes for a shorter period of time compared to those in Australia and New Zealand.]

Based on data from the National Cancer Institute's Surveillance, Epidemiology, and End Results program (SEER), Chinese women residing in the USA have a fourfold increased risk of lung cancer, and Filipino women a twofold increased risk, compared to that expected based on rates in non-Hispanic whites in the USA with a similar amount of cigarettes smoked (Epplein *et al.*, 2005). Among Chinese women, the increased risk was largely restricted to adenocarcinoma and large cell undifferentiated carcinoma. Chinese females residents of the western US mainland have a much higher risk of lung cancer than would be expected from their tobacco use patterns, just as they do in Asia (Peto *et al.*, 1999; Epplein *et al.*, 2005), the reason for these difference have not been identified. [Controlling for potential confounding factors was limited using aggregate data from SEER.]

Age, sex and race-specific risks of lung cancer mortality among lifetime non-smokers were compared in the two large ACS Cancer Prevention Study cohorts (CPS-I; CPS-II). The mortality rate was higher among African American women than among white women in CPS-II (hazard ratio (HR), 1.43; 95%CI: 1.11–1.36) (Thun *et al.*, 2006). This suggests an inherent susceptibility difference between white and black women but it could also be explained by access to care, diet, or exposure to environmental carcinogens.

The risk for lung cancer associated with cigarette smoking in 183813 African-American, Japanese-American, Latino, native Hawaiian and white men and women was examined in the Multiethnic Cohort Study in the USA (Haiman *et al.*, 2006). Information on demographic factors, smoking status, cigarettes/day smoked, years of smoking, years since quitting, diet, occupations, educational level and racial and ethnic group were collected for all subjects through a self-administered questionnaire at enrolment. Information about age of smoking initiation and cessation rates were collected on a subgroup of 5090 study subjects. Incident lung cancer cases were identified by linkage to the SEER cancer registries covering California and Hawaii. Among those who smoked no more than 10 cigarettes/day and those who smoked 11–20 cigarettes/day, relative risks ranged from 0.21 to 0.39 ($P < 0.001$) among Japanese Americans and Latinos and from 0.45

to 0.57 ($P < 0.001$) among whites as compared with African Americans. However, at levels exceeding 30 cigarettes/day, differences between racial/ethnic groups were no longer significant. The differences in lung cancer risk by racial group associated with smoking were observed for both men and women and for all histological types of lung cancer. These findings could not be explained by differences between populations in other known or suspected risk factors, including diet, occupation, and education level or by age at starting smoking or cessation of smoking.

Polymorphisms in glutathione-*S*-transferase (GST), GSTM1, GSTT1 and GSTP1 genes in humans are associated with reduction of enzymatic activity towards several substrates, including those found in tobacco smoke. In a population based case–control study involving early-onset lung cancer, African Americans carrying at least one G allele at the GSTP1 locus were more likely to have lung cancer compared with African Americans without a G allele after adjustment for age, sex, pack–years of smoking and a history of lung cancer in a first degree relative (OR, 2.9; 95%CI: 1.29–6.20). African Americans with either one or two risk genotypes at the GSTM1 (i.e. null genotype) and GSTP1 loci were at increased risk of having lung cancer compared with those having fully functional GSTM1 and GSTP1 genes (one risk genotype: OR, 2.8; 95%CI: 1.1–7.2 and two risk genotypes: OR, 4.0; 95%CI: 1.3–12.2). No significant single gene associations between GSTM1, GSTT1 and GSTP1 and early-onset lung cancer were observed in Caucasians, after adjusting for age, sex, pack–years and a family history of lung cancer (Cote *et al.*, 2005).

The cytochrome P450 (CYP) superfamily of enzymes catalyses one of the first steps in the metabolism of carcinogens such as polycylic aromatic hydrocarbons, nitroaromatics and arylamines. A population-based case–control study of lung cancer in the metropolitan Detroit area found that neither CYP1A1 MspI nor CYP1A1 Ile^{462}Val was associated with lung cancer susceptibility among Caucasians or African Americans. Among Caucasians, however, CYP1B1 Leu^{432} Val was significantly associated with lung cancer susceptibility (OR for at least one Val allele, 2.87; 95%CI: 1.63–5.07). Individuals with both this polymorphism and exposure to second-hand tobacco smoke were at particularly high risk for lung cancer. Combinations of particular CYP1B1 polymorphisms appeared to increase risk, although no combination differed significantly from the risk associated with CYP1B1 Leu^{432} Val alone (Cote *et al.*, 2005; Wenzlaff *et al.*, 2005).

The hypothesis that polymorphisms in TP53 may modulate the risk for lung cancer associated with tobacco smoke was evaluated in a case–control study of lung cancer in Baltimore, Maryland. African-Americans with Pro-T-A-G-G haplotype (combining the polymorphisms TP53_01 (rs1042522), TP53_65 (rs9895829), TP53_66 (re2909430), TP53_16 (rs1625895), and TP_11 (rs12951053)) had both an increased risk for lung cancer (HR, 2.32; 95%CI: 1.38–4.10) and a worsened lung cancer prognosis (HR, 2.38; 95%CI: 0.38–4.10) compared with those having the Arg-T-A-G-T haplotype. No association of TP53 polymorphisms with lung cancer was observed in Caucasians (Mechanic *et al.*, 2007). Common genetic variation in TP53 could modulate lung cancer pathways in African Americans. Differences in lung cancer susceptibility may exist based on race, tobacco exposure and selected genetic polymorphisms (Mechanic *et al.*, 2007).

2.2.6 Interactions

(a) Diet and exercise

Antioxidant vitamins, carotenoids, isothiocyanates, total dietary vegetables and fruit, and physical exercise have been associated with a decreased risk for cancer in some studies but the overall protective effect of diet and exercise account for only a small fraction of the total risk associated with tobacco smoking.

The association of fruit and vegetable with lung cancer incidence among both smokers and non-smokers was evaluated in the European Prospective Investigation into Cancer and Nutrition (EPIC). In current smokers lung cancer risk was significantly decreased with higher vegetable consumption, the hazard ratio being 0.78 (95%CI: 0.62–0.98) per 100 g increase in daily vegetable consumption, and 0.90 (95%CI: 0.81–0.99) per 100 g fruit (Linseisen *et al.*, 2007). While overall consumption of fruits and vegetables was not found to be protective of lung cancer in the NIH-AARP Diet and Health Study, higher consumption of several botanical subgroups (i.e. rosaceae, convolvulaceae, and umbelliferae) was significantly inversely associated with risk, but only in men (Wright *et al.*, 2008).

Cruciferous vegetables (i.e. broccoli, cabbage, cauliflower, Brussels sprouts, kale) are rich in isothiocyanates and have been hypothesized to have anticancer properties that may contribute to reduced risk for lung cancer. Isothiocyanates may inhibit the bioactivation of procarcinogens found in tobacco smoke such as polycyclic aromatic hydrocarbons and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (Hecht, 2000). Isothiocyanates may also enhance excretion of carcinogenic metabolites before they can damage DNA (Gasper *et al.*, 2005). Furthermore, sulforaphane, a major isothiocyanate found in broccoli, can induce cell cycle arrest and apoptosis (Seow *et al.*, 2005). GSTM1 and GSTT1 encode isoenzymes that play an important role in xenobiotic metabolism (Hecht, 2000). Individuals with homozygous deletion of GSTM1 and GSTT1, or both may metabolize isothiocyanates less efficiently and may be more intensely exposed to isothiocyanates after consumption of cruciferous vegetables. Epidemiological evidence from 30 studies on the association between lung cancer and either total cruciferous vegetable consumption (6 cohort and 12 case–control studies) or specific cruciferous vegetables (1 cohort and 11 case–control studies) was recently evaluated (Lam *et al.*, 2009). The risk for lung cancer among those in the highest category of total cruciferous vegetable intake was 22% lower in case–control studies (pooled OR, 0.78; 95%CI: 0.70–0.88) and 17% lower in cohort studies (pooled RR, 0.83; 95%CI: 0.62–1.08). The strongest inverse association of total cruciferous vegetable intake with lung cancer was seen among individuals with GSTM1 and GSTT1 double null genotypes (OR, 0.41; 95%CI: 0.26–0.65; p for interaction = 0.01). The inverse association was observed in both smokers and non-smokers.

The potential role of vitamin A in the development of lung cancer attracted early research interest (Bjelke, 1975). Carotenoids were thought to have anti-cancer properties and early evidence from case–control studies tended to support an inverse association of lung cancer incidence with β-carotene intake and with serum concentrations of β-carotene. However, the case–control design is not ideal for assessing the effect of serum carotenoids as a risk factor for lung cancer risk since the disease is likely to effect serum levels. In a meta-analysis of six randomized clinical trials and 25 prospective observational studies, Gallicchio *et al.* (2008) computed a pooled relative risk for studies comparing β-carotene supplements with placebo of 1.10 (95%CI: 0.89–1.36). Among observational studies, the pooled relative risk for total carotenoid dietary intake from six studies was 0.86 (95%CI: 0.75–0.99) among current smokers. For dietary intake of β-cryptoxanthin, data from six studies gave a pooled relative risk among smokers of 0.75 (95%CI: 0.58–0.96). No other carotenoids significantly reduced the risk in current, former or never smokers.

Based on a review of the literature, antioxidant vitamins show no clear protective effect on lung cancer risk in smokers or non-smokers, although there was some, albeit inconsistent, evidence pointing to a protective role for vitamin C and E. No clear protective role was observed for vitamin A (Ruano-Ravina *et al.*, 2006).

Increased physical activity has been associated with a reduction in the incidence and mortality from all-site cancer and some site-specific cancers in studies of non-smokers, but less is known about whether physical activity is associated with similar risk reduction in smokers. Several early studies suggested that physical activity is associated with decreased risk of lung cancer in men and women after adjusting for smoking, with risk reductions estimated from 18% (Peterson *et al.*, 2001) to 62% (Kubík *et al.*, 2001). The effect of physical activity on lung cancer risk was assessed in a sample drawn from participants in the Beta-Carotene and Retinol Efficacy Trial. The results suggested that physical activity may play a small role in reducing cancer risk and mortality among those with significant tobacco exposure. The incidence of lung cancer and of all cancer sites combined seemed to be more attenuated by exercise in men than in women, while the attenuation in lung cancer mortality was greater in women than in men. These effects may be more pronounced for younger people and may differ inconsistently by pack–years of smoking (Alfano *et al.*, 2004).

(b) Radon

In a pooled analysis of data from 13 case–control studies of residential radon and lung cancer from nine European countries (7148 cases of lung cancer and 14208 controls), the dose–response relation seemed to be linear with no threshold and remained significant in analyses limited to individuals from homes with measured radon < 200 Bq/m^3. The absolute risks of lung cancer by age 75 years at radon concentrations of 0, 100, and 400 Bq/m^3 would be about 0.4%, 0.5% and 0.7%, respectively, for lifelong non-smokers, and about 25 times greater (10%, 12% and 16%) for cigarette smokers. These studies show appreciable hazards from residential radon, particularly for smokers and recent ex-smokers (Darby *et al.*, 2005). Similar risks were identified in a pooling project of North American case–control studies (Krewski *et al.*, 2005).

(c) Asbestos

Exposure to asbestos and tobacco smoking are both known causes of lung cancer in humans (Doll & Peto, 1978; de Klerk *et al.*, 1996). Some studies suggest a multiplicative effect [where the effect of asbestos exposure is a multiple of the effect of smoking] (Hammond *et al.*, 1979; Doll & Peto, 1985), and meta-analyses have suggested that the additive model [where asbestos exposure and smoking are independent of each other] is unsound (Lee, 2001; Liddell, 2001). In a recent study of 2935 asbestos miners, persons exposed to asbestos and tobacco who subsequently quit smoking remained at a 90% increased risk of lung cancer up to 20 years after smoking cessation, compared to never-smoker asbestos workers (Reid *et al.*, 2006a).

(d) Genetic polymorphisms

Lung cancer is plausibly caused by the interplay between environmental factors and several low-risk alleles. Attempts in identifying specific single nucleotide polymorphisms (SNPs) responsible for modulating lung cancer risk have yielded few conclusive results. Recent studies have focused on mechanistically plausible polymorphisms in genes coding for enzymes involved in the activation, detoxification and repair of chemical damage caused by tobacco smoke. Genetic association studies indicate that several inherited genetic polymorphisms may be associated with lung cancer risk, but the data from individual studies with low statistical power are conflicting. Evidence from pooled or meta-analyses, along with some individual studies, is briefly summarized below.

(i) Metabolic genes

Most of the 70 carcinogens in tobacco smoke are procarcinogens that must be activated by phase I enzymes and may then be deactivated by

phase II enzymes. Polymorphisms that alter the function of the genes involved in the activation or detoxification of tobacco smoke carcinogens can potentially influence an individual's risk of developing a tobacco-related cancer.

Meta and pooled analyses of 34 case–control, genotype-based studies were conducted to assess the effect of GSTT1 genotypes and smoking on lung cancer risk. No significant interaction was observed (Raimondi *et al.*, 2006). A pooled analysis of 21 case–control studies from the International Collaborative study of Genetic Susceptibility to Environmental Carcinogens showed no evidence of increased risk for lung cancer among carriers of the GSTM1 null genotype and there was no evidence of interaction between GSTM1 genotype and either smoking status or cumulative tobacco consumption (Benhamou *et al.*, 2002). Similarly, in another pooled analysis the summary OR indicated the slow acetylator genotype of N-acetyltransferase 2 (NAT2) detoxification enzyme was not associated with lung cancer risk among Caucasians (Borlak & Reamon-Buettner, 2006). In a pooled analysis to test the hypothesis of interaction among genetic variants in increasing the individual risk for cancer, the cumulative effect of variants in three metabolic genes, CYP1A1, GSTM1 and GSTT1 was assessed. The risk for lung cancer was increased with the combination of CYP1A1*2B or CYP1A1*4 alleles and the double deletion of both GSTM1 and GSTT1 up to an OR of 8.25 (95%CI: 2.29–29.77). The combination including CY1A1*4 among never smokers was associated with an OR of 16.19 (95%CI: 1.90–137). These estimates did not change after adjustment by the number of cigarettes smoked and duration of smoking. The results were consistent across ethnicities and were approximately the same for adenocarcinoma and squamous cell carcinoma (Vineis *et al.*, 2007).

Microsomal epoxide hydrolase 1 (EPHX1) plays an important role in both the activation and detoxification of tobacco-derived carcinogens. Polymorphisms at exons 3 and 4 of the EPHX1 gene have been reported to be associated with variations in EPHX1 activity. In a meta-analysis of 13 case–control studies the low-activity (variant) genotype of EPHX1 polymorphism at exon 3 was associated with decreased risk for lung cancer (OR, 0.65; 95%CI: 0.44–0.96) among whites. In white-populations, the high activity (variant) genotype of EPHX1 polymorphism at exon 4 was associated with a modest increased risk of lung cancer (OR, 1.22; 95%CI: 0.79–1.90) and the predicted low activity was associated with a modest decrease in risk (OR, 0.72; 95%CI: 0.43–1.22) (Kiyohara *et al.*, 2006).

(ii) DNA repair and cell cycle pathways

Data from 14 studies of lung cancer were used in a pooled analysis focusing on 18 sequence variants in 12 DNA repair genes, including APEX1, OGG1, XRCC1, XRCC2, XRCC3, ERCC1, XPD, XPF, XPG, XPA, MGMT and TP53 (Hung *et al.*, 2008a). None of the variants appeared to have a large effect on lung cancer risk. In a recent meta-analysis the X-ray repair cross-complementing protein group 3 (XRCC3) and the xeroderma pigmentosum group D (XPD)/excision repair cross-complementing group 2 (ERCC2) genes were evaluated (Manuguerra *et al.*, 2006). The authors found no association between these genes and the cancer sites investigated (skin, breast and lung). A significant association was identified for XPD/ERCC2 single nucleotide polymorphisms (codons 312 and 751) and lung cancer.

(iii) Nicotine acetylcholine receptor genes

A series of large genome-wide association studies for lung cancer have identified susceptibility loci for lung cancer in chromosome arms 5p, 6p and 15q (Landi *et al.*, 2009). In particular, the susceptibility locus at chromosome region 15q25 includes several genes, including three that encode nicotinic acetylcholine receptor subunits (CHRNA5, CHRNA3 and CHRNB4). Nicotinic acetylcholine receptor subunit genes

code for proteins that form receptors present in neuronal and other tissue, in particular alveolar epithelial cells, pulmonary neuroendocrine cells, and lung cancer cell lines (Wang *et al.*, 2001; Minna, 2003) and bind to nicotine and nicotine derivatives including NNN. An association of CHRNA3 and CHRNA5 variants with nicotine dependence has been reported (Saccone *et al.*, 2007; Berrettini *et al.*, 2008). These genes may act, at least partially, upon cigarette smoking. Current smokers with one or two copies of the susceptibility variant are likely to smoke between one and two cigarettes more a day (Spitz *et al.*, 2008). Evidence for an effect of the 15q25 locus among never smokers is conflicting, with an association found in one study in Europe (Hung *et al.*, 2008b) and one in Asia (Wu *et al.*, 2009a), but not in others. Whether genes in the 15q25 locus have an effect on lung cancer beyond their propensity to increase numbers of cigarettes smoked is unclear.

Three genome-wide association studies identified genetic factors that modified disease risk. The first was a genome-wide association analysis to identify genetic polymorphisms associated with lung cancer risk in 1154 lung cancer patients of European ancestry who were current or former smokers and 1137 control subjects who were frequency matched to the lung cancer patients by age, sex, race and smoking status. Two SNPs, rs105173 and rs803419, which mapped to a region of strong linkage disequilibrium within 15q25.1, were strongly associated with risk of lung cancer, with an odds ratio for rs105173 of 1.31 ($P = 9.84 \times 10^{-6}$). This finding was replicated with an additional 711 case subjects and 632 control subjects from Texas ($P = 0.00042$) and in 2013 case subjects and 3062 control subjects in the United Kingdom ($P = 2.33 \times 10^{-10}$). The region of interest encompasses the nicotinic acetylcholine receptor subunit genes CHRNA3 and CHRNA5 (as well as CHRNB4) (Spitz *et al.*, 2008). A second genome-wide association study conducted among 1989 lung cancer cases and 2625 controls from six central European countries confirm these results (Hung *et al.*, 2008a). In a third genome-wide association study of 665 Icelandic, 269 Spanish and 90 Dutch lung cancer cases and 32244 controls a common variant in the nicotinic acetylcholine receptor gene cluster [chromosome region 15q24] was significantly associated with lung cancer risk (OR, 1.31; 95%CI: 0.1.19–1.44). The variant was observed to have a significant effect on the number of cigarettes smoked per day (Thorgeirsson *et al.*, 2008). These studies have all shown a link between this variant and lung cancer risk either through a mechanism involving nicotine dependence or a direct role in downstream signalling pathways that promote carcinogens. Together these results provide compelling evidence of a locus at 15q25 and 15q24 predisposing to lung cancer.

(iv) Alpha(1)-antitrypsin

Alpha(1)-antitrypsin deficiency (α(1)ATD) is one of the most common genetic disorders, especially among European descendents. Recent results suggest that α(1)ATD carriers are at a 70–100% increased risk of lung cancer, accounting for 11% to 12% of patients with lung cancer (Yang *et al.*, 2008). [The specific effect by smoking status was not evaluated.]

(v) Other genes

Mutations in the checkpoint CHEK2 gene have been associated with increased risk of breast, prostate and colon cancer and a decreased risk of lung cancer among those with the I157T missense variant of the CHEK2 gene. In a large Polish case–control study CHEK2 mutations were protective against lung cancer (OR, 0.3; 95%CI: 0.2–0.5) (Cybulski *et al.*, 2008).

The Swedish Family-Cancer Database was used to compare the rate of lung cancers among persons without family history of lung cancer to those with a family history (Li & Hemminki, 2004). A high risk by family history in adenocarcinoma (standardized incidence ratio (SIR),

2.03) and large cell carcinoma (SIR, 2.14) was found, a slightly lower risk among patients with squamous cell carcinoma (SIR, 1.63) and small cell carcinoma (SIR, 1.55). Among siblings, an increased risk was shown for concordant adenocarcinoma and small cell carcinoma at all ages and for all histological types when cancer was diagnosed before age 50. At young age, risks between siblings were higher than those between offspring and parents. These data suggest that a large proportion of lung cancers before age 50 are heritable and due to a high-penetrant recessive gene or genes that predispose to tobacco carcinogen susceptibility.

(e) Viral infection

Data are limited regarding lung cancer risk in human immunodeficiency virus (HIV)-infected persons with modest immune suppression, before the onset of acquired immunodeficiency syndrome (AIDS). Among 57350 HIV-infected persons registered in the USA during 1991–2002 (median CD4 counts 491 cells/mm^3), 871 cancers occurred. Risk was elevated for several non-AIDS defining malignancies, including cancer of the lung (SIR, 2.6 [n = 109]) (Engels *et al.*, 2008). [Specific evaluation with smoking status was not performed.]

2.3 Cancers of the upper aerodigestive tract

Evidence relating to cancers of the upper aerodigestive tract obtained from relevant cohort and case–control studies on specific sites is described in Sections 2.3.1 to 2.3.6; studies that looked at several subsites combined are described in Section 2.3.7. The major potential confounders for the relationship between smoking and cancers of the upper aerodigestive tract are alcohol consumption and use of any form of smokeless tobacco, and for some sites infection with human papillomavirus (HPV) (especially HPV16). In general, the studies examined by the Working Group had adjusted for these two confounders when appropriate. Some studies also adjusted for dietary intake, especially of fruits and vegetables, although few reported stratified relative risks.

2.3.1 Cancer of the oral cavity

Tobacco smoking was found to be causally related to oral cancer (IARC, 1986, 2004a). New studies on the relationship between oral cancer and cigarette smoking published since the most recent *IARC Monograph* (IARC, 2004a) include four cohort studies (Table 2.7 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.7.pdf), and eight case–control studies (Tables 2.8–2.11 online; see below).

(a) Intensity and duration of smoking

Intensity of smoking was measured in almost all cohort (Table 2.7 online) and case–control studies (IARC 2004a; Table 2.8 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.8.pdf and Table 2.9 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.9.pdf). In addition to the number of cigarettes or amount of tobacco smoked daily, cumulative exposure to cigarette smoke was also measured in terms of pack–years, tobacco-years or lifetime tobacco consumption. The link between duration of cigarette consumption and oral cancer was examined in 15 case–control studies. Seven case–control studies also considered age at starting smoking.

One cohort study (McLaughlin *et al.*, 1995) and 14 case–control studies reported a dose-dependent increase in risk with increasing number of cigarettes smoked daily or increasing daily tobacco consumption (Franceschi *et al.*, 1990, 1992, 1999; Nandakumar *et al.*, 1990; Zheng *et al.*, 1990; Choi & Kahyo, 1991; Oreggia *et al.*, 1991; Bundgaard *et al.*, 1995; Zheng *et al.*, 1997; De Stefani *et al.*, 1998; Hayes *et al.*, 1999; De Stefani

et al., 2007; Subapriya *et al.*, 2007; Muwonge *et al.* 2008). Whenever analysed, the trend was always statistically significant (Franceschi *et al.*, 1990, 1992; Oreggia *et al.*, 1991; Bundgaard *et al.*, 1995; McLaughlin *et al.* 1995; Hayes *et al.*, 1999; Subapriya *et al.*, 2007), except in the study of Muwonge *et al.* (2008) which also included bidi smokers.

Bundgaard *et al.* (1995) used lifetime tobacco consumption divided into four categories and reported a positive, significant trend after adjustment for life-time consumption of alcohol and other risk factors. A positive trend was also found in all studies that have analysed consumption in pack–years or tobacco-years (Zheng *et al.*, 1990; Maier *et al.*, 1992a; Macfarlane *et al.*, 1995; Hung *et al.*, 1997; Zheng *et al.*, 1997; De Stefani *et al.*, 1998, 2007; Applebaum *et al.*, 2007; Muwonge *et al.*, 2008), except Muwonge *et al.* (2008).

Ten studies (Franceschi *et al.*, 1990, 1992; Nandakumar *et al.*, 1990; Zheng *et al.*, 1990; Choi & Kahyo, 1991; Oreggia *et al.*, 1991; Zheng *et al.*, 1997; De Stefani *et al.*, 1998, 2007; Znaor *et al.*, 2003; Subapriya *et al.*, 2007; Muwonge *et al.*, 2008) classified the duration of smoking in up to four categories, and all but one (Nandakumar *et al.*, 1990) reported increased relative risks and a positive trend.

Of six studies that considered age at start of smoking (Franceschi *et al.*, 1990, 1992; Choi & Kahyo, 1991; Oreggia *et al.*, 1991; Zheng *et al.*, 1997; Balaram *et al.*, 2002) two reported a statistically significant trend of increasing risk with decreasing age at starting (Franceschi *et al.*, 1990, 1992).

(b) *Cessation of smoking*

Three cohort studies (McLaughlin *et al.*, 1995; Freedman *et al.*, 2007a; Friborg *et al.* 2007) and nine case–control studies (Zheng *et al.*, 1990; Choi & Kahyo, 1991; Oreggia *et al.*, 1991; Franceschi *et al.*, 1992; Ko *et al.*, 1995; Zheng *et al.*, 1997; De Stefani *et al.*, 1998, 2007; Schildt *et al.*, 1998; Balaram *et al.*, 2002; Pacella-Norman *et al.*, 2002; Muwonge *et al.* 2008) estimated risks for former smokers which were always lower than those for current smokers and in five studies almost reached unity (Zheng *et al.*, 1990; Choi & Kahyo, 1991; Zheng *et al.*, 1997; Schildt *et al.*, 1998; Muwonge *et al.*, 2008). Twelve case–control studies examined the risk by years since quitting and all reported a negative trend, with relative risks compared with those in non-smokers decreasing to near unity after 10 or more years (Franceschi *et al.*, 1990, 1992; De Stefani *et al.*, 1998, 2007; Schlecht *et al.*, 1999a; Table 2.7 online; Table 2.10 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.10.pdf).

(c) *Type of cigarette*

The effect of the type of cigarette smoked was examined in several case–control studies (Table 2.11 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.11.pdf). The characteristics of the cigarettes included the presence of a filter, the type of tobacco, the tar content and whether the product was manufactured or hand-rolled. Two studies reported a statistically significantly higher risk for black than for blond tobacco (Oreggia *et al.*, 1991; De Stefani *et al.*, 1998, 2007). Similarly, a much higher risk was found for hand-rolled cigarettes than for manufactured cigarettes, and plain cigarettes had a much higher risk than filter-tipped cigarettes (De Stefani *et al.*, 1998, 2007). In one study the differences between black and blond tobacco and between hand-rolled and manufactured cigarettes persisted after stratification by duration of smoking (De Stefani *et al.*, 1998). Smoking cigarettes with a high-tar content led to higher risks than smoking cigarettes with a low-tar content (Franceschi *et al.*, 1992) and the same trend was observed for cigarettes without filter compared to cigarettes with filter (De Stefani *et al.*, 2007).

(d) Sex

Sex-specific effects were examined in two case–control studies (Zheng *et al.*, 1990; Hayes *et al.*, 1999). In both studies, the relative risks for all categories of intensity, duration of smoking and pack–years were higher for women than for men. [The Working Group noted that the background risk of oral cancer is considerably lower in women than men. Thus, the higher relative risk estimates in women than men indicate a higher proportionate contribution from smoking in women than men, rather than higher absolute risk.]

2.3.2 Cancer of the pharynx

Tobacco smoking was considered to be an important cause of oropharyngeal and hypopharyngeal cancers in the previous *IARC Monographs* on tobacco smoking (IARC, 1986, 2004a). Since then, results available from three cohort (Table 2.12 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.12.pdf) and seven case–control studies (Table 2.13 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.13.pdf and Table 2.14 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.14.pdf) provide further support for the association. Many studies, however, combine cancers of the oral cavity and pharynx (see Section 2.3.7). This section summarizes the evidence from all eight cohort and 21 case–control studies that reported results specifically on oropharyngeal and hypopharyngeal cancer, or on pharyngeal cancer in general; the latter may include data on nasopharyngeal cancer.

The risk for pharyngeal cancer was significantly increased in smokers in four cohort studies (Doll *et al.*, 2005; McLaughlin *et al.*,1995; Freedman *et al.*, 2007a; Friborg *et al.*, 2007) and all but one of the case–control studies (Rao *et al.*, 1999). The trend of increasing risk associated with increasing daily or cumulative consumption of cigarettes was evident from all these studies, particularly those from Europe (Brugere *et al.*, 1986; Tuyns *et al.*, 1988; Franceschi *et al.*, 1990, 1999; Maier *et al.*, 1994; Escribano Uzcudun *et al.*, 2002; Vlajinac *et al.*, 2006), India (Znaor *et al.*, 2003; Sapkota *et al.*, 2007), Uruguay (De Stefani *et al.*, 1998, 2007) and the USA (McLaughlin *et al.*, 1995; Applebaum *et al.*, 2007), and less strongly so in studies from Canada (Elwood *et al.*, 1984) and the Republic of Korea (Choi & Kahyo, 1991). The multicentre study in Europe, North and South America of Hashibe *et al.* (2007c) showed increased risks according to frequency (cigarettes/day) and duration (years) in never drinkers. Applebaum *et al.* (2007) found a relationship between increasing risk of pharyngeal cancer and increased pack–years of smoking in subjects with negative HPV16 serology but not in those with positive HPV16 serology (p value for interaction = 0.007).

In two case–control studies the risk increased with decreasing age at starting smoking (Franceschi *et al.*, 1990; Choi & Kahyo, 1991,), but adjustment was not made for duration and intensity of smoking. In a case–control study from Spain (Escribano Uzcudun *et al.*, 2002) the risk increased with the age of starting smoking.

Former smokers had consistently lower relative risks than did current smokers in both cohort (McLaughlin *et al.*, 1995; Freedman *et al.*, 2007a) and case–control studies (Choi & Kahyo, 1991; De Stefani *et al.*, 1998; Vlajinac *et al.*, 2006). In comparison with non-smokers, the relative risks for former smokers who had quit smoking for more than 10 years were between 2 and 4 (Franceschi *et al.*, 1990; De Stefani *et al.*, 1998; La Vecchia *et al.*, 1999), whereas the relative risks for current smokers in these studies were 10–14. In one study in Brazil (Schlecht *et al.*, 1999a), relative risks for former smokers who had stopped smoking for more than 10 years approached 1, whereas that for current smokers was just below 6. Consumption of black tobacco, hand-rolled cigarettes or plain cigarettes resulted in a higher

risk for pharyngeal cancer than consumption of blond tobacco, manufactured cigarettes or filter-tipped cigarettes (De Stefani *et al.*, 1998; 2007).

2.3.3 Cancer of the nasal cavity and accessory sinuses

In the Life Span Study in Japan (Akiba, 1994) the association of tobacco use with sinonasal cancer was examined. A total of 26 cases of sinonasal cancer were identified among 61505 adults during follow-up. Relative risk estimates, adjusted for sex, location, population group, atomic bomb exposure, year of birth and attained age, were 2.9 (95%CI: 0.5–) and 4.0 (95%CI: 1.2–) for former and current smokers, respectively, when compared with non-smokers [upper confidence limits were not reported]. The cohort of 34439 British doctors followed up to 50 years (Doll *et al.*, 2005) showed increased risk for current smokers and smokers of more than 25 cigarettes per day, but only six deaths from nasal cavity and sinuses cancers were observed (Table 2.15 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.15.pdf).

A total of nine case–control studies of nasal cavity and sinus cancers have been conducted. When histological types were combined, all studies found an increased risk associated with cigarette smoking, but only one was statistically significant (Caplan *et al.*, 2000). In seven studies, dose–response in terms of intensity of smoking (cigarettes/day), duration of smoking or pack–years was considered. A positive significant trend was found in five studies (Brinton *et al.*, 1984; Hayes *et al.*, 1987; Fukuda & Shibata, 1990; Zheng *et al.*, 1993; Caplan *et al.*, 2000) and suggested in the other two (Strader *et al.*, 1988; Zheng *et al.*, 1992c).

One study (Zheng *et al.*, 1993a) found a significant decrease in risk for sinonasal cancer associated with increasing number of years since cessation of smoking. In a previous study, the same authors had found a negative, non-significant association (Zheng *et al.*, 1992c).

Five studies analysed squamous-cell carcinomas and adenocarcinomas separately (Brinton *et al.*, 1984; Hayes *et al.*, 1987; Strader *et al.*, 1988; Zheng *et al.*, 1992c; 't Mannetje *et al.*, 1999). In all studies, there was a significantly increased risk for squamous-cell carcinomas, whereas the risk was generally not increased for adenocarcinomas.

2.3.4 Cancer of the nasopharynx

(a) Cohort studies

The risk for nasopharyngeal carcinoma has been examined in relation to tobacco use in six cohort studies, three of them reported since the last evaluation (IARC 2004a; Table 2.16 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.16.pdf). In one study, conducted in a low-risk area (Chow *et al.*, 1993a), a significant increase in risk among smokers and suggestive positive dose–response relationships by duration of smoking and age at starting smoking were found. In another study, conducted in Province of Taiwan, China, an area in which nasopharyngeal cancer area is endemic, a similarly increased risk was found, but it was not statistically significant (Liaw & Chen, 1998). Doll *et al.* (2005) identified a risk only for smokers of more than 25 cigarettes per day, however, this result was based on only four deaths. Friborg *et al.* (2007) in Singapore found statistically significant increased risk of nasopharyngeal cancer only for those smoking for 40 years or more. Hsu *et al.* (2009) in Taiwan, China observed increased statistically significant risks only for those smoking for 30 years or more and those with cumulative exposure of 30 pack–years or more.

(b) Case–control studies

The study designs and the results of the case–control studies on the association of nasopharyngeal carcinoma with cigarette smoking

reported since the previous *IARC Monograph* (IARC, 2004a) are given in Table 2.17 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.17.pdf) and Table 2.18 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.18.pdf), one being a nested case–control analysis within a cohort study (Marsh *et al.*, 2007).

In total, 14 informative case–control studies were available. In almost all of these, the risk for nasopharyngeal carcinoma was higher in smokers than in non-smokers. In Taiwan, China (Cheng *et al.*, 1999) high risks were statistically significant only for duration of smoking of 20 years or more. In the five studies conducted in the USA (Mabuchi *et al.*, 1985; Nam *et al.*, 1992; Zhu *et al.*, 1995; Vaughan *et al.*, 1996; Marsh *et al.*, 2007), where the incidence of nasopharyngeal carcinoma is low, the relative risks for current smokers ranged between 2 and 4, but were not statistically significant in the two studies (Mabuchi *et al.*, 1985; Marsh *et al.*, 2007). In a study conducted in Shanghai, an area of China in which nasopharyngeal carcinoma is not endemic (Yuan *et al.*, 2000), the relative risk was just below 2. In one study from the Philippines there was a sevenfold increase in risk after more than 30 years of smoking (West *et al.*, 1993). The four studies (Lin *et al.*, 1973; Yu *et al.*, 1990; Ye *et al.*, 1995; Cao *et al.*, 2000) conducted in areas of China in which nasopharyngeal carcinoma is endemic (Taiwan, China, Guangzhou, and Sihui) found relative risks for ever smoking ranging between 2 and 5. In the study from the North of Africa (Feng *et al.*, 2009) the only statistically significant increased risk was found for differentiated nasopharyngeal cancer in those that had smoked more than 22 cigarettes/day. [The result, based only on three cases, is very unstable (RR, 313; 95%CI: 1.94–50336).]

A statistically significant dose–response relationship was detected in seven studies that evaluated the effects of daily or cumulative exposure to tobacco smoke (Yu *et al.*, 1990; Nam *et al.*, 1992; Zhu *et al.*, 1995; Vaughan *et al.*, 1996; Cao *et al.*, 2000; Yuan *et al.*, 2000; Feng *et al.*, 2009) and was suggestive in two others (Lin *et al.*, 1973; West *et al.*, 1993). In two studies the risk of nasopharyngeal carcinoma decreased with increasing time since quitting smoking (Nam *et al.*, 1992; Vaughan *et al.*, 1996).

In the remaining studies, six from areas in which nasopharyngeal carcinoma is endemic (Ng, 1986; Yu *et al.*, 1986; Sriamporn *et al.*, 1992; Zheng *et al.*, 1994; Cheng *et al.*, 1999; Feng *et al.*, 2009; Guo *et al.*, 2009) and seven from areas in which it was not endemic (Henderson *et al.*, 1976; Lanier *et al.*, 1980; Mabuchi *et al.*, 1985; Ning *et al.*, 1990; Armstrong *et al.*, 2000, Marsh *et al.*, 2007), the relative risks for nasopharyngeal carcinoma for ever smoking were not significantly increased (Lanier *et al.*, 1980; Mabuchi *et al.*, 1985; Cheng *et al.*, 1999) or were close to 1.0 (Henderson *et al.*, 1976; Ng, 1986; Yu *et al.*, 1986; Ning *et al.*, 1990; Sriamporn *et al.*, 1992; Zheng *et al.*, 1994; Guo *et al.*, 2009).

In the two studies that distinguished between different histological types, relative risks were higher for keratinized (squamous-cell) carcinoma than for unkeratinized carcinoma (Zhu *et al.*, 1995; Vaughan *et al.*, 1996).

In the three studies in which men and women were analysed separately (Lin *et al.*, 1973; Nam *et al.*, 1992; Yuan *et al.*, 2000), the relative risks were found to increase similarly in both sexes in two studies (Nam *et al.*, 1992; Yuan *et al.*, 2000) and were higher among women in the study of Lin *et al.* (1973).

2.3.5 Cancer of the oesophagus

In the previous *IARC Monograph* (IARC, 2004a), both histological subtypes of oesophageal cancer (squamous-cell carcinoma and adenocarcinoma) were considered to be causally related to cigarette smoking. Many more epidemiological studies have since been conducted, and results of these studies further support this conclusion.

(a) *Squamous cell carcinoma and unspecified cancer of the oesophagus*

Since the previous *IARC Monograph* (IARC, 2004a), there have been reports on 9 cohort studies (Table 2.19 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.19.pdf) and 22 case–control studies (Tables 2.20–2.23; see below), making 30 cohort and 55 case–control studies in all. All showed that the risk of oesophageal squamous cell carcinoma was associated with cigarette smoking. In one study (Li *et al.*, 1989), the elevated risk was observed only in an area with a relatively low incidence of oesophageal cancer. However, two later studies in the same area, Lin County, China, found a twofold increase in risk for oesophageal cancer among smokers (Gao *et al.*, 1994; Lu *et al.*, 2000).

In most cohort studies and in most case–control studies with relatively large sample sizes (IARC, 2004a; Table 2.19 online; Table 2.20 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.20.pdf; Table 2.21 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.21.pdf), the risk for oesophageal cancer was shown to increase with increasing duration of smoking (11 cohort and 32 case–control studies) or number of cigarettes smoked daily (18 cohort and 31 case–control studies), and to decrease with increasing age at starting smoking (12 case–control studies). In comparison with pharyngeal and laryngeal cancers, relative risks for oesophageal cancer estimated by duration and by intensity of smoking were somewhat lower (see Sections 2.3.2 and 2.3.6, respectively).

Ten cohort and 20 case–control studies (IARC, 2004a; Table 2.19 online; Table 2.22 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.22.pdf) investigated the effect of smoking cessation on risk of oesophageal cancer. Although not all studies analysed the trend, all found a decreasing relative risk with increasing number of years since quitting. In some studies, the risk first started to decrease after 10 years of cessation (Brown *et al.*, 1988; Rolón *et al.*, 1995; Gammon *et al.*, 1997; Castellsagué *et al.*, 1999; Freedman *et al.*, 2007b; Bosetti *et al.*, 2008) or after 30 years of cessation (Pandeya *et al.*, 2008).

When comparing the types of tobacco smoked (Table 2.23 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.23.pdf), consumption of black tobacco resulted in a higher risk for oesophageal cancer than did consumption of blond tobacco (De Stefani *et al.*, 1990; Rolón *et al.*, 1995; Castellsagué *et al.*, 1999; Launoy *et al.*, 2000; Vioque *et al.*, 2008). Similarly, smoking untipped cigarettes generally resulted in a higher risk than smoking filter-tipped cigarettes (Vaughan *et al.*, 1995; Gammon *et al.*, 1997; Castellsagué *et al.*, 1999).

Two studies from the USA reported risks separately for blacks and whites. After adjustment for alcohol consumption, age and income, risks were very similar for former and current smokers and for the number of cigarettes smoked per day and duration of smoking (Brown *et al.*, 1994a; Brown *et al.*, 2001).

(b) *Adenocarcinoma of the oesophagus*

Two decades ago it was noted that incidence rates for adenocarcinoma of the oesophagus and gastric cardia had increased steadily in the USA, whereas the incidence rate for squamous-cell carcinoma of the oesophagus had remained relatively stable (Blot *et al.*, 1991). An increase in the incidence of adenocarcinoma of the distal oesophagus and cardia was also noted in the United Kingdom (Powell & McConkey, 1990), and in several other countries. Since 1990, several studies have focused on the risk factors for adenocarcinoma of the oesophagus. Since the last evaluation (IARC, 2004a) one cohort study (Freedman *et al.*, 2007b) and three case–control studies (Table 2.24 available at http://monographs.iarc.fr/ENG/Monographs/

vol100E/100E-01-Table2.24.pdf; Table 2.25 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.25.pdf) have been reported, totaling 13 case–control studies on the association of cigarette smoking and adenocarcinoma of the oesophagus.

(i) Intensity and duration of smoking

Ten studies, three that included only cases of adenocarcinoma of the oesophagus (Menke-Pluymers *et al.*, 1993; Gammon *et al.*, 1997; Wu *et al.*, 2001), three that included cases of adenocarcinoma of the oesophagus, gastro-oesophageal junction and gastric cardia combined (Kabat *et al.*, 1993; Brown *et al.*, 1994b; Vaughan *et al.*, 1995), and four that stratified by histology (Lindblad *et al.*, 2005; Freedman *et al.*, 2007b; Hashibe *et al.*, 2007a; Pandeya *et al.*, 2008), showed a significant positive association of adenocarcinoma of the oesophagus with cigarette smoking. The relative risks were somewhat lower than those for squamous cell carcinoma of the oesophagus. Three studies, one in China (Gao *et al.*, 1994), one in Sweden (Lagergren *et al.*, 2000), and one in the USA (Zhang *et al.*, 1996), reported similarly elevated relative risks, but some of these risks were not statistically significant, probably because of relatively small numbers of cases.

Of those studies that reported risks adjusted for alcohol consumption, a positive, significant dose–response relationship was found with intensity of smoking (Kabat *et al.*, 1993; Brown *et al.*, 1994b; Gammon *et al.*, 1997; Hashibe *et al.*, 2007a), duration of smoking (Gammon *et al.*, 1997; Pandeya *et al.*, 2008) and/or pack–years (Vaughan *et al.*, 1995; Zhang *et al.*, 1996; Gammon *et al.*, 1997; Pandeya *et al.*, 2008).

(ii) Cessation of smoking

Ten studies provided point estimates for former smokers. In eight, relative risks were lower in former smokers than in current smokers, although they remained elevated (Kabat *et al.*, 1993; Gao *et al.*, 1994; Vaughan *et al.*, 1995; Gammon *et al.*, 1997; Wu *et al.*, 2001; Lindblad *et al.*, 2005; Freedman *et al.*, 2007b; Pandeya *et al.*, 2008), and were increased in the other studies (Lagergren *et al.*, 2000; Hashibe *et al.*, 2007a). The decrease in relative risk associated with years since cessation was weak, but a significant trend was found in two out of six studies (Gammon *et al.*, 1997; Wu *et al.*, 2001).

(iii) Confounding

With the exception of two studies (Levi *et al.*, 1990; Wu *et al.*, 2001), all studies adjusted for alcohol intake as a potential confounder. Three more recent studies also adjusted for fruit and vegetables intake (Freedman *et al.*, 2007b; Hashibe *et al.*, 2007a; Pandeya *et al.*, 2008). Ten of these studies were conducted in the USA (Kabat *et al.*, 1993; Brown *et al.*, 1994b; Vaughan *et al.*, 1995; Zhang *et al.*, 1996; Gammon *et al.*, 1997; Freedman *et al.*, 2007b) the Netherlands (Menke-Pluymers *et al.*, 1993), the United Kingdom (Lindblad *et al.*, 2005), central and eastern Europe (Hashibe *et al.*, 2007a) and Australia (Pandeya *et al.*, 2008), where chewing of betel quid with tobacco or use of other forms of smokeless tobacco are not likely confounders. One study conducted in Sweden was adjusted for snuff use (Lagergren *et al.*, 2000).

(iv) Sex

Kabat *et al.* (1993) examined risks for men and women separately and observed similar patterns in both sexes, although risks among current smokers and heavy smokers were somewhat higher for women than for men. Lindblad *et al.* (2005) also found higher risks in women than in men, but they were not statistically significant.

2.3.6 Cancer of the larynx

Laryngeal cancer is one of the cancers most strongly associated with cigarette smoking (IARC, 1986, 2004a). Since the previous *IARC*

Monograph, more epidemiological evidence has become available to strengthen this conclusion.

(a) Potential confounders

Other causes of laryngeal cancer include alcohol consumption, some occupational exposures (e.g. sulphuric acid; IARC, 2012a) and possibly some dietary habits. In investigating associations between smoking and laryngeal cancer, potential confounding by alcohol consumption has been considered in most of the studies.

(b) Intensity and duration of smoking

Cohort and case–control studies have been carried out in Asia, Europe, North and South America, and South Africa. In all, the risk for laryngeal cancer was consistently higher in smokers, and a positive significant trend was observed with increasing duration and intensity of smoking (IARC, 2004a; Table 2.26 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.26.pdf; Table 2.27 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.27.pdf; Table 2.28 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.28.pdf).

In most case–control studies, the relative risks for laryngeal cancer were near to or greater than 10 for smokers who had smoked for longer than 40 years (Falk *et al.*, 1989; Zheng *et al.*, 1992b) or had smoked more than 20 cigarettes per day (Tuyns *et al.*, 1988; Falk *et al.*, 1989; Choi & Kahyo, 1991; Zatonski *et al.*, 1991; Muscat & Wynder, 1992; Zheng *et al.*, 1992b; Hedberg *et al.*, 1994; Sokić *et al.*, 1994; Talamini *et al.*, 2002). Cancer of the larynx in non-smokers is so rare that several studies used as the reference category light smokers (Herity *et al.*, 1982; Olsen *et al.*, 1985a; De Stefani *et al.*, 1987; Zatonski *et al.*, 1991; López-Abente *et al.*, 1992; Maier & Tisch, 1997), or former smokers (Hashibe *et al.*, 2007b). Consequently, relative risks were lower in these studies, although the increases were still statistically significant.

Three case–control studies reported odds ratios for cancer of the larynx that increased with decreasing age of starting smoking (Franceschi *et al.*, 1990; Zatonski *et al.*, 1991; Talamini *et al.*, 2002).

(c) Cessation of smoking

The risk for cancer of the larynx declines rather rapidly after cessation of smoking (IARC, 2004a; Table 2.29 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.29.pdf). No detectable higher risk compared with never-smokers was seen among subjects who had quit smoking for at least 10 years (Franceschi *et al.*, 1990; Ahrens *et al.*, 1991; Schlecht *et al.*, 1999a, b; Bosetti *et al.*, 2006; Hashibe *et al.*, 2007b).

(d) Types of tobacco or of cigarette

Some investigators considered the role of type of tobacco (IARC, 2004a; Table 2.30 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.30.pdf). An average 2.5-fold higher risk was observed in smokers of black tobacco compared to smokers of blond tobacco (De Stefani *et al.*, 1987; Tuyns *et al.*, 1988; López-Abente *et al.*, 1992). Smoking untipped cigarettes also led to a higher risk than smoking filter-tipped cigarettes (Wynder & Stellman, 1979; Tuyns *et al.*, 1988; Falk *et al.*, 1989). Those that smoke cigarettes only had higher risks of larynx cancer than those that smoke cigars only (Hashibe *et al.*, 2007b).

(e) Subsites

Six studies investigated the risk for glottic and supraglottic cancer separately (Olsen *et al.*, 1985a; Tuyns *et al.*, 1988; López-Abente *et al.*, 1992; Maier *et al.*, 1992b; Muscat & Wynder, 1992; Sapkota *et al.*, 2007). The cancer risk increased with increasing amount smoked per

day and with cumulative exposure for both subsites (IARC, 2004a; Table 2.28 online). In addition, the observed relative risks were higher for supraglottic cancer than for glottic cancer (Maier *et al.*, 1992b; Sapkota *et al.*, 2007).

(f) Sex

Few studies investigated sex-specific effects. In one cohort study (Raitiola & Pukander, 1997) similar risks were found for men and women, whereas in two case–control studies (Zheng *et al.*, 1992b; Tavani *et al.*, 1994), the relative risks for women were up to 10-fold higher than for the corresponding categories in men, though a small number of cases were involved. However, Freedman *et al.* (2007a) observed higher relative risks in men than women (Table 2.26 online). One study looked at women only and found higher risks of laryngeal cancer in former and current smokers relative to non-smokers, and also according to the number of cigarettes per day with a clear dose–response effect ($P < 0.001$) (Gallus *et al.*, 2003b).

2.3.7 Cancer of the upper aerodigestive tract combined

In epidemiological studies, especially in cohort studies in which there are few cases at some sites, investigators often combine cancers of the oral cavity, pharynx, larynx and oesophagus and term these 'cancer of the upper aerodigestive tract'. This section summarizes the data from 19 cohort studies (IARC, 2004a; Table 2.31 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.31.pdf), and 40 case–control studies (IARC, 2004a; Tables 2.32–2.35; see below).

(a) Intensity and duration of smoking

In all but two cohort studies from Japan (Kono *et al.*, 1987; Akiba, 1994), the risk for cancer of the upper aerodigestive tract was strongly associated with cigarette smoking. Relative risks increased with increasing daily cigarette consumption (Hammond & Horn, 1958; Doll *et al.*, 1980, 1994; Akiba & Hirayama, 1990; Kuller *et al.*, 1991; Chyou *et al.*, 1995; Engeland *et al.*, 1996; Murata *et al.*, 1996; Yuan *et al.*, 1996; Kjaerheim *et al.*, 1998; Liaw & Chen, 1998; Yun *et al.*, 2005; Freedman *et al.*, 2007a), duration of smoking (Chyou *et al.*, 1995; Yun *et al.* 2005; Friborg *et al.*, 2007) or pack–years (Liaw & Chen, 1998; Freedman *et al.*, 2007a).

The main characteristics and results of the case–control studies are presented in IARC (2004a), and in Table 2.32 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.32.pdf) and Table 2.33 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.33.pdf), respectively. Intensity of smoking was measured in most of these studies. The link between duration of smoking and cancer of the upper aerodigestive tract was examined in 20 case–control studies (Blot *et al.*, 1988; Merletti *et al.*, 1989; Barra *et al.*, 1991; De Stefani *et al.*, 1992, 2007; Franceschi *et al.*, 1992; Day *et al.*, 1993; Mashberg *et al.*, 1993; Kabat *et al.*, 1994; Lewin *et al.*, 1998; Bosetti *et al.*, 2000a; Garrote *et al.*, 2001; Gallus *et al.*, 2003a; Lissowska *et al.*, 2003; Znaor *et al.*, 2003; Castellsagué *et al.*, 2004; Menvielle *et al.*, 2004a, b; Rodriguez *et al.*, 2004; Hashibe *et al.*, 2007c; Sapkota *et al.*, 2007). Nine also considered age at starting smoking (Blot *et al.*, 1988; Merletti *et al.*, 1989; Barra *et al.*, 1991; Franceschi *et al.*, 1992; Day *et al.*, 1993; Lewin *et al.*, 1998; Garrote *et al.* 2001; Lissowska *et al.* 2003; Menvielle *et al.* 2004a).

In all but one study (Rao *et al.*, 1999) there was an increased risk for cancer of the upper aerodigestive tract associated with cigarette smoking. A clear dose–response relationship was seen with increasing daily tobacco consumption and duration of smoking as well as with decreasing age at starting smoking in most of the studies examined.

(b) Cessation of smoking

Twelve cohort studies (Doll *et al.*, 1980, 1994; Tomita *et al.*, 1991; Akiba, 1994; Chyou *et al.*, 1995; Engeland *et al.*, 1996; Nordlund *et al.*, 1997; Kjaerheim *et al.*, 1998; Yun *et al.*, 2005; Freedman *et al.*, 2007a; Friborg *et al.*, 2007; Ide *et al.*, 2008) provided point estimates for former smokers (IARC 2004a; Table 2.31 online). The relative risks for former smokers were always lower than those for current smokers.

In 16 case–control studies the relative risk by years since quitting was examined and generally a statistically significant negative trend was found (Table 2.34 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.34.pdf).

(c) Types of cigarette

The characteristics studied in several case–control studies included the use of a filter, the type of tobacco, the tar content and whether the product was manufactured or hand-rolled (IARC, 2004a; Table 2.35 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.35.pdf). Consumption of black tobacco, cigars, untipped cigarettes, hand-rolled cigarettes, or cigarettes with a high-tar yield generally resulted in a higher risk than consumption of blond tobacco (Merletti *et al.*, 1989; Castellsagué *et al.*, 2004; De Stefani *et al.*, 2007), filter-tipped cigarettes (Merletti *et al.*, 1989; Mashberg *et al.*, 1993; Kabat *et al.*, 1994; Lissowska *et al.*, 2003; De Stefani *et al.*, 2007), manufactured cigarettes (De Stefani *et al.*, 1992, 2007) or low-tar cigarettes (Franceschi *et al.*, 1992). Two studies from India (Znaor *et al.*, 2003; Sapkota *et al.* 2007) revealed higher risks of *bidi* smoking related to cigarettes smoking.

(d) Sex

Sex-specific effects were analysed in four cohort studies (IARC 2004a; Table 2.31 online). In three cohort studies (Hammond & Seidman, 1980; Akiba & Hirayama, 1990; Freedman *et al.*, 2007a) a higher relative risk was found for male smokers than for female smokers; however, Ide *et al.* (2008) detected a higher risk among women in a study with a small number of cases.

In three case–control studies (Blot *et al.*, 1988; Kabat *et al.*, 1994; Muscat *et al.*, 1996) the relative risks were higher for women than for men in all categories of intensity of smoking (number of cigarettes per day), cumulative exposure (cumulative tar consumption, pack–years, duration of smoking) and age at starting smoking, as well as for former smokers. However, the trends in men were always in the same direction and of the same order of magnitude. An exception to the pattern was that in one study (Merletti *et al.*, 1989) the relative risk for smoking filter-tipped cigarettes was higher than that for smoking untipped cigarettes for women.

Overall, the strength of association by sex was generally similar, especially when taking into account the fact that women generally under-report levels of smoking and that most studies included many fewer women than men.

(e) Ethnicity

Relative risks were reported separately for blacks and whites in a large case–control study from the USA (Day *et al.*, 1993). Relative risks adjusted for alcohol consumption, sex and other relevant variables were very similar for the number of cigarettes smoked per day, years of cigarette smoking, age at starting smoking and number of years since stopping smoking.

2.4 Cancer of the stomach

2.4.1 Overview of studies

In the previous *IARC Monograph* (IARC, 2004a) it was concluded that there was *sufficient evidence* that tobacco smoking causes cancer of the stomach. Three meta-analyses have since examined the evidence for gastric cancer in 42

independent cohort studies published between 1958 and July 2007 (Ladeiras-Lopes *et al.*, 2008), in 46 case–control studies published between 1997 and June 2006 (La Torre *et al.*, 2009), and in 10 cohort and 16 case–control studies conducted in Japanese populations published between 1966 and March 2005 (Nishino *et al.*, 2006; Table 2.36 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.36.pdf). For current smokers compared to never smokers, the risk for stomach cancer was found to be statistically significantly increased by 53% (Ladeiras-Lopes *et al.*, 2008), 56% (Nishino *et al.*, 2006), and 57% when considering high quality case–control studies (La Torre *et al.*, 2009), with moderate to high heterogeneity.

Since the previous *IARC Monograph* (IARC, 2004a), the association between cigarette smoking and stomach cancer risk (15 studies) and mortality (4 studies) has been examined in 19 cohort studies (Table 2.37 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.37.pdf). Eleven of these were conducted in Asia (Sasazuki *et al.*, 2002; Jee *et al.*, 2004; Koizumi *et al.*, 2004; Wen *et al.*, 2004; Fujino *et al.*, 2005; Sauvaget *et al.*, 2005; Tran *et al.*, 2005; Kurosawa *et al.*, 2006; Kim *et al.*, 2007; Sung *et al.*, 2007; Shikata *et al.*, 2008), seven in Europe (Simán *et al.*, 2001; González *et al.*, 2003; Doll *et al.*, 2005; Lindblad *et al.*, 2005; Sjödahl *et al.*, 2007; Batty *et al.*, 2008; Zendehdel *et al.*, 2008) and one in the USA (Freedman *et al.*, 2007a). Only the updated British Doctors' study (Doll *et al.*, 2005) and the most recent studies (Shikata *et al.*, 2008; Zendehdel *et al.*, 2008) were not included in the meta-analysis of cohort studies (Ladeiras-Lopes *et al.*, 2008). Elevated risks in current smokers were found in all studies. The reported association of current smoking with mortality in the four cohort studies conducted in Taiwan, China (Wen *et al.*, 2004), Japan (Kurosawa *et al.*, 2006) and the United Kingdom (Doll *et al.*, 2005; Batty *et al.*, 2008) was comparable to that with incidence.

In addition, the association between smoking and stomach cancer risk has been reported in 37 case–control studies since the previous *IARC Monograph*, of which 22 are hospital-based and 15 population-based. With the exception of three studies (Campos *et al.*, 2006; García-González *et al.*, 2007; Suwanrungruang *et al.*, 2008; Table 2.38 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.38.pdf), all these studies were included in the meta-analysis conducted by (La Torre *et al.*, 2009).

2.4.2 Factors affecting risk

(a) Intensity and duration

Clear evidence has been provided by the meta-analyses as well as by the additional cohort studies that the risk for stomach cancer increases significantly with increasing daily cigarette consumption, duration or pack–years of smoking, although individual studies did not always find statistically significant dose–response relationships. In one meta-analysis based on 21 cohort studies, the risk for stomach cancer increased statistically significantly by 53% with consumption of approximately 20 cigarettes per day (Ladeiras-Lopes *et al.*, 2008). Using trend estimation analysis as proposed by Greenland & Longnecker (1992), the authors found an increase in relative risk from 1.3 for the lowest consumption to 1.7 for smoking 30 cigarettes per day.

(b) Cessation of smoking

Risk for stomach cancer has been generally found to be lower in former smokers than in current smokers. In six of the cohort studies decreasing risk with increasing years since stopping smoking was found although none found statistically significant dose–response relationships (González *et al.*, 2003; Koizumi *et al.*, 2004; Sauvaget *et al.*, 2005; Freedman *et al.*, 2007a; Kim *et al.*, 2007; Zendehdel *et al.*, 2008). Risk in former smokers was comparable to never smokers after quitting for 5 years (Kim *et al.*,

2007), 10 years (González *et al.*, 2003; Sauvaget *et al.*, 2005; Freedman *et al.*, 2007a) or 15 years (Koizumi *et al.*, 2004).

2.4.3 Subsites

The effect of current smoking on the risk for stomach cancer by subsite was assessed in ten cohort studies. Elevated risks were found for both cardia and non-cardia cancers. In six studies higher risks were found for cancer of the gastric cardia than for cancer of the distal stomach (Simán *et al.* 2001; González *et al.*, 2003; Freedman *et al.*, 2007a; Sung *et al.*, 2007; Shikata *et al.*, 2008; Zendehdel *et al.*, 2008), three studies found no difference (Sasazuki *et al.*, 2002; Lindblad *et al.*, 2005; Tran *et al.*, 2005), and in one study higher risk for cancer in the antrum rather than the body or the cardia was found (Koizumi *et al.*, 2004). A meta-analysis yielded statistically significant summary relative risks of 1.87 for cardia cancers and 1.60 for non-cardia cancers based on nine cohort studies (Ladeiras-Lopes *et al.*, 2008). However, there was substantial heterogeneity across studies for cardia cancers. For case–controls studies, the corresponding odds ratios were 2.05 (95%CI: 1.50–2.81) and 2.04 (95%CI: 1.66–2.50), respectively, with greater heterogeneity for non-cardia cancers. Criteria for the classification by subsite were not always described (Simán *et al.*, 2001; Koizumi *et al.*, 2004; Lindblad *et al.*, 2005; Tran *et al.*, 2005) and some studies included tumours located in the upper third of the stomach in the group of cardia cancer (Sasazuki *et al.*, 2002; Sung *et al.*, 2007; Shikata *et al.* 2008).

In three studies risk estimates for smoking associated stomach cancer were estimated by histological type (Sasazuki *et al.*, 2002; Koizumi *et al.*, 2004; Shikata *et al.*, 2008). The relative risks were 2.1 (95%CI: 1.2–3.6), 1.6 (95%CI: 1.1–2.3) and 2.3 (95%CI: 1.3–4.1) for the differentiated type, respectively, and 0.6 (95%CI: 0.3–1.1), 2.1 (95%CI: 1.1–4.1), and 1.3 (95%CI: 0.5–3.5) for the non-differentiated type, respectively.

2.4.4 Population characteristics

In four of the additional cohort studies risk was reported separately for men and women (González *et al.*, 2003; Jee *et al.*, 2004; Fujino *et al.*, 2005; Kim *et al.*, 2007), in three studies only for men (Koizumi *et al.*, 2004; Tran *et al.*, 2005; Sung *et al.*, 2007) and in one mortality study for men as well as for women (Wen *et al.*, 2004). Generally, the relative risks were smaller in women than in men. For all stomach cancers, risk in current smokers compared to never smokers was found to be significantly increased by 62% in men (based on 18 studies) and by 20% in women (based on nine studies) in the meta-analysis of cohort studies (Ladeiras-Lopes *et al.*, 2008). The men–women differences were independent of exposure level but could be explained by the sex difference in the distribution by histological type and other factors associated with socioeconomic status.

Ethnicity does not appear to modify the effect of smoking on stomach cancer risk. In the meta-analysis of case–control studies risk in current smokers was increased by 78% in Caucasians and by 48% in Asians (La Torre *et al.*, 2009). The summary risk based on the cohort studies increased by 46% and 47% in Caucasian and Asian studies, respectively. In a meta-regression analysis including the variables sex, population, and fruit and vegetable consumption, sex but not origin of the population showed significant differences in risk estimates (Ladeiras-Lopes *et al.*, 2008).

2.4.5 Bias and confounding

Generally, most cohort studies have relied on baseline information and did not update the exposure information, possibly leading to misclassification of smoking status. Most of

the recent cohort studies have accounted for confounding by alcohol consumption (Fujino *et al.*, 2005; Lindblad *et al.*, 2005; Sjödahl *et al.*, 2007; Sung *et al.*, 2007) as well as fruit and vegetable consumption (González *et al.*, 2003; Koizumi *et al.*, 2004; Freedman *et al.* 2007a) and still observed significantly increased risk of stomach cancer in current smokers.

2.4.6 Helicobacter pylori infection

The association between tobacco smoking and stomach cancer could be confounded or modified by the effect of *H. pylori* infection, an established risk factor for stomach cancer. In three case–control studies (Zaridze *et al.*, 2000; Brenner *et al.*, 2002; Wu *et al.* 2003), and two cohort studies (Simán *et al.*, 2001; Shikata *et al.*, 2008) the joint effects and possible interaction between *H. pylori* status and smoking in relation to risk for stomach cancer was investigated. Among subjects who had *H. pylori* infection, the risk for stomach cancer was higher in current smokers than in non-smokers by 1.6 to 2.7 fold, providing evidence for a causal effect of tobacco smoking independently of *H. pylori* infection. Smoking was associated with risk elevations of the same order of magnitude among subjects without *H. pylori* infection. Smoking and *H. pylori* therefore may act synergistically, leading to very high risks in current smokers with *H. pylori* infection compared to non-smokers without *H. pylori* infection. In one study that examined risk by subsite an effect of smoking independent of *H. pylori* infection for gastric cardia as well as distal gastric cancer was found (Wu *et al.*, 2003). In none of the studies was there statistically significant evidence for interaction.

2.5 Cancer of the pancreas

2.5.1 Overview of studies

Previous *IARC Monographs* (IARC, 1986, 2004a) concluded that exposure to tobacco smoke caused cancer of the pancreas. Additional evidence has come from a pooled analysis of eight cohort studies with almost 1500 incident cases of pancreatic cancer and an equal number of controls (Lynch *et al.*, 2009) as well as a meta-analysis of 82 independent studies (42 case–control studies, 40 cohort studies) published between 1950 and 2007 (Iodice *et al.*, 2008; Table 2.39 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.39.pdf). In the meta-analysis 74% and 20% significant increased risks for current and former smokers, respectively, were found with significant heterogeneity of effect regarding current smoking across studies. Adjustment for confounders explained some of the heterogeneity (Iodice *et al.*, 2008). A similar significant risk elevation of 77% for current smokers was found in the pooled analysis, without study heterogeneity (Lynch *et al.*, 2009). For former smokers, risk was increased non-significantly by 9%.

Since the previous *IARC Monograph* (IARC, 2004a), a total of 15 cohort studies have reported on the association between cigarette smoking and pancreatic cancer incidence (8 studies) and mortality (5 studies) or both (one study) (Table 2.40 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.40.pdf), two of which were included in the pooled analysis (Coughlin *et al.*, 2000; Vrieling *et al.*, 2009). Excluding case–control studies that did not report odds ratios for current smokers, there were three additional case–control studies (Duell *et al.*, 2002; Inoue *et al.*, 2003; Alguacil & Silverman, 2004; Table 2.41 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.41.pdf). The effect of cigar and pipe smoking on pancreatic cancer was

also examined in the ACS Cancer Prevention Study II regarding mortality (Shapiro *et al.*, 2000; Henley *et al.*, 2004) and in the Kaiser Permanente Medical Care Program regarding incidence (Iribarren *et al.*, 1999). All the additional studies showed an increased risk for pancreatic cancer associated with tobacco smoking, generally higher in current than in former smokers. The reported risk estimates were not always statistically significant, predominantly due to the small size of some studies and therefore lack of statistical precision.

2.5.2 Factors affecting risks

(a) Intensity and duration

Clear evidence has been provided by the meta-analysis, the pooled analysis as well as the additional studies that the risk for cancer of the pancreas increases significantly with increasing daily cigarette consumption, duration and pack–years of smoking (Coughlin *et al.*, 2000; Gapstur *et al.*, 2000; Nilsen & Vatten, 2000; Nilsson *et al.*, 2001; Isaksson *et al.*, 2002; Doll *et al.*, 2005; Yun *et al.*, 2005; Ansary-Moghaddam *et al.*, 2006; Gallicchio *et al.*, 2006; Vrieling *et al.*, 2009). In the meta-analysis risk of pancreatic cancer increased significantly by 62% with an increase of 20 cigarettes per day (based on 45 studies) and by 16% with a 10-year increase in smoking duration (based on 16 studies), but with significant study heterogeneity. In the pooled analysis, the excess odds ratio per pack–years generally declined with increasing smoking intensity (Lynch *et al.*, 2009).

(b) Cessation of smoking

A reduction in risk in former smokers who had stopped smoking for at least 10 years was found in the meta-analysis (Iodice *et al.*, 2008) and the pooled study (Lynch *et al.*, 2009). In some cohort studies risk was already comparable to never smokers five years after quitting (Boyle *et al.*, 1996; Fuchs *et al.*, 1996; Nilsen & Vatten, 2000; Vrieling *et al.*, 2009).

(c) Types of tobacco

In non–cigarette smokers, mortality from pancreatic cancer was increased although not statistically significantly so in cigar smokers in the CPS-II cohort study (Shapiro *et al.*, 2000) as well as a large case–control study (Alguacil & Silverman, 2004) but was less clearly elevated in the smaller Kaiser Permanente cohort study (Iribarren *et al.*, 1999). There was a significantly increased mortality for current cigar smokers who reported inhaling cigar smoke (Shapiro *et al.*, 2000). Pipe smoking was also found to be associated with an increased risk of cancer of the pancreas, which was stronger in those who reported that they inhaled the smoke (Henley *et al.*, 2004). A limitation of the cohort studies is that smoking habits were reported only at baseline, misclassification of smoking exposure is likely to underestimate the associated risks. In the meta-analysis there was a significant increase in risk of 47% associated with current cigar and/or pipe smoking (18 studies) and a non-significant risk elevation of 29% with former cigar and/or pipe smoking (5 studies) (Iodice *et al.*, 2008).

2.5.3 Population characteristics

The effect of sex on pancreatic cancer risk was investigated in two cohort studies (Nilsen & Vatten, 2000; Larsson *et al.*, 2005) and on pancreatic cancer mortality in four cohort studies (Coughlin *et al.*, 2000; Gapstur *et al.*, 2000; Nilsson *et al.*, 2001; Lin *et al.*, 2002a). The relative risks were comparable between men and women and no consistent evidence for an effect modification by sex was observed.

Ethnicity does not appear to modify the association of smoking with pancreatic cancer risk. The roughly twofold elevated risk in current smokers compared to never smokers was observed both in studies of Caucasians (Lynch *et al.*, 2009) and of Asians (Lin *et al.*, 2002a; Jee *et al.*, 2004; Yun *et al.*, 2005; Li *et al.*, 2006). In populations of the Asia-Pacific Region, there

was also no difference in the strength of association between Asia and Australia/New Zealand (Ansary-Moghaddam *et al.*, 2006).

2.5.4 Confounding factors

In two large cohort studies the risk estimates for pancreatic cancer associated with cigarette smoking were not substantially influenced by adjustment for further potential confounding factors, including diabetes, body mass index (BMI), alcohol and dietary intake (Coughlin *et al.*, 2000; Vrieling *et al.*, 2009).

2.6 Cancer of the colorectum

2.6.1 Overview of studies

In the previous *IARC Monograph* (IARC, 2004a) it was not possible to conclude that the association between tobacco smoking and colorectal cancer is casual, principally because of concern about confounding by other risk factors. That evaluation was based on a total of 60 epidemiologic studies, although only few were specifically designed to study the effects of smoking. Studies have however shown consistently that cigarette smoking is a risk factor for colorectal adenomatous polyps, which are recognized precursor lesions of colorectal cancer (Hill, 1978). To explain this discrepancy, Giovannucci *et al.* (1994) hypothesized that a long induction period is required for tobacco to play a role in colorectal carcinogenesis, which would not be captured by studies with shorter follow-up time.

Four recent meta-analyses consistently showed a strong association between cigarette smoking and colorectal cancer (Botteri *et al.*, 2008a; Liang *et al.*, 2009; Huxley *et al.*, 2009; Tsoi *et al.*, 2009).

2.6.2 Cohort studies

Since the previous *IARC Monograph* (IARC, 2004a), 22 additional cohort studies have investigated the association between tobacco smoke and colorectal cancer (Table 2.42 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.42.pdf). [Studies that did not provide point estimates of risk (Andersen *et al.*, 2009; Hansen *et al.*, 2009; Murphy *et al.*, 2009) and included prevalent colorectal cancer in patients with other diagnosis (Chan *et al.*, 2007) are excluded from this review]. Seven of the studies were conducted in Europe, nine in Asia and five in the USA. In eleven studies, risk estimates were reported solely for colorectal cancer (Tiemersma *et al.*, 2002a; Limburg *et al.*, 2003; Otani *et al.*, 2003; Colangelo *et al.*, 2004; Sanjoaquin *et al.*, 2004; Lüchtenborg *et al.*, 2005a; Kim *et al.*, 2006; Akhter *et al.*, 2007; Huxley, 2007a; Kenfield *et al.*, 2008; Hannan *et al.*, 2009), five studies separately for colon cancer and rectal cancer (Shimizu *et al.*, 2003; Wakai *et al.*, 2003; Jee *et al.*, 2004; Yun *et al.*, 2005; Batty *et al.*, 2008) and five studies both for colorectal cancer as well as for colon and rectal cancers (Terry *et al.*, 2002a; van der Hel *et al.*, 2003a; Doll *et al.*, 2005; Paskett *et al.*, 2007; Tsong *et al.*, 2007; Gram *et al.*, 2009). Six studies were restricted to women (Terry *et al.*, 2002a; Limburg *et al.*, 2003; van der Hel *et al.*, 2003a; Paskett *et al.*, 2007; Kenfield *et al.*, 2008; Gram *et al.*, 2009), and two studies to men (Doll *et al.*, 2005; Yun *et al.*, 2005; Akhter *et al.*, 2007). One study reported both colorectal incidence and mortality (Limburg *et al.*, 2003) and three studies only reported colorectal cancer mortality (Doll *et al.*, 2005; Huxley, 2007a; Batty *et al.*, 2008; Kenfield *et al.*, 2008).

(a) Smoking status

Virtually all studies reported elevated risk associated with smoking, although results were not always statistically significant. The largest meta-analysis based on 36 prospective studies

with data from a total of 3007002 subjects found that compared to never smokers, current smokers had a 15% significantly higher risk of developing colorectal cancer and 27% significantly higher risk of colorectal cancer mortality (Liang *et al.*, 2009; Table 2.43 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.43.pdf). In former smokers, colorectal cancer risk was also significantly elevated by 20% whereas colorectal cancer mortality was non-significantly increased by 20%. The risk estimates were not significantly different between colon and rectal cancer for current smokers (RR, 1.10 versus 1.19) and for former smokers (RR, 1.10 versus 1.20). There was no heterogeneity among colorectal cancer studies and no evidence for publication bias. Comparable risk elevations in current and former smokers were found in the other meta-analyses. For current smokers, the risk for colorectal cancer increased significantly by 16% when using data from 22 cohort studies (Huxley *et al.*, 2009), by 20% based on 28 cohort studies (Tsoi *et al.*, 2009), and by 7% based on data from 45 cohort and case–control studies (Botteri *et al.*, 2008a). In the latter meta-analysis a 17% significantly higher risk of colorectal cancer in former smokers was found.

(b) *Intensity of smoking*

All but three of the recent 21 cohort studies (van der Hel *et al.*, 2003a; Jee *et al.*, 2004; Sanjoaquin *et al.*, 2004) investigated dose–response relationships, using at least one of number of cigarettes smoked, duration of smoking, pack–years of smoking, age at smoking initiation, time since smoking cessation. In two further studies (Tiemersma *et al.*, 2002a; Batty *et al.*, 2008) these parameters were examined separately in current and former smokers, as by Chao *et al.* (2000). Statistically significant dose–response trends with amount smoked daily were reported for colorectal cancer (Lüchtenborg *et al.*, 2005a; Akhter *et al.*, 2007; Paskett *et al.*, 2007; Kenfield *et al.*, 2008), for colon cancer (Paskett *et al.*, 2007), and for rectal cancer (Paskett *et al.*, 2007; Tsong *et al.*, 2007). The dose–response of daily cigarette consumption and colorectal cancer was assessed in two meta-analyses (Liang *et al.*, 2009; Tsoi *et al.*, 2009) and both found statistically significant relationships. Based on eleven studies, Liang *et al.* (2009) found that risk for colorectal cancer increased significantly by 17% with an increase of 20 cigarettes/day and by 38% with an increase of 40 cigarettes/day, while colorectal cancer mortality increased by 41% and 98%, respectively (Table 2.43 online). The risk elevation associated with an increase of 20 cigarettes/day was greater for rectal than for colon cancer (13% versus 3%) but this difference was not statistically significant.

(c) *Duration of smoking*

In addition to two previously reported studies (Hsing *et al.*, 1998; Chao *et al.*, 2000), thirteen studies have examined duration of smoking and colorectal cancer risk. A statistically significant trend of increasing risk with increasing duration was found for colorectal (Limburg *et al.*, 2003; Kim *et al.*, 2006; Paskett *et al.*, 2007; Gram *et al.*, 2009), for colon cancer (Paskett *et al.*, 2007) and for rectal cancer (Terry *et al.*, 2002a; Paskett *et al.*, 2007; Tsong *et al.*, 2007). In one study, increasing duration of smoking was significantly associated with risk for colorectal cancer solely in former smokers (Tiemersma *et al.*, 2002a). Based on eight studies (Terry *et al.*, 2002a; Tiemersma *et al.*, 2002a; Limburg *et al.*, 2003; Lüchtenborg *et al.*, 2005a; Kim *et al.*, 2006; Akhter *et al.*, 2007; Paskett *et al.*, 2007; Tsong *et al.*, 2007), a meta-analysis for duration of smoking and colorectal cancer incidence yielded highly significant results (Liang *et al.*, 2009). Risk was increased by 9.4% with a 20-year increase in smoking duration and 19.7% with a 40-year increase. Smoking duration was also significantly associated with risk for rectal cancer but not for colon cancer. In another meta-analysis where dose–response relationship was modelled, a nonlinear increase in risk with

increasing duration was observed (Botteri *et al.*, 2008a). The risk started to increase after approximately 10 years of smoking and reached statistical significance after 30 years.

(d) Pack–years

Since the previous *IARC Monograph*, the association of colorectal cancer with pack–years of cigarette smoking has been evaluated in six studies (Limburg *et al.*, 2003; Otani *et al.*, 2003; Shimizu *et al.*, 2003; Wakai *et al.*, 2003; Kim *et al.*, 2006; Gram *et al.*, 2009). In addition to the previously reported significant results (Giovannucci *et al.*, 1994; Heineman *et al.*, 1994; Chao *et al.*, 2000; Stürmer *et al.*, 2000), a statistically significant trend of increasing risk with increasing pack–years was found for colorectal cancer in two studies (Limburg *et al.*, 2003; Gram *et al.*, 2009), and for colon cancer in one study (Gram *et al.*, 2009). In their dose–response analysis of pack–years and colorectal incidence, Liang *et al.* (2009) included five studies (Giovannucci *et al.*, 1994; Stürmer *et al.*, 2000; Limburg *et al.*, 2003; Otani *et al.*, 2003; Kim *et al.*, 2006) and found a statistically significant trend of increasing risk with increasing pack–years of smoking for colorectal cancer but not specifically for colon or rectal cancer. Risk for colorectal cancer increased by 27% for an increase of 35 pack–years and by 50% for an increase of 60 pack–years.

(e) Age at initiation

In nine of the cohort studies the age at smoking initiation in relation to colorectal cancer (eight studies) or colon and rectal cancer (four studies) was investigated. In four studies a statistically significant trend of increasing risk with decreasing age at initiation of smoking for colorectal cancer was found (Limburg *et al.*, 2003; Kim *et al.*, 2006; Akhter *et al.*, 2007; Gram *et al.*, 2009) and for colon cancer (Gram *et al.*, 2009) and rectal cancer (Tsong *et al.*, 2007). In one meta-analysis (Liang *et al.*, 2009), a highly significant association was found for age at smoking initiation and colorectal cancer incidence based on six studies (Limburg *et al.*, 2003; Kim *et al.*, 2006; Akhter *et al.*, 2007; Paskett *et al.*, 2007; Tsong *et al.*, 2007; Gram *et al.*, 2009). Risk for colorectal cancer was reduced by 2.2% for a 5-year delay in smoking initiation and by 4.4% for a 10-year delay.

(f) Smoking cessation

The effect of smoking cessation by years since stopping was assessed in seven studies, six for colorectal cancer (Tiemersma *et al.*, 2002a; Lüchtenborg *et al.*, 2005a, 2007; Paskett *et al.*, 2007; Kenfield *et al.*, 2008; Gram *et al.*, 2009; Hannan *et al.*, 2009) and three for colon and/or rectal cancer (Wakai *et al.*, 2003; Paskett *et al.*, 2007; Gram *et al.*, 2009). In one study a statistically significant trend in risk reduction with years since quitting was found both overall as well as separately for men and for women (Hannan *et al.*, 2009).

(g) Population characteristics

It has been suggested that the association between smoking and colorectal cancer may be stronger in men than in women. In the three recent cohort studies reporting sex–specific results (Shimizu *et al.*, 2003; Wakai *et al.*, 2003; Colangelo *et al.*, 2004), this was only observed in studies in Japan (Shimizu *et al.*, 2003; Wakai *et al.*, 2003), but could be attributed to the very low prevalence of smoking in women. The studies restricted to women have generally shown associations with cigarette smoking that were of comparable magnitude to those observed in men (Terry *et al.*, 2002a; Limburg *et al.*, 2003; van der Hel *et al.*, 2003a; Paskett *et al.*, 2007; Kenfield *et al.*, 2008; Gram *et al.*, 2009).

Recent studies have been carried out either in Europe and in USA, with predominantly Caucasian study subjects, or in Asia, mostly in Japan and in the Republic of Korea. The results from these studies suggest no differences in the association between tobacco smoking and

colorectal cancer between different ethnic groups.

(h) Subsites

Smoking and risks for colon cancer and for rectal cancer were investigated in eleven of the 21 additional studies. Risk patterns are generally consistent between colon and rectal cancer (Otani *et al.*, 2003; van der Hel *et al.*, 2003a; Wakai *et al.*, 2003; Jee *et al.*, 2004; Yun *et al.*, 2005; Batty *et al.*, 2008). In some studies, dose–response relationships were stronger for rectal cancer than for colon cancer (Terry *et al.*, 2002a; Paskett *et al.*, 2007) or were statistically significant only for rectal cancer (Shimizu *et al.*, 2003; Doll *et al.*, 2005; Tsong *et al.*, 2007). In a meta-analysis (Liang *et al.*, 2009) the association was stronger for rectal cancer than for colon cancer in the subset of cohort studies that differentiated cancer by site. Most dose–response variables were not associated with colon cancer incidence whereas associations were stronger for rectal cancer incidence and statistically significant with longer duration of smoking, albeit based only on a small number of studies. In one cohort study the increased risk associated with smoking was more apparent for proximal than for distal colon cancer (Lüchtenborg *et al.*, 2005a), which was not found in an earlier study (Heineman *et al.*, 1994).

(i) Confounding and effect modification

Smokers have been shown to be more likely than non-smokers to be physically inactive, to use alcohol, to have lower consumption of fruits and vegetables and higher consumption of fat and meat, and they are less likely to be screened for colorectal cancer (Le Marchand *et al.*, 1997; Ghadirian *et al.*, 1998; Nkondjock & Ghadirian, 2004; Reid *et al.*, 2006b; Mutch *et al.*, 2009).

Few potential confounders were considered in the cohort studies evaluated in the previous *IARC Monograph* (IARC, 2004a). Of the cohort studies published since, all except three (van der Hel *et al.*, 2003a; Jee *et al.*, 2004; Doll *et al.*, 2005) considered two or more potential confounders. In eleven of the recent studies adjustments were made for physical activity, alcohol consumption, overweight/obesity (Terry *et al.*, 2002a; Limburg *et al.*, 2003; Otani *et al.*, 2003; Wakai *et al.*, 2003; Yun *et al.*, 2005; Akhter *et al.*, 2007; Ashktorab *et al.*, 2007; Paskett *et al.*, 2007; Tsong *et al.*, 2007; Kenfield *et al.*, 2008; Hannan *et al.*, 2009), and seven also adjusted for dietary habits (e.g. intake of fruits and vegetables, dietary fibres, fat, red meat). Among the studies with the latter adjustments, eight (Giovannucci *et al.*, 1994; Chao *et al.*, 2000; Stürmer *et al.*, 2000; Limburg *et al.*, 2003; Yun *et al.*, 2005; Akhter *et al.*, 2007; Paskett *et al.*, 2007; Hannan *et al.*, 2009) found significant dose–response relationships with at least one of the smoking variables. In two studies a significant association of smoking with colorectal cancer risk was observed after accounting for history of colonoscopy (Paskett *et al.*, 2007; Hannan *et al.*, 2009). Risk factors in multivariable analyses in several studies were level of education, use of menopausal hormone therapy, family history and regular aspirin use. The association between smoking and colorectal cancer was not modified by these other characteristics, or by alcohol consumption in two studies (Otani *et al.*, 2003; Tsong *et al.*, 2007). Therefore, confounding factors do not seem to affect the observed significant increase in risk for colorectal cancer associated with tobacco smoking and the dose–response relationships with smoking variables.

When considering other types of smoking, it is generally found that cigar and pipe smoking are less associated with socioeconomic class and other life-style habits than cigarette smoking. Therefore, it is logical to assume that, for these types of smoking, risk associations derived from epidemiologic studies may be less prone to potential confounding. In all the cohort studies reviewed in the previous *IARC Monograph* (IARC, 2004a) an elevated, though not always statistically significant, risk was consistently reported for cancers of the colon and the rectum

associated with exclusive pipe and/or cigar smoking.

Infection with JC virus has been proposed as a potential risk factor for colon cancer (Rollison *et al.*, 2009) but results still need further validation.

Three cohort studies assessed possible modifying effects by genetic susceptibility. Rapid acetylator phenotype (as determined by polymorphisms of the *NAT2* gene involved in metabolism of heterocyclic aromatic amines) was found to increase the risk for colorectal cancer in smokers, in one (van der Hel *et al.*, 2003a) but not in another study (Tiemersma *et al.*, 2002a). For genes involved in the metabolism of polycyclic aromatic hydrocarbons such as *GSTM1* or *GSTT1,* no statistical contribution to the risk of colorectal cancer associated with smoking was observed (Tiemersma *et al.*, 2002a; Lüchtenborg *et al.*, 2005a).

2.6.3 Case–control studies

Thirty-one case–control studies were included in the previous *IARC Monograph* (IARC, 2004a). Although results were inconsistent with respect to risk association in ever versus former and current smokers, a dose–response relationship with smoking variables was found in some studies. Since then, seventeen case–control studies investigating the association between tobacco smoke and colorectal cancer risk have been published, seven carried out in Asia, four in Europe, five in North America and one in Hawaii (Table 2.44 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.44.pdf). Six studies reported solely for colorectal cancer (Ateş *et al.*, 2005; Chia *et al.*, 2006; Verla-Tebit *et al.*, 2006; Lüchtenborg *et al.*, 2007; Steinmetz *et al.*, 2007; Wu *et al.*, 2009b), four separately for colon and rectal cancer (Ji *et al.*, 2002; Sharpe *et al.*, 2002; Minami & Tateno, 2003; Goy *et al.*, 2008), two for colorectal cancer as well as for colon and rectal cancer (Ho *et al.*, 2004; Gao *et al.*, 2007; Wei *et al.*, 2009), three for colon cancer only (Diergaarde *et al.*, 2003; Kim *et al.*, 2003; Hu *et al.*, 2007) and one for rectal cancer only (Slattery *et al.*, 2003). Nine of the studies reported risk estimates separately for men and for women.

(a) Smoking status

Most case–control studies considered the effects of current and former smoking separately. A positive association between smoking and colorectal cancer was found in virtually all the studies, although the results were generally not statistically significant. Statistically significant increased risk was reported in current smokers for colorectal cancer (Chia *et al.*, 2006; Wu *et al.*, 2009b), for rectal cancer (Slattery *et al.*, 2003; Ho *et al.*, 2004), and in former smokers for colorectal cancer both in men and women combined (Chia *et al.*, 2006) and in women only (Lüchtenborg *et al.*, 2007). Five studies, which did not focus on the main effects of smoking, only evaluated risks for ever smoking (Diergaarde *et al.*, 2003; Kim *et al.*, 2003; Ateş *et al.*, 2005; Gao *et al.*, 2007; Hu *et al.*, 2007); none of these reported significant risk estimates.

(b) Intensity of smoking

Nine case–control studies investigated dose–response relationships considering at least one smoking variable. Number of cigarettes smoked daily was evaluated in seven studies, three for colorectal cancer (Verla-Tebit *et al.*, 2006; Lüchtenborg *et al.*, 2007; Wu *et al.*, 2009b), two for colon and rectal cancer (Ji *et al.*, 2002; Minami & Tateno, 2003), one for rectal cancer (Slattery *et al.*, 2003) and one for colorectal cancer and both subsites (Ho *et al.*, 2004). Statistically significant positive trends of increasing risk with increasing number of cigarettes smoked daily were found for colorectal cancer in only one study (Wu *et al.*, 2009b).

(c) Duration of smoking, pack–years, age at initiation, smoking cessation

Duration of smoking was examined in several studies in relation to colorectal cancer (Ho *et al.*, 2004; Chia *et al.*, 2006; Verla-Tebit *et al.*, 2006; Lüchtenborg *et al.*, 2007; Wu *et al.*, 2009b) and/or to colorectal cancer by subsite (Ji *et al.*, 2002; Minami & Tateno, 2003; Ho *et al.*, 2004). A statistically significant trend with increasing number of years smoked was found in two of the five studies of colorectal cancer (Chia *et al.*, 2006; Wu *et al.*, 2009b). In one study, increasing duration of smoking was significantly associated with risk for rectal cancer in ever smokers but not in current smokers (Ho *et al.*, 2004). In only one earlier case–control study was a significant association in ever smokers with increasing number of years of smoking for colon as well as rectal cancer found (Newcomb *et al.*, 1995).

Duration of smoking exposure was assessed by pack–years of smoking in seven studies (Ji *et al.*, 2002; Slattery *et al.*, 2003; Chia *et al.*, 2006; Verla-Tebit *et al.*, 2006; Lüchtenborg *et al.*, 2007; Goy *et al.*, 2008; Wu *et al.*, 2009b) and by age at smoking initiation in three studies (Ji *et al.*, 2002; Slattery *et al.*, 2003; Wu *et al.*, 2009b). All four studies that evaluated pack–years of smoking with respect to colorectal cancer risk found statistically significant associations. Two studies found a significant association with increasing pack–years in men and women combined; when investigated separately, the increasing trend was statistically significant only in women (Verla-Tebit *et al.*, 2006) or only in men (Wu *et al.*, 2009b). In one study a statistically significant trend with pack–years of smoking in both men and women was found only with non-filtered cigarettes (Lüchtenborg *et al.*, 2007); the relative risk was significant for colon as well as rectal cancer and was greater for rectal cancer.

In two studies a non-significant trend of decreasing risk with increasing time since stopped smoking was found (Verla-Tebit *et al.*, 2006; Lüchtenborg *et al.*, 2007).

(d) Subsites and molecular subtypes

A stronger association between tobacco smoking and rectal cancer compared with colon cancer has generally been observed in the studies that reported risk estimates by cancer site. In a recent meta-analysis including both cohort and case–control studies, higher smoking-related risk estimates for rectal cancer were found than for proximal and distal colon cancer (Botteri *et al.*, 2008a). Stronger relative risk in ever smokers, but not in current smokers, was found for proximal compared to distal tumours in one recent study (Hu *et al.*, 2007).

Colorectal cancer is a multipathway disease. A molecular approach to its classification utilizes: (1) the type of genetic instability, specifically microsatellite instability, and (2) the presence of DNA methylation or the CpG island methylator phenotype (CIMP) (Jass, 2007). Smoking has been associated with microsatellite instability in sporadic colon cancer. Higher risk for microsatellite-unstable than for microsatellite-stable tumours was found in four studies (Slattery *et al.*, 2000; Yang *et al.*, 2000; Chia *et al.*, 2006; Campbell *et al.*, 2009). The observed twofold risk elevation for colorectal cancer showing microsatellite instability is similar in order of magnitude to that found for colorectal polyps. In only one small study similar risk estimates for stable and unstable tumours were found (Diergaarde *et al.*, 2003). Microsatellite instability is characteristic of hereditary nonpolyposis colorectal cancer syndrome and smoking has been associated with colorectal cancer in patients with this syndrome (Watson *et al.*, 2004; Diergaarde *et al.*, 2007). Among sporadic colorectal tumours with microsatellite instability, about 11–28% carry somatic genetic mutations. In addition, the association of colon cancer with smoking was increased two to threefold when widespread CIMP and/or *BRAF* mutation, irrespective of microsatellite instability

status, was present (Samowitz *et al.*, 2006). These data indicate that the association with MSI-high tumours may be attributed to the association of smoking with CIMP and *BRAF* mutation.

(e) Effect modification

Effect modification by genetic polymorphisms in enzymes metabolizing tobacco smoke constituents could provide further evidence for a causal association between smoking and colorectal cancer. Most studies that have investigated modification of colorectal cancer risk associated with smoking by genetic polymorphisms of xenobiotic enzymes were too small to be informative (Inoue *et al.*, 2000; Smits *et al.*, 2003; Jin *et al.*, 2005; Tranah *et al.*, 2005; van den Donk *et al.*, 2005; Tijhuis *et al.*, 2008). Studies on the possible differential effect by acetylation status have reported stronger association of tobacco smoking (in terms of pack–years) with colorectal cancer risk in slow acetylators phenotypes (Lilla *et al.*, 2006), and with rectal cancer in rapid acetylators phenotypes (Curtin *et al.*, 2009). Furthermore, *CYP1A1* and *GSTM1* variant alleles were found to greatly affect colon cancer or rectal cancer risk in smokers (Slattery *et al.*, 2004).

2.6.4 Colorectal polyps

Colorectal adenomas and possibly some hyperplastic polyps are considered precursors of colorectal cancer. The epidemiologic evidence on the relationship between cigarette smoking and colorectal polyps has been generally consistent. Since the previous *IARC Monograph* (IARC, 2004a), twelve further independent studies have investigated this association (Table 2.45 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.45.pdf). All studies found a significantly increased risk for polyps in association with one or more smoking variables. A recent meta-analysis including 42 studies reported a statistically significant positive association between smoking and colorectal adenomas (Botteri *et al.*, 2008b). The meta-analysis, which included several studies that did not explicitly report relative risks for tobacco smoking (Cardoso *et al.*, 2002; Voskuil *et al.*, 2002; Sparks *et al.*, 2004; Gong *et al.*, 2005; Jiang *et al.*, 2005; Kim *et al.*, 2005; Mitrou *et al.*, 2006; Otani *et al.*, 2006; Skjelbred *et al.*, 2006), found a twofold risk elevation for colorectal adenomas in current smokers and a 50% increase in former smokers. The association had been previously found to be equally strong in men and women. In one of two recent studies, there was no difference in the results for men and women separately (Tranah *et al.*, 2004) but significantly greater effects in women were found in the other (Hermann *et al.*, 2009).

Significant positive trends with number of cigarettes per day were found in four (Ji *et al.*, 2006; Larsen *et al.*, 2006; Stern *et al.*, 2006; Shrubsole *et al.*, 2008) of five studies (Tiemersma *et al.*, 2004). Dose–response with duration of smoking was assessed in four studies (Ji *et al.*, 2002; Tiemersma *et al.*, 2004; Stern *et al.*, 2006; Shrubsole *et al.*, 2008) and with pack–years of smoking in five studies (Hoshiyama *et al.*, 2000; Ulrich *et al.*, 2001; Tranah *et al.*, 2004; Ji *et al.*, 2006; Shrubsole *et al.*, 2008; Omata *et al.*, 2009). All nine studies found statistically significant trends, which were consistent with those for adenomas and hyperplastic polyps when reported separately (Ulrich *et al.*, 2001; Ji *et al.*, 2006; Shrubsole *et al.*, 2008). Ever smokers were estimated to have a 13% (95%CI: 9–18%) increasing risk of presenting with adenomatous polyps for every additional 10 pack–years smoked in comparison to never smokers, based on data from 19 studies (Botteri *et al.*, 2008b).

Decreasing risks with years since quitting smoking were found in four studies (Ulrich *et al.*, 2001; Tiemersma *et al.*, 2004; Ji *et al.*, 2006; Shrubsole *et al.*, 2008), statistically significant so in the latter three studies. In comparison to never smokers, former smokers retained moderately elevated risk for colorectal polyps even 20

years after quitting smoking. One study examined both dose metrics (cigarettes per day, duration, and pack–years) and recency of tobacco use: in subjects who had quit smoking for at least 20 years, only the heaviest users of tobacco still had modest excess risks (Ji *et al.*, 2006).

It has been proposed that the association between cigarette smoking and polyps may be stronger with non-progressing adenomas, such as those that are smaller and less villous but the hypothesis is not supported in most studies (Anderson *et al.*, 2003; Toyomura *et al.*, 2004; Ji *et al.*, 2006; Skjelbred *et al.*, 2006). In one study a clearly higher risk for large and multiple adenomas in every anatomic site of the colon was found in a dose–response manner (Toyomura *et al.*, 2004). A meta-analysis found that the combined risk estimate for high-risk adenomas associated with smoking was greater than that for low-risk adenomas and that the difference was statistically significant for current smokers but not former smokers (Botteri *et al.*, 2008b). In addition, a stronger association of smoking with hyperplastic polyps than with adenomas was found in some studies (Ulrich *et al.*, 2001; Ji *et al.*, 2006; Shrubsole *et al.*, 2008) but not in another (Erhardt *et al.*, 2002). The risk associated with smoking may be even higher in subjects presenting with concurrent benign hyperplastic and adenomatous polyps (Ji *et al.*, 2006; Shrubsole *et al.*, 2008).

Relative risk estimates for tobacco smoking and polyps generally range between 2 and 3 whereas those for colorectal cancer range between 1.2 and 1.4. One possible explanation is the effect dilution due to the inclusion of a high proportion of individuals with precursor lesions in the unscreened control groups in most colorectal cancer studies (Terry & Neugut, 1998). Some indirect evidence for this hypothesis is provided by the meta-analysis of colorectal adenomas, which showed that the smoking-associated risk for adenomas was significantly higher in studies including subjects who had undergone complete colonoscopy in comparison to those in which some or all controls had undergone incomplete examination (i.e. only sigmoidoscopy) (Abrams *et al.*, 2008; Botteri *et al.*, 2008b).

It is also possible that smoking is associated with a subset of colorectal cancers so that relative risk estimates for colorectal cancer as a whole are diluted. The pattern of risk observed for colorectal cancer by microsatellite instability status and for type of colorectal polyps suggests that the traditional (non-serrated) adenoma-carcinoma sequence may proceed through a hyperplastic polyps-mixed polyps-serrated adenoma progression and that smoking may be more strongly related to the development of these subtypes (Jass *et al.*, 2000; Hawkins & Ward, 2001). More recently, a *BRAF* mutation was shown to be a specific marker for the serrated polyp neoplasia pathway originating from a hyperplastic polyp, in which the CIMP-high develops early and the microsatellite instability carcinoma develops late (O'Brien *et al.*, 2006). The findings of strong associations between smoking and colon cancer with CIMP and/or BRAF mutation, irrespective of microsatellite status, are compatible with this observation (Samowitz *et al.*, 2006).

2.7 Hepatocellular carcinoma

2.7.1 Overview of studies

In the previous *IARC Monograph* (IARC, 2004a), a causal relationship between liver cancer (hepatocellular carcinoma) and smoking was established. Two case–control and one cohort studies have been published since (Table 2.46 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.46.pdf). Overall, most cohort studies and the largest case–control studies, most notably those that included community controls, showed a moderate association between tobacco smoking and risk for hepatocellular carcinoma.

Confounding from alcohol has been addressed in the best studies. The association between alcohol drinking and hepatocellular carcinoma is strong, and alcohol intake is frequently misclassified, leading to potential residual confounding. However an association with smoking has been demonstrated also among non-drinkers.

A meta-analysis was based on 38 cohort studies and 58 case–control studies (Lee *et al.*, 2009). Compared to never smokers, the meta-relative risks adjusted for appropriate confounders were 1.51 (95%CI: 1.37–1.67) for current smokers and 1.12 (0.78–1.60) for former smokers. The increased liver cancer risk among current smokers appeared to be consistent in strata of different regions, study designs, study sample sizes, and publication periods. The association with smoking was observed in non-alcohol-drinkers (RR, 1.34; 95%CI: 0.92–1.94 in men and 1.31; 95%CI: 0.70–2.44 in women). Further supportive evidence is provided by the association between smoking and liver cancer observed among Chinese women and Japanese women, in whom alcohol drinking is extremely rare (Li *et al.*, 2011). One difficulty is that sometimes studies do not specify the histology of liver cancer (hepatocellular versus intra-hepatic biliary tract).

In the update of the Whitehall study (Batty *et al.*, 2008) (a cohort of 17363 government employees in London, followed-up for 38 years), the hazard ratio for death from liver cancer was 1.03 (0.49–2.16) in former smokers and 1.43 (0.69–2.95) in current smokers (based on 57 deaths). In the 50-year follow-up of the British doctors cohort (Doll *et al.*, 2005), there were 74 deaths from liver cancer. Death rates per 100000 per year were 4.4 in never smokers, 10.7 in smokers of 1–14 cigarettes/day, 2.6 in smokers of 15–24 cigarettes/day, and 31.3 in smokers of ≥ 25 cigarettes/day.

2.7.2 Factors affecting risks

(a) Dose–response relationship

Most studies, including the recent ones (Table 2.46 online), show a dose–response relationship with the number of cigarettes smoked and with smoking duration, with exceptions such as Franceschi *et al.* (2006) and some older studies from Asia. Relative risk estimates increased to 2.0 after 20 years of smoking.

(b) Cessation

Though former smokers tend to have lower relative risks than current smokers, there were no consistent patterns of risks after cessation, including in the recent studies (Table 2.46 online).

2.7.3 Interaction with hepatitis B or C

Infection with hepatitis B virus (HBV) is one of the major causes of liver cancer worldwide, whereas hepatis C virus (HCV) infection causes a large fraction of liver cancer in Japan, Northern Africa and southern Europe. While many studies, most notably from Asia, have found no attenuation of the association between smoking and liver cancer after adjustment/stratification for markers of HBV or HCV infection, an apparent interaction between smoking and HBV or HCV infection has been reported. The increase in risk for liver cancer associated with cigarette smoking appears to be greater among HBV carriers than among uninfected persons in some studies (Tu *et al.*, 1985), but not in others (Kuper *et al.*, 2000a). Two recent reports (Franceschi *et al.*, 2006; Hassan *et al.*, 2008a) studied possible interactions between smoking and hepatitis virus infection and both reported an apparent interaction between smoking and hepatitis C infection. Interactions between smoking and hepatitis B infection were not found among men in one study (Hassan *et al.*, 2008a) and the rarity of HBsAg prevented the evaluation of HBV and smoking in the other (Franceschi *et al.*, 2006;

Table 2.46 online). In the meta-analysis by Lee *et al.* (2009) adjustment for HBV reduced the relative risks in both men and women, while adjustment for HCV did not change the risk in women and increased it in men.

2.8 Renal cell carcinoma

2.8.1 Overview of studies

The previous *IARC Monograph* (IARC, 2004a) concluded that renal-cell carcinoma is associated with tobacco smoking in both men and women. Four case–control studies and no cohort studies have become available since then (Table 2.47 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.47.pdf). Overall these confirm the previous evidence, though with some conflicting results. In particular, both the study by Hu *et al.* (2005) in Canada and the multicentre European study by Brennan *et al.* (2008) do not show a clear effect of smoking. In contrast, the study by Theis *et al.* (2008) shows an increased risk with smoking duration (irregular, levelling-off after 40 years) and a statistically significant dose–response relationship with pack–years.

In the update of the Whitehall study (Batty *et al.*, 2008) (a cohort of 17363 government employees in London, followed for 38 years), the hazard ratio for deaths from kidney cancer was 0.64 (0.32–1.26) for former smokers, and 1.29 (0.69–2.41) for current smokers (based on 68 deaths). In the 50-year follow-up of the British doctor cohort (Doll *et al.*, 2005) there were 140 deaths from kidney cancer. Mortality rates per 100000 per year were 9.3 in never smokers, 16.4 in smokers of 1–14 cigarettes/day, 16.6 in smokers of 15–24 cigarettes/day, and 15.5 in smokers of ≥ 25 cigarettes/day (age-adjusted).

Hunt *et al.* (2005) performed a meta-analysis based on 19 case–control studies and 5 cohort studies (total 8032 cases in case–control and 1326 in cohort studies). The relative risk for smoking men was 1.54 (1.42–1.68), and for smoking women was 1.22 (1.09–1.36). A dose–response relationship was found in both men and women. The association observed was more convincing in population-based compared to hospital-based studies.

2.8.2 Confounding

Hypertension is a well established risk factor for kidney cancer but the association with smoking is only indirect. Potential confounding from hypertension was considered only by Brennan *et al.* (2008).

Other potential confounders such as BMI have been appropriately addressed in most studies.

2.8.3 Cessation

Most studies reviewed in the previous *Monograph* showed a lower risk for former smokers compared to current smokers, with a significant negative trend with increasing number of years since quitting (IARC, 2004a). In case–control study on smoking cessation and renal-cell carcinoma, the decrease in risk became significant only after 30 years of quitting (Parker *et al.*, 2003). In the meta-analysis (Hunt *et al.*, 2005), former smokers were at reduced risk after 10 years or more of quitting. A clear decline in risk after cessation was also reported by Theis *et al.* (2008). [The Working Group noted the poor quality of the study, considering the low response rate among controls.]

2.9 Cancer of the lower urinary tract (including cancer of the bladder, ureter, and renal pelvis)

2.9.1 Overview of studies

The previous *IARC Monograph* (IARC, 2004a) clearly identified a causal relationship of smoking with transitional-cell carcinomas and squamous-cell carcinomas of the bladder, ureter and renal pelvis both in men and women. Two new case–control studies (Cao *et al.*, 2005; Samanic *et al.*, 2006; Table 2.48 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.48.pdf) and two cohort studies (Bjerregaard *et al.*, 2006; Alberg *et al.*, 2007; Table 2.49 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.49.pdf) have been reported since then in addition to updates of cohort studies with longer follow-up.

In the update of the Whitehall study (Batty *et al.*, 2008) (a cohort of 17363 government employees in London, followed-up for 38 years), the hazard ratio for death from bladder cancer was 0.98 (0.62–1.54) in former smokers and 1.66 (1.06–2.59) in current smokers (based on 164 deaths). In the 50-year follow-up of the British doctors cohort (Doll *et al.*, 2005), there were 220 deaths from bladder cancer. Death rates per 100000 per year were 13.7 in never smokers, 37.7 in smokers of 1–14 cigarettes/day, 31.8 in smokers of 15–24 cigarettes/day, and 51.4 in smokers of ≥ 25 cigarettes/day. All the new studies confirm the existence of a dose–response relationship with the number of cigarettes smoked and with duration, and a decline in relative risk with time since quitting smoking, compared to non-quitters.

2.9.2 Types of tobacco

The risk of lower urinary tract cancer was more strongly associated with smoking air-cured (black) tobacco than smoking flue-cured (blond) tobacco in several studies (IARC, 2004a). The stronger association with air-cured (black) than blond tobacco among current smokers has not been clearly confirmed in a re-analysis of the Spanish multicentre case–control study (Samanic *et al.*, 2006). Relative risks in current smokers were 7.3 (4.9–10.9) in black tobacco smokers and 5.8 (3.4–10.0) in blond tobacco smokers; in former smokers, 4.2 (2.9–6.0) for black tobacco and 1.8 (1.0–3.2) for blond tobacco (Table 2.48 online). The effect of cessation was more pronounced in blond tobacco smokers than in black tobacco smokers, suggesting potentially different mechanisms of action of the two types of tobacco. Air-cured (black) tobacco is richer in arylamines.

2.9.3 Gene–environment interactions

A large number of studies have considered gene–environment interactions between tobacco smoking and genetic polymorphisms, including DNA repair genes (Vineis *et al.*, 2009) and genes involved in carcinogen metabolism (Malats, 2008; Dong *et al.*, 2008). Overall, there is evidence that the slow acetylator variant of the *NAT2* gene is involved in bladder carcinogenesis and may interact with smoking. The meta-relative risk for *NAT2* slow acetylator and bladder cancer was 1.46 (95%CI: 1.26–1.68; $P = 2.5 \times 10^{-7}$), based on 36 studies and 5747 cases (Dong *et al.*, 2008). Similar but weaker evidence has been provided for *GSTM1* (Malats, 2008).

The extent of interaction between *NAT2* variants and smoking is still unclear. In one study the *NAT2* acetylation status was found to modulate the association of bladder cancer and cigarette smoking through smoking intensity and not smoking duration (Lubin *et al.*, 2007). Studies are not consistent concerning the three-way association between smoking intensity, *NAT2* and bladder cancer. Some studies found greater effects at a lower level of exposure and others the opposite (Malats, 2008). Genome-wide

association studies have indicated 8q24 as a region that may confer high risk for bladder cancer (Kiemeney *et al.*, 2008).

2.10 Myeloid leukaemia (acute and chronic)

Myeloid leukaemia in adults was observed to be causally related to cigarette smoking in the previous *IARC Monograph* (IARC, 2004a). Risk increased with amount of tobacco smoked in a substantial number of adequate studies, with evidence of a dose–response relationship. Biological plausibility for a causal relationship of smoking with myeloid leukaemia is provided by the finding of known leukaemogens in tobacco smoke, one of which (benzene) is present in relatively large amounts. No evidence was found for an association with acute lymphocytic leukaemia.

One recently published cohort study included information on acute and chronic myeloid leukaemias (Fernberg *et al.*., 2007), based on 372 incident cases. A weak association was found between acute myeloid leukaemia and intensity of smoking, and a statistically significant association with current smoking (RR, 1.5; 95%CI: 1.06–2.11). No association was found with chronic myeloid leukaemia.

In the update of the Whitehall study (Batty *et al.*., 2008) (a cohort of 17363 government employees in London, followed-up for 38 years), the hazard ratio for mortality from myeloid leukaemias (acute plus chronic) was 5.08 (95%CI: 1.78–14.5) for current smokers, and 3.84 (95%CI: 1.35–11.0) for former smokers (based on 66 deaths). In the 50-year follow-up of the British doctors cohort (Doll *et al.*., 2005), there were 100 deaths from myeloid leukaemias. The mortality rates per 100000 per year were 6.3 in never smokers, 2.8 in smokers of 1–14 cigarettes/day, 14.0 in smokers of 15–24, and 18.3 in smokers of ≥ 25 cigarettes/day (age-adjusted).

2.11 Other leukaemias and lymphomas

2.11.1 Non-Hodgkin lymphoma

Six cohort studies have been published on the association between non-Hodgkin lymphoma and smoking, all reviewed in the previous *IARC Monograph* (IARC, 2004a). In five of these, no increased risk among smokers was evident (Doll *et al.*, 1994; McLaughlin *et al.*, 1995; Adami *et al.*, 1998; Herrinton & Friedman, 1998; Parker *et al.*, 2000). However, in one study, men who had ever smoked cigarettes had a twofold increase in risk for non-Hodgkin lymphoma, and the risk was still higher among the heaviest smokers (Linet *et al.*, 1992). Data from case–control studies generally also fail to support an effect of smoking on the incidence of non-Hodgkin lymphoma (Peach & Barnett, 2001; Stagnaro *et al.*, 2001; Schöllkopf *et al.*, 2005; Bracci & Holly, 2005; Table 2.50 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.50.pdf). Reanalysis of data of an Italian study (Stagnaro *et al.*, 2004) found a statistically significant association (OR, 1.4; 95%CI: 1.1–1.7) for blond tobacco exposure and non-Hodgkin lymphoma risk.

Three studies and a pooled analysis have examined histological subtypes of non-Hodgkin lymphoma. In one cohort study in women, smoking was associated with increased risk for follicular non-Hodgkin lymphoma (Parker *et al.*, 2000). Similarly, two other studies reported a weak positive association between smoking and risk for follicular lymphoma, but no effect for other histological types (Herrinton & Friedman, 1998; Stagnaro *et al.*, 2001). A large pooled analysis based on nine North-American and European case–control studies found an overall odds ratio of 1.07 (95%CI: 1.0–1.15) for smokers; the association was particularly strong for follicular lymphoma (OR, 1.31; 95%CI: 1.12–1.52) (Morton *et al.*, 2005).

2.11.2 Hodgkin lymphoma

In the previous *IARC Monograph* (IARC, 2004a) seven studies on the association between Hodgkin lymphoma and smoking were examined and null or weakly positive associations were noted. Among studies published since, a positive association was observed in two case–control (Willett *et al.*, 2007; Kanda *et al.*, 2009) and three cohort studies (Nieters *et al.*, 2006; Lim *et al.*, 2007; Nieters *et al.*, 2008), while one study found no clear association (Monnereau *et al.*, 2008). Several other recent studies also reported a positive association, but with some internal inconsistencies. In a European multicentre case–control study, no association was observed between tobacco and Hodgkin lymphoma for subjects below age 35 years, whereas for older subjects, ever-smokers experienced a doubled risk of Hodgkin lymphoma as compared to never smokers (Besson *et al.*, 2006). In contrast, a positive association was observed in young adults participating in the International Twin Study (Cozen *et al.*, 2009). A positive association was observed in a Scandinavian case–control study, but without a clear dose–response (Hjalgrim *et al.*, 2007). In a case–control study addressing infectious precursors, particularly Epstein-Barr virus (EBV), an increased risk for EBV-positive Hodgkin lymphoma was found among current smokers (Glaser *et al.*, 2004; Table 2.50 online).

Several of the above studies found positive associations for Hodgkin lymphoma while also demonstrating null or inverse associations with non-Hodgkin lymphoma (Nieters *et al.*, 2006; Lim *et al.*, 2007; Nieters *et al.*, 2008; Kanda *et al.*, 2009).

2.11.3 Multiple myeloma

In the previous *IARC Monograph* (IARC, 2004a), the large majority of studies on tobacco smoking and risk for multiple myeloma evaluated showed no clear association. More recently, two case–control studies found a positive association (Vlajinac *et al.*, 2003; Nieters *et al.*, 2006), whereas no clear association was observed in another case–control study (Monnereau *et al.*, 2008) or in a cohort study in Sweden (Fernberg *et al.*, 2007; Table 2.51 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.51.pdf).

2.12 Cancer of the breast

Approximately 150 epidemiological studies have been published on the relationship between breast cancer and active and passive smoking. The results from these studies have been comprehensively examined in peer-reviewed literature (Palmer & Rosenberg, 1993; Terry *et al.*, 2002a; Johnson *et al.*, 2002; Johnson, 2005; Terry & Goodman, 2006; Miller *et al.*, 2007). The previous *IARC Monograph* (IARC, 2004a) considered studies conducted through June 2002 and concluded that there is evidence suggesting lack of carcinogenicity of tobacco smoking in humans for cancers of the female breast.

Other consensus reviews have drawn different conclusions, based partly on the availability of new data, and partly on differences in interpretation:

- The 2001 US Surgeon General Report on Women and Smoking (Department of Health & Human Services, 2001) concluded that tobacco smoking does not appear to appreciably affect breast cancer risk overall. However, several issues were not entirely resolved, including whether starting to smoke at an early age increases risk, whether certain subgroups defined by genetic polymorphisms are differentially affected by smoking, and whether exposure to second-hand smoke affects risk.
- The 2004 US Surgeon General report on "The Health Consequences of Smoking" (Department of Health & Human

Services, 2004) concluded the evidence is suggestive of no causal relationship between tobacco smoking and breast cancer.

- The 2009 Canadian Expert Panel on Tobacco Smoke and Breast Cancer Risk (Collishaw *et al.*, 2009) concludes that based on the weight of evidence from epidemiological and toxicological studies and understanding of biological mechanisms, the associations between tobacco smoking and both pre- and post-menopausal breast cancer are consistent with causality.

The lack of agreement in the conclusions from these groups is not surprising, given that the observed associations are weaker and less consistent for breast cancer than for other tobacco-related cancers. Furthermore, several methodological considerations could either obscure a small increase in risk caused by tobacco smoking, or alternatively introduce a spurious association where no causal relationship exists.

2.12.1 Methodological and related issues

The principal concerns about studies of tobacco smoking and breast cancer are the following: timing of exposure, the relevant disease endpoint, the potential for confounding by factors associated with both smoking and the occurrence/detection of breast cancer, the hypothesis that tobacco smoking may have opposing effects on breast cancer risk (protective and detrimental), and the hypothesis that some women may be genetically more susceptible to develop breast cancer from smoking, and that increased risk in these subgroups may be obscured in analyses of average risk in the population.

(a) Misclassification of exposure

Self-reported information on tobacco smoking is generally considered more reliable than questionnaire information on exposure to second-hand tobacco smoke. However, studies of tobacco smoking have not uniformly considered the duration of smoking (years), the average amount smoked (cigarettes/day), or the timing of initiation in relation to first full-term pregnancy. Only one (Al-Delaimy *et al.*, 2004) of the seven available cohort studies updated the information on smoking behaviour during follow-up. Whereas some exposure variables, such as age at initiation and age at first full-term pregnancy remain constant over time, others, such as smoking status, duration and age at cessation do not. Furthermore, the average age at initiation and duration of smoking are highly correlated with birth cohort and attained age. While the number of years of smoking before first full term pregnancy has been proposed as a potentially relevant measure of exposure, the range of this variable is constrained except among women whose first pregnancy occurs at an older age, which is itself an independent risk factor for breast cancer.

(b) Specificity of disease endpoints

Breast cancer is not a single disease. Accordingly, some researchers have postulated that exposure to tobacco smoke (from tobacco smoking or second-hand tobacco smoke) could differentially affect certain clinical subtypes of breast such as pre- or post-menopausal cancers or tumours with or without hormonal receptors. It is also possible that smoking might affect the survival of women with breast cancer, whether or not it affects incidence rates. Most published studies have measured incidence rates as the endpoint, although some have measured mortality rates or effects on survival.

(c) Confounding

Alcohol consumption is positively correlated with tobacco smoking (Marshall *et al.*, 1999) and is an established cause of breast cancer (IARC, 2010a; *Monograph* on Consumption of Alcoholic Beverages in this Volume). Most epidemiologic studies attempt to control for alcohol consumption using questionnaire information on usual drinking patterns. This approach is vulnerable to residual confounding, because self-reported data on lifetime alcohol consumption leave room for misclassification. Potential confounding by alcohol consumption is of greater concern for current than for former smokers, since, on average, current smokers drink more than former smokers (Reynolds *et al.*, 2004a, b). One study by the Collaborative Group on Hormonal Factors and Breast Cancer (Hamajima *et al.*, 2002) controlled rigorously for alcohol consumption by restricting the analysis of smoking and breast cancer to women who reported drinking no alcohol.

Conversely, mammography screening can be a negative confounder in studies of tobacco smoking and breast cancer incidence. Few studies of tobacco smoking in relation to breast cancer have controlled for mammography screening. Current smokers report a lower frequency of mammographic screening than never-smokers, whereas health conscious former smokers report higher screening rates (Gross *et al.*, 2006). Mammography screening affects the detection rather than the occurrence of breast cancer; it detects some tumours that might otherwise never have been recognized and allows earlier diagnosis of others, thereby increasing breast cancer incidence in the short-term. The consequence of uncontrolled confounding by mammography screening would be to underestimate an association between current smoking and breast cancer incidence, and to overestimate the association in former smokers. Confounding by screening would be expected to have the opposite effect in studies of breast cancer mortality.

Other correlates of tobacco smoking might also confound a potential association between tobacco smoking and breast cancer, although their net effect is likely to be smaller and harder to predict than confounding by alcohol and mammography screening. Women who smoke undergo menopause about two to three years earlier than never-smokers (Baron *et al.*, 1990). The effect of this may be partly or wholly offset by the greater likelihood of girls who experience early menarche to initiate smoking in early adolescence (Jean *et al.*, 2011). There is no documentation that smokers and never-smokers differ with respect to average years of ovulation. Tobacco smoking also has a complex relationship to body mass index. Post-menopausal women who smoke are less likely to be overweight or obese than former or never smokers, but overweight adolescent girls are more likely to begin smoking for weight control (Fine *et al.*, 2004). Similarly complex relationships exist between smoking and physical activity. Current smokers report less physical activity than either former or never smokers (Kaczynski *et al.*, 2008; Trost *et al.*, 2002), but only a small proportion of the population engages in the vigorous physical activity that is needed to protect against breast cancer. The socioeconomic correlates of smoking have changed over time. Women who attended college during the 1960s and 1970s were more likely to initiate smoking than less educated women, but subsequently college-educated women have been more likely to quit. Thus, the potential for confounding by reproductive patterns and use of post-menopausal hormone treatment varies by birth cohort and differs for current and former smokers.

Most epidemiological studies have attempted to control for factors that might confound the relationship between breast cancer and tobacco smoking using questionnaire information collected on these factors. None of the published

studies have been able to control for all of the potential confounders, however. Most studies lack data on screening behaviour and have limited information on alcohol consumption, use of post-menopausal hormones, and physical activity.

(d) Potential anti-estrogenic effects of tobacco smoking

Indirect evidence suggests that tobacco smoking may have anti-estrogenic effects that might offset the adverse effects of tobacco smoke carcinogens on breast cancer risk. Baron *et al.* (1990) pointed to observations suggesting lower estrogen activity levels in women who smoke compared to those who do not. Smokers have lower risk of endometrial cancer (Department of Health & Human Services, 2004), higher risk of osteoporosis (Jensen *et al.*, 1985; Jensen & Christiansen, 1988), earlier age at natural menopause (Baron *et al.*, 1990) and lower mammography density (Roubidoux *et al.*, 2003) than women who do not smoke. Smoking also attenuates the effects of hormone replacement therapy (HRT) on lipid profiles (Jensen & Christiansen, 1988) and serum estrone (McDivit *et al.*, 2008). No difference in serum concentrations of estradiol and estrone between post-menopausal smokers and non-smokers have been reported in several studies (Cassidenti *et al.*, 1992; Khaw *et al.*, 1988; Berta *et al.*, 1991; Longcope *et al.*, 1986; Berta *et al.*, 1992; Cauley *et al.*, 1989; Friedman *et al.*, 1987; Key *et al.*, 1991). However, smokers have been observed to have higher levels of androgens (Cassidenti *et al.*, 1992) (specifically androstenedione) (Khaw *et al.*, 1988; Cauley *et al.*, 1989; Friedman *et al.*, 1987; Key *et al.*, 1991), prolactin (Berta *et al.*, 1991), and unbound serum estradiol (Cassidenti *et al.*, 1992).

(e) Genetically susceptible subgroups

Certain subgroups of women may have greater risk of breast cancer when exposed to tobacco smoke because of genetic or other factors affecting cancer susceptibility. Potential interactions between inherited polymorphisms and tobacco smoking have been studied for selected candidate genes that affect carcinogen metabolism, modulation of oxidative damage, immune responses, and DNA repair (see Sections 2.12.4b and 4.2).

2.12.2 Analytical studies

Over 130 epidemiological studies on tobacco smoking and breast cancer were reviewed.

(a) Incidence in current and former smokers

Since the previous *IARC Monograph* (IARC, 2004a), seven reports on cohort studies (Al-Delaimy *et al.*, 2004; Reynolds *et al.*, 2004a; Gram *et al.*, 2005; Hanaoka *et al.*, 2005; Olson *et al.*, 2005; Cui *et al.*, 2006; Ha *et al.*, 2007) have been published on breast cancer incidence in relation to tobacco smoking (Table 2.52 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.52.pdf). Breast cancer incidence was significantly associated with current tobacco smoking in three studies (Reynolds *et al.*, 2004a; Olson *et al.*, 2005; Cui *et al.*, 2006), with relative risk estimates among the larger studies ranging from 1.12 (95%CI: 0.92–1.37) (Al-Delaimy *et al.*, 2004) to 1.32 (95%CI:1.10–1.57) (Reynolds *et al.*, 2004a). Former smoking was significantly associated with risk in only one cohort (Al-Delaimy *et al.*, 2004), with relative risk estimates across all of the cohorts ranging from 1.00 (95%CI: 0.93–1.08) (Cui *et al.*, 2006) to 1.18 (95%CI: 1.02–1.36) (Al-Delaimy *et al.*, 2004). The association with breast cancer is stronger in current than in former smokers in four of the seven cohort studies (Reynolds *et al.*, 2004a; Hanaoka *et al.*, 2005; Olson *et al.*, 2005; Cui *et al.*, 2006), although the confidence intervals overlap widely in all but one (Cui *et al.*, 2006). [The Working group noted that three cohort studies (Gram *et al.*, 2005; Hanaoka *et al.*, 2005; Olson *et al.*, 2005) provided data on both

the age-adjusted and the multivariate-adjusted risk estimates for current and former smoking. None of these showed attenuation of the estimate associated with current smoking, and two (Hanaoka *et al.*, 2005; Olson *et al.*, 2005) reported somewhat stronger estimates when adjusted for established risk factors besides age. None of the studies adjusted for the frequency of mammography screening. Residual confounding by screening and incomplete control for other risk factors would be expected to cause underestimation of the association with current smoking, and overestimation of the association with former smoking.]

Since the previous *IARC Monograph* (IARC, 2004a), a total of 12 case–control studies on tobacco smoking and breast cancer incidence have been published (Table 2.53 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.53.pdf). Results from the case–control studies are less consistent than those from the cohort studies. Six studies (Li *et al.*, 2004; Mechanic *et al.*, 2006; Magnusson *et al.*, 2007; Prescott *et al.*, 2007; Roddam *et al.*, 2007; Slattery *et al.*, 2008) differentiated between current and former smokers, while the six other reports (Band *et al.*, 2002; Lash & Aschengrau, 2002; Gammon *et al.*, 2004; Rollison *et al.*, 2008; Ahern *et al.*, 2009; Young *et al.*, 2009) specify only ever or never smokers. Only one study (Li *et al.*, 2004) reported a borderline significant increase in risk associated with current smoking, and two studies (Band *et al.*, 2002; Rollison *et al.*, 2008) with ever smoking.

None of the six case–control studies that presented data on breast cancer incidence separately for current and former smokers found a significant difference in risk between the two smoking categories; the relative risk estimates were higher for former than for current smokers in four of the studies (Mechanic *et al.*, 2006; Prescott *et al.*, 2007; Roddam *et al.*, 2007; Slattery *et al.*, 2008) and identical in the fifth (Magnusson *et al.*, 2007).

(b) Years of cessation

When the relative risk for breast cancer incidence in former smokers is examined by years since cessation in cohort studies (Table 2.54 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.54.pdf), the point estimates do not consistently decrease with longer time since cessation. In none of the four cohort studies (London *et al.*, 1989b; Egan *et al.*, 2002; Reynolds *et al.*, 2004a; Cui *et al.*, 2006) and in only one (Li *et al.*, 2005) of the five case–control studies (Chu *et al.*, 1990; Gammon *et al.*, 1998; Johnson *et al.*, 2000; Kropp & Chang-Claude, 2002; Li *et al.*, 2005) that formally tested for trend (Table 2.55 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.55.pdf) was there a statistically significant decrease in relative risk observed with longer time since cessation. Only one study has reported data on breast cancer mortality in relation to years since quitting or age at cessation (Calle *et al.*, 1994). A statistically significant inverse trend in the relative risk estimates was reported with both years since quitting (p trend = 0.04) and younger age at cessation (p trend = 0.02). [The Working Group noted that the inverse trends in the relative risk of dying from breast cancer observed in this study are weaker than those observed with most other cancers designated as causally associated with smoking.]

(c) Duration of smoking and age at initiation

Tables 2.56–2.61 (see below for links) list the published epidemiologic studies that relate breast cancer incidence to duration of tobacco smoking, age at initiation and/or timing relative to first full term pregnancy.

Longer duration of smoking is associated with higher breast cancer incidence in five of seven cohort studies (Table 2.56 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.56.pdf). A similar trend is seen inconsistently among the 33 case–control

studies that report relative risk estimates by duration of smoking (Table 2.57 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.57.pdf). Among the 18 studies that reported a formal test of trend, eight studies (Gammon *et al.*, 1998; Johnson *et al.*, 2000; Reynolds *et al.*, 2004a; Gram *et al.*, 2005; Li *et al.*, 2005; van der Hel *et al.*, 2005; Cui *et al.*, 2006; Mechanic *et al.*, 2006) reported a statistically significant or borderline increase in the relative risk of incident breast cancer with the duration of smoking; seven studies (Ewertz, 1990; Palmer *et al.*, 1991; Egan *et al.*, 2002; Al-Delaimy *et al.*, 2004; Lissowska *et al.*, 2006; Magnusson *et al.*, 2007; Prescott *et al.*, 2007) reported no trend, and one study (Brinton *et al.*, 1986) reported an inverse relationship.

Thirty studies, including cohort (Tables 2.58 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.58.pdf) and case–control studies (Table 2.59 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.59.pdf) related breast cancer incidence to age at smoking initiation. Fifteen of these (Chu *et al.*, 1990; Ewertz, 1990; Palmer *et al.*, 1991; Nordlund *et al.*, 1997; Gammon *et al.*, 1998; Johnson *et al.*, 2000; Egan *et al.*, 2002; Kropp & Chang-Claude, 2002; Gram *et al.*, 2005; Cui *et al.*, 2006; Lissowska *et al.*, 2006; Ha *et al.*, 2007; Lissowska *et al.*, 2007; Magnusson *et al.*, 2007; Prescott *et al.*, 2007; Slattery *et al.*, 2008) reported a formal test of trend. Among these, only two (Gram *et al.*, 2005; Ha *et al.*, 2007) found a statistically significant or borderline significantly higher risk in women who began smoking at a younger ages; twelve studies (Chu *et al.*, 1990; Ewertz, 1990; Palmer *et al.*, 1991; Nordlund *et al.*, 1997; Gammon *et al.*, 1998; Johnson *et al.*, 2000; Egan *et al.*, 2002; Cui *et al.*, 2006; Lissowska *et al.*, 2006; Magnusson *et al.*, 2007; Prescott *et al.*, 2007; Slattery *et al.*, 2008) found no relationship with age at initiation, and one (Kropp & Chang-Claude, 2002) reported higher risk among women who began smoking later. [The Working Group noted that at least two studies (Cui *et al.*, 2006; Slattery *et al.*, 2008) appear to have included never-smokers in the tests of trend and that the categories that define age at initiation differ across studies.]

The relative risk of incident breast cancer according to the timing of smoking initiation relative to first full-term pregnancy was reported in 21 studies, of cohort (Table 2.60 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.60.pdf) and case–control (Table 2.61 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.61.pdf) design. For nine studies (Hunter *et al.*, 1997; Egan *et al.*, 2002; Al-Delaimy *et al.*, 2004; Reynolds *et al.*, 2004a; Li *et al.*, 2005; Cui *et al.*, 2006; Prescott *et al.*, 2007; Rollison *et al.*, 2008; Young *et al.*, 2009) categorical data on years of smoking before first pregnancy are presented, whereas for 12 (Lash & Aschengrau, 1999; Innes & Byers, 2001; Band *et al.*, 2002; Kropp & Chang-Claude, 2002; Lash & Aschengrau, 2002; Fink & Lash, 2003; Lawlor *et al.*, 2004; Gram *et al.*, 2005; Olson *et al.*, 2005; Lissowska *et al.*, 2006; Magnusson *et al.*, 2007; Slattery *et al.*, 2008) whether smoking was initiated before or after the initial pregnancy was considered. Breast cancer incidence is consistently higher when smoking began before or during first pregnancy in most (Hunter *et al.*, 1997; Lash & Aschengrau, 1999; Innes & Byers, 2001; Band *et al.*, 2002; Egan *et al.*, 2002; Al-Delaimy *et al.*, 2004; Reynolds *et al.*, 2004a; Gram *et al.*, 2005; Li *et al.*, 2005; Olson *et al.*, 2005; Cui *et al.*, 2006; Slattery *et al.*, 2008; Young *et al.*, 2009) but not all (Kropp & Chang-Claude, 2002; Lash & Aschengrau, 2002; Fink & Lash, 2003; Prescott *et al.*, 2007) studies that tested this. [The Working Group noted that the number of years of smoking before first pregnancy is highly correlated with age at first full-term pregnancy, which is itself an independent risk factor for breast cancer.]

It has been argued that some studies, and especially cohort studies, may underestimate

the true association between tobacco smoking and breast cancer risk by ignoring or underestimating lifetime exposure to second-hand tobacco smoke of those in the referent group (California Environmental Protection Agency, 2005; Johnson, 2005; Collishaw *et al.*, 2009). This criticism is based on the hypothesis that exposure to second-hand smoke may confer almost the same degree of breast cancer risk as tobacco smoking. Under this hypothesis, the inclusion of women exposed to second-hand smoke in the referent group dilutes the contrast between exposed and unexposed women in studies of tobacco smoking, and causes underestimation of the association between tobacco smoking and breast cancer. In several case–control studies the association between breast cancer and tobacco smoking strengthened when the referent group was defined as women with "never active, never-passive" exposure to tobacco smoke (Morabia *et al.*, 1996; Lash & Aschengrau, 1999; Johnson *et al.*, 2000; Kropp & Chang-Claude, 2002). In contrast, a stronger association between tobacco smoking and breast cancer risk, when women exposed only to second-hand smoke are excluded from the referent group, has not been observed in cohort studies (Egan *et al.*, 2002; Reynolds *et al.*, 2004a). Debate continues over whether the case–control studies should be considered "of highest quality" because they provide "lifetime exposure assessment" (Collishaw *et al.*, 2009) or whether the cohort studies are more credible, because prospectively-collected exposure data are not susceptible to the recall bias that can affect retrospective studies.

(d) Survival and mortality from breast cancer

The relationship between smoking and the natural history of breast cancer has been examined in several studies (Daniell, 1988; Ewertz *et al.*, 1991; Daniell *et al.*, 1993; Scanlon *et al.*, 1995; Yu *et al.*, 1997; Manjer *et al.*, 2000; Murin & Inciardi, 2001; Holmes *et al.*, 2007). In cross-sectional analyses, Daniell *et al.* (1993) found that smokers with breast cancer had more and larger lymph node metastases than non-smokers, after controlling for primary tumour size and other variables. Further, a case–control study (Murin & Inciardi, 2001) and a retrospective cohort study (Scanlon *et al.*, 1995) found smoking to be associated with an increased risk of developing pulmonary metastases from breast cancer. However, these studies could not definitively distinguish lung metastases from primary lung cancers.

Five cohort studies have focused specifically upon the association of tobacco smoking with either breast cancer survival (Ewertz *et al.*, 1991; Yu *et al.*, 1997; Manjer *et al.*, 2000; Holmes *et al.*, 2007) or breast cancer death rates (Calle *et al.*, 1994). A study of 1774 Danish women showed no association between smoking and breast cancer survival (Ewertz *et al.*, 1991), as did a study of 5056 women with breast cancer in the Nurse's Health Study (Holmes *et al.*, 2007). In contrast, follow-up of 792 women with in situ or invasive breast cancer detected in a screening study in Malmø, Sweden found a crude relative risk for smokers and ex-smokers, compared to never smokers, of 1.44 (95%CI: 1.01–2.06) and of 1.13 (95%CI: 0.66–1.94), respectively (Manjer *et al.*, 2000). The relative risk associated with smoking remained significant after adjustment for age and stage at diagnosis (RR, 2.14; 95%CI: 1.47–3.10). A study based on the ACS Cancer Prevention Study II reported an association between current smoking and increased breast cancer death rates after six years of follow-up (Table 2.56 online; Calle *et al.*, 1994). Risk of death attributed to breast cancer was positively and significantly related to the duration of current smoking reported at the time of enrolment. However, the authors acknowledge that mortality studies cannot exclude biases arising from the effect of smoking on overall death rates, which could increase the potential for prevalent breast cancer to be coded as the underlying cause of death on the death certificate (Calle *et al.*, 1994).

2.12.3 Subtypes

(a) Pre- versus post-menopausal

Since the previous *IARC Monograph* (IARC, 2004a), 19 case–control studies have published data on tobacco smoking in relation to pre- and post-menopausal breast cancer (Table 2.62 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.62.pdf). The results are inconsistent. Of the 12 studies that provide information separately for current smokers (Schechter *et al.*, 1985; Brinton *et al.*, 1986; Rohan & Baron, 1989; Ewertz, 1990; Baron *et al.*, 1996; Gammon *et al.*, 1998; Millikan *et al.*, 1998; Johnson *et al.*, 2000; Zheng *et al.*, 2002; Magnusson *et al.*, 2007; Slattery *et al.*, 2008), only five (Schechter *et al.*, 1985; Johnson *et al.*, 2000; Magnusson *et al.*, 2007; Slattery *et al.*, 2008) found a stronger association with pre- than with post-menopausal breast cancer. The other analyses show either similar associations (Brinton *et al.*, 1986; Ewertz, 1990; Baron *et al.*, 1996; Gammon *et al.*, 1998; Millikan *et al.*, 1998; Zheng *et al.*, 2002) or a stronger association with post-menopausal breast cancer (Rohan & Baron, 1989; Millikan *et al.*, 1998; Johnson *et al.*, 2000; Zheng *et al.*, 2002).

(b) Hormone receptor status

Two cohort studies (London *et al.*, 1989a; Manjer *et al.*, 2001), one case–control study (Morabia *et al.*, 1998) and a case series (Yoo *et al.*, 1997) have examined the association between quantitative measures of cigarette smoking and breast cancer risk according to estrogen receptor (ER) status. In one of the cohort studies (Manjer *et al.*, 2001), a statistically significant increased risk (RR, 1.6) of ER negative tumours associated with current smoking was found but no clear association between smoking and ER positive tumours, and no difference in the association with progestogen receptor (PR)-positive and PR-negative tumours. In the other three studies there was no clear difference in the association related to ER or PR receptor status.

2.12.4 Susceptible populations

More than 30 studies and meta-analyses (Alberg *et al.*, 2004; Terry & Goodman, 2006; Ambrosone *et al.*, 2008; Collishaw *et al.*, 2009) have evaluated whether a family history of breast cancer and/or inherited polymorphisms in various genes may confer greater susceptibility to develop breast cancer from exposure to tobacco smoke. These are described below in relation to the measure indicating potential susceptibility.

(a) Family history

In two studies, whether a family history of breast cancer modifies susceptibility to develop breast cancer from tobacco smoking has been examined. Couch *et al.* (2001) measured breast cancer incidence among female family members in a cohort of breast cancer cases diagnosed between 1944 and 1952 at the University of Minnesota. Sisters and daughters in families with at least three breast and/or ovarian cancers were at 2.4 fold higher risk for breast cancer (95%CI: 1.2–5.1) if they smoked compared to never-smokers. No dose–response was observed in relation to pack–years of smoking.

Suzuki *et al.* (2007) reported a statistically significant interaction between family history of breast cancer and smoking history in a hospital-based case–control study of 3861 breast cancer cases treated at a large cancer centre in Japan between 1988 and 2000. A family history of breast cancer in the absence of smoking was associated with a relative risk of 1.44 (95%CI: 1.21–1.71); the relative risk estimate was 1.95 (95%CI: 1.36–2.81) in women who reported < 30 pack–years of tobacco smoking, and 4.33 (95%CI: 1.65–11.40) in women who reported > 30 pack–years of smoking.

[The Working group noted that Japanese women who smoked during this time period

may have differed from never-smokers in other characteristics related to breast cancer. Besides its strong correlation with female smoking, "Westernization" might be associated with delayed childbearing, smaller families, higher body mass index, and greater use of post-menopausal hormones.]

(b) Genetic polymorphisms

Studies of breast cancer, smoking and low penetrance genetic polymorphisms are summarized in Table 2.63 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.63.pdf). The candidate genes in these studies are involved in carcinogen metabolism [*N*-acetyltransferases (*NAT1, NAT2*), cytochrome P450s (*CYP1A1, CYP1B1, CYP2E2*), GSTs], host responses to oxidative stress (superoxide dismutase) or to infectious organisms (myeloperoxidase and immunoglobulin binding protein) and DNA repair (O^6-methylguanine DNA methyltransferase, nucleotide excision repair).

The most consistent associations with breast cancer risk have been observed among long-term smokers with the *NAT2* slow acetylation genotype (Terry & Goodman, 2006). *NAT2* slow acetylation genotype is thought to confer less capability to detoxify tobacco smoke carcinogens and is associated with an increase in breast cancer risk (Ambrosone *et al.*, 1996, 2008). Approximately 50–60% of Caucasian women are reported to be slow acetylators.

Table 2.63 (online) lists 15 studies of polymorphisms in *NAT2*, of which 9 were included in a pooled analysis and 13 in a meta-analysis (Ambrosone *et al.*, 2008). [The study by Delfino *et al.* (2000) was excluded from these analyses because cases included women with benign breast disease; the study by Lilla *et al.* (2005) was not considered because it is based on the same population as that by Chang-Claude *et al.* (2002).] The meta-analysis found a statistically significant association between ever tobacco smoking and breast cancer risk among women with the *NAT2* slow acetylator genotype (meta-RR, 1.27; 95%CI: 1.16–1.40) but not in those with rapid acetylator genotype (meta-RR, 1.05; 95%CI: 0.95–1.17). Pack–years of tobacco smoking was significantly associated with increasing breast cancer risk among women with *NAT2* slow acetylator genotype (meta-RR for ever smokers, 1.44; 95%CI: 1.23–1.68, for > 20 pack–years versus never smokers), but not among rapid acetylators (Ambrosone *et al.*, 2008). No main effect was seen between *NAT2* status and breast cancer risk (meta-RR, 1.0; 95%CI: 0.93–1.07). In contrast to an earlier meta-analysis (Alberg *et al.*, 2004), this study observed no difference in risk for pre- or post-menopausal breast cancer. The pooled analysis of nine studies (Ambrosone *et al.*, 2008) reported pooled risk estimates for pre- and post-menopausal women of 1.49 (95%CI: 1.08–2.04) and 1.42 (95%CI: 1.16–1.74), respectively, among women with slow *NAT2* genotype and at least 20 pack–years of smoking compared to never-smokers. The corresponding values for women with rapid acetylator genotype were 1.29 (95%CI: 0.89–1.86) and 0.88 (95%CI: 0.69–1.13). A statistically significant interaction was observed between pack–years of smoking as a continuous variable and *NAT2* genotype (p interaction = 0.03).

A population-based case–control study published after the meta-analysis by Ambrosone *et al.* compared the prevalence of the *NAT2* genotypes and their joint effect with smoking on breast cancer risk in Hispanic and non-Hispanic white women (Baumgartner *et al.*, 2009). Non-Hispanic white women were more likely ($P < 0.001$) than Hispanics to have a slow (41.7% versus 33.5%) or very slow (19.0% versus 11.1%) *NAT2* acetylator status. Breast cancer risk was significantly increased in non-Hispanic smoking white women with a very slow acetylator genotype (RR, 2.46; 95%CI: 1.07–5.65 for current versus never).

[The Working Group noted that publication bias remains a concern in the studies of *NAT2* published to date. All of the studies included in the meta-analysis by Ambrosone *et al.* were published between 1996 and 2006; some among them (Morabia *et al.*, 2000; Sillanpää *et al.*, 2007) reported very strong associations that seem inconsistent with the rest of the data. Because genetic studies often examine multiple genes, it is plausible that studies that find no main effect with *NAT2* have not examined this association or that null results for smoking have not been published.]

Fewer studies with less consistent findings have been published on polymorphisms in other genes such as *NAT1*, *CYP1A1*, *GST*, *NOS3*, *MPO*, *MnSOD2* and various DNA repair genes (Table 2.63 online).

2.12.5 High penetrance genes & prognosis

At least seven studies have examined the hypothesis that tobacco smoking may modify breast cancer risk among women who carry *BRCA1* and *BRCA2* mutations (Brunet *et al.*, 1998; Ghadirian *et al.*, 2004; Colilla *et al.*, 2006; Gronwald *et al.*, 2006; Nkondjock *et al.*, 2006; Breast Cancer Family Registry, 2008; Ginsburg *et al.*, 2009). The results have been inconsistent. A recent case–control study of women under age 50 years who were carriers of mutations in *BRCA1* or *BRCA2* reported increased risk for breast cancer associated with as little as five pack–years of smoking. Compared to non-smokers, the risk associated with five or more pack–years of smoking was 2.3 (95%CI: 1.6–3.5) for *BRCA1* mutation carriers and 2.6 (95%CI: 1.8–3.9) for *BRCA2* mutation carriers (Breast Cancer Family Registry, 2008). In contrast, six other studies reported no increased risk among *BRCA1* or *BRCA2* carriers who smoke. The Canadian Panel review (Collishaw *et al.*, 2009) postulated that the five previous studies (Brunet *et al.*, 1998; Ghadirian *et al.*, 2004; Colilla *et al.*, 2006; Gronwald *et al.*, 2006; Nkondjock *et al.*, 2006) may have failed to observe a relationship because they included prevalent cases. However, a sixth study published since the Canadian panel review is also negative (Ginsburg *et al.*, 2009).

2.13 Cancer of the cervix

The association between smoking and cervical cancer has been examined in many epidemiological studies over the past few decades.

Since the previous *IARC Monograph* (IARC, 2004a), additional epidemiological studies have been published. Study design and results of the case–control studies restricted to HPV positive women or that adjusted for HPV status are presented in Table 2.64 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.64.pdf) and Table 2.65 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.65.pdf). Cohort studies and pooled analyses are presented in Table 2.66 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.66.pdf) and Table 2.67 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.67.pdf), respectively. Table 2.68 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.68.pdf) and Table 2.69 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.69.pdf) present additional cohort studies and pooled analyses on tobacco smoking and cervical, cervical intraepithelia neoplasia and carcinoma in situ, with our without controlling for HPV status, respectively.

2.13.1 Dose–response relationship

A positive association between smoking and incidence of cervical squamous-cell carcinoma, which account for approximately 90% of all cervical cancers, has been shown consistently over several decades in many epidemiological studies

of various designs conducted across different geographic regions. Dose–response associations with smoking intensity and duration were noted in many of the studies where such associations were examined (Berrington de González *et al.*, 2004; Appleby *et al.*, 2006). Conversely, no clear association was found among former smokers. For adenocarcinoma of the cervix, which usually account for less than 10% of the total of all types of cervical cancer, there appears to be no clear association with smoking (Berrington de González *et al.*, 2004).

2.13.2 Interaction with HPV positivity

Epidemiological studies of smoking and cervical cancer increasingly have considered the effects of HPV infection, which is recognized as the main etiological factor for invasive and pre-invasive cervical neoplasia worldwide (IARC, 1995, 2012b). HPV infection has been considered not only with respect to possible effect modification (Hellberg & Stendahl, 2005; Gunnell *et al.*, 2006), but also to confounding, as both HPV infection and smoking habits are directly associated with number of sexual partners and other indications of high-risk sexual behaviours (Sikström *et al.*, 1995; Wang *et al.* 2004; Hellberg & Stendahl, 2005; McIntyre-Seltman *et al.*, 2005; Syrjänen *et al.*, 2007). Although there have been exceptions (Syrjänen *et al.*, 2007), recent studies have generally continued to show that statistical adjustment for the potential confounding effects of HPV infection, or restricting studies to women with high risk HPV infection (Plummer *et al.*, 2003), does not appreciably alter the finding of a positive association or its magnitude (McIntyre-Seltman *et al.*, 2005; Appleby *et al.*, 2006; Tolstrup *et al.*, 2006; Tsai *et al.*, 2007; Nishino *et al.*, 2008; Kapeu *et al.*, 2009).

Statistical adjustment for the potentially confounding effect of HPV infection was usually based on the measured presence of HPV DNA in cervical cells or anti-HPV serum antibodies in multivariate analytical models; as noted above, studies have also restricted their analyses to HPV-positive cases and controls. As there is currently no reliable marker of persistent HPV infection, case–control studies based on a cross-sectional measurement of HPV cannot distinguish between transient and persistent infections (Franco *et al.*, 1999). Tobacco smoking is suspected to facilitate acquisition or persistence of an HPV infection through a reduced number of Langerhans cells and CD4 lymphocytes, which are markers of local immune response in the cervix (Vaccarella *et al.*, 2008). In addition, smoking may affect innate immunity (Ferson *et al.*, 1979). Current smokers have been shown to have a slightly higher HPV prevalence than non-smokers in a broad range of world populations after adjustment for life-time number of sexual partners (OR, 1.18; 95%CI: 1.01–1.39) (Vaccarella *et al.*, 2008). Studies have evaluated the effect of smoking on HPV persistence. One study shows lower probability of HPV clearance among ever smokers (Giuliano *et al.*, 2002) but a few others found no relationship (Molano *et al.*, 2003; Richardson *et al.*, 2005).

2.14 Cancer of the endometrium

2.14.1 Overview of studies

To date, at least 42 epidemiological studies have examined the association between smoking and endometrial cancer, 25 reviewed in the previous *IARC Monograph* (IARC, 2004a) and 17 published since then (Petridou *et al.*, 2002; Folsom *et al.*, 2003; Furberg & Thune, 2003; Newcomb & Trentham-Dietz, 2003; Beral *et al.*, 2005; Matthews *et al.*, 2005; Viswanathan *et al.*, 2005; Okamura *et al.*, 2006; Strom *et al.*, 2006; Trentham-Dietz *et al.*, 2006; Weiss *et al.*, 2006a; Al-Zoughool *et al.*, 2007; Bjørge *et al.*, 2007; Lacey *et al.*, 2007; Loerbroks *et al.*, 2007; Setiawan *et al.*, 2007; Lindemann *et al.*, 2008). Study design and results of the additional studies

are presented separately for the case–control studies (Table 2.70 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.70.pdf and Table 2.71 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.71.pdf, respectively) and for the cohort studies (Table 2.72 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.72.pdf and Table 2.73 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.73.pdf, respectively).

(a) Cohort studies

The majority of the 13 cohort studies (Engeland *et al.*, 1996; Terry *et al.*, 1999, 2002b; Folsom *et al.*, 2003; Furberg & Thune 2003; Beral *et al.*, 2005; Viswanathan *et al.*, 2005; Al-Zoughool *et al.*, 2007; Bjørge *et al.*, 2007; Lacey *et al.*, 2007; Loerbroks *et al.*, 2007; Setiawan *et al.*, 2007; Lindemann *et al.*, 2008) suggest a decreased risk among current smokers, including the largest study with over 9000 cases (Bjørge *et al.*, 2007). In five of these studies quantitative smoking measures have been examined in relation to endometrial cancer risk (Terry *et al.*, 1999, 2002b; Viswanathan *et al.*, 2005; Al-Zoughool *et al.*, 2007; Loerbroks *et al.*, 2007). Of these, one (Terry *et al.*, 1999) found a 50% reduced risk among current smokers in the highest level of intensity (11 cigarettes per day or more) compared with non-smokers, but the number of cases was low and the confidence intervals correspondingly wide. A more recent and larger cohort study (Terry *et al.*, 2002b) found a statistically significant 40% reduced risk among current smokers of more than 20 cigarettes per day, but showed somewhat weaker and statistically non-significant reductions in risk with smoking of long duration or high cumulative consumption (i.e. pack–years). In contrast, the risk among former smokers was similar to that among never smokers. The largest of these studies generally showed decreasing risk of endometrial cancer with increasing smoking intensity, duration, and pack–years of consumption (Viswanathan *et al.*, 2005). Three studies examined the association between time since smoking cessation and endometrial cancer risk. Two of these studies suggested a positive association with time since quitting (compared with non-smokers) (Viswanathan *et al.*, 2005; Loerbroks *et al.*, 2007), whereas one found no association (Terry *et al.*, 2002b).

(b) Case–control studies

The results of 17 population-based case–control studies (Smith *et al.*, 1984; Tyler *et al.*, 1985; Franks *et al.*, 1987; Elliott *et al.*, 1990; Rubin *et al.*, 1990; Brinton *et al.*, 1993; Goodman *et al.*, 1997; Shields *et al.*, 1999; Jain *et al.*, 2000; McCann *et al.*, 2000; Newcomer *et al.*, 2001; Weiderpass & Baron, 2001; Newcomb & Trentham-Dietz, 2003; Matthews *et al.*, 2005; Strom *et al.*, 2006; Trentham-Dietz *et al.*, 2006; Weiss *et al.*, 2006a), that have included between 46 and 1304 endometrial cancer cases, generally have shown reductions in risk among current smokers compared with never smokers (although the magnitude of the reduction in risk has varied somewhat); results among former smokers compared with never smokers were equally variable, albeit somewhat weaker overall. The results of eight hospital-based case–control studies (Kelsey *et al.*, 1982; Lesko *et al.*, 1985; Levi *et al.*, 1987; Stockwell & Lyman, 1987; Koumantaki *et al.*, 1989; Austin *et al.*, 1993; Petridou *et al.*, 2002; Okamura *et al.*, 2006), which included between 83 and 1374 endometrial cancer cases, are somewhat consistent with those of population-based studies. They showed moderate (e.g. 30–40%) reduction in risks among current compared with never smokers, and unaltered risks (or perhaps a small 10–20% reduction in risk) in former compared with never smokers. The largest of the hospital-based studies (Stockwell & Lyman, 1987), with 1374 cases and 3921 controls, found both former and current smokers to be at moderately (approximately 30%) reduced risk

of endometrial cancer. To date, six population-based case–control studies (Tyler *et al.*, 1985; Lawrence *et al.*, 1987, 1989; Brinton *et al.*, 1993; Newcomer *et al.*, 2001; Weiderpass & Baron, 2001) have examined quantitative measures of smoking in relation to endometrial cancer risk, generally showing inverse associations to be strongest among current smokers of high intensity or long duration.

2.14.2 Confounders

Whereas the majority of these studies adjusted their relative risk estimates for potentially confounding variables, such as BMI, HRT, parity, diabetes, and age at menopause, studies that did not adjust for these variables tended to show similar inverse associations. Within individual studies, statistical adjustment for the effects of BMI and other covariates often made little difference, although some attenuation of relative risk estimates has been noted (Weiderpass & Baron, 2001; Terry *et al.*, 2002c).

2.14.3 Effect modification

The association between smoking and endometrial cancer risk according to factors that are known determinants of endogenous hormone concentrations, and which may counteract or augment possible tobacco-related hormonal changes, have been examined in several studies. These factors include menopausal status, HRT and BMI. Effect modification can reflect true underlying differences in the association across strata (for example, if cigarette smoking acts to reduce or modify estrogen concentrations differently in one group compared with another), but can also reflect methodological factors, such as differences that occur by chance or through the varying prevalence of confounding variables.

(a) Menopausal status

Although endometrial cancer is rare among pre-menopausal women, several studies have examined the association between cigarette smoking and endometrial cancer risk according to menopausal status, because the effect of smoking (if any) might vary according to the underlying hormonal milieu. The studies have included two cohort studies (Terry *et al.*, 2002b; Al-Zoughool *et al.*, 2007), five population-based case–control studies (Smith *et al.*, 1984; Franks *et al.*, 1987; Lawrence *et al.*, 1987; Brinton *et al.*, 1993; Weiderpass & Baron, 2001), and four hospital-based case–control studies (Lesko *et al.*, 1985; Levi *et al.*, 1987; Stockwell & Lyman, 1987; Koumantaki *et al.*, 1989). In all but one of these studies, a study of early stage endometrial cancer (Lawrence *et al.*, 1987), the inverse association was (to varying degrees) stronger among post-menopausal than pre-menopausal women. Among pre-menopausal women, the relative risk estimates for cigarette smoking have been inconsistent, sometimes showing increased risks with certain measures of cigarette smoking (Smith *et al.*, 1984; Stockwell & Lyman, 1987; Koumantaki *et al.*, 1989; Brinton *et al.*, 1993; Al-Zoughool *et al.*, 2007), sometimes showing decreased risks (Lawrence *et al.*, 1987; Levi *et al.*, 1987; Brinton *et al.*, 1993; Terry *et al.*, 2002b), and sometimes showing practically no association (Lesko *et al.*, 1985; Weiderpass & Baron, 2001; Al-Zoughool *et al.*, 2007). In analyses limited to post-menopausal women, on the other hand, all showed between 10% and 80% reduced risks of endometrial cancer with the various smoking measures.

(b) Hormone replacement therapy

Given the possibility that cigarette smoking affects hormone concentrations mostly among women who are taking HRT (Jensen *et al.*, 1985; Jensen & Christiansen, 1988; Cassidenti *et al.*, 1990), the inverse association between tobacco

smoking and endometrial cancer risk might be stronger among HRT users than among non-users. However, the results of studies that have examined the association between smoking and endometrial cancer risk according to HRT use have been equivocal (Weiss *et al.*, 1980; Franks *et al.*, 1987; Lawrence *et al.*, 1987; Levi *et al.*, 1987; Terry *et al.*, 2002b; Beral *et al.*, 2005). Whereas in two studies (Franks *et al.*, 1987; Levi *et al.*, 1987) a larger reduction in risk among smokers taking HRT than among smokers not taking HRT was observed, in two other studies (Lawrence *et al.*, 1987; Terry *et al.*, 2002b) there was no difference in the association according to HRT status. A cohort study that examined associations only among women using HRT showed no clear association among users of continuous combined HRT and cyclic combined HRT, but some suggestion of increased risk among smokers who used tibolone (perhaps more clearly among former smokers) (Beral *et al.*, 2005). Thus, although effect modification by HRT status is biologically plausible, the available epidemiological evidence is equivocal.

(c) Relative body weight

Obesity is an established risk factor for endometrial cancer (IARC, 2002). Smokers tend to have a lower BMI than non-smokers, although former smokers tend to have a higher BMI than current or never smokers (Baron *et al.*, 1990). Two case–control studies have examined the association between cigarette smoking and endometrial cancer risk according to BMI, one population-based (Elliott *et al.*, 1990) and one hospital-based (Levi *et al.*, 1987). Neither of these studies found clear differences in the association between smoking and endometrial cancer risk according to BMI. In a population-based case–control study of early stage endometrial cancer (Lawrence *et al.*, 1987), the inverse association with cigarette smoking tended to become stronger with increasing absolute rather than relative body weight.

2.14.4 Gene polymorphisms

Cigarette smoking and estrogen are both thought to influence cancer risk through pathways that are under the control of specific genes, such as those involved in the formation of bulky DNA adducts by estrogen metabolites (Cavalieri *et al.*, 2000) and both bulky and non-bulky adducts formed by carcinogens in tobacco smoke (Terry & Rohan, 2002). Therefore, studies have been conducted to examine the association between smoking and endometrial cancer risk according to genes that repair these types of DNA damage. In a moderately-sized population-based case–control study no clear effect modification according to certain polymorphisms in the *XPA* and *XPC* genes, both of which are involved in the nucleotide excision repair of bulky DNA adducts and may influence endometrial cancer risk, were found (Weiss *et al.*, 2005, 2006b). A nested case–control study also showed no clear effect modification according to three polymorphisms in *CYP1A1* (McGrath *et al.*, 2007), a gene that encodes microsomal CYP1A1, which contributes to aryl hydrocarbon hydroxylase activity, catalysing the metabolism of PAHs and other carcinogens found in tobacco smoke (Masson *et al.*, 2005). In another nested case–control study some evidence was found that the association between smoking and endometrial cancer may vary according to a polymorphism (Ile^{143}Val) in O^6-methylguanine DNA methyltransferase (*MGMT*). Overall, studies that address the association between smoking and endometrial cancer risk according to genotype are scarce.

2.15 Cancer of the prostate

Many epidemiological studies have examined the association between cigarette smoking and prostate cancer risk, and most have shown no consistent association (Hickey *et al.*, 2001; Levi & La Vecchia, 2001; Batty *et al.*, 2008; Butler

et al., 2009; Huncharek *et al.*, 2010; Table 2.74 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.74.pdf; Table 2.75 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.75.pdf). However, questions remain regarding whether smoking may alter risk in various population subgroups, for example, those defined by certain genotypes, and whether any association with smoking may be stronger for, or limited to, advanced tumours or prostate cancer mortality. Regarding this latter issue, the majority of epidemiological studies, including several large, long-term cohort studies, have reported a positive association between smoking and prostate cancer mortality (Rohrmann *et al.*, 2007; Zu & Giovannucci, 2009). Several studies that examined smoking in relation to both prostate cancer incidence and mortality tend to show positive results only for the latter (Rohrmann *et al.*, 2007; Zu & Giovannucci, 2009). Given the largely null results with respect to prostate cancer incidence, the latter findings suggest that smoking is less likely to be a causal agent in prostate cancer initiation than an agent that acts on existing tumours to promote their progression (Zu & Giovannucci, 2009).

A recent review of smoking and prostate cancer that focused specifically on aggressive and fatal tumours, considered the findings from 14 cohort studies (Zu & Giovannucci, 2009). Nine of these studies showed statistically significant increased risk with at least one smoking measure, and five showed increased risks that were not statistically significant for any measure. Only one study showed no association with any measure of tobacco consumption. Seven studies of various designs examined smoking with respect to indicators of cancer aggressive behaviour at the time of diagnosis. In these studies smoking was associated positively with tumour grade, risk of regional, distant, extraprostatic or metastatic disease, Gleason score, and biochemical outcome (failure) after prostate brachytherapy and in several dose–response associations with the respective endpoint were demonstrated. In one study smoking cessation was associated with a decline in risk compared with that among current smokers.

The association between smoking and prostate cancer risk according to genotype and other potentially effect-modifying factors have been examined in several studies. For example, in a population-based case–control study tobacco use was a risk factor for prostate cancer primarily among men with high BMI (Sharpe & Siemiatycki, 2001). The results of a cohort study in Switzerland suggest that risk of prostate cancer mortality is increased in smokers, particularly those with low plasma vitamin E levels (Eichholzer *et al.*, 1999). These latter associations, as well as those regarding several genotypes that may modify the association (Mao *et al.*, 2004; Nock *et al.*, 2006; Quiñones *et al.*, 2006; Yang *et al.*, 2006; Iguchi *et al.*, 2009; Kesarwani *et al.*, 2009), have yet to be fully clarified.

[The Working Group noted that several of the studies of smoking and prostate cancer mortality did not demonstrate clear dose–response associations with risk, and noted the possibility of bias due to confounding by screening behaviour. However, in the Health Professionals Follow-up Study, screening behaviour was not found to differ appreciably between smokers and non-smokers. In an analysis limited to men with a negative digital rectal examination in the prior two years, stronger associations were found between smoking and metastatic prostate cancer risk among high intensity smokers (RR, 4.2; 95%CI: 1.6–10.9) (Zu & Giovannucci, 2009). This finding was evidence against bias from screening behaviour.]

2.16 Cancer of the ovary

2.16.1 Overview of studies

A total of over 30 epidemiological studies have investigated the association between tobacco smoking and ovarian cancer risk. Of these, 24 were case–control studies (IARC, 2004a; Table 2.76 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.76.pdf; Table 2.77 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.77.pdf) and six were cohort studies (IARC, 2004a; Table 2.78 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.78.pdf; Table 2.79 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.79.pdf). Most studies showed no statistically significant association between a measure of smoking and risk for ovarian cancer overall (Newhouse *et al.*, 1977; Smith *et al.*, 1984; Tzonou *et al.*, 1984; Baron *et al.*, 1986; Stockwell & Lyman, 1987; Whittemore *et al.*, 1988; Hartge *et al.*, 1989; Polychronopoulou *et al.*, 1993; Engeland *et al.*, 1996; Goodman *et al.*, 2001; Goodman & Tung, 2003; Pan *et al.*, 2004; Zhang *et al.*, 2004; Kurian *et al.*, 2005; Niwa *et al.*, 2005; Baker *et al.*, 2006; Huusom *et al.*, 2006; Fujita *et al.*, 2008; Lurie *et al.*, 2008; Nagle *et al.*, 2008; Tworoger *et al.*, 2008); some showed positive associations (Doll *et al.*, 1980; Tverdal *et al.*, 1993; Kuper *et al.*, 2000b; Marchbanks *et al.*, 2000; Green *et al.*, 2001; Modugno *et al.*, 2002; Gram *et al.*, 2008; Rossing *et al.*, 2008) and one (Riman *et al.*, 2004) showed an inverse association.

2.16.2 Histological subtypes

Differences in ovarian cancer risk factor profiles have been observed according to histological type, on the basis of which it has been suggested that mucinous and non-mucinous tumours are etiologically distinct diseases (Risch *et al.*, 1996). Epidemiological studies that have considered histological type tend to support a positive association primarily between cigarette smoking and mucinous ovarian tumours (Kuper *et al.*, 2000b; Marchbanks *et al.*, 2000; Green *et al.*, 2001; Modugno *et al.*, 2002; Pan *et al.*, 2004; Zhang *et al.*, 2004; Kurian *et al.*, 2005; Tworoger *et al.*, 2008). In contrast, two studies showed no clear association between smoking and risk of mucinous or non-mucinous ovarian tumours (Riman *et al.*, 2004; Baker *et al.*, 2006). In addition, one early case–control study (Newhouse *et al.*, 1977), with 300 ovarian cancer cases and with both population and hospital controls, found no clear association with "ever" compared with "never" smoking, and reported no differences according to histological type.

A pooled analysis of 10 case–control studies (Kurian *et al.*, 2005) with 254 cases of mucinous and 1580 non-mucinous tumours found an increased risk of mucinous tumours among current smokers (RR, 2.4; 95%CI: 1.5–3.8), a positive association that was not observed with other histological types. Former smokers in that analysis did not have an increased risk of any histological type of ovarian cancer. This type of dose–response, whereby current smokers have a higher risk than former smokers, was observed in most, but not all, studies of mucinous ovarian cancer (Tables 2.77 and 2.79 online). Overall, the positive association between cigarette smoking and risk of mucinous ovarian tumours is generally consistent across both case–control and cohort studies conducted among various populations. In contrast, associations with smoking have been mostly null with respect to non-mucinous ovarian tumours, suggesting that recall bias is unlikely to explain the association with mucinous tumours.

[The Working Group considered the possibility that women who smoke may come to medical attention more frequently. This raises the possibility of detection bias, because mucinous tumours, benign or malignant, tend to be quite large and could be more easily detected on routine physical exam or testing. However, the

Working Group felt that detection bias would not account for the association entirely.

2.17 Cancer of the thyroid

The previous *IARC Monograph* (IARC, 2004a) noted inconsistent associations between smoking and thyroid cancer risk. In 2003, a pooled analysis of 14 case–control studies showed that smoking was inversely associated with thyroid cancer risk (Mack *et al.*, 2003; Table 2.80 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.80.pdf; Table 2.81 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.81.pdf). The sample consisted of 2725 thyroid cancer cases (2247 women, 478 men) and 4776 controls (3699 women, 1077 men). The inverse association was stronger among current smokers (RR, 0.6; 9% CI: 0.6–0.7) than former smokers (RR, 0.9; 9% CI: 0.8–1.1) and were similar in both men and women, for both papillary and follicular thyroid cancers, as well as by age and region. An inverse association between smoking and thyroid cancer risk was also found in a subsequent case–control study (Nagano *et al.*, 2007). In contrast, two case–control studies (Zivaljevic *et al.*, 2004; Bufalo *et al.*, 2006) reported no clear association between smoking and thyroid cancer risk (no risk ratio estimates were reported; hence, data are not shown in the tables) and a cohort study with 169 incident cases of thyroid cancer, also found no clear association with any qualitative or quantitative smoking measure (Navarro Silvera *et al.*, 2005; Table 2.82 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.82.pdf; Table 2.83 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.83.pdf).

2.18 Other cancers

The cancers reviewed in this section generally have low incidence and mortality rates and are not considered to be strongly associated with cigarette smoking. This raises the possibility of preferential reporting of positive associations in epidemiological studies.

2.18.1 Cancer of the salivary gland

Studies of smoking and cancers of the salivary gland reviewed in the previous *IARC Monograph* (IARC, 2004a) were sparse and their results were inconsistent (Spitz *et al.*, 1990; Swanson & Burns, 1997; Hayes *et al.*, 1999). A few additional studies also show inconsistent results (Kotwall, 1992; Pinkston & Cole, 1996; Horn-Ross *et al.*, 1997; Vories & Ramirez, 1997; Muscat & Wynder, 1998). Studies that focused specifically on Warthin's tumour [papillary cystadenoma lymphomatosum or adenolymphoma, a benign tumour of the parotid gland] tend to show strong positive associations with smoking (Kotwall, 1992; Pinkston & Cole, 1996; Vories & Ramirez, 1997). One study (Pinkston & Cole, 1996) compared the risk for Warthin's tumour with that for other salivary gland tumours and found that smoking increased risk significantly only for Warthin's tumour.

2.18.2 Cancer of the small intestine

Epidemiological studies (all of case–control design) reviewd in the previous *IARC Monograph* (IARC, 2004a) have been inconsistent in showing a positive association between smoking and cancers of the small intestine (Chow *et al.*, 1993b; Chen *et al.*, 1994; Wu *et al.*, 1997; Negri *et al.*, 1999; Kaerlev *et al.*, 2002). A more recent study showed no clear association (Hassan *et al.*, 2008b).

2.18.3 Cancers of the gallbladder and extra-hepatic bile ducts

Epidemiological studies of smoking and risk of cancers of the gallbladder and extra-hepatic bile ducts reviewed in the previous *IARC Monograph* (IARC, 2004a) tended to show null, weak, or moderately strong positive associations. More recent studies also tend to show either no clear association with biliary tract carcinoma/extra-hepatic cholangiocarcinoma (Shaib *et al.*, 2007; Welzel *et al.*, 2007) or suggest positive associations with gallbladder/biliary cancers (Pandey & Shukla, 2003; Yagyu *et al.*, 2008; Grainge *et al.*, 2009). Attention should be paid to potential confounders, especially BMI, when considering the results of epidemiological studies of risk of cancers of the gallbladder and extra-hepatic bile ducts. Recent studies that statistically adjusted for BMI, on gallbladder disease risk (Grainge *et al.*, 2009) or on extrahepatic biliary tract carcinoma risk (Ahrens *et al.*, 2007), showed a positive and null association with smoking, respectively. To date, there are too few studies with adequate control for potentially confounding factors to determine any clear pattern.

2.18.4 Soft-tissue sarcoma

As reported in the previous *IARC Monograph* (IARC, 2004a), one cohort study found an association between cigarette smoking and mortality from soft-tissue sarcoma after 26 years of follow-up but no dose–response relationship with the number of cigarettes/day, duration of smoking or pack–years (Zahm *et al.*, 1992). No effect of cigarette smoking was detected in an Italian hospital-based case–control study (Franceschi & Serraino, 1992).

2.18.5 Cancer of the skin

(a) Melanoma

Several case–control studies found no difference in the prevalence of tobacco smoking between patients with malignant melanoma and controls, and one study found an inverse association (IARC, 2004a). An inverse association with smoking was also found in the US Radiologic Technologists cohort Study (Freedman *et al.*, 2003a). In that study, smoking for at least 30 years compared with never smoking was inversely related to melanoma risk (RR, 0.6; 95%CI: 0.3–1.3), though risk was not associated with number of cigarettes/day. An inverse association was also observed in a cohort of Swedish construction workers (Odenbro *et al.*, 2007). In this study, the risk for malignant melanoma was reduced in a dose-dependant manner for both cigarette and pipe smokers. The possibility that smoking may reduce the risk for melanoma should, therefore, be considered.

(b) Non-melanoma skin cancer

Four studies showed a positive association between smoking and non-melanoma skin cancer risk (De Stefani *et al.*, 1995; Wojno, 1999; Smith & Randle, 2001; Boyd *et al.*, 2002), and two found no clear association (van Dam *et al.*, 1999; Corona *et al.*, 2001). When distinguishing between histological subtypes, tobacco smoking was linked to the incidence of squamous-cell carcinoma of the skin in most studies, whereas the results for basal cell carcinoma remain inconsistent (Zak-Prelich *et al.*, 2004). No clear association between smoking and risk for basal cell carcinoma was found in a cohort study (Freedman *et al.*, 2003b).

2.18.6 Cancer of the penis

Case–control studies of smoking and penile cancer (Hellberg *et al.*, 1987; Daling *et al.*, 1992, 2005; Maden *et al.*, 1993; Harish & Ravi, 1995;

Table 2.84 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.84.pdf; Table 2.85 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.85.pdf) and reviews of studies of smoking and penile cancer and population surveys (Dillner *et al.*, 2000; Favorito *et al.*, 2008; Bleeker *et al.*, 2009; Table 2.86 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.86.pdf; Table 2.87 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.87.pdf) consistently showed a positive association. In most studies there was a dose–response relationship, with higher risks among those with increased smoking intensity and/or duration. A study in Brazil showed a positive correlation with penile tumour grade (Favorito *et al.*, 2008). Based on the two reviews (Dillner *et al.*, 2000; Bleeker *et al.*, 2009), relative risks were generally increased twofold to fivefold among smokers.

Most studies did not adjust for HPV infection. In one case–control study (Daling *et al.*, 2005), current smoking was associated with a 160% increased risk of HPV-positive penile cancer (n = 75), and a 180% increased risk of HPV-negative penile cancer (n = 19), suggesting no important effect modification.

2.18.7 Cancer of the testis

Studies reviewed in the previous *IARC Monograph* (IARC, 2004a) showed no association between cigarette smoking and risk for testicular cancer. More recently, two case–control studies showed positive associations with smoking, one in Canada (Srivastava & Kreiger, 2004) and one in the Czech Republic (Dusek *et al.*, 2008).

2.18.8 Cancer of the central nervous system

A recent meta-analysis was conducted on smoking in relation to glioma risk (Mandelzweig *et al.*, 2009), which included 17 epidemiological studies (6 cohort and 11 case–control). It was concluded that smoking is not associated with risk of glioma, despite a small significant increased risk seen in cohort studies. A recent cohort study found no association between smoking and carcinoma of the brain (Batty *et al.*, 2008). There have been no consistent associations of smoking with other CNS tumours (IARC, 2004a). In a population-based case–control study in the USA, smoking was associated with increased risk of intracranial meningioma in men (OR, 2.1; 95%CI: 1.1–4.2) but not in women (Phillips *et al.*, 2005).

2.18.9 Cancer of the adrenal gland

Data on risk factors for adrenal carcinoma are sparse. In the US Veterans' Study there was a fivefold increase in risk among current cigarette smokers during 26 years of follow-up, with risk being particularly high among those who smoked most intensely (Chow *et al.*, 1996). Other forms of tobacco use were associated with a statistically non-significant increase in risk. A case–control study in the USA found a twofold increase in risk for adrenal cancer among heavy smokers in men, but not in women (Hsing *et al.*, 1996).

2.19 Bidi smoking

2.19.1 Cancer of the oral cavity

(a) Overview of studies

The association between cancers of oral cavity and bidi smoking has been examined in 10 case–control studies conducted in India (Sankaranarayanan *et al.*, 1989a, b, 1990a; Rao *et al.*, 1994; Rao & Desai, 1998; Dikshit & Kanhere, 2000; Balaram *et al.*, 2002; Znaor *et al.*, 2003; Subapriya *et al.*, 2007; Muwonge *et al.*, 2008; Table 2.88 available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.88.pdf). In these studies both cases and controls were interviewed and analyses were restricted

to men, except for the studies by Balaram *et al.* (2002) and Subapriya *et al.* (2007), because very few women smoked among study subjects.

Three hospital-based case–control studies considered cancers of subsites of the oral cavity (gingiva, tongue and floor of the mouth, buccal and labial mucosa) (Sankaranarayanan *et al.*, 1989a, b, 1990a). All three studies showed a higher oral cancer risk for bidi smoking. In one early study an unadjusted relative risk of 1.6 (95%CI: 1.3–2.0) for oral cancer in bidi smokers was reported (Rao *et al.*, 1994). [The Working Group noted that the study had several deficiencies, particularly in the selection of controls that resulted in cigarette smoking apparently being protective for oral cancer.] In another early study (Rao & Desai, 1998) relative risks were estimated after stratification by age and place of residence. Bidi smoking was a significant risk factor for cancer of the base of the tongue (RR, 5.9; 95%CI: 4.2–8.2) but not significant for cancer of the anterior tongue. Relative risk for bidi smoking adjusted for alcohol drinking, illiteracy, non-vegetarian diet and tobacco chewing showed significant risk for cancer of the base of the tongue (RR, 4.7; 95%CI: 3.5–6.3) but not for cancer of the anterior tongue. In a population-based case–control study a relative risk of 1.5 (95%CI: 0.9–2.4), adjusted for age and tobacco quid chewing for smokers (bidis and/or cigarettes), was found (Dikshit & Kanhere, 2000).

Two hospital-based multi centre case–control studies on cancer of the oral cavity were conducted in southern India. One included 309 cases and 292 controls (Balaram *et al.*, 2002). The risk for oral cavity cancer among those who smoked < 20 bidis per day was 2.0 (95%CI: 1.1–3.8) and 2.5 (95%CI: 1.4–4.4) for ≥ 20 per day. The second study included 1563 cases and 3638 controls and found a risk for bidi smoking only of 2.2 (95%CI: 1.75–2.63) compared to never smokers, adjusted for age, centre, level of education, alcohol consumption and chewing (Znaor *et al.*, 2003).

In a hospital-based case–control study with 388 oral squamous cell carcinoma cases (202 men and 186 women) and an equal number of age and sex-matched controls the effect of lifestyle factors (tobacco chewing, smoking and alcohol drinking, diet and dental care) on the risk of oral cancer was evaluated (Subapriya *et al.*, 2007). Both cases and controls were interviewed using a structured questionnaire. The risk estimate for bidi smoking based on 22 cases (84 cases included in the model) and 22 controls was 4.6 (95%CI not given).

Data from a randomized control trial conducted between 1996 and 2004 in Trivandrum, southern India were used in a nested case–control analysis with 282 (163 men and 119 women) incident oral cancer cases and 1410 matched population controls aged 35 years and over (Muwonge *et al.*, 2008). Oral cancer risk among men, adjusted for education and religion, was 1.9 (95%CI: 1.1–3.2) for bidi smokers only compared to never smokers. No association was found between mixed smoking of bidi and cigarette and risk of oral cancer.

Rahman *et al.* (2003) performed a meta-analysis to investigate the relationship between bidi smoking and oral cancer. They identified 12 case–control studies published in English during 1996–2002 with quantitative information on bidi smoking and oral cancer. Of these, ten studies were conducted in India, one in Sri Lanka and one in Pakistan. All cases were confirmed histologically and exposure data were collected by direct interview. In these studies ORs were not adjusted for tobacco chewing or alcohol drinking. The OR for bidi smokers compared to never smokers based on random effects model was 3.1 (95%CI: 2.0 –5.0). The ORs ranged from 2.0 to 3.6 in different regions of India: studies conducted in Mumbai had an OR of 3.6 (95%CI: 1.6 –7.9), in central India 2.7 (95%CI: 1.6–4.6), in Kerala 2.0 (95%CI: 1.5–2.9) and in Bangalore 2.0 (95%CI: 1.1–3.7).

(b) Dose–response evidence

The trends in relative risks by intensity and duration of bidi smoking were both statistically significant in two studies (Rao *et al.*, 1994; Rao & Desai, 1998). A meta-analysis based on three studies on duration of bidi smoking and on five studies on number of bidi sticks per day, showed a dose–response relationship for duration of bidi smoking but not for number of sticks used per day (Rahman *et al.*, 2003).

In a nested case–control analysis (Muwonge *et al.*, 2008) a dose–response relationship was observed for duration of bidi smoking ($P = 0.045$). [It is not clear if the analysis was restricted to bidi smokers only (n = 40 men) and if smokers with combined smoking habits (bidi and cigarette) were excluded. Moreover, ORs for the dose–response analysis were not reported.]

2.19.2 Cancer of the pharynx

Five case–control studies, two hospital-based (Wasnik *et al.*, 1998; Rao *et al.*, 1999), one population-based (Dikshit & Kanhere, 2000) and two multicentric studies (Znaor *et al.*, 2003; Sapkota *et al.*, 2007) were conducted on cancers of oropharynx and hypopharynx in India (Table 2.88 online). In all these studies, analyses were restricted to men because very few women smoked among study subjects.

Wasnik *et al.* (1998) conducted a case–control study on oropharyngeal cancers with cases and controls were matched on age and sex. Odds ratios for tobacco smoking, predominantly in the form of bidi and/or chillum, were 2.3 (95%CI: 1.2–3.7) after adjustment for tobacco chewing and outdoor occupation. [The Working Group noted some problems with the data analysis.]

Rao *et al.* (1999) reported a relative risk for bidi smoking adjusted for alcohol, illiteracy, diet and tobacco chewing of 4.7 (3.6–6.3) for oropharyngeal cancer and of 2.8 (2.1–3.7) for cancer of the hypopharynx. Dikshit & Kanhere (2000) found an odds ratio for oropharyngeal cancer among bidi smokers only of 7.9 (95%CI: 5.1–12.4).

Znaor *et al.* (2003) reported a risk for bidi smoking only for pharyngeal cancer of 4.7 (95%CI: 3.5–6.3) and for combined bidi and cigarette smoking of 3.6 (95%CI: 2.55–4.98). Sapkota *et al.* (2007) reported an odds ratio for hypopharyngeal cancer of 6.8 (95%CI: 4.6–10.0) for bidi smokers compared to never smokers.

A dose–response relationship was observed for intensity and duration of bidi smoking for both cancers of oropharynx and hypopharynx (Rao *et al.*, 1999; Dikshit & Kanhere, 2000; Sapkota *et al.*, 2007).

2.19.3 Cancer of the lung

One cohort study (Jayalekshmy *et al.*, 2008), one population-based case–control study (Dikshit & Kanhere, 2000) and two hospital-based case–control studies (Gupta *et al.*, 2001; Gajalakshmi *et al.*, 2003) in India (Table 2.88 online) have investigated the relationship between bidi smoking and lung cancer. In all these studies both cases and controls were interviewed and analyses were restricted to men because very few women smoked among study subjects. One hospital-based case–control study in Chiang Mai, Thailand, looked at the association between lung cancer and *khii yoo,* hand-rolled cigars. The risk for lung cancer for *khii yoo* smoking was 1.2 in men and 1.5 in women, $P > 0.05$ (Simarak *et al.*, 1977).

In the population based case–control study by Dikshit & Kanhere (2000) the age-adjusted relative risk for lung cancer among bidi smokers only was 11.6 (95%CI: 6.4–21.3).

Gupta *et al.* (2001) reported an odds ratio for bidi smoking of 5.8 (95%CI: 3.4–9.7) from a hospital-based case–control study of lung cancer conducted in Chandigarh. Gajalakshmi *et al.* (2003) conducted a case–control study in two centres in which all subjects were interviewed by trained social investigators with standard

questionnaires. Odds ratios were adjusted for age, educational level, centre, chewing and alcohol habit. The odds ratios of lung cancer for former and current bidi smokers were 3.4 (95%CI: 2.1–5.4) and 5.3 (95%CI: 3.8–7.3), respectively. Odds ratios for former and current smokers of cigarette and bidi combined were 4.0 (95%CI: 2.5–6.6) and 9.1 (95%CI: 6.2–13.2), respectively.

Baseline data of a cohort of 359 619 residents in Kerala, India was collected by direct interview using standardized questionnaires during 1990–97 (Jayalekshmy *et al.*, 2008). After excluding rare earth workers, those who died, were diagnosed with cancer before 1997 or died within three years of interview, there were 65 829 bidi-smoking men aged 30–84 years old. Two hundred and twelve lung cancer cases were identified by the Karunagappally Cancer Registry between 1997 and 2004. The relative risk for lung cancer for current compared to never bidi smokers calculated by Poisson regression analysis and adjusted for age, religion and education was 3.9 (95%CI: 2.6–6.0; $P < 0.001$). The risk was lower among former than among current smokers.

(a) Dose–response evidence

Lung cancer risks increased with increasing bidi smoking intensities. The highest odds ratio was found for 9 pack–years (3.9; 95%CI: 2.1–7.1) (Gupta *et al.*, 2001). In a cohort study Jayalekshmy *et al.* (2008) found increased lung cancer incidence with increasing number of bidi sticks smoked per day ($P < 0.001$) and with increasing duration of bidi smoking ($P < 0.001$). [The number of lung cancer cases was small in each category, resulting in wide confidence intervals.] Gajalakshmi *et al.* (2003) also reported increased risk with duration and intensity of bidi smoking.

(b) Cessation of smoking

In two case–control studies (Gupta *et al.*, 2001; Gajalakshmi *et al.*, 2003) there was a clear decreasing trend in risk for years since quitting. Gajalakshmi *et al.* (2003) reported that lung cancer risk of former bidi smokers fell to 0.4 (0.1–1.2) after quitting for more than 15 years. The cohort study conducted in Kerala did not have the power to assess the risk associated with stopping bidi smoking (Jayalekshmy *et al.*, 2008).

2.19.4 Cancer of the larynx

Two hospital based case–control studies (Sankaranarayanan *et al.*, 1990b; Rao *et al.*, 1999) showed a higher risk for bidi smokers (Table 2.88 online). The relative risk was adjusted for age and religion in Sankaranarayanan *et al.* (1990b) study and for alcohol use, illiteracy, vegetarian/non-vegetarian diet and tobacco chewing in Rao *et al.* (1999) study. A multicentre case–control study on laryngeal cancer was conducted in four Indian centres using standardized questionnaires adjusting risks for centre, age, socio-economic status, alcohol consumption, tobacco snuffing and tobacco chewing (Sapkota *et al.*, 2007). Compared to never smokers bidi smokers had a higher risk for cancers of the supraglottis (OR, 7.5; 95%CI: 3.8–14.7), glottis (OR, 5.3; 95%CI: 3.2–8.9) and rest of larynx (OR, 9.6; 95%CI: 5.6–16.4).

All levels of intensity and duration of bidi smoking were associated with significant relative risk estimates and dose–response for laryngeal cancer (Sankaranarayanan *et al.*, 1990b; Rao *et al.*, 1999). A strong dose–response relationship was observed for duration and frequency of bidi smoking for cancers of supraglottis, glottis and rest of larynx (Sapkota *et al.*, 2007).

2.19.5 Cancer of the oesophagus

Three hospital-based case–control studies and one multicentre study (Sankaranarayanan *et al.*, 1991; Nandakumar *et al.*, 1996; Nayar *et al.*, 2000; Znaor *et al.* 2003) showed increased risk for oesophageal cancer among bidi smokers in India (Table 2.88 online). A significantly elevated

risk for all three segments of the oesophagus was reported (Nandakumar *et al.*, 1996). One study (Nayar *et al.*, 2000) adjusted for chewing of betel leaf with tobacco and low consumption of vegetables other than leafy vegetables. The multicentre case–control study conducted in two centres in South India found an increased risk for oesophageal cancer for bidi smoking only (OR, 3.3; 95%CI: 2.45–4.39) (Znaor *et al.*, 2003). Odds ratios were adjusted for age, centre, level of education, alcohol consumption and chewing. Only men were analysed in all the above studies.

Significant effects were noted in men for all levels of intensity and for duration of more than 20 years of bidi smoking (Sankaranarayanan *et al.*, 1991).

2.19.6 Cancer of the stomach

In a hospital-based case–control study the association between stomach cancer and bidi smoking was analysed as part of a multicentre study (Gajalakshmi & Shanta, 1996). Cases and controls were matched on age, sex, religion and mother tongue. The odds ratio for stomach cancer for current bidi smokers only was 3.2 (95%CI: 1.8–5.7) and for current smokers of any type of tobacco was 2.7 (95%CI: 1.8–4.1).

Table 2.88 (online) summarizes the studies published since the last *IARC Monograph* (IARC, 2004a). A hospital-based case–control study of stomach cancer included 170 stomach cancer cases (121 men and 49 women) and 2184 controls (1309 men and 875 women) aged 30–75 years (Rao *et al.*, 2002). The association between bidi smoking and stomach cancer was not significant (RR, 0.8; 95%CI: 0.5–1.2) in a univariate analysis. The risk increased with increase in lifetime exposure to bidi smoking and was highly significant ($P < 0.001$).

One study investigated stomach cancer risk in association with smoking of *meiziol*, a local cigarette in Mizoram, India (Phukan *et al.*, 2005). Statistically significant higher risks were seen for smokers of combined users of tobacco (cigarette and *meiziol*), with an odds ratio of 3.1 (95%CI: 2.0–11.1). Among users of a single type of tobacco, higher risks were seen for *meiziol* smokers (OR, 2.2; 95%CI: 1.3–9.3) in the multivariate model in comparison to cigarette smokers. Overall, the excess risk was limited to smokers of > 10 *meiziols* per day.

2.20 Synergistic effects of tobacco smoking and alcohol drinking

This section addresses the combined effects of smoking and alcohol consumption on cancers of oral cavity, pharynx, larynx and oesophagus, which have been examined extensively. For the purposes of this report interdependence of effects is termed *effect modification*, and *synergism* and *antagonism* are used to describe the consequences of the interdependence of disease risk when both risk factors are present (Rothman & Greenland, 1998). The studies varied in their methods and in the approaches used to assess effect modification, which ranged from descriptive to formal estimation of interaction terms in multivariate models. Study designs of the case–control and cohort studies are presented in Table 2.89 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.89.pdf) and Table 2.90 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.90.pdf), respectively; and the results for both study designs are presented in Table 2.91 (available at http://monographs.iarc.fr/ENG/Monographs/vol100E/100E-01-Table2.91.pdf).

2.20.1 Cancers of the upper aerodigestive tract

It was noted in the previous *IARC Monograph* (IARC, 2004a) with relatively large numbers of cases and controls that the pattern of increasing cancer risk with increasing alcohol consumption is strong (Mashberg *et al.*, 1993; Kabat *et al.*, 1994).

For cancers of the oral cavity, recent evidence comes from seven case–control studies and one cohort study. The pattern of odds ratios for smoking, across categories of alcohol consumption, is consistent with synergism. In four case–control studies with relatively large numbers of cases and controls (more than 200 cases and equivalent number of controls), the pattern of increasing cancer risks with increasing alcohol consumption was strong (Schlecht *et al.*, 1999b; Znaor *et al.*, 2003; Castellsagué *et al.*, 2004; Hashibe *et al.*, 2009). In the cohort study from Taiwan, China (Yen *et al.*, 2008) similar strong risks were also observed. In all four case–control studies in which the estimate of formal statistical interaction was examined, the tests were statistically significant (Schlecht *et al.*, 1999b; Znaor *et al.*, 2003; Castellsagué *et al.*, 2004; Hashibe *et al.*, 2009). In two case–control studies from India (Znaor *et al.*, 2003; Muwonge *et al.*, 2008) and in the cohort study from Taiwan, China (Yen *et al.*, 2008) the interaction of tobacco smoking, alcohol and betel quid chewing was examined. In general, the results suggested increasing risks when betel quid chewing was included in the model.

Five case–control studies and one cohort study examined the effect of interaction between tobacco and alcohol in pharyngeal cancer. The results from case–control studies were similar to those observed for oral cancer (Olsen *et al.*, 1985b; Choi & Kahyo, 1991; Schlecht *et al.*, 1999b; Znaor *et al.*, 2003; Hashibe *et al.*, 2009). In a Singapore cohort study (Friborg *et al.*, 2007) the pattern of odds ratios for smoking across categories of alcohol consumption was consistent with synergism for oropharyngeal but not for nasopharyngeal cancer.

Two cohort and fourteen case–control studies reported on joint effects of tobacco smoking and alcohol drinking on the risk for oesophageal cancer. Since multiple logistic regression models were used for analysing most of these studies, some of them tested likelihood ratio test for departure from multiplicativity of the individual effects of tobacco and alcohol. Generally, the positive results were stronger for squamous cell carcinoma. However, these tests for interaction are inadequate to assess synergy. Four studies from India and Taiwan, China, included betel quid chewing to the joint effect analysis of tobacco smoking and alcohol consumption and the results suggested increasing risks of oesophageal cancer.

Most of the twenty case–control studies of laryngeal cancer provided strong evidence for synergism of tobacco smoking and alcohol consumption. Only Zheng *et al.* (1992) did not find consistent evidence with synergism. In several studies, tests for interaction were carried out and reported to be 'non significant.' These were tests for departure from the multiplicative models, typically multiple logistic regression models, used to analyse the case–control data, and not tests for departure from additive model.

Several studies (14 case–control, 3 cohort) reported on cancer of the 'mixed upper aerodigestive tract', comprising studies on squamous cell carcinomas, regardless of specific sites. These studies also provided strong evidence for synergism.

The Working Group considers that there is strong evidence of tobacco smoking and alcohol consumption interaction on the incidence of upper aerodigestive tract cancers, as well as with regard to cancer of specific subsites of this anatomical region.

2.21 Synthesis

2.21.1 Lung

Tobacco smoking is the major cause of lung cancer, primarily from cigarettes. Duration of smoking is the strongest determinant of lung cancer in smokers. Risk also increases in proportion to the number of cigarettes smoked. The strong dose– and duration–response

relationships between lung cancer and tobacco smoking have been confirmed more recently in both questionnaire-based and biomarker-based studies. Tobacco smoking increases the risk of all histological types of lung cancer.

Differences in the intensity and/or duration of tobacco smoking may explain, in part, the lower lung cancer risks in Asian populations relative to whites. However, several studies of genetic polymorphisms among African-American and Caucasian populations provide some preliminary evidence supporting the hypothesis of a racial/ethnic disparity in susceptibility.

The results from observational studies do not provide strong support that a higher intake or a greater circulating concentration of carotenoids reduce lung cancer risk, particular in light of the elevated risk of lung cancer observed in the randomized trials of β-carotene supplementation. Residual confounding from smoking and the possibility that carotenoid measurements are serving as markers for a diet rich in total fruit and vegetables mitigate the likelihood of any protective role for total carotenoids or β-cryptoxanthins.

The specific genes that are responsible for enhanced lung cancer risk remain poorly understood, in spite of hundreds of candidate gene studies. Single-gene studies conducted to date have several limitations which contribute to inconclusive results, including small sample size and associated low power to detect moderate risks when allele frequencies are low.

2.21.2 Upper areodigestive tract

(a) Oral cavity

Tobacco smoking is causally associated with cancer of the oral cavity in both men and women. Since the previous *Monograph*, additional evidence has accumulated that further confirms the association. Risk increases with duration and intensity of smoking, and decreases after quitting.

(b) Pharynx

Tobacco smoking is an important cause of oropharyngeal and hypopharyngeal cancers. The risk increases with increasing duration and intensity of smoking and decreases with increasing time since quitting.

(c) Nasal cavity and accessory sinuses

The evidence of an association between tobacco smoking and sinonasal cancer is based on the results from case–control studies, each of which may be subject to different sources of bias. However, presence of a dose–response relationship in most studies, the decrease in risk associated with time since quitting, the consistently higher risks for squamous-cell carcinoma than for adenocarcinoma and the lack of potential confounders support the existence of a causal association.

(d) Nasopharynx

Although the interpretation of the results is complicated by small sample sizes in several studies, by different criteria used for the selection of controls and by the control groups in some studies including smoking-related diseases, the combined evidence shows an association between tobacco smoking and nasopharyngeal carcinoma in both endemic and non-endemic areas. Most studies that adjusted for known and suspected causes of nasopharyngeal carcinoma such as intake of Chinese-style salted fish, other dietary factors, alcohol drinking and family history of nasopharyngeal carcinoma, suggested only a limited confounding effect of these factors. Adjustment for infection with Epstein–Barr virus (human herpes virus 4), a major cause of nasopharyngeal carcinoma worldwide, was possible in just one of the available studies. However, it is unlikely that confounding by infection with Epstein–Barr virus would explain the observed association between tobacco smoking and risk for nasopharyngeal carcinoma.

(e) Oesophagus

Several well conducted case–control studies found a statistically significant higher risk for adenocarcinoma of the oesophagus in smokers than in nonsmokers. Positive dose–response relationships obtained using various indicators of amount smoked support a causal association, which is further corroborated by the findings of decreasing risks after smoking cessation. Several of these studies reported relative risks adjusted for alcohol consumption and other potential confounders. Further risk factors, such as chewing betel quid with tobacco or use of other forms of smokeless tobacco, have not been considered in these populations, but are not likely to be strong confounders. Studies from Australia, China and Europe also found increased risks for smokers.

(f) Larynx

Laryngeal cancer is one of the cancers most strongly associated with cigarette smoking. Recent epidemiological evidence strengthens this conclusion.

2.21.3 Stomach

The additional epidemiologic data showing a consistent association of stomach cancer with tobacco smoking in both men and women greatly strengthens the previous conclusion of a causal association. There was insufficient evidence for differential risks between cardia and non-cardia stomach cancer. Confounding and effect modification by H. pylori has not been found.

2.21.4 Pancreas

The additional data supports the previous evaluation that cancer of the pancreas is causally associated with tobacco smoking. The risk increases with increasing daily consumption levels and duration of smoking and decreases with increasing time since cessation of smoking. The risk remains elevated after accounting for potential confounding factors.

2.21.5 Colorectum

At the previous evaluation, there was already some evidence from prospective cohort and case–control studies that the risk of colorectal cancer is increased among tobacco smokers. However, inadequate adjustment for various potential confounders was considered to possibly account for some of the small increase in risk that appears to be associated with smoking. Since then, an appreciable amount of data has accumulated to support a causal association with smoking. In virtually all the cohort studies published since elevated risk associated with smoking was found, although not always statistically significant. More than half of the cohort studies that assessed dose–response relationships found statistically significant increasing risks with increasing daily cigarette consumption, duration of smoking and/or pack–years of smoking. Risk of colorectal cancer decreased with increasing delay in smoking initiation and years since cessation of smoking. A meta-analysis based on 36 cohort studies with data from a total of 3 million subjects found a significantly 15% increased risk of colorectal cancer and 27% higher risk of colorectal cancer mortality in current smokers compared to never smokers. A stronger association with smoking for rectal cancer than for colon cancer was found in the meta-analysis of the subset of cohort studies that differentiated colorectal cancer by site. Risk for colorectal cancer increased significantly by 17% and by 38% with 20 cigarettes and 40 cigarettes/day, respectively, and was elevated by 9.4% and by 19.7% with a 20-year and a 40-year duration of smoking, respectively. While these results are persuasive, this meta-analysis could not correct for the potential confounders in the individual studies. Convincing evidence has been provided by three large cohort studies that adjusted for at

least four important potential confounders (i.e. physical activity, alcohol consumption, body mass index and dietary intake of fruits and vegetables and/or meat); two studies also adjusted for history of colonoscopy. Significant dose–response relationships were found with one or more of the smoking variables, for risk of colorectal cancer and/or colon cancer and/or rectal cancer. Earlier cohort studies may not have been able to establish the association because of insufficient follow-up time and a limited number of cases. Updated results of several large cohort studies, which now show clearly significant increased risk of colorectal cancer associated with smoking, provide support for the lag-time hypothesis for smoking and colorectal risk.

Recent evidence suggests that smoking may be associated with the subtype of colorectal cancer characterized by microsatellite instability, and by CIMP status and BRAF mutation. For this subtype, the magnitude of risk associated with smoking reaches the twofold risk elevation consistently observed for colorectal adenomas and supported by a recent meta-analysis. Smoking has been associated with a stronger risk for hyperplastic polyps than for adenomas. Also, CIMP positivity and BRAF mutations have been associated with hyperplastic polyps, particularly serrated polyps. These data suggest that smoking may be associated primarily with a subtype of colorectal cancer that develops through a hyperplastic (serrated) polyp progression. The association with smoking may therefore be diluted when considering colon cancers overall.

2.21.6 Liver

Recent studies on smoking and hepatocellular carcinoma supports the established causal relationship. Supporting evidence comes from the consistency of the findings across regions (with the best evidence coming from Asian studies), and the observations of an association among non-drinkers and after controlling for hepatitis B or C virus infection.

2.21.7 Kidney

Recent evidence supports a causal association between kidney cancer and smoking. After adjustment for body mass index and hypertension, current and former smokers still had a greater risk for renal-cell cancer. A dose–response relationship with the number of cigarettes smoked has been noted in most studies, and a few also noted a reduction in risk after cessation.

2.21.8 Urinary bladder

Tobacco smoking is causally associated to bladder cancer, based on a large number of case–control and cohort studies that showed statistically significant associations not explained by confounding or bias. Risk increased with the duration of smoking and the number of cigarettes smoked. Also, stopping smoking at any age avoids the further increase in risk incurred by continued smoking. The evidence supporting a modulating role by *NAT2* polymorphisms is convincing.

2.21.9 Myeloid leukaemia

There is evidence for a causal association of tobacco smoking with myeloid leukaemia.

2.21.10 Breast

New evidence from cohort and case–control studies and from meta-analyses of genetic polymorphisms has become available since the previous *IARC Monograph* (IARC, 2004a). Results from seven new cohort studies consistently show a small overall association between current smoking and breast cancer incidence, with relative risk estimates ranging from 1.1–1.3 in studies with at least 100 exposed cases. The overall association is weaker than that observed with other cancers that have been designated as causally related to smoking, and the dose–response relationships (with years of smoking,

cigarettes smoked per day, age at initiation) are correspondingly small.

Emerging evidence from case–control studies suggests that inherited polymorphisms in the *NAT2* gene, which encode the slow acetylator phenotype, may modify (increase) the association between smoking and breast cancer. The p-value for interaction with pack–years of smoking as a continuous variable is statistically significant (P = 0.03) and another small study published since this meta-analysis supports the conclusion. The potential for publication bias remains of concern.

It is biologically plausible that tobacco smoke could be causally related to breast cancer risk. There are multiple chemicals in tobacco smoke that are known to cause mammary cancer in rodents. These substances reach the breast in humans; some are stored in adipose tissue, and some can be detected in nipple aspirate and DNA adducts.

Hypotheses have been proposed to explain why numerous well conducted epidemiological studies have generally not observed strong or consistent associations between tobacco smoking and breast cancer. Underlying all of these is the theory that tobacco smoking may have both protective and detrimental effects on breast cancer risk, which cancel each other out and which could explain the atypical dose–response relationship that has been reported between tobacco smoke and breast cancer from some studies.

2.21.11 Cervix

The largely positive findings observed in studies of cohort design, the relatively high consistency of positive associations found for squamous-cell carcinoma of the cervix (but not adenocarcinomas) across all epidemiological studies, including those with adjustment for a wide range of potentially confounding variables, and the positive associations observed in studies restricted to HPV-positive individuals, all argue against the observed positive association being due to recall or selection bias or confounding.

2.21.12 Endometrium

The results of epidemiological studies to date, including recent studies, largely show inverse associations of smoking with risk of postmenopausal endometrial cancer. However, the Working Group noted the few studies of premenopausal cancer that were less consistent, as well as indications of an increased risk among smokers in a recent multicentre European study.

2.21.13 Prostate

Many epidemiological studies have examined the association between cigarette smoking and prostate cancer risk, and most have shown no consistent association. The question remains whether smoking may alter risk in various population subgroups.

2.21.14 Ovary

A causal association between cigarette smoking and risk for mucinous ovarian tumours is indicated by 1) the consistency of the positive association across the large majority of ten pooled case–control studies and ten additional independent epidemiological studies of both case–control and cohort design, 2) the relatively strong magnitude of the association (typically greater than a doubling of risk among current smokers), 3) the tendency to show dose–response associations with risk, such that current smokers generally have higher risk than former smokers and the dose–response observed with measures of smoking intensity in some (but not all) studies, and 4) the specificity of the positive association with the mucinous histological type, which argues against recall bias as an explanation of the findings.

2.21.15 Thyroid

A pooled analysis of 14 case–control studies showed that smoking was inversely associated with thyroid cancer risk. Similar inverse associations were also observed in two subsequent case–control studies.

2.21.16 Other sites

There is inconsistent or sparse evidence for an association between tobacco smoking and other cancer sites that were considered by the Working Group.

2.21.17 Bidi smoking

Overall, bidi smoking increases the risk for cancers of the oral cavity, oropharynx, hypopharynx, larynx, lung, oesophagus and stomach.

3. Cancer in Experimental Animals

3.1 Mainstream tobacco smoke

3.1.1 Mouse

There have been multiple studies of the carcinogenic potential of tobacco smoke in mice (Table 3.1). Lifetime exposure of several mouse strains to cigarette smoke failed to result in the production of lung tumours (Harris & Negroni, 1967; Otto & Elmenhorst, 1967; Henry & Kouri, 1986). However, studies involving lifetime exposure of C57BL mice to a mixture of flue-cured or air-cured cigarette smoke or to the gas phase of flue-cured cigarette smoke led to significant increases in the number of lung tumours (adenomas) (Harris *et al.*, 1974). Similarly, lifetime exposure of Snell's mice to the gas phase of cigarette smoke led to an increased incidence of lung adenocarcinomas (Leuchtenberger & Leuchtenberger, 1970). Exposure of $B6C3F_1$ female mice to smoke for lifetime led to increased incidence of lung adenomas, bronchiolar papillomas and lung adenocarcinomas in smoke-exposed mice. In addition, the occurrence of squamous cell carcinomas of the nasal cavity in smoke-exposed mice was increased (Hutt *et al.*, 2005). In a recent study, Swiss mice were exposed whole-body to cigarette smoke for 120 days, starting within 12 hours of the birth. Smoke-exposed mice developed microscopic lung tumours beginning only 75 days after birth and reaching an overall incidence of 78.3% after 181–230 days. The mean lung tumour multiplicity was 6.1 and 13.6 tumours per mouse in males and females, respectively. In addition, malignant tumours, some of which may have had a metastatic origin, were detected in the urinary tract of smoke-exposed mice (Balansky *et al.*, 2007).

3.1.2 Rat

Several studies have evaluated the carcinogenic potential of mainstream tobacco smoke in rats (Table 3.1). Exposure of Wistar rats to cigarette smoke for lifetime did not increase the lung tumour incidence (Davis *et al.*, 1975). In contrast, exposure of Fischer 344 rats to a mixture of non-filter cigarette smoke for 128 weeks resulted in an increased incidence of nasal and lung tumours. There was also an increase in subcutaneous sarcomas at forelimb ulceration sites (Dalbey *et al.*, 1980). CDF rats were exposed to low-dose cigarette smoke (LCS) or high-dose cigarette smoke (HCS) for 126 weeks. The incidence of lung tumours was significantly higher only in female rats that received HCS (Finch *et al.*, 1995). In a recent study, Fischer 344 rats received whole body exposure to smoke containing either 100 mg (LCS) or 250 mg (HCS) total particulate matter/m^3 for 30 months. This led to significant increases in the incidence of lung and nasal cavity tumours in male rats treated with HCS but not with LCS. In female rats, there were significant increases in the incidence of lung adenomas

in animals treated with HCS and of all lung tumours in animals treated with both LCS and HCS. There was also a significant increase in the occurrence of nasal cavity tumours in female rats treated with HCS (Mauderly *et al.*, 2004).

3.1.3 Hamster

Four studies have evaluated the ability of mainstream tobacco smoke to induce tumours in hamsters (Table 3.1). Syrian golden hamsters were exposed to either a mixture of German reference cigarette smoke or of dark air-cured cigarette smoke for lifetime. There were increases in the incidence of laryngeal carcinomas in hamsters exposed to both smoke preparations (Dontenwill *et al.*, 1973). In a subsequent study, hamsters were exposed to a mixture of German reference cigarette smoke containing 1.5 mg nicotine, 0.173 mg phenol and 12.7 mL carbon monoxide/cigarette for lifetime. The incidence of laryngeal tumours in smoke-exposed hamsters was higher than in controls (Dontenwill *et al.*, 1977). BIO male hamsters exposed to a mixture of US reference smoke for 100 weeks developed laryngeal and nasopharyngeal tumours (Bernfeld *et al.*, 1974). In a subsequent study, male BIO hamsters exposed to smoke from commercial British filter cigarettes developed higher incidence of laryngeal tumours than controls (Bernfeld *et al.*, 1979).

3.2 Co-administration of tobacco smoke with known carcinogens and other agents

Study design and results of the studies on co-administration of tobacco smoke with known carcinogens and other agents are summarized in Table 3.2.

3.2.1 Rat

(a) Benzo[a]pyrene

Wistar rats received a single intratracheal instillation of 2 mg benzo[*a*]pyrene followed by lifetime exposure to cigarette smoke. This treatment led to a low incidence of lung tumours that was not significantly higher than in controls (Davis *et al.*, 1975). In another study Wistar rats were given intratracheal instillations of benzo[*a*]pyrene mixed with ferric oxide and exposed to cigarette smoke either during initiation and post-initiation or only after treatment with benzo[*a*]pyrene/ferric oxide (post-initiation). Inhalation of cigarette smoke during the initiation and post-initiation phases of carcinogenesis resulted in a higher lung tumour (squamous-cell carcinoma) multiplicity than that seen in rats exposed during the post-initiation phase only (Gupta *et al.*, 1990).

(b) Radon progeny

Sprague-Dawley rats were exposed to radon progeny at cumulative doses of 4000, 500 or 100 work-level-months (WLM), with or without concurrent exposure to cigarette smoke by inhalation for one year. Rats exposed to 4000 WLM radon progeny, without exposure to smoke, developed lung carcinomas (17/50). Thirty four carcinomas were seen in 50 rats exposed to radon and cigarette smoke. The 500 WLM radon progeny group exposed to radon only had 2/28 lung carcinomas as compared with 8/30 rats exposed to radon and cigarette smoke. No tumours were observed in rats treated with 100 WLM radon and one carcinoma was seen among 30 rats exposed to 100 WLM radon and cigarette smoke. Seventy five percent of the lung tumours were squamous-cell carcinomas, 20% were adenocarcinomas, and the remainder were undifferentiated carcinomas (Chameaud *et al.*, 1982).

Table 3.1 Carcinogenicity in response to mainstream tobacco smoke in animals

Species, strain (sex) Reference	Animals/group at start Dosing regimen Duration	Lung burden	Results Target organ Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mice, C57BL (M, F) Harris & Negroni (1967)	100 animals/sex/group Nose-only, mixture of fresh non-filter cigarette smoke/air (1/39, v/v), nicotine, 0.1 mg/mL; CO, 0.064% (v/v), 12 min/every other d; lifetime	Nicotine, 14–17 µg	Alveologenic adenocarcinomas: M–4/100 (alveologenic AdC) Controls–0/100 F–4/100 (alveologenic AdC) Controls–0/100	 $P = 0.06$ $P = 0.06$	
Mice, C57BL and BLH (sex, NR) Otto & Elmenhorst (1967)	126 animals/group Whole-body, gas phase of 12 cigarettes puffed 2 sec/min, concentration (NR), 90 min/d; lifetime (~27 mo)	NR	Lung (adenomas): C57BL–7/126 (5.5%) Controls–3/90 (3%) BLH–40/126 (32%) Controls–19/60 (32%)	 NS NS	
Mice, (C57BL/Cum × C3H/Anf Cum)F_1 (F) Henry & Kouri (1986)	2053, 1 014 sham Nose-only, 10% smoke from US reference cigarettes, concentration (NR), smoke 20 sec/min, 6–8 min/d, 5 d/wk for 110 wk; 116 wk	Particulate deposition, 125–200 µg	Alveolar adenocarcinomas: 19/978 (2%) Sham-exposed controls–7/651 (1%)	 $P = 0.10$	Shorter latency of tumour occurrence in smoke-exposed group suggested
Mice, C57BL (M, F) Harris *et al.* (1974)	100 animals/sex/group Nose-only, mixture of fresh flue-cured or air-cured cigarette smoke/air (1/39, v/v), concentration (NR), 12 min/d on alternate d; lifetime	NR	M: 9/162[a] (5%, flue-cured), 7/189[a] (4%, air-cured) Controls–3/160[a] (2%) F: 7/164[a] (4%, flue-cured), 0/173 (air-cured) Controls–1/159[a] (1%)	$P = 0.07$, flue-cured $P > 0.05$, air-cured $P = 0.04$, flue-cured	
	Nose-only, gas phase of flue-cured cigarette smoke, concentration (NR), 12 min/d on alternate d; lifetime	NR	M: 3/8[a] (37%) Controls–3/160[a] (2%) F: 2/88[a] (2%) Controls–1/159[a] (1%)	$P > 0.05$ $P > 0.05$	

Table 3.1 (continued)

Species, strain (sex) Reference	Animals/group at start Dosing regimen Duration	Lung burden	Results Target organ Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mice, Snell (M, F) Leuchtenberger & Leuchtenberger (1970)	160 M, 118 F Whole-body, whole fresh cigarette smoke, concentration (NR), 2 puffs, 1 × /d, lifetime (26 mo)	Nicotine, 5 µg	M: Lung A–7/107 (6.5%) Controls–8/106 (7.5%)	NS	
			Lung AdC–11/107 (10%) Controls–5/106 (4.7%)	$P = 0.15$	
			F: Lung A–2/65 (3%) Controls–1/78 (1.2%)	$P = 0.475$	
			Lung AdC–5/65 (7.7%) Controls–3/78 (3.8%)	$P = 0.035$	
	100 M, 89 F Whole-body, gas phase of fresh cigarette smoke, concentration (NR), 2 puffs, 1 × /d, lifetime (26 mo)	NR	M: Lung A–1/44 (2%) Controls–8/106 (7%)	NS	
			Lung AdC–10/44 (23%) Controls–5/106 (5%)	$P = 0.005$	
			F: Lung A–3/44 (7%) Controls–1/78 (1%)	$P = 0.15$	
			Lung AdC–5/44 (11%) Controls–3/78 (4%)	$P = 0.15$	
Mice, B6C3F1 (F) Hutt *et al.* (2005)	330, 326 controls Whole-body, smoke from Kentucky 2R1 unfiltered reference cigarettes, 250 mg total particulate matter/m^3, 6 h/d, 5 d/wk for 30 mo; 30 mo or lifetime	NR	Lung A: 93/330 (28%) Sham-exposed controls–22/326 (7%)	$P < 0.001$ $P < 0.007$	
			Bronchiolar papillomas: 15/330 (4%) Controls–0/326	$P < 0.001$	
			Lung AdC: 67/330 (20%) Controls–9/326	$P < 0.001$	
			All lung tumours: 148/330 (45%) Controls–31/326	$P < 0.001$[b]	
			Nasal cavity tumours: 20/330 (6%) Controls–0/326	$P = 0.002$, one-tailed Fisher	
			Squamous-cell carcinomas: 9/330 (3%) Controls–0/326		

Table 3.1 (continued)

Species, strain (sex) Reference	Animals/group at start Dosing regimen Duration	Lung burden	Results Target organ Incidence and/or multiplicity of tumours (%)	Significance	Comments
Mice, Swiss (M, F) Balansky *et al.* (2007)	38, 36 controls (neonatal mice, 21 h of age) Whole-body, cigarette smoke/air, 818 mg total particulate matter/m^3, 65 min/d for 120 d	NR	Lung A: 15/38(19%) Sham-exposed controls–0/36	$P < 0.001$	
			Lung AdC: 7/38 (18%) Controls–0/38	$P < 0.01$	
			Kidney A: 6/16 (16%) (F only) Controls–0/21	$P < 0.01$	
			Liver carcinomas: 2/16 (5%) (F only) Controls–0/21		
Rats, Wistar (F) Davis *et al.* (1975)	408, 102 untreated, 102 sham Nose-only, mixture of cigarette smoke/air (1/5), concentration (NR), 15 sec/min, 2 × 11 min/d, 5 d/wk, lifetime	NR	4/408 (1%) (1 lung C and 3 lung neoplasms of uncertain malignancy) Controls–0/102 Sham treated controls–0/102	NS	
Rats, F344 (F) Dalbey *et al.* (1980)	80, 63 untreated, 30 sham Nose-only, mixture of non-filter cigarette smoke/air (1/10), 18.4 mg smoke particulate and 0.89 mg nicotine/cigarette, 1 cigarette/h, 7 cigarettes/d, 5 d/wk for 128 wk; 160 wk	Particulate deposition, 1.75 mg/d	10/80 (12%) (1 nasal AdC, 1 nasal C, 5 pulmonary A, 1 pulmonary C, 2 alveologenic C) Controls–3/93 (3%)	$P < 0.05$	
			Subcutaneous sarcomas at forelimb ulceration sites: 21/80 (26%) Controls–0/93	$P < 0.05$	
Rats, CDF$^{\circ}$ (F344)/CrlBR (M, F) Finch *et al.* (1995)	2165 animals Whole-body, cigarette smoke/air, 100 mg (LCS) or 250 mg (HCS) total particulate matter/m^3, 6 h/d, 5 d/wk for 30 mo; lifetime	NR	Lung tumours: M: LCS 3/173 (2%) HCS 7/78 (9%) Filtered air 3/119 (2%)	$P < 0.05$	
			F: LCS–4/145 (3%) HCS–6/83 (7%) Filtered air–0/113	$P < 0.05$	

Table 3.1 (continued)

Species, strain (sex) Reference	Animals/group at start Dosing regimen Duration	Lung burden	Results Target organ Incidence and/or multiplicity of tumours (%)	Significance	Comments
Rats, F344 (M, F) Mauderly *et al.* (2004)	M: 178 LCS, 82 HCS F: 175 LCS, 81 HCS Whole-body, smoke from Kentucky 1R3 unfiltered reference cigarettes, 100 mg (LCS) or 250 mg (HCS) total particulate matter/m^3, 6 h/d, 5 d/wk for 30 mo; lifetime	NR	M: Lung A– LCS 4/178 (2%) HCS 2/82 (2%) Sham-exposed controls 1/118 (1%)	NS	
			Lung AdC– LCS 1/178 (1%) HCS 5/82 (6%) Controls 3/118 (3%)	NS	
			All lung tumours– LCS 4/178 (2%) HCS 7/82 (8%) Controls 4/118	NS; trend, $P = 0.055$	
			Nasal cavity (all tumour types)– LCS 1/178 (1%) HCS 5/82 (6%) Controls 1/118 (1%)	$P = 0.032$ (HCS); trend, $P = 0.010$	
			F: Lung A– LCS 7/175 (6%) HCS 7/81 (9%) Sham-exposed controls 0/119	NS (LCS); $P < 0.001$ (HCS)	
			AdC– LCS 4/175 (2%) HCS 4/81 (5%) Controls 0/119	NS	
			All lung tumours– LCS 10/175 (6%) HCS 11/81 (13%) Controls 0/119	$P = 0.023$ (LCS); $P = 0.001$ (HCS)	
			Nasal cavity (all tumour types)– LCS 0/175 HCS 3/81 Controls 0/119	$P = 0.020$ (HCS); trend, $P = 0.003$	

Table 3.1 (continued)

Species, strain (sex) Reference	Animals/group at start Dosing regimen Duration	Lung burden	Results Target organ Incidence and/or multiplicity of tumours (%)	Significance	Comments
Hamsters, Syrian golden (M, F) Dontenwill *et al.* (1973)	80 animals/sex/group Whole-body, mixture of German reference cigarette smoke/air (1/15), concentration (NR), smoke of 30 cigarettes for 7–10 min; 1, 2 or 3 × /d, 5 d/wk, lifetime	NR	Laryngeal carcinomas: 1/160 (1%), 17/160 (11%) and 11/160 (7%) Controls–0/80		
	Whole-body, mixture of dark air-cured cigarette smoke/air (1/15), concentration (NR), Smoke of 30 cigarettes for 7–10 min: twice/d, 5 d/wk, lifetime	NR	Laryngeal carcinomas: 2/160 (1%) Controls–0/80		
Hamsters, Syrian golden (M, F) Dontenwill *et al.* (1977)	80 animals/group Whole-body, mixture of German reference cigarette smoke/air (1/15), 1.5 mg nicotine, 0.173 mg phenol and 12.7 mL CO/cigarette, smoke of 30 cigarettes for 7–10 min; 1, 2 or 3 × /d, 5 d/wk, lifetime	NR	M: Laryngeal C–0, 4, 6 and 11%, Controls–0% F: Laryngeal C–0, 1, 2 and 7% Controls–0%		
Rats, Inbred BIO 15.16 & Inbred BIO 87.20 (M) Bernfeld *et al.* (1974)	102 animals/group Whole-body, mixture of US reference cigarette smoke/air (1/5), concentration (NR), duration (NR)		Inbred BIO 15.16: Laryngeal tumours–9/84 (10%) Nasopharyngeal tumours–2/84 (2%) Sham-exposed controls 0/42 Controls 0/40 Inbred BIO 87.20: Laryngeal tumours–2/87 (2%) Sham-exposed controls 0/44 Controls 0/48		
Rats, Inbred BIO 15.16 (M) Bernfeld *et al.* (1979)	Number at start (NR) Whole-body, 11 or 22% smoke from commercial British filter cigarettes, concentration (NR), 2 × 12 min/d, 7 d/wk for 35–42 wk; up to 74–80 wk	NR	Laryngeal carcinomas: 11% smoke–3/44 (7%) 22% smoke–27/57 (47%) Sham-exposed controls 0/36; Controls 0/50		

[a] Most of these lung tumours are adenomas

[b] Nasal cavity tumours included 14 squamous cell carcinomas (5 in situ), 5 hemangiomas, and 1 respiratory papilloma

A, adenoma; AdC, adenocarcinoma; C, carcinoma; CO, carbon monoxide; d, day or days; F, female; h, hour or hours; HCS, high cigarette smoke; LCS, low cigarette smoke; M, male; min, minute or minutes; mo, month or months; NR, not reported; NS, not significant; sec, second or seconds; wk, week or weeks; yr, year or years

Table 3.2 Carcinogenicity in response to exposure to mainstream tobacco smoke in conjunction with exposure to known carcinogens or other agents in animals

Species, strain (sex) Reference	Animals/group at start Dosing regimen Duration	Results Target organ Incidence and/or multiplicity of tumours (%)	Significance
Rats, Wistar (F) Davis *et al.* (1975)	84 or 408 animals/group A single intratracheal instillation of benzo[*a*]pyrene (2 mg) + infusine + carbon black followed by British reference cigarette smoke/air (1/5); 1 cigarette, twice/d, 5 d/wk, lifetime	3/84 (4%, lung C), 1/84 (1%, lung C; benzo[*a*]pyrene alone), 4/408 (1%, 3 A + 1 malignant neoplasm; cigarette smoke only), 0/204 (controls + sham-exposed controls)	NS
Rats, Wistar (M) Gupta *et al.* (1990)	35 animals/group Cigarette smoke; 5 cigarettes/8.2 L air; 1 h/d during 2nd–24th wk or 10th–24th wk of the study Benzo[*a*]pyrene (20 mg) + Fe_2O_3; intratracheally (3 weekly doses) during 6th–8th wk of the study; 24 wk	Conventional diet: 2nd–24th wk, 2.14 lung C/animal; 10th–24th wk, 1.33 lung C/animal; benzo[*a*]pyrene control, 1.22 lung C/animal. Vitamin A-deficient diet: 2nd–24th wk, 2.86 lung C/animal; 10th–24th wk, 1.67 lung C/animal; benzo[*a*]pyrene control, 1.83 lung C/animal	
Rats, Sprague-Dawley, sex NR) Chameaud *et al.* (1982)	28–50 animals/group French reference cigarette smoke (9 cigarettes/500 L air); 10–15 min session, 4 d/wk for 1 yr Radon progeny (4 000, 500 or 100 WLM) Lifetime	4000 WLM: 34/50 (68%, lung C); 17/50 (34%, lung C; radon progeny alone) 500 WLM: 8/30 (27%, lung C); 2/28 (7%, lung C; radon progeny alone) 100 WLM: 1/30 (3%, lung C); 0/50 (radon progeny alone)	$P = 0.0015$
CDF*(F344)/CrlBR (M, F) Finch *et al.* (1995)	Number at start (NR) Cigarette smoke/air, 100 mg (LCS) or 250 mg (HCS) total particulate matter/m^3; 6 h/d, 5 d/wk for 126 wk $^{239}PuO_2$ aerosol, 1 wk (6th wk of the study); > 52 wk	49–61% (lung tumours, LCS + $^{239}PuO_2$) 72–74% (HCS + $^{239}PuO_2$) 20–33% ($^{239}PuO_2$) 2–3% (LCS) 7–8% (HCS)	
Syrian golden (M, F) Dontenwill *et al.* (1973)	80 animals/sex/group German reference cigarette smoke/air (1/15); Smoke of 30 cigarettes for 7–10 min; twice/d, 5 d/wk, lifetime DMBA (0.5 mg); intratracheally 10 d before the beginning of smoke exposure	32/160 (20%, laryngeal C), 17/160 (11%, laryngeal C; smoke only), 0/160 (DMBA alone)	

Table 3.2 (continued)

Species, strain (sex) Reference	Animals/group at start Dosing regimen Duration	Results Target organ Incidence and/or multiplicity of tumours (%)	Significance
Syrian golden, (sex NR) Hoffmann *et al.* (1979)	20 or 40 animals/group Cigarette smoke/air (1/7); Cigarette smoke 2 × 10 min/d, 5 d/wk, 48 wk DMBA (0.24 mg); intralaryngeally	3/40 (7%, laryngeal C), 0/20 (smoke only), 0/20 (DMBA alone)	
Syrian golden (M) Takahashi *et al.* (1992)	10 or 30 animals/group Cigarette smoke/air (1/7); Smoke of 30 cigarettes for 9 min; twice/d, 5 d/wk, 12 wk NDEA (100 mg/kg bw); subcutaneously	Non-filter cigarettes (2.10 ± 1.74 P+H/animal) and filter cigarettes (1.93 ± 1.55 P + H/animal) versus sham-exposed (0.97 ± 1.03 P + H/animal)	$P < 0.01$ $P < 0.01$
Syrian golden (M) Harada *et al.* (1985)	30 animals/group Non-filter cigarette smoke/air (1/7); Smoke of 30 cigarettes for 6 min: twice/d, 5 d/wk, 58 wk NDEA (10 mg/hamster); subcutaneously (12 weekly doses)	Nasal cavity tumours 14/30 (47%, smoke + NDEA), 5/30 (17%, NDEA alone)	$P < 0.05$

A, adenoma; bw, body weight; C, carcinoma; d, day or days; DMBA, 7,12-dimethylbenz[*a*]anthracene; F, female; h, hour or hours; HCS, high cigarette smoke; LCS, low cigarette smoke; M, male; min, minute or minutes; mo, month or months; NDEA, *N*-nitrosodiethylamine; NR, not reported; NS, not significant; P + H, epithelial hyperplasias and papillomas; sec, second or seconds; wk, week or weeks; WLM, work-level-months; yr, year or years

(c) Plutonium oxide

CDF®/CrlBR rats were exposed to either filtered air or mainstream cigarette smoke at concentrations of either 100 or 250 mg total particulate matter/m^3 (LCS and HCS groups, respectively). At 12 weeks, rats were removed from smoke chambers and exposed nose-only to plutonium oxide ($^{239}PuO_2$) then returned to the smoke chambers one week later for 30 months of continuous exposure to either filtered air or cigarette smoke. The incidence and multiplicity of lung tumours (adenocarcinomas, squamous-cell carcinomas, adenosquamous carcinomas) in animals exposed to both concentrations of cigarette smoke and $^{239}PuO_2$ were higher than those in animals exposed to $^{239}PuO_2$, LCS or HCS alone (Finch *et al.*, 1995).

3.2.2 Hamster

(a) 7,12-Dimethylbenz[a]anthracene

Groups of 160 Syrian golden hamsters received 7,12-dimethylbenz[*a*]anthracene (DMBA) intratracheally, followed by cigarette smoke for life, or treated with cigarette smoke or DMBA only. A total of 32 squamous-cell carcinomas of the larynx were observed in animals treated with both DMBA and cigarette smoke, in comparison with 17 in hamsters exposed to cigarette smoke only and none in hamsters treated with DMBA alone (Dontenwill *et al.*, 1973). Similar results were reported from other experiments in which Syrian golden hamsters were exposed to DMBA and cigarette smoke (Hoffmann *et al.*, 1979).

(b) N-Nitrosodiethylamine

Groups of hamsters received a single subcutaneous injection of *N*-nitrosodiethylamine (NDEA) and then were exposed to smoke from unfiltered cigarettes, filtered cigarettes and sham smoke. Controls were exposed to either unfiltered cigarette smoke, filtered cigarette smoke or sham smoke. In the NDEA-smoke-treated groups, epithelial hyperplasias and/or papillomas of the larynx were induced at higher frequency than in controls (Takahashi *et al.*, 1992). Hamsters exposed to cigarette smoke in air also received 12 weekly subcutaneous injections of NDEA (total dose, 10 mg/hamster). Treatment with NDEA only resulted in both benign and malignant tumours of the respiratory tract, and co-exposure to cigarette smoke potentiated the development of tumours in the nasal cavity (Harada *et al.*, 1985).

3.3 Smoke condensates

Study design and results of the studies on administration of tobacco smoke condensates are summarized in Table 3.3.

3.3.1 Skin application

(a) Mouse

Cigarette-smoke condensate produces both benign and malignant tumours on mouse skin. The carcinogenic potency of the cigarette-smoke condensate depends upon tobacco variety, composition of cigarette paper and the presence of additives (Wynder *et al.*, 1957; Gargus *et al.*, 1976; Gori, 1976).

(b) Rabbit

Cigarette-smoke condensate induced skin papillomas and carcinomas when applied to the ears of rabbits for lifetime (Graham *et al.*, 1957).

3.3.2 Intrapulmonary administration

Injection of 24 mg cigarette-smoke condensate into the lungs of female Osborne Mendel rats led to the development of squamous cell carcinomas (Stanton *et al.*, 1972). These observations were confirmed by Dagle *et al.* (1978) who observed a dose-dependent incidence of lung carcinomas when cigarette-smoke condensate prepared from two types of cigarettes were given.

Table 3.3 Carcinogenicity in response to exposure to cigarette-smoke condensate in animals

Species, strain (sex) Reference	Animals/group at start Dosing regimen Duration	Results Target organ Incidence and/or multiplicity of tumours (%)	Significance
Mice CAF1 (M, F) Wynder *et al.* (1953)	112, 44 controls Skin painting (dorsal) of CSC, CSC/acetone solution (40 mg CSC/ application), 3 × /wk, lifetime	36/81 (44%, skin epidermoid C), 0/30 (acetone controls)	
Mice ICR Swiss (F) Gargus *et al.* (1976)	5200 Skin painting (dorsal) of CSC, CSC/acetone solution (150 mg or 300 mg CSC/wk), 6 × /wk, 78 wk	482/5200 (9%, skin C), 3/800 (0.4%, acetone controls)[a]	
Mice ICR Swiss (F) Gori (1976)	4900 Skin painting (dorsal) of CSC, CSC/acetone solution (25 mg or 50 mg CSC/application), 6 × /wk, 78 wk	1157/4900 (24%, skin C), 0/800 (acetone controls)	
Mice, ICR/Ha Swiss (F) Hoffmann & Wynder (1971)	30 animals/group Skin painting (dorsal) with CSC active fraction with or without subsequent painting of the skin with croton oil, CSC active fraction/acetone (2.5 mg of 0.6% CSC/ application), 10 times on alternate d Croton oil (2.5%), 3 × /wk, up to 12 mo, 10 d after last CSC active fraction application; 15 mo	After 12 and 15 mo: 4/30 (13%, skin C), 0/65 (croton oil controls)	
Mice, Swiss (F) Wynder & Hoffmann (1961)	30–50 animals/group Skin painting (dorsal) of CSC with or without initiation by DMBA application; DMBA (75 µg); CSC/acetone (75 mg CSC/application, start: 1 wk after DMBA application), 2–3 × /wk, 12 mo; 15 mo	DMBA: 2/30 (7%, skin C) 2 × CSC: 1/40 (3%, skin C) DMBA + 2 × CSC: 8/30 (27%, skin C) 3 × CSC: 11/50 (22%, skin C) DMBA + 3 × CSC: 11/30 (37%, skin C)	
Mice, SENCAR (F) Meckley *et al.* (2004a)	40 animals/group Skin painting (dorsal) of CSC from Kentucky 1R4F reference cigarettes, with or without initiation by DMBA application; DMBA (75 µg) or acetone, 1x. Then starting 1 wk after DMBA or acetone: CSC in acetone, 0, 10, 20 or 40 mg/application, 3 × /wk, 29 wk; 31 wk	Mean mice with tumours/mice per group at 31 wk[e]: No DMBA: 0/40 acetone-acetone, 9/40 (22%) acetone-CSC 40 mg/treatment DMBA/CSC: 0/40, 3/40 (1.0), 16/40 (75 tumours/16 mice = 4.7), 32/40 (200 tumours/32 mice = 6.3)	
Mice, SENCAR (F) Meckley *et al.* (2004b)	40 animals/group Skin painting (dorsal) of CSC from Kentucky 1R4F reference cigarettes or ECLIPSE (non-burned) cigarettes, with or without initiation by DMBA application; DMBA (75 µg) or acetone, 1 × . Then starting 1 wk after DMBA or acetone: CSC in acetone, 0, 10, 20 or 40 mg/application, 3 × /wk, 29 wk; 31 wk	No DMBA: acetone/acetone 0/40 (0); acetone/1R4F CSC 40 mg, 9/40 (1.8); acetone/ECLIPSE CSC 40 mg, 0/40 (0) DMBA/CSC: acetone, 0/40 (0); 1R4F CSC 10 mg, 6/40 (1.8); 1R4F CSC 12 mg, 28/40 (6.6); 1R4F CSC 40 mg, 36/40 (6.8); ECLIPSE CSC, 0/40 (0); ECLIPSE CSC 10 mg, 1/40 (1); ECLIPSE CSC 20 mg, 2/40 (5.5); ECLIPSE CSC 40 mg, 12/40 (2.6)	

Table 3.3 (continued)

Species, strain (sex) Reference	Animals/group at start Dosing regimen Duration	Results Target organ Incidence and/or multiplicity of tumours (%)	Significance
Mice, SENCAR (F) Hayes *et al.* (2007)	40 or 50 animals/group Skin painting (dorsal) of CSC from heat-exchanged flue cured tobacco (HE; low TSNA) or direct-fire (DF) cured tobacco, with or without initiation by DMBA application; DMBA (75 µg) or acetone, 1 × . Then starting 1 wk after DMBA or acetone: CSC/acetone, 0, 9, 18, or 36 mg/application, 3 × /wk, 29 wk; 31 wk	No DMBA: acetone/acetone 0/40; acetone/DF CSC 36 mg, 5/50 (1.4); acetone/HE CSC 36 mg, 8/50 (1.3) DMBA/CSC: DF CSC, 0/40, DF CSC 9 mg, 15/40 (5.5); DF CSC 18 mg, 30/40 (10.0); DF CSC 36 mg, 43/50 (8.2); HE CSC, 0/40, HE CSC 9 mg, 17/40 (4.8); HE CSC 18 mg, 32/40 (7.3); HE CSC 36 mg, 42/50 (8.5)	$P < 0.05$ $P < 0.05$ $P < 0.05$
Mice, Swiss albino (M) Pakhale *et al.* (1988)	20 animals/group Oral gavage of Indian bidi smoke condensate; 1 mg bidi smoke condensate/0.1 mg DMSO, 5 d/wk, 55 wk; 90 wk	4/15 (27%, hepatic haemangiomas); 1/15 (7%, stomach papilloma); 1/15 (7%, stomach carcinoma); 1/15 (7%, oesophageal carcinoma); 0/15 (untreated or DMSO-treated controls)	
Rats, Osborne Mendel (F) Stanton *et al.* (1972)	Number/group at start (NR) Intrapulmonary administration of CSC pellet; CSC/beeswax:tricaprylin (24 mg CSC/injection), up to 107 wk after implantation	14/40[c] (35%, lung squamous-cell C), 0/63[c] (beeswax:tricaprylin controls)	
Rats, OM/NCR (F) Dagle *et al.* (1978)	120[d] Intrapulmonary administration of CSC pellet; CSC/beeswax:tricaprylin (5, 10, 20 or 67 mg CSC/injection), 120 wk after implantation	4, 10, 20 and 42% pulmonary C prevalence; 0% C prevalence for 3 control groups of about 190 rats each	
Rabbits, Albino New Zealand (M, F) Graham *et al.* (1957)	38, 7 controls Skin painting of CSC (both ears); CSC/acetone solution (100 mg CSC/application/ear), 5 × /wk, lifetime (4–6 yr)	4/38 (11%, 2 skin C + 1 skin liposarcoma + 1 skin fibrosarcoma), 0/7 (acetone controls)	

[a] Skin papillomas
[b] Mostly adenomas
[c] Incidence in animals that died 43–107 weeks after injection
[d] 4 × 10 rats/group terminated before 120 weeks
[e] Total visually identified and histologically confirmed skin tumours included mostly squamous papillomas and carcinomas [Tumour incidences and multiplicities estimated from graphs]
C, carcinoma; CSC, cigarette-smoke condensate; d, day or days; DMBA, 7,12-dimethylbenz[*a*]anthracene; DMSO, dimethyl sulfoxide; F, female; M, male; NR, not reported; TSNA, tobacco-specific *N*-nitrosamines; wk, week or weeks

3.3.3 Initiation-promotion skin painting studies

Cigarette-smoke condensate and its fractions can act as skin co-carcinogens in Swiss and SENCAR mice when tested in conjunction with croton oil (Hoffmann & Wynder, 1971) or DMBA (Wynder & Hoffmann, 1961; Meckley *et al.*, 2004a, b; Hayes *et al.*, 2007).

3.3.4 Bidi smoke

Swiss albino mice administered 1 mg bidi smoke condensate in dimethyl sulfoxide (DMSO) by oral gavage developed haemangiomas (4/15), stomach carcinoma (1/15), and esophageal carcinoma (1/15), whereas no tumours were observed in controls (Pakhale *et al.*, 1988).

3.4 Synthesis

Mainstream tobacco smoke induced lung tumours in mice, lung and nasal cavity tumours in rats and laryngeal carcinomas in hamsters.

Co-administration of tobacco smoke with benzo[*a*]pyrene, radon progeny and plutonium resulted in higher lung tumour responses in rats than administration of either agent alone. Hamsters exposed to cigarette smoke and either DMBA or NDEA had higher lung tumour responses compared to cigarette smoke, DMBA or NDEA alone.

Topical application of cigarette-smoke condensate led to the development of skin tumours in mice and rabbits; intrapulmonary administration of cigarette-smoke condensate induced squamous cell carcinomas in rat lung.

4. Other Relevant Data

4.1 Overview of the mechanistic evidence for the carcinogenicity of tobacco

4.1.1 Conceptual model of the carcinogenesis of tobacco and tobacco smoke

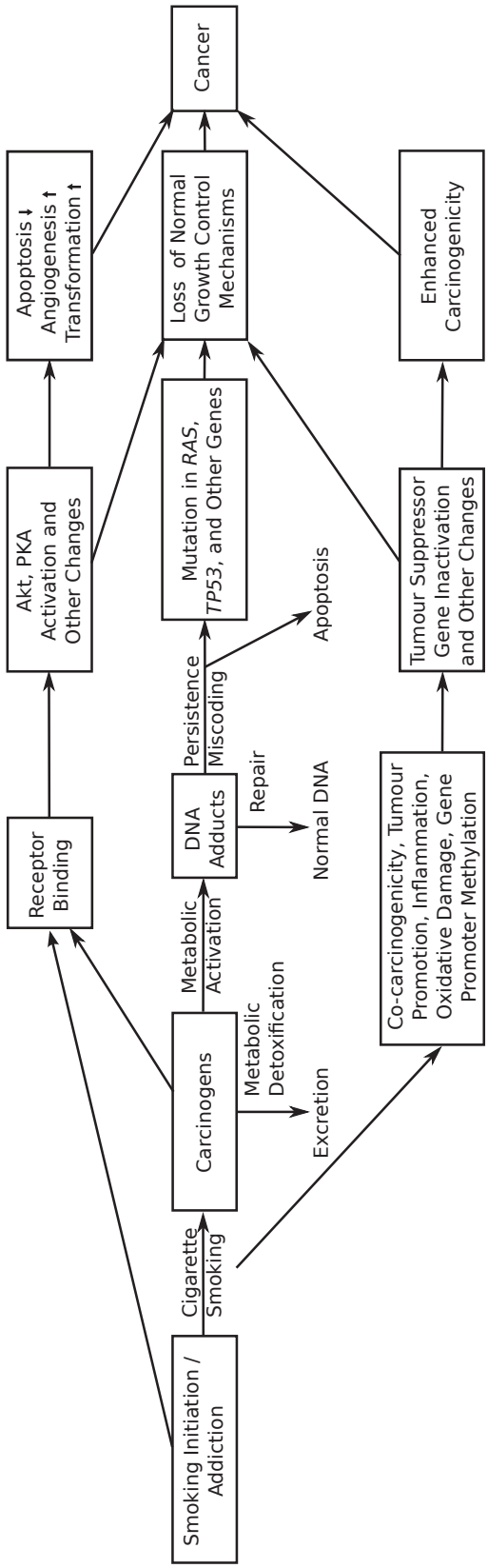
A conceptual model for understanding mechanisms by which tobacco smoke causes cancer is shown in Fig. 4.1 (Hecht, 1999, 2003). This model also applies to smokeless tobacco and other forms of smoked tobacco and, in theory, to second-hand tobacco smoke since it contains all of the same carcinogens and toxicants as mainstream cigarette smoke, although at lower doses.

The major accepted mechanistic pathway is summarized in the central track of Fig. 4.1. Smokers inhale carcinogens which, either directly or after metabolism, covalently bind to DNA, forming DNA adducts. DNA adducts are central to chemical carcinogenesis because they can cause miscoding and permanent mutations. If these mutations occur in critical regions of oncogenes and tumour suppressor genes, which are essential in growth control, the result can be loss of normal cellular proliferation mechanisms, genomic instability, and cancer. A study that sequenced 623 cancer-related genes in 188 human lung adenocarcinomas validated this premise by finding multiple somatic mutations in critical growth control genes, consistent with the chronic bombardment of cellular DNA by tobacco smoke carcinogens and their metabolically activated forms (Ding *et al.*, 2008).

Each step of this conceptual model is considered in detail below.

Most people begin smoking cigarettes when they are teenagers, and become addicted to nicotine. Nicotine is not generally considered to be a carcinogen (Schuller, 2009), but it is accompanied in each puff of each cigarette by a complex

Fig. 4.1 Conceptual model for understanding mechanisms of tobacco carcinogenesis



There may be overlap between these three tracks: e.g. mutation in *TP53* leading to tumour suppressor gene inactivation.

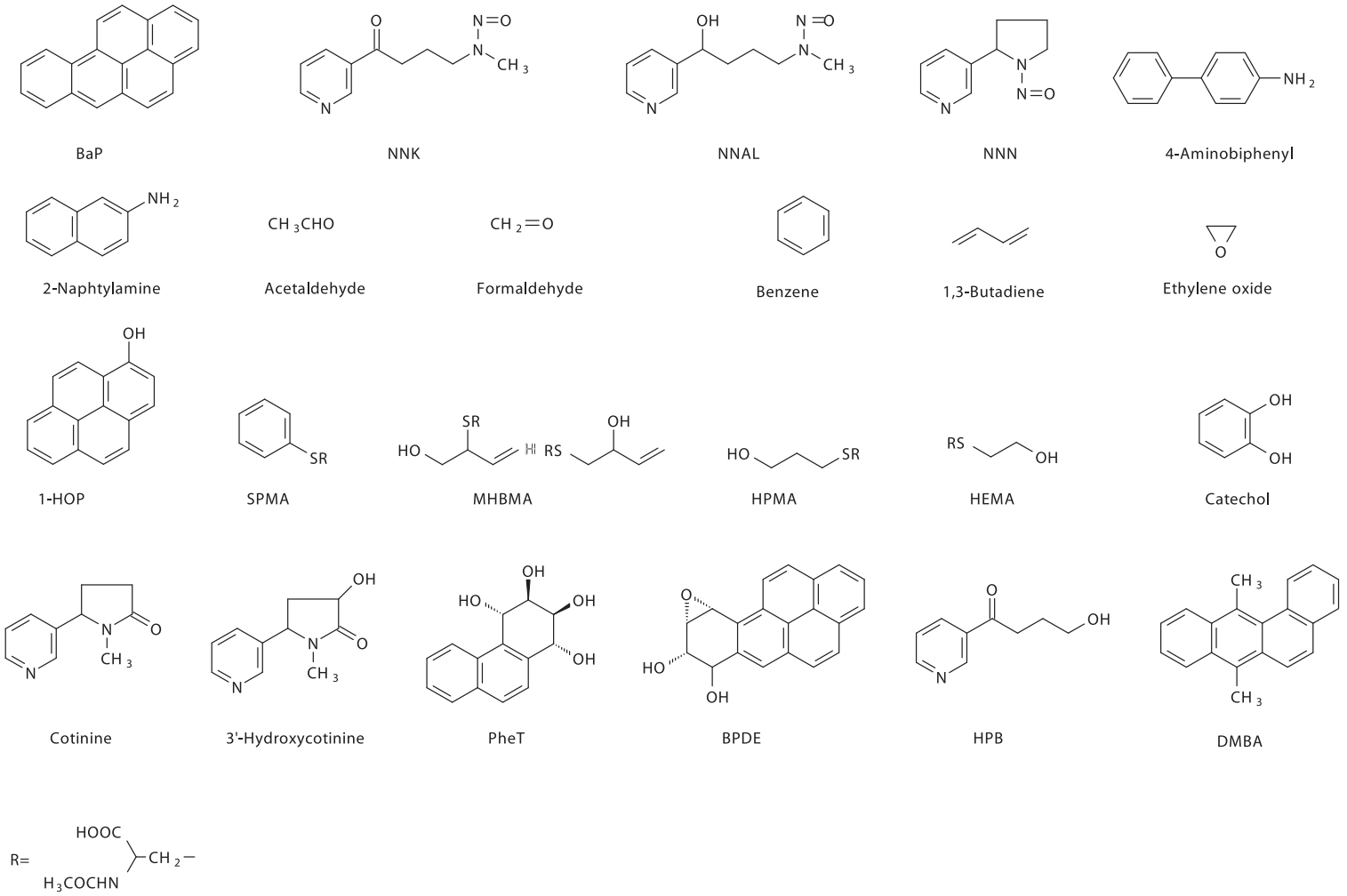
mixture of carcinogens and toxicants. There are over 60 carcinogens in cigarette smoke that have been evaluated in the previous *IARC Monograph* as having *sufficient evidence* for carcinogenicity in laboratory animals (IARC, 2004a), sixteen of which are considered to be *carcinogenic to humans (Group 1)*. There are also many other carcinogens and potential carcinogens in cigarette smoke that have not been evaluated (Rodgman & Perfetti, 2006; see Section 1.1). Structures of tobacco smoke constituents and biomarkers discussed here are presented in Fig. 4.2.

Numerous studies demonstrate the uptake of tobacco smoke carcinogens and toxicants by smokers, and showed higher levels of their metabolites in urine and blood of smokers than non-smokers (Sections 4.1.1 and 4.1.2). There are substantial differences in carcinogen exposure among people because of the number and types of cigarettes they smoke and the ways in which they smoke them. These differences can be monitored in part by biomarkers of exposure such as urinary metabolites or haemoglobin adducts (Section 4.1.2). Haemoglobin adducts of multiple aromatic amines and volatile carcinogens have been demonstrably related to tobacco (Hatsukami *et al.*, 2006a). There may also be differences in carcinogen exposure due to genetic variations (Section 4.2).

The body's response to cigarette smoke constituents is similar to its response to pharmaceutical agents and other foreign compounds. Drug metabolizing enzymes, most frequently CYPs, convert these compounds to more water soluble forms, facilitating excretion. During this natural protective attempt, some reactive intermediates are formed. These intermediates are frequently electrophilic (electron seeking, or bearing a partial or full positive charge). Electrophilic intermediates may react with water, generally resulting in detoxification, or may covalently bind to nucleophilic (electron rich) sites in DNA, forming DNA adducts (Guengerich, 2001; Jalas *et al.*, 2005), which are critical in the carcinogenic process (see Section 4.1.3c). CYP1A1 and CYP1B1, repeatedly shown to be inducible by cigarette smoke via interactions of smoke compounds with the aryl hydrocarbon receptor (AhR), are particularly important in the metabolic activation of PAHs, while CYP2A13 is critical for the metabolism of NNK (Nebert *et al.*, 2004; Jalas *et al.*, 2005). The inducibility of certain CYPs may be a critical aspect of cancer susceptibility in smokers (Nebert *et al.*, 2004). CYP1A2, CYP2A6, CYP2E1 and CYP3A4 are also important in the metabolism of cigarette smoke carcinogens to DNA binding intermediates (Jalas *et al.*, 2005), and aldo-keto reductase enzymes, also induced by tobacco smoke (Quinn *et al.*, 2008), are involved in the metabolism of NNK, BaP and other tobacco smoke carcinogens. Competing with this process of "metabolic activation" resulting in DNA binding is the intended metabolic detoxification, which leads to harmless excretion of carcinogen metabolites, and is also catalysed by CYPs and a variety of other enzymes including GSTs, uridine diphosphate-glucuronosyl transferases (UGTs), and arylsulfatases. The relative amounts of carcinogen metabolic activation and detoxification differ among individuals. It is widely hypothesized that this balance will affect cancer risk with those having higher activation and lower detoxification capacity being the most susceptible. This premise is supported in part by molecular epidemiologic studies of polymorphisms, or variants in more than 1% of the population, in certain genes coding for these enzymes (Vineis *et al.*, 2003; Carlsten *et al.*, 2008).

DNA adducts are thought to be a critical lesion in carcinogenesis. Many investigations demonstrate the presence of DNA adducts in human tissues, and some of these are summarized in Section 4.1.2c. There is massive evidence, particularly from studies which use relatively non-specific DNA adduct measurement methods, that DNA adduct levels in the lung and other tissues of smokers are higher than in non-smokers, and some epidemiologic data link

Fig. 4.2 Structures of compounds discussed in the text



BaP, Benzo[*a*]pyrene; BPDE, Benzo[*a*]pyrene diol epoxyde; DMBA, dimethylbenz[*a*]anthracene; 1-HOP, 1-hydroxypyrene; HEMA, 2-hydroxyethyl-mercapturic acid; HPMA, 3-hydroxypropyl-mercapturic acid; HPB, 4-hydroxy-1-(3-pyridyl)-1-butanone; HPMA, 3-hydroxypropyl-mercapturic acid; MHBMA, monohydroxybutyl-mercapturic acid; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*'-nitrosonornicotine; PheT, phenanthrenetetrol; SPMA, S-phenyl-mercapturic acid

these higher adduct levels to increased cancer risk (IARC, 2004b; Veglia *et al.*, 2008). However, there is much more limited evidence from studies using specific carcinogen-derived DNA adducts as biomarkers (Pfeifer *et al.*, 2002). Oxidative DNA damage has also been observed, and this may result partially from exposure to metals in cigarette smoke (Stavrides, 2006).

Cellular DNA repair systems can excise DNA adducts and restore normal DNA structure (Christmann *et al.*, 2003). These complex multiple systems include direct base repair by alkyltransferases, removal of DNA damage by base and nucleotide excision repair, mismatch repair, and double strand repair. If these DNA repair systems are unsuccessful in fixing the damage, then the DNA adducts can persist, increasing the probability of a permanent mutation. There are polymorphisms in genes coding for some DNA repair enzymes. If these variants lead to deficient DNA repair, the probability of cancer development can increase (Vineis *et al.*, 2009).

DNA adducts can cause miscoding during replication when DNA polymerase enzymes misread the DNA adduct and consequently insert the wrong base opposite to it. There is some specificity in the relationship between specific DNA adducts formed from cigarette smoke carcinogens and the types of mutations which they cause. G to T and G to A mutations have often been observed (Section 4.1.3) (Hecht, 1999). Extensive studies have characterized the mutations which occur because of specific carcinogen-DNA adducts (Delaney & Essigmann, 2008). Mutations have been reported in the *KRAS* oncogene in lung cancer and in the *TP53* tumour suppressor gene in a variety of cigarette smoke-induced cancers (Ahrendt *et al.*, 2001; Pfeifer *et al.*, 2002; Ding *et al.*, 2008). The cancer causing role of these genes has been firmly established in animal studies (Lubet *et al.*, 2000; Johnson *et al.*, 2001). A selection and promotion process may also play a role in the final mutation spectrum seen in genes in smoking-associated tumours (Rodin & Rodin, 2005; Sudo *et al.*, 2008).

Urinary mutagenicity, sister chromatid exchanges, micronuclei in buccal cells, and other genetic effects have been consistently observed in smokers at higher levels than in non-smokers (IARC, 2004a; Proia *et al.*, 2006). In addition to mutations, numerous cytogenetic changes are observed in lung cancer, and chromosome damage throughout the field of the aerodigestive tract is strongly associated with cigarette smoke exposure. Mutations resulting from DNA adducts can cause loss of normal cellular growth control functions, via a complex process of signal transduction pathways, ultimately resulting in genomic instability, cellular proliferation and cancer (Ding *et al.*, 2008). Apoptosis, or programmed cell death, is a protective process, and can remove cells which have DNA damage, thus serving as a counterbalance to these mutational events. The balance between apoptotic mechanisms and those suppressing apoptosis will have a major impact on tumour growth.

While the central track of Fig. 4.1 is the major pathway by which tobacco smoke carcinogens cause cancer, other mechanisms also contribute, as indicated in the top and bottom tracks (Hecht, 2003). Nicotine, NNK, and NNN bind to nicotinic and other cellular receptors, resulting in activation of serine/threonine kinase Akt (also known as protein kinase B), protein kinase A, and other changes. Nicotine and NNK increase expression of survivin, an inhibitor of apoptosis in normal human bronchial epithelial cells, and survivin mRNA is detected in bronchial brush samples from heavy smokers (Jin *et al.*, 2008). This can cause decreased apoptosis, increased angiogenesis, and increased transformation (Heeschen *et al.*, 2001; West *et al.*, 2003). Thus, although nicotine is not carcinogenic, it may enhance carcinogenicity in various ways (Schuller, 2009). Cigarette smoke also contains well established oxidants, co-carcinogens, tumour promoting fractions, and inflammatory agents, as well as

cilia-toxic compounds such as acrolein, which impede clearance. Many studies demonstrate the co-carcinogenic and cytotoxic effects of catechol, an important constituent of cigarette smoke. An epigenetic pathway frequently observed in tobacco-induced cancers is enzymatic methylation of promoter regions of genes such as *p16* and *FHIT* [fragile histidine triad gene, a gene coding for a dinucleoside 5′, 5‴- P^1, P^3-triphosphate hydrolase, a putative tumour suppressor protein] resulting in gene silencing, which are also strongly implicated in tobacco-induced lung cancer (D'Agostini *et al.*, 2006; Bhutani *et al.*, 2008). When this occurs in tumour suppressor genes, the result can be unregulated proliferation (Belinsky, 2005). Inflammation due to smoking is associated with tumour promotion and activation of factors such as NFκB. Inflammation also plays a role in chronic obstructive pulmonary disease (COPD), which in turn is an independent risk factor for lung cancer (Smith *et al.*, 2006; Turner *et al.*, 2007; Lee *et al.*, 2008a).

This conceptual model can be applied to smokeless tobacco products. Smokeless tobacco products have much lower levels of carcinogens and toxicants that result from combustion, so the effects of these agents are not seen to a significant extent. The most prevalent strong carcinogens in smokeless tobacco are the tobacco-specific nitrosamines; other nitrosamines, PAHs, aldehydes and metals are also present, and there are large amounts of some inorganic salts that may contribute to inflammation (IARC, 2007a; Stepanov *et al.*, 2008). An additional factor in carcinogenesis by betel quid with tobacco is the basic pH resulting from addition of slaked lime to the quid, leading to oxidative damage and inflammation (IARC, 2004b).

Multiple studies demonstrate that tobacco-specific nitrosamines are absorbed and metabolised in smokeless tobacco users (IARC, 2007a).

There is evidence for DNA adduct formation in oral tissues of smokeless tobacco users, and sister chromatid exchanges, chromosomal aberrations, and micronuclei – consequences of DNA adduct formation – have been reported (Proia *et al.*, 2006; Warnakulasuriya & Ralhan, 2007). Many studies have demonstrated *RAS* and *TP53* mutations in smokeless tobacco users (Warnakulasuriya & Ralhan, 2007) consistent with the conceptual framework.

Oxidative stress and reactive oxygen species could play a significant role in cancer induction in smokeless tobacco users, particularly at high pH (Boffetta *et al.*, 2008). Chronic local inflammation and irritation induced by smokeless tobacco and its constituents could have a tumour promoting or co-carcinogenic effect (Boffetta *et al.*, 2008). Upregulation of cyclooxygenase-2, involved in prostaglandin synthesis and inflammation, has been observed in animal studies upon exposure to smokeless tobacco (Boffetta *et al.*, 2008). Smokeless tobacco products have relatively high levels of sodium chloride (NaCl), which could contribute to inflammation, tumour promotion, and co-carcinogenesis. Cancer of the oral cavity is strongly associated with tobacco smoking (IARC, 2004a) or chewing (IARC, 2007a) and alcoholic beverage drinking (IARC, 2010a) However only a fraction of exposed subjects develop tumours, which suggests that other exposures such as HPV may be independently involved or act as cofactors. HPV is known to infect the oral cavity of healthy individuals and several HPV-related lesions have been characterized (IARC, 2007b). Herpes simplex virus has also been shown to enhance the carcinogenicity of smokeless tobacco products in animal studies (Park *et al.*, 1986). These factors may contribute significantly to the local carcinogenic effects characteristic of smokeless tobacco use.

4.1.2 Absorption, distribution, metabolism and excretion

There are examples of toxicant and carcinogen metabolism and excretion for representatives of virtually every major class of compounds;

some of these are summarized in Table 4.1. Nicotine and five of its urinary metabolites – cotinine, 3′-hydroxycotinine and their glucuronides, and nicotine glucuronide – comprise about 73–96% of the nicotine dose (Hukkanen *et al.*, 2005), and are found in blood, sweat, hair and toenails (Al Delaimy, 2002; Hukkanen *et al.*, 2005; Stepanov *et al.*, 2007; Al Delaimy & Willett, 2008). Metabolites of various polycyclic aromatic hydrocarbons including pyrene, phenanthrene, fluorene, and benzo[*a*]pyrene have been quantified in human urine and are higher in smokers than in non-smokers (Hecht, 2002; Hecht *et al.*, 2005a; Jacob *et al.*, 2007; Hansen *et al.*, 2008). Metabolites of tobacco-specific nitrosamines – NNAL and its glucuronides (total NNAL) from NNK; and NNN and its glucuronides (total NNN) from NNN – are present in human urine (Hecht, 2002; Stepanov & Hecht, 2005; Hecht *et al.*, 2008a; Stepanov *et al.*, 2008). Total NNAL has also been quantified in blood and toenails (Hecht *et al.*, 2002; Stepanov *et al.*, 2007). Aromatic amine-haemoglobin adducts have been frequently measured in human blood, and their levels increase with smoking (Hecht, 2002; Hatsukami *et al.*, 2006a). Mercapturic acids of several tobacco smoke compounds such as benzene, 1,3-butadiene, acrolein, and ethylene oxide are present in human urine and are related to smoking (Carmella *et al.*, 2009). Haemoglobin adducts of acrylonitrile and related compounds are elevated in smokers' blood, and levels of metals such as Cd are increased in smokers' urine (Carmella *et al.*, 2002; IARC, 2004b).

All of the metabolites listed in Table 4.1 are elevated in cigarette smokers; in studies of second-hand smoke exposure, only nicotine metabolites and urinary total NNAL are consistently increased in exposed versus non-exposed subjects, although one very large study also observed an increase in PAH metabolites (Pirkle *et al.*, 2006; Hecht, 2008; Suwan-ampai *et al.*, 2009). Smokeless tobacco users have significantly raised levels of nicotine metabolites and tobacco-specific nitrosamine metabolites compared to non-tobacco users (Hecht *et al.*, 2007).

4.1.3 Biomarkers

Tobacco carcinogen biomarkers are quantifiable entities that can be *specifically* related to tobacco carcinogens. Specificity to a given carcinogen is critical because tobacco carcinogens vary widely in their potency and target organs.

Considering the mechanistic framework outlined in Fig. 4.1, one could visualize various types of biomarkers. Currently, biomarkers of carcinogen/toxicant dose, reflecting the second box of the central track of Fig. 4.1, are by far the most extensively used and validated. The second most common are measurements of DNA adducts (or protein adducts as their surrogates), but fewer of these have both practical utility and validation with respect to tobacco carcinogen specificity.

The use of tobacco carcinogen biomarkers bypasses many uncertainties in estimation of dose. The most commonly used estimation of dose is self-reported number of cigarettes/day, but this is not a very good marker. It may not be reported accurately and it provides no information on the way in which the cigarettes were smoked, which is critical when one considers the common phenomenon of smoker's compensation. Brand information together with machine smoking measurements of specific components is another way of obtaining a measure of dose. However, machine smoking measurements are known to have limitations and the application of a given machine smoking protocol to a given smoker requires smoking topography measurements for that smoker. A disadvantage of tobacco carcinogen biomarkers is that they are affected to some extent by individual differences in metabolism, which may complicate interpretation of dose.

Table 4.1 Examples of toxicant or carcinogen metabolites in tobacco users

Toxicant or carcinogen	Examples of metabolites in tobacco users	References
Nicotine	Cotinine, 3′-hydroxycotinine and their glucuronides in urine, blood or saliva; nicotine and cotinine in toenails	Al Delaimy (2002), Hukkanen *et al.* (2005), Al Delaimy & Willett (2008), Stepanov *et al.* (2007)
Polycyclic Aromatic Hydrocarbons (PAHs)	1-hydroxypyrene, phenanthrols, phenanthrene tetraols, fluorenols, benzo[*a*]pyrenols, benzo[*a*]pyrene tetraols in urine	Hecht (2002), Hecht *et al.* (2005a), Hansen *et al.* (2008), Jacob *et al.* (2007)
Tobacco-specific nitrosamines	NNAL and its glucuronides (total NNAL) in urine or blood, total NNN in urine; NNAL and NNN in toenails	Hecht (2002), Hecht *et al.* (2002, 2008a), Stepanov & Hecht (2005), Stepanov *et al.*, (2007, 2008)
Aromatic amines	Parent amines in urine and haemoglobin adducts in blood	Hecht (2002), Hatsukami *et al.* (2006a)
Volatile hydrocarbons		
Benzene 1,3-Butadiene	Muconic acid and S-phenyl-mercapturic acid (SPMA) in urine; Monohydroxybutyl-mercapturic acid (MHBMA) in urine	Hecht (2002), Carmella *et al.* (2009)
Acrolein	3-hydroxypropyl-mercapturic acid (HPMA) in urine	Carmella *et al.* (2009)
Ethylene oxide	2-hydroxyethyl-mercapturic acid (HEMA) in urine, haemoglobin adducts in blood	Bono *et al.* (2002), Carmella *et al.* (2009)
Acrylonitrile	Haemoglobin adducts in blood	Carmella *et al.* (2002)
Metals	Cadmium in urine	IARC (2004a)

(a) Urinary biomarkers

Probably the most practical and, to date, the most extensively applied tobacco carcinogen biomarkers are urinary metabolites of tobacco carcinogens, and these have been comprehensively reviewed (Hecht, 2002; IARC, 2004a). Advantages include the ready availability of samples, and concentrations in urine that are easily quantifiable using modern analytical chemistry methods, most frequently liquid chromatography-tandem mass spectrometry (LC-MS/MS). The urinary metabolites listed in Table 4.1 have all been used as biomarkers and all are validated with respect to exposure in cigarette smokers (Carmella *et al.*, 2009). Total nicotine equivalents (the sum of nicotine and the five metabolites in Table 4.1) is a particularly effective way of estimating nicotine dose from tobacco products.

Total NNAL, the sum of NNAL and its glucuronides, is a highly useful biomarker of NNK exposure (Hecht, 2002, 2003; Hatsukami *et al.*, 2006a). The tobacco-specificity of NNK, and therefore total NNAL, is a key feature of this biomarker because studies in which it is applied are not confounded by other environmental or dietary exposures. It also has a considerably longer half-life than cotinine and several other urinary biomarkers. Total NNAL has been used in numerous studies that estimated uptake of NNK in smokers under varying circumstances. In one example, smokers reduced their number of cigarettes smoked per day, but there was not a corresponding decrease in NNK uptake due to compensation (Hecht *et al.*, 2004). In another study, NNK and PAH uptake, estimated by total NNAL and 1-hydroxypyrene, respectively, were compared in smokers of regular, light, and ultra-light cigarettes, and found to be similar, consistent with epidemiologic studies that

demonstrate no protection against lung cancer in smokers of light compared to regular cigarettes (Hecht *et al.*, 2005b). Other studies evaluated NNK uptake in smokers who switched from their current cigarette brand to products advertised as being less hazardous, but the results generally did not support these claims (Hatsukami *et al.*, 2004). One of the most useful applications of total NNAL has been in studies of non-smokers exposed to second-hand tobacco smoke (Hecht, 2003). The sensitivity and specificity of this biomarker are ideal for such studies, and it is the most commonly elevated tobacco carcinogen biomarker in non-smokers exposed to second-hand smoke. Total NNAL has also found utility in establishing NNK uptake in smokeless tobacco users (Hecht *et al.*, 2002, 2007, 2008a, b; Hecht, 2008)

The relationship of urinary total NNAL to lung cancer was demonstrated in a study of stored urine samples collected years before diagnosis of lung cancer from smokers in Shanghai, China and Singapore (Yuan *et al.*, 2009). There was a significant relationship between total NNAL and lung cancer incidence, after correction for numbers of cigarettes smoked per day and duration of smoking. An 8.5 fold increased risk for lung cancer was observed for those smokers in the highest tertile of total NNAL and cotinine, relative to smokers with the same smoking history but in the lowest tertiles of total NNAL and cotinine. Urinary biomarkers were also used to demonstrate higher uptake of nicotine and NNK per cigarette in smokers with polymorphisms in the nicotinic acetylcholine genes associated with lung cancer in genome-wide association studies (see Section 4.2; Le Marchand *et al.*, 2008). Collectively, these results indicate that urinary total NNAL is not only a biomarker of exposure, but also a biomarker of risk for lung cancer.

(b) Serum and saliva metabolites

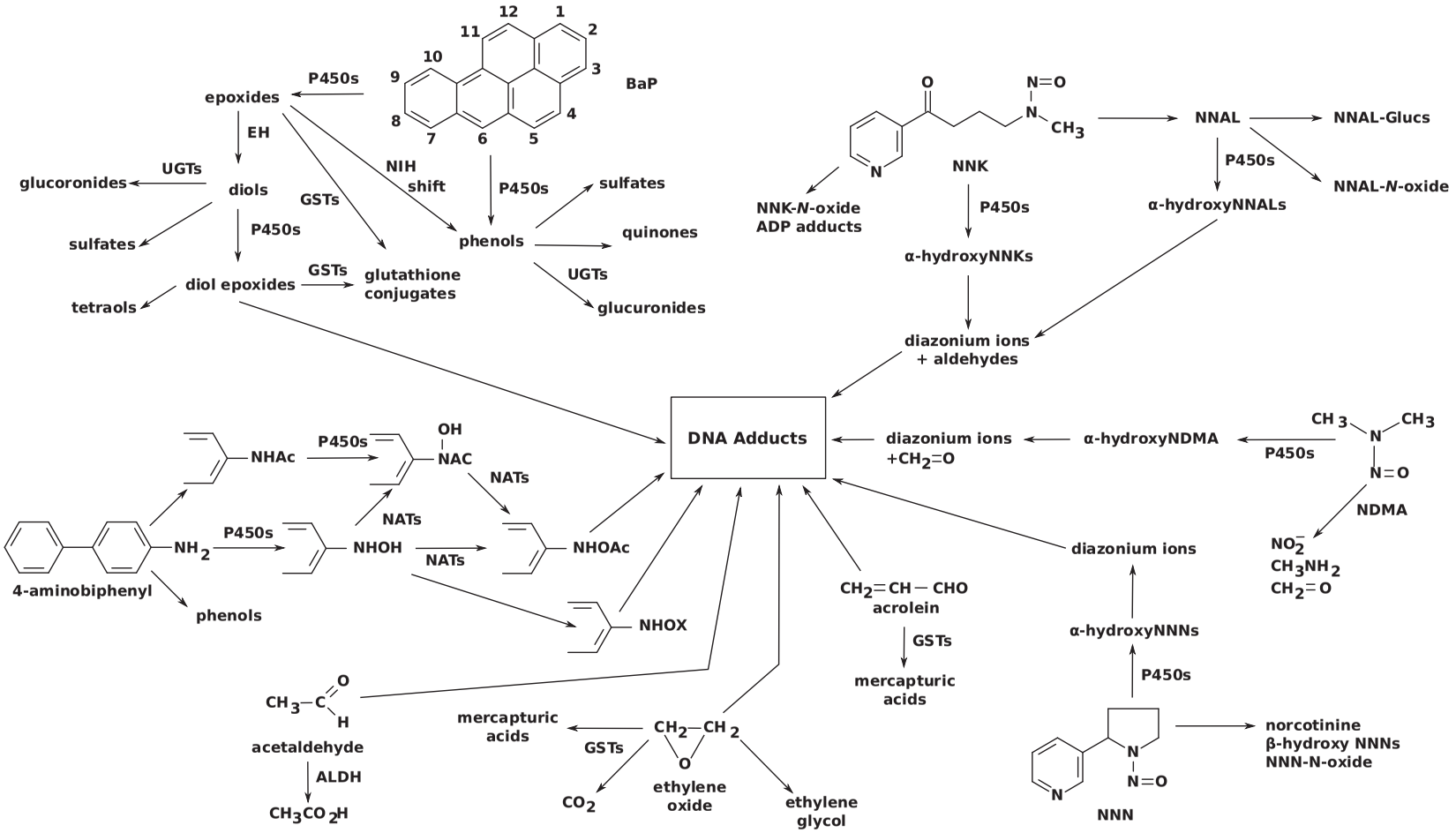
Serum and saliva metabolites have been used as biomarkers much less often than urine metabolites. The most frequently measured tobacco smoke toxicant in serum and saliva is cotinine, documented as a useful biomarker of cigarette smoking in many studies (Lee, 1999; Hukkanen *et al.*, 2005). Total NNAL can be readily quantified in serum and its levels remain relatively constant in a given smoker sampled at bimonthly intervals over a one year period. Consistent with the results described above, one study showed a significant relationship between total NNAL in prospectively collected serum samples from smokers and lung cancer risk (Church *et al.*, 2009). Other biomarkers that have been measured in serum include cadmium, benzene, styrene and *r*-1,*t*-2,3,*c*-4-tetrahydroxy-1,2,3,4-tetrahydrophenanthrene (PheT) (IARC, 2004a; Church *et al.*, 2009).

(c) DNA adducts

Fig. 4.3 presents an overview of metabolism and DNA adduct formation from eight tobacco smoke compounds (clockwise from top left): BaP, NNK, *N*-nitrosodimethylamine (NDMA), NNN, acrolein, ethylene oxide, acetaldehyde and 4-aminobiphenyl. Evidence exists for DNA adduct formation from each of these carcinogens in smokers, based on studies carried out with tissues or blood cells. DNA adduct biomarkers have been applied mainly in studies of smokers, and there is far less evidence from studies of second-hand tobacco smoke or smokeless tobacco use.

The structures of DNA adducts of tobacco smoke carcinogens have been characterized in detail, but a complete description of these structures is beyond the scope of this section. Selected DNA adduct structures are shown in Fig. 4.4. A major DNA adduct of BaP results from *trans*- addition of the benzo[*a*]pyrene diol epoxide (BPDE) to the N^2-position of dG (Szeliga

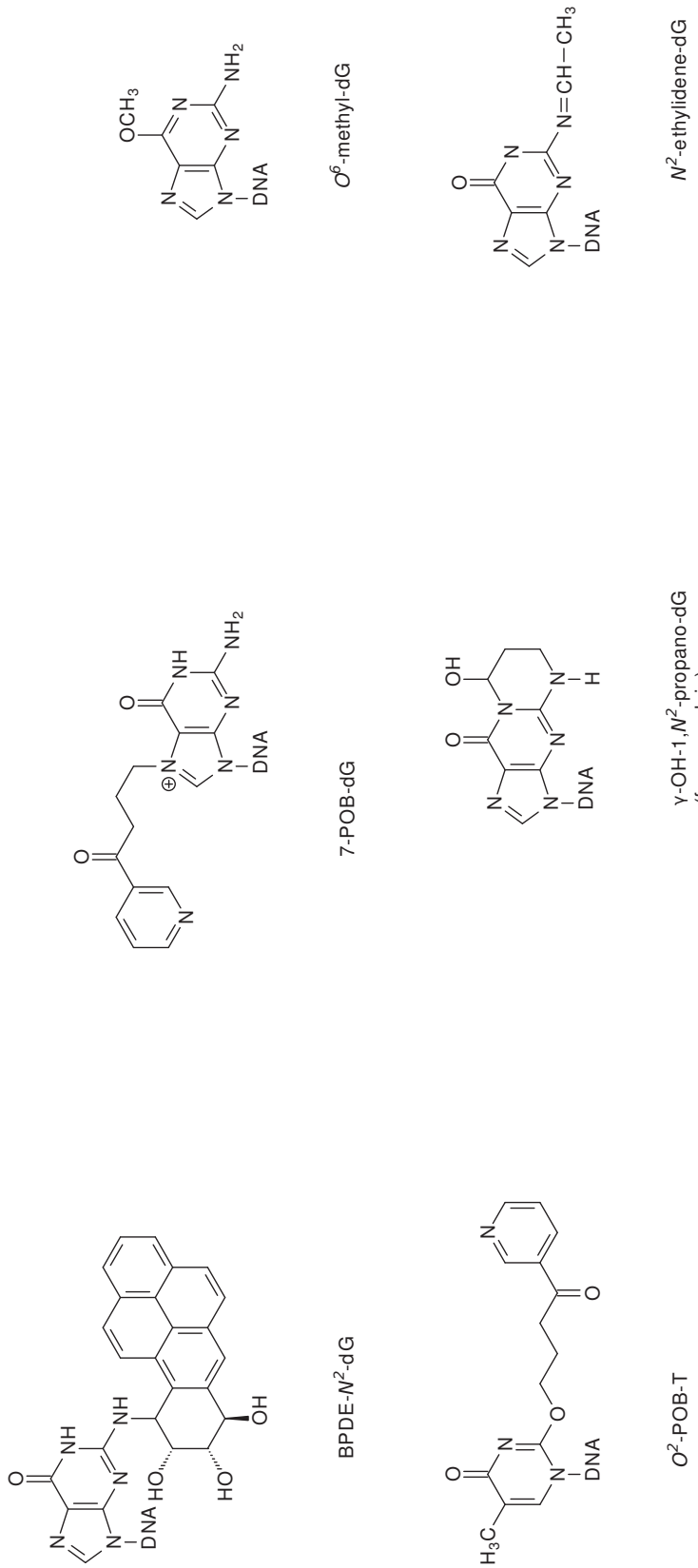
Fig. 4.3 Overview of metabolism and DNA adduct formation from eight tobacco smoke constituents



4-ABP, 4-aminobiphenyl; AC, acetyl; ADP, adenosine diphosphate; ALDH, aldehyde dehydrogenase; AKR, aldo-ketoreductase; B[*a*]P, benzo[*a*]pyrene; EH, epoxide hydrolase; Gluc, glucuronide; GSTs, glutathione *S*-transferases; NATs, *N*-acetyltransferases; NDMA, *N*-nitrosodimethylamine; NIH shift, phenomenon of hydroxylation-induced intramolecular migration; NNAL, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NNN, *N*'-nitrosonornicotine; P450s, cytochrome P450 enzymes; UGTs, uridine-5′-diphosphate-glucuronosyl transferases

Adapted from Cooper *et al.* (1983); Preussmann & Stewart (1984); Kadlubar & Beland (1985); Hecht (1998, 1999); Penning & Drury (2007); IARC (2008, 2010b).

Fig. 4.4 Structures of some DNA adducts of tobacco smoke constituents



BPDE-N^2-dG, benzo[*a*]pyrene diol epoxide-N^2-deoxyguanosine; 7-POB-dG, pyridyloxobutyl-deoxyguanosine; O^2-POB-T, O^2-pyridyloxobutyl-thymidine

& Dipple, 1998). Pyridyloxobutyl (POB)-DNA adducts of NNK and NNN are formed at the 7- and O^6-positions of deoxyguanosine dG, the O^2-position of thymidine, and the O^2-position of deoxycytidine (Hecht, 2008). They can be measured in part as 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB) released upon hydrolysis. Metabolic activation of NNK also leads to 7-methyl-dG and O^6-methyl-dG, identical to the DNA adducts formed from NDMA and other DNA methylating agents (Hecht, 2008). Ethylating agents and ethylene oxide in cigarette smoke also alkylate dG (Zhao *et al.*, 1999; Singh *et al.*, 2005). Acrolein and crotonaldehyde react with DNA to produce exocyclic 1,N^2-dG adducts, while acetaldehyde forms a Schiff base adduct with the exocyclic N^2 amino group of dG. There is evidence for the presence of all these DNA adducts in tissues or blood cells of smokers, but there are also many studies in which these specific adducts have been sought but not found (Boysen & Hecht, 2003).

Measurement of these DNA adducts as biomarkers potentially can provide the most direct link between cellular exposure and cancer, because DNA adducts are so critical in carcinogenesis. However, it is challenging because their levels are extremely low, frequently ranging from 1 per 10^6 to 1 per 10^8 normal bases, and the tissue or blood samples containing them are usually available in only small quantities. Fortunately, the routine detection of amol levels [attomole, equivalent to 10 moles] of DNA adducts by conventional LC-MS/MS techniques is now feasible (Singh & Farmer, 2006). There are still relatively few examples of quantitation of *specific* DNA adducts of tobacco carcinogens in tissues of smokers using mass spectrometry, high pressure liquid chromatography (HPLC)-fluorescence, HPLC with electrochemical detection, or postlabelling techniques (Pfeifer *et al.*, 2002). A much larger body of work has used the highly sensitive, but relatively non-specific ^{32}P-postlabelling and immunoassay methods of DNA adduct detection. Although the adducts detected using ^{32}P-postlabelling are often referred to as "aromatic DNA adducts," there is strong evidence that they are not related to PAHs (Arif *et al.*, 2006). Adduct levels are generally higher in lung tissues of smokers than non-smokers while studies using blood DNA have produced varied results. Adducts have also been detected in the larynx, oral and nasal mucosa, bladder, cervix, breast, pancreas, stomach, placenta, foetal tissue, cardiovascular tissues, sputum, and sperm of smokers (IARC, 2004a). A meta-analysis of the relationship of DNA adduct levels in smokers to cancer, as determined by ^{32}P-postlabelling in the majority of studies or enzyme linked immunosorbent assay (ELISA), demonstrated a positive relationship in current smokers (Veglia *et al.*, 2003; 2008).

(d) Protein adducts

Carcinogen-haemoglobin (Hb) and serum albumin adducts are regarded as surrogates for DNA adduct measurements. Although these proteins are not targets for carcinogenesis, virtually all carcinogens that react with DNA will also react with protein. Advantages of haemoglobin adducts include the ready availability of haemoglobin from blood and the relatively long lifetime of the erythrocyte in humans – 120 days –,which provides an opportunity for adducts to accumulate. Studies on protein adducts in smokers have been comprehensively reviewed (IARC, 2004a).

Haemoglobin adducts of aromatic amines are a highly informative type of carcinogen biomarker, with levels that are consistently higher in smokers than non-smokers, particularly for 3-aminobiphenyl and 4-aminobiphenyl-Hb adducts. Haemoglobin binds aromatic amines efficiently because heme accelerates the rate of nitrosoarene formation from the hydroxylamine, which is produced metabolically from the aromatic amine by CYP1A2 (Fig. 4.3; Skipper & Tannenbaum, 1990). Binding of the nitrosoarene occurs at the β-93 cysteine residue of human

haemoglobin; the adduct is hydrolysed releasing the free amine, which is quantified by GC-MS (Skipper & Tannenbaum, 1990). Adduct levels are clearly related to cigarette smoking (Skipper & Tannenbaum, 1990). Adducts that form at the terminal valine of haemoglobin are also useful biomarkers: examples include those derived from ethylene oxide, acrylonitrile and acrylamide (Bergmark, 1997; Fennell *et al.*, 2000). Ethylated N-terminal valine of haemoglobin is also higher in smokers than in non-smokers (Carmella *et al.*, 2002).

HPB-releasing Hb adducts of NNK and NNN have been quantified in studies of smokers and smokeless tobacco users (IARC, 2004a, 2007a). These adducts are thought to be tobacco-specific, but some studies report their presence in non-smokers (Falter *et al.*, 1994; Schlöbe *et al.*, 2008).

4.1.4 Genetic and related effects

(a) Mutagenicity and cytogenetic effects

Tobacco smoke and its condensates are mutagenic in a wide variety of test systems from bacteria to mammalian cells in culture to rodents and humans (DeMarini, 2004; IARC, 2004a; Husgafvel-Pursiainen, 2004). In bacterial systems, the heterocyclic amines and aromatic amines in condensates account for much of the frameshift mutagenicity, whereas the PAHs and nitrosamines may account for some of the base-substitution mutagenicity (DeMarini *et al.*, 1995). G to T is the predominant class of base-substitution mutation induced by condensates in experimental systems and found in oncogenes and tumour-suppressor genes in smoking-associated lung tumours (IARC, 2004a). The genotoxic potencies of a variety of condensates in several genotoxicity assays likely have only qualitative value with regard to health risk assessment (DeMarini *et al.*, 2008). This is consistent with findings that smokers of low- or high-tar cigarettes have similar urinary levels of lung carcinogens (Hecht *et al.*, 2005b; Hatsukami *et al.*, 2006b) and similar risks for lung cancer (Harris *et al.*, 2004).

In rodents, cigarette smoke induces sister chromatid exchange and micronuclei in bone marrow and lung cells. Human newborns of smoking mothers have increased frequencies of *HPRT* mutations, chromosomal translocations, and DNA strand breaks. Sperm of smokers has increased frequencies of aneuploidy, DNA adducts, strand breaks, and oxidative damage. Cigarette smoke also causes germ-cell mutations in mice (Yauk *et al.*, 2007). Collectively, these data suggest that smoking is likely a germ-cell mutagen in humans. Smoking produces mutagenic urine and somatic-cell mutations in humans, including *HPRT* mutations, sister chromatid exchange, microsatellite instability and DNA damage in a variety of tissues. Genotoxic effects have been found in eight organ sites at which tobacco smoke causes cancer in humans (DeMarini, 2004; IARC, 2004a).

(b) Mutations in TP53, KRAS and related genes

Gene mutation data from a variety of databases, including the IARC Cancer *TP53* Mutation Database (http://www-p53.iarc.fr/), have been collated in the Genetic Alterations in Cancer (GAC) database (http://dir-apps.niehs.nih.gov/gac/) so that mutations in a variety of genes in various cancerous tissues can be compared. An assessment of the Gene Alterations in Cancer database showed that at least three genes were mutated more frequently in lung tumours from smokers than non-smokers (Lea *et al.*, 2007): *TP53* (39 versus 26%), *K-RAS* (20 versus 3%), and loss of heterozygosity at *FHIT* (57 versus 27%). Thus, genes in the cell cycle (*TP53*), cell signalling (*KRAS*) and apoptotic (*FHIT*) pathways are mutated more frequently in smoking- rather than in nonsmoking-associated lung tumours. Genomic sequencing of lung tumours has identified other mutated genes that are associated with smoking; ten times more genes are mutated in

lung tumours from smokers compared to non-smokers (Ding *et al.*, 2008).

GC to TA transversions were the predominant class of base-substitution mutation found in *TP53* and *KRAS* genes in lung tumours from smokers, with the frequency of this mutation in *TP53* being 30% in smokers versus 22% in non-smokers. In smoking-associated oral cancers, the percentage of GC to TA mutations in *TP53* was 15% versus 2%, respectively. This mutation spectrum is consistent with that produced by a variety of known carcinogens present in tobacco smoke (IARC, 2004a). At the codon level, the most frequently mutated codons in *TP53* in lung tumours of smokers were 157, 175, 245, 248, and 273, all of which occur in the DNA-binding domain of the protein; among these codons, only 273 was mutated in lung tumours from non-smokers. Only three of these codons (157, 245 and 273) were mutated in smoking-associated larynx tumours, and only codon 157 was mutated in smoking-associated oral tumours. Thus, the mutational specificity at *TP53* is different among smoking- and nonsmoking-associated tumours and among smoking-associated tumours at various organs (Lea *et al.*, 2007). Thus, different pathways are involved in the development of different types of tumours (Le Calvez *et al.*, 2005; Mounawar *et al.*, 2007; Subramanian & Govindan, 2008).

4.1.5 Effects on gene expression profile

As indicated in a review by Sen *et al.* (2007) involving microarray analysis of 18 studies in human smokers, 7 in smoke-exposed rodents, and 3 in condensate-exposed mammalian cells, smoking generally upregulated a wide variety of genes, especially those involved in the stress response, phase I metabolism, and immune response. Genes that were consistently expressed differentially in smokers (as assessed in alveolar macrophages, lung cells or peripheral lymphocytes) included metallothioneins, heat-shock proteins, superoxide dismutase, glutathione transferase, heme oxygenase, *CYP* genes (*1A2*, *1A1* and *1B1*), interleukins and chemokines.

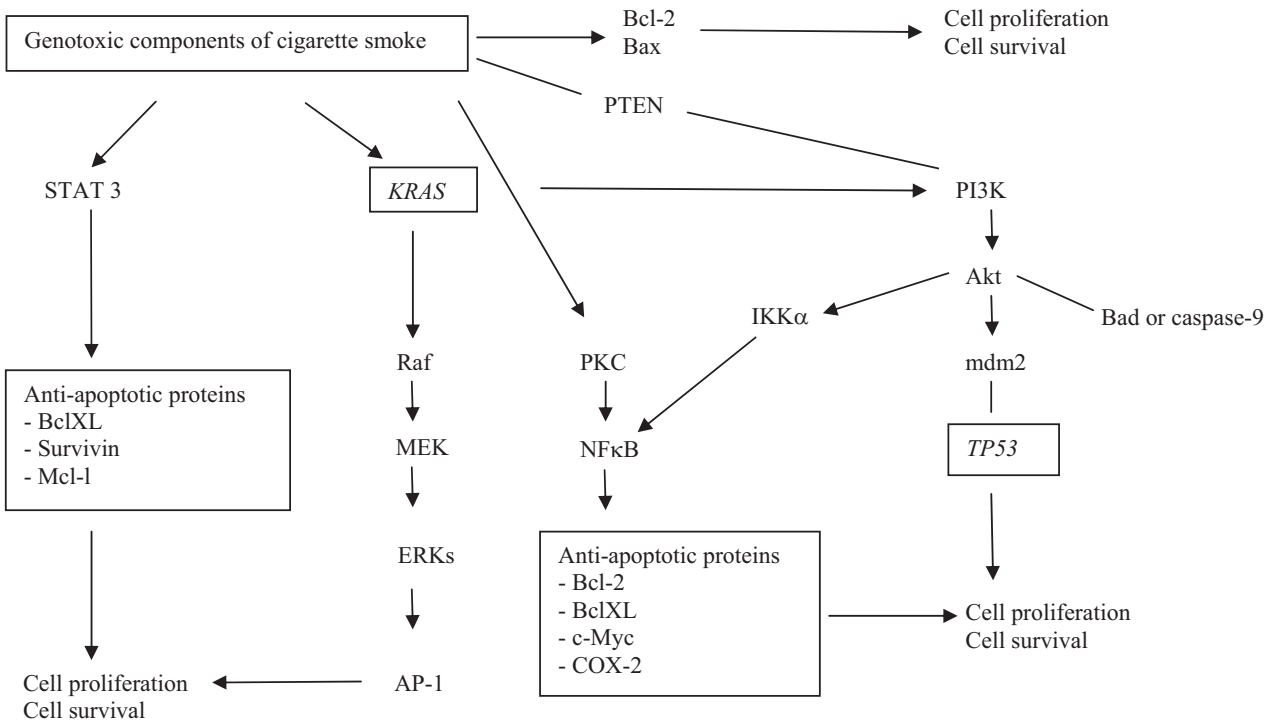
Spira *et al.* (2004) analysed global gene expression in bronchial epithelial cells and found that the expression levels of metabolizing and antioxidant genes had reverted to control levels after two years of smoking cessation. However, expression of potential oncogenes and tumour-suppressor genes never reverted to never-smoker levels even after years of smoking cessation. Consistently, expression of microRNAs is generally downregulated by cigarette smoke (Izzotti *et al.*, 2009). As discussed below, smoking also altered methylation patterns and gene expression in smoking-associated tumours.

4.1.6 Other effects associated with carcinogenesis

(a) Proliferation, differentiation, apoptosis, and inflammation

As noted above, the signal-transduction pathways in lung tumours from smokers are distinctly different from those of non-smokers (Mountzios *et al.*, 2008). Fig 4.5 shows details of signalling pathways that are deregulated by tobacco smoke. The involvement of high frequencies of mutated *K-RAS* and *TP53* genes in smoking-associated lung tumours results in altered regulation of cell proliferation, differentiation, cytoskeletal organization and protein trafficking. Cigarette smoking activates *NF-κB*, which induces pro-inflammatory cytokine expression and induces growth factors and proliferative signals (Mountzios *et al.*, 2008). This gene also influences the expression of the anti-apoptotic gene *BCL2* and pro-apoptotic gene *BAX*. Smoking produces chronic inflammation, which promotes cancer (Walser *et al.*, 2008). Smoking results in high levels of reactive oxygen species, which damage epithelial and endothelial cells and impair their function. In smoking-associated lung cancer, elevated levels of cyclooxygenase-2 (COX-2) and

Fig. 4.5 General scheme of some cell-signalling pathways that are deregulated by tobacco smoke in lung carcinogenesis



Akt, serine/threonine protein kinase; ERKs, extracellular regulated kinases; MEK, mitogen-activated protein kinase; Bad, Bcl2-associated agonist of cell death; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; NF-κB, nuclear factor κB; IKKα, inhibitor of nuclear factor κ-B kinase; PTEN, phosphatase and tensin homologue; STAT3, signal transducer and activator of transcription, COX-2, cyclooxygenase-2

prostaglandin (PGE_2) indicate apoptosis resistance, proliferation, immunosuppression, angiogenesis, invasion, and epithelial-mesenchymal transition (Walser *et al.*, 2008).

(b) Endogenous nitrosation

Intragastric formation of *N*-nitroso compounds, measured using urinary nitrosamino acids excreted in urine, was increased in smokers compared to non-smokers (Hoffmann & Brunnemann, 1983). Two recent studies demonstrated that NNN forms endogenously in some users of nicotine replacement therapy products (Stepanov *et al.*, 2009a, b).

(c) Hormonal changes

These are described in Section 4.3.2a.

4.2 Polymorphisms in carcinogen-metabolizing genes

4.2.1 Introduction

It has been long proposed that the known variation among individuals in their capacity to activate and detoxify carcinogens may be associated with increased susceptibility to cancer, and that polymorphisms of carcinogen-metabolising genes may play a significant role. The most intensively studied genes involved in the metabolism of carcinogens include the various *CYP* genes, the *GST* genes and the *NAT* genes. Other relevant xenobiotic-metabolising genes, such as *EPHX*, sulfotransferase (*SULT*), *UGT*, myeloperoxidase (*MPO*), and NAD(P)H quinone oxidoreductase-1 (*NQO1*) genes, have also been studied. Recently, extensive pooled studies and

reviews have been published on polymorphisms of carcinogen-metabolising genes and their role in cancer susceptibility, especially in tobacco-related lung cancer and cancers at other sites. Similarly, various biomarkers of exposure and genotoxicity that are presumed to provide a mechanistic basis for such associations have been comprehensively investigated in relation to these polymorphisms. A brief overview based largely on reviews and the meta- and pooled analyses is presented here.

4.2.2 Genetic polymorphisms of carcinogen metabolism: some central genes

(a) CYP genes

CYPs comprise the principal enzyme system catalysing various phase I oxidation reactions, including metabolic activation and detoxification of many carcinogenic substances in tobacco smoke such as PAHs. Of the various CYP enzymes expressed in humans, many of those belonging to CYP1 to CYP3 families play a role in carcinogen metabolism, producing highly reactive DNA-damaging metabolites as well as detoxified metabolites (Guengerich & Shimada, 1998; Lang & Pelkonen, 1999; Ingelman-Sundberg, 2004). CYPs have evolved into a wide superfamily with close to 60 different active genes currently identified; most of these genes exhibit polymorphism (www.cypalleles.ki.se).

(i) CYP1A1

Several allelic variants of the human *CYP1A1* gene are currently known (www.cypalleles.ki.se). The major variant forms of the *CYP1A1* gene (wildtype allele *CYP1A1*1*) mostly frequently studied for association to cancer susceptibility include the following two alleles: (i) *CYP1A1*2A* allele (m1 allele; *Msp* I) and (ii) *CYP1A1*2B* (Cascorbi *et al.*, 1996) or *CYP1A1*2C* (www.cypalleles.ki.se) allele (m2 allele; Ile^{462}Val). Importantly, the *CYP1A1* m1 allele and m2 allele are in complete linkage disequilibrium in Caucasians (Kawajiri, 1999; Bartsch *et al.*, 2000). In addition, *CYP1A1*4* allele (m4; Thr^{461}Asn) (Cascorbi *et al.*, 1996), and *CYP1A1*3* (m3) allele found in African-Americans but not in Caucasians or Asians (Crofts *et al.*, 1993) are included in some studies (Bartsch *et al.*, 2000).

In smoking-related lung cancer, the various *CYP1A1* polymorphisms as well as the differences in the frequencies of the rare variant alleles between ethnicities contribute to the differences in findings. There are collective analyses of data predominantly indicating an overall mild to moderate effect of *CYP1A1* polymorphisms on lung cancer risk (Kawajiri, 1999; Bartsch *et al.*, 2000; Houlston, 2000; Le Marchand *et al.*, 2003; Vineis *et al.*, 2003; Vineis *et al.*, 2004; Lee *et al.*, 2008a; Shi *et al.*, 2008). In many reviews and meta- or pooled analyses the increased risk associated with *CYP1A1* polymorphism has most clearly been seen in Asian populations (Kawajiri, 1999; Le Marchand *et al.*, 2003; Vineis *et al.*, 2003; Lee *et al.*, 2008a; Shi *et al.*, 2008).

Multiple studies have also analysed the gene-gene interactions between *CYP1A1*, *GSTM1* and *GSTT1* polymorphisms and lung cancer (d'Errico *et al.*, 1999; Houlston, 1999; Benhamou *et al.*, 2002; Bolt & Thier, 2006; Raimondi *et al.*, 2006; Ye *et al.*, 2006; Carlsten *et al.*, 2008). Some of the analyses have indicated that the elevated risk for lung cancer may be more pronounced for some *CYP1A1/GSTM1* null genotype combinations (Le Marchand *et al.*, 1998; Bartsch *et al.*, 2000; Vineis *et al.*, 2004, 2007; Lee *et al.*, 2008a; Shi *et al.*, 2008).

(ii) CYP1A2

CYP1A2 is highly inducible and metabolises, including deacetylation reactions, many tobacco smoke carcinogens such as aromatic and heterocyclic amines and nitro-aromatic compounds, and tobacco-specific nitrosamines such as NNK (Nebert *et al.*, 2004; Jalas *et al.*, 2005; IARC, 2007a). A few major variant alleles have been described (www.cypalleles.ki.se), some of which

may have been reported to influence inducibility (Nakajima *et al.*, 1999; Ingelman-Sundberg *et al.*, 2007). Overall, the phenotype-genotype relations have not been well established for *CYP1A2*, although current evidence points towards contribution of genetic variation (Murayama *et al.*, 2004; Ingelman-Sundberg *et al.*, 2007); data on possible associations with tobacco related cancer are sparse (Agundez, 2004; Nebert & Dalton, 2006).

(iii) CYP2A6

Several aspects of smoking behaviour are likely to be influenced by *CYP2A6* genetic variation, which influences nicotine metabolism (Malaiyandi *et al.*, 2005; Mwenifumbo & Tyndale, 2007). The most important functionally altered allele is *CYP2A6*4* (gene deletion), which confers a poor-metabolizer phenotype in homozygous individuals (Malaiyandi *et al.*, 2005; Ingelman-Sundberg *et al.*, 2007; Mwenifumbo & Tyndale, 2007). In some studies, polymorphic variants of *CYP2A6* gene have been implicated in susceptibility to smoking-related cancers (Gambier *et al.*, 2005; Malaiyandi *et al.*, 2005; Nakajima, 2007). In line with this, the accumulated data have suggested that *CYP2A6* polymorphism may affect cancer risk in smokers but not in non-smokers (Tan *et al.*, 2001; Kamataki *et al.*, 2005; Malaiyandi *et al.*, 2005; Canova *et al.*, 2009).

(iv) CYP2A13

From human CYPs, CYP2A13 is the primary form involved in the metabolic activation of the tobacco-specific nitrosamines NNK and NNN (Jalas *et al.*, 2005; IARC, 2007a). The *CYP2A13* gene exhibits polymorphism in humans (Zhang *et al.*, 2002; Jalas *et al.*, 2005), and experimental studies suggest that some of the polymorphisms may affect the hydroxylation of NNN and NNK (Jalas *et al.*, 2005; Schlicht *et al.*, 2007). However, the data on possible effects of these polymorphisms on the risk of tobacco-related cancers in humans are still limited (Wang *et al.*, 2003; Song *et al.*, 2009; Timofeeva *et al.*, 2009).

(v) CYP2D6

The *CYP2D6* gene shows high variability in expression. The enzyme is not inducible, and therefore genetic variation largely contributes to the interindividual variation in enzyme activity. Currently, more than 100 different functional CYP2D6 gene variants have been described, and these are divided into alleles causing abolished, decreased, normal, and ultrarapid enzyme activity (Ingelman-Sundberg, 2005; Ingelman-Sundberg *et al.*, 2007). The most important null alleles leading to poor-metabolizer phenotype are *CYP2D6*4* (splice defect) and *CYP2D6*5* (gene deletion) (Ingelman-Sundberg, 2005; Ingelman-Sundberg *et al.*, 2007).

A large series of studies have been carried out over the past 20 years on the association between *CYP2D6* polymorphism and susceptibility to lung cancer and to some other tobacco-related cancers (Wolf & Smith, 1999). Despite some indication of an association between CYP2D6 poor-metabolizer and decreased risk for lung cancer, no major role for CYP2D6 in carcinogen metabolism or a molecular basis for such an association have been discovered (Wolf & Smith, 1999; Ingelman-Sundberg, 2005).

(vi) Other CYP genes

CYP1B1 allelic variants that affect the catalytic activity have been described but they have been studied to a lesser extent for the association with susceptibility to smoking-related cancers (Thier *et al.*, 2003). Some positive findings have been reported on head and neck cancer (Ko *et al.*, 2001), and lung cancer (Zienolddiny *et al.*, 2008).

Several polymorphisms have been characterized in the *CYP2E1* gene and several positive associations with the risk of different cancers have been reported, in particular for cancers of the upper aerodigestive tract, lung and gastrointestinal tract (Section 2.19). *CYP2E1* may also

play an important role in the interaction of the carcinogenic effects of alcohol and tobacco (Section 4.4).

From the human *CYP3A* locus (*CYP3A4*, *CYP3A5* and *CYP3A7*), the *CYP3A4*1B* allele has been associated with lung cancer and prostate cancer in some studies but not in all (Dally *et al.*, 2003; Rodriguez-Antona & Ingelman-Sundberg, 2006). However, the role of these variants in relation to tobacco smoking is unknown.

(b) *GSTM1 and other GST genes*

Polymorphic *GST* genes have long been proposed to modify susceptibility to lung cancer (Seidegård *et al.*, 1986; Ketterer *et al.*, 1992). The polymorphic genes encoding the various classes of cytosolic GST enzymes include the *GSTM1* and *GSTM3* genes (mu class), the *GSTP1* gene (pi class), and the *GSTT1* gene (theta class). The gene deletion (null) allele of the *GSTM1* gene (*GSTM1*0*) and of the *GSTT1* gene (*GSTT1*0*) have been the most intensively studied polymorphisms in relation to increased susceptibility to cancer (Strange *et al.*, 2001; Bolt & Thier, 2006; McIlwain *et al.*, 2006). For the *GSTP1* gene, the form most abundantly present in lung tissue, genetic variation in exon 5 (*GSTP1*2*; *Ile*^{105}*Val*), in exon 6 (*Ala*^{114}*Val*), as well as a combination of these, are the variations most frequently studied for cancer susceptibility (Watson *et al.*, 1998; Cote *et al.*, 2009).

Numerous reviews, meta- and pooled analyses have been published over the past 15 years or so for the *GST* genes with systematic assessments covering altogether tens of thousands of cases and controls. For the *GSTM1* null genotype, such analyses have largely provided negative, suggestive or at most moderately positive results for an association with an increased risk for lung cancer (d'Errico *et al.*, 1999; Houlston, 1999; Benhamou *et al.*, 2002; Ye *et al.*, 2006; Carlsten *et al.*, 2008). The larger the studies, the less significant the estimates for the role of *GSTM1* emerge in systematic analysis (Ye *et al.*, 2006; Carlsten *et al.*, 2008). Also the varying allele frequencies related to ethnic background affect the findings for *GSTM1* as well as for many other genes (Garte *et al.*, 2001; Ye *et al.*, 2006; Carlsten *et al.*, 2008; Lee *et al.*, 2008a).

In a meta-analysis of the association between the *GSTT1* gene polymorphism and lung cancer no association between *GSTT1* null genotype and risk for lung cancer in Caucasians was observed, but a positive association was found for Asians (Raimondi *et al.*, 2006). A significant association for either Caucasians or Asians was also not found in a pooled analysis (Raimondi *et al.*, 2006). A meta-analysis found no significant association between lung cancer risk and the *GSTP1 Ile*^{105}*Val* polymorphism; but the pooled analysis suggested an overall statistically significant mild association between lung cancer and homozygosity or heterozygosity for the Val^{105} allele (Cote *et al.*, 2009).

A recent body of epidemiologic data suggests an inverse association between cruciferous vegetables/isothiocyanates intake and cancers of the colorectum, lung and breast; the studies also provide evidence that this protective effect is greater among individuals who possess the *GSTM1* or *T1* null genotype, who would be expected to accumulate higher levels of isothiocyanates at the target tissue level, a pre-requisite for their enzyme-inducing effects (Seow *et al.*, 2005). The association between isothiocyanates and cancer, and its modification by *GSTM1* and *GSTT1* status, is most consistent for lung cancer and appears to be strongest among current smokers who possess the combined *GSTM1* and *GSTT1* null genotypes (London *et al.*, 2000a; Spitz *et al.*, 2000; Zhao *et al.*, 2001; Brennan *et al.*, 2005; Seow *et al.*, 2005).

(c) *NAT1 and NAT2 genes*

The pooled and meta-analyses carried out on *NAT1* and *NAT2* polymorphisms and bladder cancer risk have consistently reported significantly increased risk for *NAT2* slow acetylators

(Dong *et al.*, 2008; Malats, 2008; see also Section 2.9). Data on *NAT1* fast acetylators are inconsistent, as are the studies suggesting an increased risk for *NAT2* rapid acetylator status. Additionally, genotypes for other genes, specially *GSTM1*, have also been implicated (Vineis *et al.* 2001; García-Closas *et al.*, 2005; Hein, 2006; Sanderson *et al.*, 2007; Dong *et al.*, 2008; Malats, 2008).

In a recent large study on tobacco-related lung cancer and upper aerodigestive cancers, the *NAT* genes, in particular *NAT*10* haplotype, emerged from a set of 16 genes as involved in the risk (McKay *et al.*, 2008). When more than one hundred single nucleotide polymorphisms for 31 genes involved in phase I or phase II metabolism or in antioxidant defence were investigated, only four of the previously reported polymorphisms of the *GSTP1*, *EPHX1* and superoxide dismutase *SOD2* genes and the *NAT1* fast acetylator phenotype remained significantly associated with risk of non-small cell lung cancer after correction for multiple testing (Zienolddiny *et al.*, 2008).

In breast cancer, several recent meta-analyses of epidemiological studies have suggested increased risk among smokers with the *NAT2* slow acetylator genotype; such an association has been observed especially among long-term smokers and post-menopausal women (Terry & Goodman, 2006; Ambrosone *et al.*, 2008; Ochs-Balcom *et al.*, 2007; Baumgartner *et al.*, 2009).

In all, the role of the *NAT* gene polymorphisms in tobacco-related cancers, with the exceptions of increased risk of bladder cancer and possibly breast cancer in *NAT2* slow acetylators, remains largely open due to the incomplete understanding of phenotype-genotype relationships, and the interplay between these two genes and their polymorphisms (Hein, 2002, 2006).

(d) Others

Genes coding for EPHX, UGT and SULT enzymes, mainly but not exclusively involved in detoxification reactions, exhibit polymophisms with numerous gene variants discovered (Mackenzie *et al.*, 1997; London *et al.*, 2000b; Glatt *et al.*, 2001; Burchell, 2003). Additional polymorphic genes studied for their significance in cancer susceptibility are the *NQO1* and *MPO* genes, with NQO1 playing a dual role in the detoxification and activation of procarcinogens, and MPO converting lipophilic carcinogens into hydrophilic forms (Nebert *et al.*, 2002). All these genes have been studied for their possible association with tobacco-related cancer risk to a varying extent and with variable outcomes (London *et al.*, 2000b; Bamber *et al.*, 2001; Garte, 2001; To-Figueras *et al.*, 2001; Tiemersma *et al.*, 2002b; Guillemette, 2003; Wells *et al.*, 2004; Kiyohara *et al.*, 2005; Moreno *et al.*, 2005; Nagar & Remmel, 2006; Gallagher *et al.*, 2007).

4.2.3 Biomarkers of tobacco carcinogenesis and polymorphic genes of carcinogen metabolism

A myriad of studies have investigated association between various biomarkers of tobacco-related carcinogenesis and genetic variation of genes involved in carcinogen metabolism. For involvement in increased cancer susceptibility, a large variety of intermediate biomarker have been studied, including PAH metabolites in urine, urinary mutagenicity, DNA and protein adducts, cytogenetic alterations, *HPRT* mutant lymphocytes, as well as somatic mutations of the tumour suppressor gene *TP53* and *KRAS* oncogene occurring in cancer tissue.

(a) PAH metabolites and mutagenicity in urine

(i) PAH metabolites in urine

Increased excretion of 1-hydroxypyrene in urine in association with the *GSTM1* null genotype has been reported in many studies on individuals with occupational or environmental exposure to PAHs (Yang *et al.*, 1999; Alexandrie *et al.*, 2000; Lee *et al.*, 2001; Kuljukka-Rabb *et al.*, 2002; Kato *et al.*, 2004). The associations seen between *GSTT1* polymorphism and the

PAH metabolites are somewhat more variable. Similarly, the joint effect of *GSTM1* and *GSTT1* null genotypes, as well as the effects of some other genes of xenobiotic metabolism, such as *EPHX*, *CYP1A1*, *CYP1A2* and the aryl hydrocarbon receptor (*AhR*) gene have been either positive or negative (Yang *et al.*, 1999; Alexandrie *et al.*, 2000; Lee *et al.*, 2001; Zhang *et al.*, 2001; Kuljukka-Rabb *et al.*, 2002; Yang *et al.*, 2003; Chen *et al.*, 2007; Cocco *et al.*, 2007; Bin *et al.*, 2008).

Another PAH metabolite studied in this context is phenanthrene, the simplest PAHs with a bay region, a feature closely associated with carcinogenicity. A study quantified ratios of urinary products of metabolic activation (such as PheT) and detoxification (such as phenanthrols, HOPhe) of phenanthrene in 346 smokers, who were also genotyped for 11 polymorphisms in genes involved in PAHs metabolism, including the *CYP1A1* and *GSTM1* genes. A significant association between the presence of the *CYP1A1* $Ile^{462}Val$ polymorphism and high PheT/3-HOPhe ratios was found, particularly in combination with the *GSTM1* null polymorphism (Hecht *et al.*, 2006).

Overall, the data on the influence of genetic variation in PAHs metabolism on the levels of the urinary metabolite biomarkers are variable, and currently inconclusive.

(ii) Urinary mutagenicity

One relatively early line of research investigated the relationship between urinary mutagenicity and genetic variation in activation or detoxification genes. These studies, however, have seldom been focused on smokers only but rather on other sources of exposure (Pavanello & Clonfero, 2000).

In some studies, *NAT2* slow acetylator genotype either alone or in combination with *GSTM1* null genotype has been associated with increased urinary mutagenicity in the *Salmonella* test in individuals with occupational, environmental or medicinal PAH-related exposure, or in smokers (Vineis & Malats, 1999; Pavanello & Clonfero, 2000). In another study, CYP1A2 activity, but not *NAT2*, *GSTM1* or *GSTT1* genotypes influenced urinary mutagen excretion in smokers (Pavanello *et al.*, 2002). A further study also suggested contribution of the *CYP1A2* gene variation to increased urinary mutagenicity in heavy smokers (Pavanello *et al.*, 2005). Associations with variants of other xenobiotic-metabolising genes (such as *EPHX1*) have also been reported, with somewhat complex results (Kuljukka-Rabb *et al.*, 2002).

(b) DNA adducts

The relationship between the variants of polymorphic genes of carcinogen metabolism and tobacco smoke-related DNA adduct formation has been addressed in an abundant number of studies among smokers, occupationally exposed groups, and patients with smoking-related cancer. In addition, multiple *in vitro* studies on this relationship have been carried out (Bartsch *et al.*, 2000; Pavanello & Clonfero, 2000; Alexandrov *et al.*, 2002; Wiencke, 2002).

The intensive efforts to study the relationship between *CYP1A1* and *GSTM1* gene polymorphism and the level of aromatic-hydrophobic/bulky PAH-DNA adducts in human lungs have so far provided little evidence for a role of a single metabolic genotype or their combinations on DNA adduct formation, with largely weak, non-significant or contradictory results. However, a trend of increasing adduct levels in subjects with the *CYP1A1*2-GSTM1*0* genotype combination has been observed, which was reinforced when BPDE-DNA adducts were specifically assessed. These results suggest a gene-gene interaction, supported by biological data from other studies (Bartsch *et al.*, 2000; Alexandrov *et al.*, 2002; Wiencke, 2002). Such gene-gene interaction lends support to the increased risk for lung cancer found in carriers of these genotypes in Japanese, among whom the frequency of the

variant *CYP1A1* allele is much higher (Bartsch *et al.*, 2000; Alexandrov *et al.*, 2002).

A wide selection of genes and genotypes included in the various studies have made it difficult to assess the overall role of the polymorphisms of *GSTM1* and other genes alone or in combination. Differences between the studies in the types of adducts determined, the various tissues, cell types and cancers studied, detection methods, variation in sources and types of exposure, sample size, gender differences, and sometimes poor knowledge regarding the alleles, genotypes and haplotypes under study also contribute to the large variability seen in these studies (d'Errico *et al.*, 1999; Hemminki *et al.*, 2001; Alexandrov *et al.*, 2002; Wiencke, 2002).

(c) Cytogenetic biomarkers of genotoxicity

(i) Chromosome aberrations and sister chromatid exchanges

Early studies investigating whether homozygosity for the *GSTM1* null allele affects prevalence of cytogenetic changes in lymphocytes of smokers reported positive results (Seidegård *et al.*, 1990; van Poppel *et al.*, 1992; Cheng *et al.*, 1995). Since then, studies have investigated the association between genetic polymorphisms of xenobiotic-metabolising genes and cytogenetic biomarkers in smokers and in some occupational groups (Rebbeck, 1997; Autrup, 2000; Pavanello & Clonfero, 2000; Norppa, 2003, 2004).

Collectively, the reported findings are in support of increased susceptibility of smokers to chromosomal effects in association with *GSTM1* and *GSTT1* null variants deficient in detoxification of tobacco smoke carcinogens. Exposure to genotoxicants generated from other environmental sources (e.g. polluted air, diet, endogenous sources such as reactive oxygen species) may contribute to the observed associations, and it is likely that other polymorphic metabolic genes such as *NAT2* may be involved (Pavanello & Clonfero, 2000; Norppa, 2001, 2003).

(ii) Micronucleus induction

The relationship between formation of micronuclei and genetic polymorphisms of carcinogen metabolism has been addressed in a wide range of human population studies (Norppa, 2003, 2004). Induction of micronuclei in smokers may be little, if at all, affected by *GSTM1*, *GSTT1* or *NAT2* genotypes. In contrast, the *NAT1* rapid genotype appears to show an association with increased susceptibility to smoking-related micronuclei (Norppa, 2004).

A recent review evaluated more than seventy human studies on genetic polymorphisms and micronucleus frequency detected either in peripheral blood lymphocytes or exfoliated cells in populations exposed to various genotoxic agents. There were no significant genotype effects involved in micronucleus induction in smokers (Iarmarcovai *et al.*, 2008). The relationship between genetic polymorphisms and micronucleus formation is complex, and is influenced to a variable extent by several genes of xenobiotic metabolism and DNA repair, as well as the variety of chromosomal alterations known to contribute to micronucleus formation (Iarmarcovai *et al.*, 2008).

(iii) Chromosomal damage induced in vitro

The effects of genotypes or genotype combinations *in vitro* on the induction of various cytogenetic endpoints by tobacco-smoke carcinogens and their metabolites have been studied, initially focused on the *GSTM1* and *GSTT1* null genotypes (Norppa, 2001, 2004). In a study investigating NNK *in vitro*, lymphocytes from *GSTM1* null donors were more sensitive to induction of chromosomal aberrations and sister chromatid exchanges by NNK than lymphocytes from *GSTM1* positive donors (Salama *et al.*, 1999).

(d) Gene mutations

(i) HPRT mutant lymphocytes

Associations between the frequencies of *HPRT* mutant T-lymphocytes in populations exposed to genotoxic agents, such as smokers, and the polymorphism of xenobiotic-metabolising genes have been studied. In the early studies, positive, weak, or negative associations were reported for *GSTM1* null genotype, and negative findings were published for *NAT2* slow acetylator genotype in occupationally exposed or non-exposed subjects (Rebbeck, 1997; Vineis & Malats, 1999). When healthy, non-smoking and occupationally non-exposed young adults were studied for HPRT mutant frequency and polymorphisms in *CYP1A1*, *GSTM1* and *NAT2* genes, none of these polymorphisms, analysed individually, were found to influence the *HPRT* mutant frequency (Davies *et al.*, 1999). A significant interaction between the *GSTM1* null genotype and *NAT2* slow acetylator was associated with higher mutant frequency, but no other genotype combinations (Davies *et al.*, 1999). Some later studies have reported variable associations between *HPRT* mutant frequency and polymorphisms for either individual genes (*GSTM1*, *GSTT1* or *EPHX1*) or some of the genotypes in combination among exposed (Viezzer *et al.*, 1999; Abdel-Rahman *et al.*, 2001, 2003).

(ii) Mutations of the TP53 gene and other cancer-related genes

Whether the frequency of somatic mutations detected in tumour tissue in cancer-related genes, primarily the *TP53* tumour suppressor gene and *KRAS* oncogene, may be modified by polymorphisms in carcinogen metabolizing genes was first investigated assessing the effects of the *GSTM1* genotype, alone or in combination with other genetic polymorphisms. Several, but not all, such studies showed significant association between *GSTM1* null genotype and either the frequency or type of *TP53* mutations in smoking-induced lung cancer or other cancer type (Rebbeck, 1997; Vineis & Malats, 1999; Autrup, 2000). Fewer studies examined the association between *TP53* mutations and *GSTT1* polymorphism, and some results suggested the involvement of both null genotypes (Vineis & Malats, 1999; Autrup, 2000).

In smokers with non-small cell lung cancer, the risk of mutation was found to be the highest among the homozygous carriers of the *CYP1A1* rare allele *CYP1A1 MspI* (*Ile*^{462}*Val*) who also exhibited the *GSTM1* null genotype (Kawajiri *et al.*, 1996). Similarly, positive associations between *K-RAS* mutations and homozygosity for the *CYP1A1* rare allele were observed; the risk of mutation was enhanced when the *CYP1A1* susceptible genotype was combined with *GSTM1* null genotype (Kawajiri *et al.*, 1996). In another study, also carried out in a Japanese study population, *K-RAS* mutations occurred with greater frequency in lung adenocarcinoma smoking patients and of the *GSTM1* null genotype as compared with the *GSTM1* positive genotype (Noda *et al.*, 2004).

Many of the studies that assessed *NAT2* acetylator genotypes have found non-significant associations with the frequency or type of *TP53* mutation in bladder, lung, or other cancers (Vineis & Malats, 1999; Autrup, 2000). A study on bladder cancer did not find an overall association between *TP53* mutation frequency and *GSTM1*, *GSTT1*, *GSTP1* or *NAT2* genotypes. However, among patients with *TP53* mutations, transversion mutations were more frequent in those with *GSTM1* null genotype as compared to those with GSTM1 positive genotype; no significant associations were found for the *NAT2* gene (Ryk *et al.*, 2005).

In rectal cancer, overall negative results for an association between *TP53* or *KRAS* mutations and *GSTM1* and *NAT2* polymorphisms among smokers and non-smokers exposed to tobacco smoke were found (Curtin *et al.*, 2009). An interaction of second-hand tobacco smoke and *NAT2*

was found in *TP53* mutation positive tumours but not in smokers (Curtin *et al.*, 2009). Earlier, an increased risk of *TP53* transversion mutations among *GSTM1* positive individuals who smoked cigarettes was found in colon cancer (Slattery *et al.*, 2002).

A statistically significant association was observed between the *GSTT1* null genotype and *TP53* mutation status of breast tumour in one study (Gudmundsdottir *et al.*, 2001), while in another larger study none of the genotypes for *CYP1B1*, *GSTM1*, *GSTT1* and *GSTP1* genes alone were associated with somatic *TP53* mutations (Van Emburgh *et al.*, 2008).

In summary, data from various cancer types on the association between genetic polymorphisms of carcinogen-metabolizing genes and somatic mutations of the *TP53* and *K-RAS* genes vary widely and do not permit to conclude (Rebbeck, 1997; Vineis & Malats, 1999; Autrup, 2000).

4.3 Site-specific mechanisms of carcinogenicity of tobacco smoke

4.3.1 Sites with sufficient evidence of carcinogenicity of tobacco smoking

(a) Lung

The conceptual model presented in Section 4.1 (Fig. 4.1) depicts the main mechanistic steps by which cigarette smoke causes cancer. Smokers inhale into their lungs carcinogens which, either directly or after metabolism, covalently bind to DNA, forming DNA adducts (see Section 4.1, Fig. 4.3). Tobacco smoke contains multiple strong lung carcinogens such as NNN, NNK, PAHs, 1,3-butadiene and cadmium. Levels of tobacco smoke-related DNA adducts, mainly ^{32}P-postlabelled aromatic-hydrophobic/PAH-related bulky DNA adducts, in the lung are higher in smokers than in non-smokers (Phillips, 2002; IARC, 2004a; Hecht, 2008). Higher levels of DNA adducts have further been linked to increased risk for cancer in pooled and meta-analyses (IARC, 2004a; Veglia *et al.*, 2008).

Mutations in *TP53* and *K-RAS* genes, two central genes of human carcinogenesis, are more frequently mutated in smokers' lung cancer as compared to lung cancer from non-smokers (DeMarini, 2004; IARC, 2004a; Lea *et al.*, 2007; Ding *et al.*, 2008; see Section 4.1.3). In particular, *TP53* but also to some extent *K-RAS* mutations found in smoking-associated lung tumours exhibit mutational specificity that is consistent with the pattern produced by PAH diol epoxides in experimental studies and different from that observed in non-smokers' lung cancer (Pfeifer *et al.*, 2002; DeMarini, 2004; IARC, 2004a; Le Calvez *et al.*, 2005; Section 4.1.3). Keeping with such exposure-specific mutation profile, lung cancer in non-smokers exposed to second-hand tobacco smoke shows mutational similarity to smokers' lung cancer, although less data are available (Husgafvel-Pursiainen, 2004; IARC, 2004a; Le Calvez *et al.*, 2005; Subramanian & Govindan, 2008). The different pathways of lung carcinogenesis for smokers and non-smokers are likely to involve somatic mutations and other genetic alterations in a larger set of genes that are critical in controlling normal cellular growth via signal transduction (Bode & Dong, 2005; Lea *et al.*, 2007; Ding *et al.*, 2008).

Smoking-related lung carcinogenesis also involves a multitude of other alterations influencing the complex pathogenic pathways involved in lung cancer development, such as increased inflammation, aberrant apoptosis, increased angiogenesis, tumour progression and tumour metastasis (Wolff *et al.*, 1998; Heeschen *et al.*, 2001; Schuller, 2002; West *et al.*, 2003; Smith *et al.*, 2006; Lee *et al.*, 2008b; Section 4.1.5). Continued exposure to toxicants, genotoxicants, carcinogens, co-carcinogens and tumour promoters present in tobacco smoke has major effects on biological processes at all steps of multistep tumourigenesis of human lung (Hecht,

2003, 2008; Section 4.1). For example, nicotine in tobacco smoke is currently not described as a full carcinogen, but it exerts its biological effects via binding to nicotinic and other cellular receptors and likely enhances cell transformation and carcinogenicity through mechanisms not yet defined (Heeschen *et al.*, 2001; West *et al.*, 2003).

Numerous studies have provided evidence that the human genome may contain one or several loci that confer susceptibility to lung cancer. There are low-penetrance genes involved in the metabolism of tobacco smoke carcinogens, DNA repair and cell cycle control that may influence individual susceptibility to lung cancer (Spitz *et al.*, 2006). The role of the polymorphisms of these various classes of genes in lung carcinogenesis requires a systematic evaluation of the genetic evidence with stringent criteria (Ioannidis, 2008; Risch & Plass, 2008; Vineis *et al.*, 2009; Sections 4.1 and 4.2). Recently, genome-wide association studies have identified a susceptibility locus at chromosome 15q25.1 (Amos *et al.*, 2008; Hung *et al.*, 2008; Thorgeirsson *et al.*, 2008). The identity or function of the gene is not yet known, nor is the mechanism through which it may predispose to lung cancer. It is however likely that lung cancer susceptibility is related to the nicotine receptor gene residing at 15q25.1, and there is some evidence suggesting that it may be related to increased uptake of nicotine and NNK per cigarette (Le Marchand *et al.*, 2008).

In addition to genetic alterations, a growing body of evidence shows that epigenetic mechanisms, such as aberrant DNA methylation, histone modifications and RNA-mediated gene silencing are involved in cancer development (Jones & Baylin, 2007; Cortez & Jones, 2008). In lung carcinogenesis, gene promoter-associated (CpG island-specific) hypermethylation is an early and frequent event causing transcriptional inactivation of genes involved in regulation of cellular growth and differentiation (Belinsky, 2004). For example, several studies have indicated that the tumour suppressor gene *p16* ($p16^{INK4a/CDKN2A}$), a cell cycle regulator, is among the genes most frequently inactivated by aberrant methylation in lung cancer from smokers (Belinsky, 2004), with differences seen between smokers and never-smokers (Toyooka *et al.*, 2006). Significant associations have been established between smoking and promoter hypermethylation of tumour suppressor genes in lung tumours from smokers, and in plasma, serum or sputum DNA from cancer-free smokers (Belinsky, 2004; Belinsky *et al.*, 2005, 2006; Toyooka *et al.*, 2006).

(b) Oral cavity

PAHs can be carcinogenic at the site of application, which could include the human oral cavity. DMBA, a highly carcinogenic PAH not present in tobacco or tobacco smoke, is a standard model compound for induction of oral tumours in the hamster cheek pouch; less is known about the effects on the oral cavity of PAHs that do occur in tobacco products (Shklar, 1972; Rao, 1984; Vairaktaris *et al.*, 2008). A mixture of NNN and NNK induced oral tumours in rats when applied locally (Hecht *et al.*, 1986), and DNA adduct formation from NNN, NNK and NNAL has been observed in the rat oral cavity (Zhang *et al.*, 2009a, b). HPB-releasing DNA adducts from NNK and/or NNN have been reported in exfoliated oral cells from smokers and smokeless tobacco users (Heling *et al.*, 2008) and HPB-releasing heamoglobin adducts are elevated in smokeless tobacco users (IARC, 2007a). Unidentified DNA adduct levels are consistently elevated in oral cells and tissues from smokers compared to non-smokers (IARC, 2004a). Mutations in the *TP53* gene have been observed in oral tumours from smokers and smokeless tobacco users (IARC, 2006b, 2007a; Warnakulasuriya & Ralhan, 2007). Tobacco-associated genetic mutations including micronuclei, gene mutations, DNA polymorphisms, and chromosomal abnormalities have been reported in studies of buccal cells from smokers and smokeless tobacco users (Proia *et al.*, 2006). The use of lime by betel quid chewers is associated

with enhanced oxidative damage that could play a role in inflammation or tumour promotion (IARC, 2004b).

(c) Larynx and nasopharynx

Hamsters exposed to cigarette smoke by inhalation consistently developed benign and malignant tumours of the larynx; tumours were produced by inhalation of the particulate phase, but not the gas phase of cigarette smoke (IARC, 1986). In related studies in which hamsters were treated with DMBA by intratracheal instillation followed by exposure to cigarette smoke, a significantly higher incidence of laryngeal tumours was observed than in hamsters exposed only to cigarette smoke or to DMBA (IARC, 1986). Collectively, these results indicate an initiation-promotion mechanism for the production of laryngeal tumours, and are consistent with the results of experiments in which tobacco smoke condensate is applied to mouse skin (IARC, 1986). The combined data implicate PAHs and tumour promoters in tobacco smoke as potential etiologic agents for cancer of the larynx in hamsters. Levels of DNA adducts measured by non-specific methods were higher in larynx tissue from smokers than from non-smokers (IARC, 2004a). Analyses of mutations in the *TP53* gene from tumours of the larynx in smokers show a pattern similar to that observed in lung tumours, and both are consistent with the pattern produced by PAH diol epoxides (IARC, 2006b). The available data are consistent with the conceptual framework illustrated in Fig. 4.1 (Szyfter *et al.*, 1999).

Formaldehyde, a constituent of cigarette smoke, causes nasopharyngeal cancer in humans (IARC, 2006a). A recent study demonstrates a 10-fold higher level of the formaldehyde-DNA adduct N^6-hydroxymethyldeoxyadenosine in leukocytes of smokers compared to non-smokers, suggesting its possible involvement in nasopharyngeal cancer in smokers (Wang *et al.*, 2009). Acetaldehyde, another carcinogenic constituent of tobacco smoke, which also forms genotoxic adducts (Section 4.1), may also contribute to the development of these forms of head and neck cancer.

(d) Oesophagus

Nitrosamines are probably the most effective oesophageal carcinogens known, with particularly strong activity in the rat (Lijinsky, 1992). NNN and NDEA are both present in cigarette smoke, and levels of NNN greatly exceed those of NDEA (IARC, 2004a). NNN is also present in considerable quantities in smokeless tobacco and betel quid containing tobacco (IARC, 2004a, 2007a). Thus, NNN is a likely candidate as a causative agent for esophageal cancer in smokers, smokeless tobacco users, and chewers of betel-quid with tobacco. While considerable mechanistic data are available from studies of NNN in laboratory animals (Hecht, 1998; Wong *et al.*, 2005; Lao *et al.*, 2007; Zhang *et al.*, 2009a), there are little comparable data in humans.

Increased acetaldehyde production derived both from tobacco smoke and from microbial alcohol oxidation may play a role in the synergistic carcinogenic action of alcohol and smoking on oesophagus, as well as on other upper aerodigestive locations (Homann *et al.*, 2000; Salaspuro & Salaspuro, 2004; Lee *et al.*, 2007a).

(e) Stomach

Hypermethylation of the E-cadherin 1 gene (*CDH1*) was observed preferentially in gastric tumours from smokers rather than non-smokers (Poplawski *et al.*, 2008). *CDH1* can act as a tumour-suppressor gene, preventing cells from growing and dividing in an uncontrolled way to form a cancerous tumour. Because the protein encoded by this gene helps cells stick together, altered regulation may lead to metastasis.

Boccia *et al.* (2007) found an increased risk for stomach cancer among smokers who had the *SULT1A1 His* genotype, and Lee *et al.* (2006) found an increased risk for those who had the *m2* allelic variant of *CYP1A1*. A nested case–control

study found that smokers had an increased risk of gastric cancer if they carried at least one variant allele A in Ex7+129 C > A (*Thr*^{461}*Asn*, *m4*) of *CYP1A1* (Agudo *et al.*, 2006). Stomach cancer tissue from smokers had higher levels of stable DNA adducts than did those from non-smokers; however, the number of non-smokers was quite small (Dyke *et al.*, 1992).

(f) Pancreas

NNK and its metabolite NNAL are the only pancreatic carcinogens known to be present in tobacco and tobacco smoke. NNK was detected in the pancreatic juice of 15 of 18 samples from smokers, at levels significantly higher than in non-smokers; NNAL and NNN were also detected in some samples (Prokopczyk *et al.*, 2002). DNA adducts of NNK and NNAL were present in pancreatic tissue of rats treated with these nitrosamines (Zhang *et al.*, 2009b), but were not detected in most human pancreatic tissue samples (Prokopczyk *et al.*, 2005).

(g) Colorectum

Tobacco smoke contains heterocyclic amines, such as 2-amino-1-methyl-6-phenylimidazo[4,5,6]pyridine (PhIP), which are intestinal carcinogens in rats and mutate the adenomatous polyposis coli (*Apc*) gene in mice (Møllersen *et al.*, 2004). The *APC* gene is frequently mutated and has altered expression in human colon cancer (Samowitz *et al.*, 2007; Samowitz, 2008). A recent model of colon cancer by Sweeney *et al.* (2009) suggests that this disease can develop via at least three independent mechanistic pathways. One pathway is initiated by methylation of *MINT* (methylation in tumour) markers that proceeds down a pathway predisposing to microsatellite instability, followed by methylation of the mismatch repair gene mutL homologue 1 (*MHL1*) and the tumour-suppressor gene *TP16*, followed by mutation in *BRAF* (a homologue of a viral raf oncogen). A second independent pathway is initiated with a mutation in the *APC* gene, followed by a mutation in the *TP53* gene. A third independent pathway involves only *KRAS2* mutations. One study found BPDE-DNA adducts at a higher frequency in colon DNA from smokers than from non-smokers (Alexandrov *et al.*, 1996). Mutations or epigenetic changes in some or all of these genes have been found in smoking-associated colon or colorectal tumours.

Microsatellite instability, which is the expansion or contraction of short nucleotide repeats, occurs in approximately 10–15% of sporadic colorectal cancer, and is usually associated with smoking and hypermethylation of the promoter of the mismatch repair gene *MLH1* (Samowitz, 2008). Smoking-associated colorectal tumours also have high frequencies of methylation at CpG islands (Samowitz, 2008).

In a case–control study of colorectal cancer, Kasahara *et al.* (2008) found that the genetic polymorphism *APEX1/APE1* (apurinic/apyrimidinic endonuclease-1) Asp^{148}Glu, which is a gene involved in DNA repair, was associated with risk for colorectal cancer among smokers but not non-smokers. Other studies have also found associations between polymorphisms in the DNA repair genes *XRCC1* and smoking and risk for colorectal cancer (Stern *et al.*, 2007; Campbell *et al.*, 2009).

(h) Liver

Tobacco smoke contains liver carcinogens such as furan and certain nitrosamines. Liver tumours exhibit increased expression of *C-MYC*, *P16*^{INK4A}, epidermal growth factor receptor (*EGFR*), telomerase, transforming growth factor-α (*TGF-α*), insulin-like growth factor-2 (*IGF-2*) and *RAF* oncogene (Abou-Alfa, 2006). Smokers show altered expression of some of these genes or of genes in the same or similar pathways (Sen *et al.*, 2007). A genome-wide association study found that SNP rs1447295 in the 8q24 chromosome was positively associated with liver cancer among ever-smokers (Park *et al.*, 2008). Thus, tobacco smoke appears to have epigenetic

effects on the liver that may contribute to hepatocellular carcinoma.

(i) Urinary bladder

Tobacco smoke contains aromatic amines such as 4-aminobiphenyl and 2-naphthylamine, which are human bladder carcinogens (see IARC, 2012a). In bladder tumours, smoking was associated with a more than twofold increase risk of methylation of the promoter region of the *P16*^{INK4A} gene and of the soluble Frizzled receptor protein (*SFRP*) gene (Marsit *et al.*, 2006). In addition, Tang *et al.* (2009) suggested that epigenetic silencing of *Wnt* antagonists through hypermethylation may play a role in smoking-related invasive bladder cancer (Tang *et al.*, 2009). SNP rs6983267 of the 8q24 chromosome was inversely associated with bladder cancer among ever-smokers (Park *et al.*, 2008). Smokers generally have mutagenic urine and smoking is associated with specific cytogenetic changes and DNA breaks in bladder tumours (DeMarini, 2004). Smoking-associated stable DNA adducts have been found in bladder tissue or exfoliated urothelial cells, supporting a role for DNA damage in smoking-associated bladder cancer (Phillips, 2002).

(j) Cervix

The cervical mucus of smokers is more mutagenic than that of non-smokers, and cervical epithelia of smokers have higher frequencies of micronuclei than those of non-smokers (DeMarini, 2004). Several studies have found increased levels of DNA adducts in cervical tissue from smokers relative to non-smokers, suggesting a role for smoking-associated DNA damage in cervical cancer (Phillips, 2002).

(k) Ovary

It has been observed that the inverse associations reported for serous and endometrioid tumours with respect to parity and oral contraceptives did not hold for the mucinous tumours. Based on these observations, Risch *et al.* (1996) suggested that mucinous ovarian tumours may be etiologically unrelated to the other types of epithelial tumours. Whereas mucinous elements such as gastric or intestinal type glands may be seen in mature teratomas, a form of germ cell neoplasia, overall mucinous tumours are classified as surface epithelial tumours because transitions among the subtypes may be observed. The major difference between mucinous and serous tumours is their biologic behaviour. Mucinous carcinomas of the ovary are slow growing tumours that appear to develop from their benign counterparts. The fact that the transitions between the benign, borderline, and malignant form of the disease can be seen in the same tumour suggests that over time, there is a progression from benign to malignant (Riopel *et al.*, 1999). *K-ras* mutational analysis, for example, demonstrates a heterogeneous distribution of the mutation within different parts of the same neoplasm, suggesting that acquisition of the *K-ras* mutation occurs in malignant transformation (Mandai *et al.*, 1998). Serous carcinomas seem to develop de novo rather than from a benign pre-existing lesion; alternatively, the rate of progression is rapid and the precursor lesion is obliterated before the detection of the tumour. In some data, current smoking is associated with a shorter interval to detection of mucinous than non-mucinous tumours. Because the mucinous tumour is slow growing, smoking could contribute to the malignant progression of the adenoma-carcinoma sequence, as the benign form of the tumour may have been present for some time.

(l) Leukaemia

Tobacco smoke contains known leukaemogens such as benzene, 1,3-butadiene and formaldehyde (IARC, 2012a). The mechanisms of leukaemogenesis are currently not well understood. Data indicate that leukaemogenic agents, such as benzene, cause toxicity to the

haemotopoietic system, as well as genotoxicity at low levels, and that genetic polymorphisms may be involved in these processes (Aksoy, 1989; Lan *et al.*, 2004; Garte *et al.*, 2008; Hosgood *et al.*, 2009; Lau *et al.*, 2009; Rappaport *et al.*, 2009). Recent studies suggest the importance in carcinogen-related leukaemogenesis of damage to haematopoietic stem/progenitor cells circulating in the peripheral blood, or, alternatively, damage to primitive pluripotent progenitor cells present in other tissues (Zhang *et al.*, 2009c). In these two models, damaged stem/progenitor cells would then travel to the bone marrow and become initiated leukaemic stem cells. Mechanisms considered central in these models are: disruption of bone marrow DNA, through e.g. formation of DNA adducts, DNA–protein crosslinks, the action of free radicals or active states of oxygen; intercalation of metals within the DNA structure; or inhibition of enzymes involved in cell division (Zhang *et al.*, 2007, 2009c).

4.3.2 Sites with limited evidence of carcinogenicity or evidence suggesting lack of carcinogenicity

(a) Breast

(i) Carcinogenic pathway

Carcinogens found in tobacco smoke pass through the alveolar membrane and into the blood stream, by means of which they can be transported to the breast via plasma lipoproteins (Yamasaki & Ames, 1977; Shu & Bymun, 1983; Plant *et al.*, 1985). Tobacco smoke contains known rodent mammary carcinogens, including PAHs and aromatic amines (IARC, 1986, 2004a; el-Bayoumy, 1992; Ambrosone & Shields, 1999; Ambrosone, 2001; Hoffmann *et al.*, 2001) which, due to their lipophilicity, can be stored in breast adipose tissue (Obana *et al.*, 1981; Morris & Seifter, 1992) and then metabolized and activated by human mammary epithelial cells (MacNicoll *et al.*, 1980). Tobacco smoke constituents reach the breast as demonstrated by the detection of cotinine in breast fluid (Petrakis *et al.*, 1978). There is evidence suggesting the presence of mutagenic arylamines (Thompson *et al.*, 2002) and PAHs (Zanieri *et al.*, 2007) in human breast milk. Cigarette smoke condensate has been shown to transform normal human breast epithelial cells *in vitro* (Narayan *et al.*, 2004), perhaps by blocking long-patch base excision repair (Kundu *et al.*, 2007). Transformation and cytogenetic effects have been observed in human mammary epithelial cells after exposure to chemical carcinogens such as PAHs or arylamine (Mane *et al.*, 1990; Eldridge *et al.*, 1992; Calaf & Russo, 1993).

The formation of specific adducts from PAHs and aromatic amines has been observed in human breast epithelial cells *in vitro*, and unspecified-DNA adducts have been found in exfoliated ductal epithelial cells in human breast milk (Gorlewska-Roberts *et al.*, 2002; Thompson *et al.*, 2002).

Mutations in the *TP53* tumour suppressor gene have been found in 15–30% of breast cancers (Goldman & Shields, 1998; Olivier & Hainaut, 2001). An increased prevalence and altered spectrum of *TP53* mutations in breast tumours have been observed among current smokers compared with never smokers (Conway *et al.*, 2002). The breast tumours with the most pronounced smoking-related mutational pattern (for example, a greater number of G:C→T:A transversions) were from women who had smoked for more than 20 years, although total *TP53* mutations were not associated with smoking duration (Conway *et al.*, 2002). This increased frequency of G to T transversions in smokers versus non-smokers is also observed in the IARC TP53 database (IARC, 2006b; Van Emburgh *et al.*, 2008).

Recent meta-analyses of epidemiological studies tend to show positive associations of breast cancer with long-term smoking among *NAT2* slow acetylators, especially among post-menopausal women (who are more likely than pre-menopausal women to be very long-term

smokers). Firozi *et al.* (2002) showed that breast tissue from *NAT2* slow acetylators had significantly higher levels of the diagonal radioactive zone (smoking-related) DNA adduct pattern than that from fast acetylators.

High rates of breast cancer in women exposed to ionizing radiation during adolescence (aged 10–19 years at exposure) (Tokunaga *et al.*, 1987) suggested that the adolescent breast may also be sensitive to the DNA-damaging effects of other exposures. This might also be true for the genotoxic compounds contained in tobacco smoke. Although some studies have supported such association, the results have been sparse and mixed. In addition, it is difficult to separate the effects of early life exposure to tobacco and smoking duration (Terry & Rohan, 2002).

Early age at first full-term pregnancy has been associated with reduced breast cancer risk (Kelsey *et al.*, 1993), hypothetically due to terminal differentiation of the breast epithelium that occurs late in the first trimester. It has been suggested that in the early stages of pregnancy, when growth-promoting hormone levels are high, but before terminal differentiation (Montelongo *et al.*, 1992), the breast may be particularly susceptible to the cancer-promoting chemicals in tobacco smoke. Several epidemiological studies compared measures of smoking before and after a first full-term pregnancy. Although suggestive, the data did not consistently show an increased risk for breast cancer among women who smoked before a first full-term pregnancy (Adami *et al.*, 1988; Hunter *et al.*, 1997; Band *et al.*, 2002; Egan *et al.*, 2003; Gram *et al.*, 2005; Li *et al.*, 2005; Olson *et al.*, 2005; Cui *et al.*, 2006). Smoking was associated with a 50% increased risk among women with slow *NAT2* acetylation genotype (Egan *et al.*, 2003). Overall, studies of risk in association with the timing of smoking relative to a first pregnancy are inconclusive; nevertheless, the breast tissue appears to have a greater susceptibility to the carcinogenic chemicals in tobacco smoke before compared to after terminal differentiation of breast epithelium.

(ii) Estrogenic pathway

The "anti-estrogenic" mechanism through which tobacco smoking may inhibit breast cancer progression is unclear. Estrogen is a known risk factor for breast cancer and several hypotheses have been proposed: earlier age at menopause among smokers, a reduction in the gastrointestinal absorption or distribution of estrogen, enhanced metabolism of estradiol to inactive catechol estrogens, increased binding of estrogens by serum sex hormone-binding globulin, lowered levels of estrogen derived from adipose tissue (Baron, 1984; Baron *et al.*, 1990; Terry & Rohan, 2002). Several studies of cigarette smoking and mammographically-defined breast density showed lower measures of breast density in current smokers than in non-smokers (Sala *et al.*, 2000; Vachon *et al.*, 2000; Warwick *et al.*, 2003; Jeffreys *et al.*, 2004; Modugno *et al.*, 2006; Bremnes *et al.*, 2007; Butler *et al.*, 2008). Since exposure to estrogen has been associated positively with breast density, a strong risk factor for breast cancer (McCormack & dos Santos Silva, 2006), the results of these studies are consistent with an anti-estrogenic effect of cigarette smoking. Although smokers and non-smokers may have the same concentrations of estrogens overall, it may be the type rather than the absolute levels of circulating estrogens that is important. Smokers might have a lower concentration of more biologically active estrogens, primarily 16-α-hydroxyestrone (16α-OHE1) (Michnovicz *et al.*, 1986, 1988; Berta *et al.*, 1992; Berstein *et al.*, 2000; Terry *et al.*, 2002b). Estrogen can be metabolized along three pathways, to 16α-OHE1 or to 2-OHE1 or to 4-OHE1. 16α-OHE1 and 4-OHE1 have been observed to increase mammary epithelial cell proliferation rates in experimental studies (Schütze *et al.*, 1993, 1994; IARC, 2007c). In contrast, 2-OHE1 might decrease epithelial cell proliferation rates (Bradlow *et al.*, 1996;

Muti *et al.*, 2000). If cigarette smoking increases estradiol 2-hydroxylation, as has been suggested (Michnovicz *et al.*, 1986), thereby increasing the ratio of 2-OHE1:16-α-OHE1, an inverse association between smoking and breast cancer risk might be observed. However, only one study has directly examined 2-hydroxylation in relation to cigarette smoking (Michnovicz *et al.*, 1986). Using injected radiolabelled estradiol, a 50% increased estradiol 2-hydroxylation was found in premenopausal women who smoked at least 15 cigarettes/day compared with non-smokers. Two studies of urinary estrogens found increased excretion of 2-OHE1 and decreased excretion of estriol among smokers (Michnovicz *et al.*, 1988; Berstein *et al.*, 2000), which may also support the hypothesis that smoking decreases the formation of active estrogen metabolites along the 16α-hydroxylation pathway. However, the ratio of urinary 2-OHE1:16α-OHE1 was not related to breast cancer risk in the one case–control study that examined the association (Ursin *et al.*, 1999). The 4-hydroxylation of estrogens is catalysed by CYP1B1, which is induced by tobacco smoke (Nebert *et al.*, 2004). This has been postulated as an additional pathway that could lead to formation of DNA adducts via catechol estrogen-quinones (Gaikwad *et al.*, 2008) and oxidative/DNA damage via redox-cycling (Zhu & Conney, 1998). The ratio of 2-OHE1:4-OHE1 has been studied in relation to breast cancer risk and smoking in one study (Berstein *et al.*, 2000). Smokers carrying the *CYP1B1 Val* allele [associated with high hydroxylation activity] had a significantly higher risk for breast cancer compared to never smokers with the *Leu/Leu* [wildtype] genotype (Saintot *et al.*, 2003).

(b) Endometrium

Exogenous estrogens unopposed by progesterone have been shown to increase the risk for endometrial cancer through increased mitotic activity of endometrial cells, increased number of DNA replication errors, and somatic mutations resulting in the malignant phenotype (IARC, 2007c, 2012c). Hence, factors associated with estrogen absorption or metabolism may alter the risk of this malignancy. Several investigators have hypothesized that cigarette smoking might be have anti-estrogenic effects, and through this mechanism reduce the risk of endometrial cancer (Baron, 1984; Baron *et al.*, 1990; Terry *et al.*, 2002b, 2004a).

Whether mediated through changes in the amount of adipose tissue, altered age at menopause, or anti-estrogenic effects, blood hormone concentrations might be an important link between smoking and the reduced risk of endometrial cancer observed in most epidemiological studies. The estrogens that have typically been studied in relation to cigarette smoking include estrone, sex hormone binding globulin (SHBG)-bound estradiol, and estriol. Blood concentrations of androgens, typically androstenedione and dehydroepiandrosterone sulfate (DHEAS), have also been studied, because these are biological precursors of estrone. Studies that have examined blood concentrations of SHBG are less common, and studies of unbound (free) estradiol are scarce.

Studies of cigarette smoking and blood hormone concentrations have been conducted mostly among post-menopausal women who were not taking HRT. Of these studies, nine examined serum (Friedman *et al.*, 1987; Cauley *et al.*, 1989; Slemenda *et al.*, 1989; Schlemmer *et al.*, 1990; Cassidenti *et al.*, 1992; Austin *et al.*, 1993; Law *et al.*, 1997) or plasma (Khaw *et al.*, 1988; Longcope & Johnston, 1988) estrone, ten examined serum (Friedman *et al.*, 1987; Cauley *et al.*, 1989; Slemenda *et al.*, 1989; Schlemmer *et al.*, 1990; Key *et al.*, 1991; Cassidenti *et al.*, 1992; Austin *et al.*, 1993; Law *et al.*, 1997) or plasma (Khaw *et al.*, 1988; Longcope & Johnston, 1988) estradiol, and two examined serum (Cassidenti *et al.*, 1992) or plasma (Longcope & Johnston, 1988) free estradiol. These studies consistently showed little or no association between smoking

and blood estrogen concentrations among post-menopausal women who were not taking hormone replacement therapy. Among pre-menopausal women, three studies (Longcope & Johnston, 1988; Key *et al.*, 1991; Berta *et al.*, 1992) found no clear association between cigarette smoking and estrogen concentrations. Studies that adjusted hormone measurements for the effects of BMI (and other covariates) showed similar results to those that did not, suggesting that BMI is not a strong confounder of this association.

In two studies the association between cigarette smoking and blood estrogen concentrations after randomization of women to groups receiving either estradiol or placebo were examined (Jensen & Christiansen, 1988; Cassidenti *et al.*, 1990). In a small study of 25 post-menopausal women, unbound estradiol was significantly lower among smokers than non-smokers both at baseline and shortly after taking micronized estradiol orally (Cassidenti *et al.*, 1990). No important differences were observed between smokers and non-smokers in serum concentrations of either estrone or bound estradiol. In contrast, in a study in which 110 post-menopausal women were randomized to take hormones (either orally or percutaneously) or a placebo (Jensen & Christiansen, 1988), smokers had lower concentrations of both estrone and bound estradiol than non-smokers after oral (but not percutaneous) hormone treatment for at least one year (concentrations of free estrogens were not examined). These results indicate that smoking might affect the absorption or metabolism of hormones used in replacement therapy.

Of the five studies that have examined the association between cigarette smoking and serum (Lapidus *et al.*, 1986; Cassidenti *et al.*, 1992; Law *et al.*, 1997) or plasma (Khaw *et al.*, 1988; Longcope & Johnston, 1988) SHBG, none found any clear association. However, one of these studies (Khaw *et al.*, 1988) found an inverse association between smoking and the ratio of bound estradiol to SHBG, a measure of estrogen activity. In this context, Cassidenti *et al.* (1990) found unbound (but not SHBG-bound) estradiol was significantly lower among smokers than non-smokers both at baseline and after taking oral estradiol, suggesting an increased SHBG-binding capacity in the women who smoked.

In post-menopausal women, androgens are the major source of estrone, converted through aromatization in fat deposits. Thus, adiposity is positively correlated with estrogen concentrations in post-menopausal women. Of the nine studies in which blood concentrations of androstenedione were examined in smokers (Friedman *et al.*, 1987; Khaw *et al.*, 1988; Longcope & Johnston, 1988; Cauley *et al.*, 1989; Slemenda *et al.*, 1989; Schlemmer *et al.*, 1990; Cassidenti *et al.*, 1992; Austin *et al.*, 1993; Law *et al.*, 1997), higher circulating concentrations were found among current than among never or former smokers in all studies. However, there was no clear variation in blood estrone concentrations by smoking status, suggesting a reduced conversion of androstenedione to estrone among smokers. Of the five studies where cigarette smoking and DHEAS concentrations were examined, three (Khaw *et al.*, 1988; Cassidenti *et al.*, 1992; Law *et al.*, 1997) found increased blood concentrations among current smokers, one (Friedman *et al.*, 1987) found also an increase that was not statistically significant, whereas another (Key *et al.*, 1991) found no clear differences according to smoking status.

Cigarette smoking and urinary estrogen concentrations have been examined in seven studies (MacMahon *et al.*, 1982; Michnovicz *et al.*, 1986; Trichopoulos *et al.*, 1987; Michnovicz *et al.*, 1988; Berta *et al.*, 1992; Key *et al.*, 1996; Berstein *et al.*, 2000). Of these, three found no major differences according to smoking status (Trichopoulos *et al.*, 1987; Michnovicz *et al.*, 1988; Berta *et al.*, 1992). The remaining four studies all showed lower urinary estriol concentrations among smokers than among non-smokers, but mixed results for urinary estrone and estradiol.

Two of these studies (Michnovicz *et al.*, 1988; Berstein *et al.*, 2000) showed higher concentrations of 2-hydroxyestrone among smokers, than non-smokers but only after estrogen treatment in Berstein *et al.* (2000).

Age at natural menopause varies substantially under the influence of genetic and environmental factors (McKinlay, 1996). A relatively early age at menopause has been associated with reduced risk of endometrial cancer (Kelsey *et al.*, 1982; Baron, 1984; Baron *et al.*, 1990; Akhmedkhanov *et al.*, 2001). A one year decrease in age at menopause has been associated approximately with a 7% decrease in risk (Kelsey *et al.*, 1982). It has been proposed that cigarette smoking decreases the age at natural menopause (Baron *et al.*, 1990), more clearly with qualitative than quantitative smoking measures (Parente *et al.*, 2008), and thus might reduce endometrial cancer risk through reduced exposure to endogenous estrogens. On average, smokers have menopause approximately 1 to 1.5 years earlier than non-smokers (Terry *et al.*, 2002b, 2004a). Adjustment for obesity and other covariates did not alter the results (Terry *et al.*, 2002b).

4.4 Mechanistic considerations of the interaction of ethanol and tobacco carcinogens

The combined effects of alcoholic beverages and tobacco on the risk for cancer incidence and mortality have been widely studied in human populations. When tested for multiplicative and additive interactions, synergistic effects of alcoholic beverages and tobacco have been found, especially for oropharyngeal and oesophageal cancers (Homann *et al.*, 2000; Castellsagué *et al.*, 2004; Salaspuro & Salaspuro, 2004; Lee *et al.*, 2005a; Lee *et al.*, 2007b).

Data support at least four possible mechanisms for the modifying effects of alcoholic beverages on cancer risk due to tobacco.

1. Alcohol may have a local permeabilizing effect on penetration of the oral mucosa by tobacco carcinogens (Du *et al.*, 2000), particularly important in the case of oropharyngeal and oesophageal cancer.
2. CYP2E1 and other CYPs may both activate and detoxify carcinogens present in tobacco smoke, including NDMA, NDEA, NNK, benzene and other tobacco-derived carcinogens in two ways: CYP induction increases metabolic activation of tobacco carcinogens leading to enhanced formation of proximate reactive chemical species at target sites; and alteration of phase II conjugation/detoxification enzymes by ethanol may also occur, changing the effective dose at the target site.
3. Competitive inhibition of CYP metabolism leads to reduced central hepatic and gastrointestinal clearance thus increasing dose delivery of carcinogens to peripheral target tissues (reviewed in Meskar *et al.*, 2001).
4. Effects of acetaldehyde derived by microbial alcohol oxidation and from the tobacco smoke (Homann *et al.*, 2000; Salaspuro & Salaspuro, 2004).

Supportive evidence for ii) and iii) is briefly presented below.

4.4.1 Effects of induction of CYPs by ethanol

(a) CYP2E1

Ethanol induces CYP2E1 in the human liver and in all species tested. Over 70 substrates of CYP2E1 have been compiled (Raucy & Carpenter, 1993; Guengerich *et al.*, 1994; Djordjević *et al.*, 1998; Klotz & Ammon, 1998; Cederbaum, 2006). Among those are tobacco carcinogens such as benzene, vinyl chloride, NDMA, NDEA and *N*-nitrosopyrrolidine, as well as many low-molecular-weight compounds. Induction of CYP2E1 by ethanol generated increased levels of toxic metabolites from the metabolism of many of these chemicals (Novak & Woodcroft, 2000). Pyridine, a constituent of tobacco smoke and

substrate of CYP2E1, generates DNA damaging products by redox-cycling (Kim & Novak, 1990).

In humans, in addition to the prominent CYP2E1 expression in the centrilobular regions of the liver, the enzyme is also detectable in the kidney cortex and, at lower levels, in organs such as the oropharynx, nasal mucosa, ovary, testis, small intestine, colon and pancreas (Ingelman-Sundberg *et al.*, 1994; Lieber, 1999, 2004).

In rats, ethanol induced CYP2E1 in epithelia of the cheek, tongue and oesophagus (Shimizu *et al.*, 1990). As a result of CYP2E1 induction by ethanol in the upper respiratory tract and possibly of inhibition of carcinogen clearance, hamsters had a significant increase of nasal cavity and tracheal tumours after intraperitoneal injection of *N*-nitrosopyrrolidine (McCoy *et al.*, 1981). Thus, induction of CYP2E1 by ethanol may participate in the genesis of cancers at several sites via metabolic activation of tobacco carcinogens into reactive species in target tissues.

(b) Other xenobiotic-activating CYPs

In addition to CYP2E1, several CYPs, including CYP3A4 and probably CYP1A2 in humans, and CYP1A1, 2B1 and 3A in rat liver, may be induced by ethanol. Of particular interest are members of the CYP3A family, which have wide substrate specificity and have been implicated in the activation of several known or suspected human carcinogens, including those derived from tobacco (Wojnowski & Kamdem, 2006). Both CYP3A4 and CYP1A2 metabolize NNK (Jalas *et al.*, 2005). Based on the Michaelis constant (Km) data (IARC, 2007a), the relative efficiencies in NNK metabolism by human CYP are (from greatest catalyst to least): 2A13 > 2B6 > 2A6 > 1A2 ~1A1 > 2D6 ~2E1 ~3A4. As the amount of CYP enzymes with overlapping substrate specificity that participate in nitrosamine metabolism varies according to organ and species, it is difficult to determine their individual contribution at target sites.

4.4.2 Effects of inhibition of CYPs by ethanol

Ethanol is a competitive inhibitor of CYP2E1 (reviewed in Anderson, 1992). It also inhibits the activities of CYP1A1, 2B6 and 2C19 but not those of CYP1A2.

Direct inhibition of CYPs by ethanol in target tissues may reduce metabolic activation of xenobiotics and hence local toxic and tumorigenic effects. Thus CYP inhibition in the liver could increase extrahepatic exposure to genotoxic metabolites from tobacco carcinogens that are substrates for these CYP enzymes. This mechanism is supported by several studies.

Ethanol caused a fivefold increase in oesophageal DNA adducts in rats induced by NDEA (Swann, 1984). In monkeys, O^6-methylguanine-DNA adducts after an oral dose of NDMA with or without ethanol were increased by co-exposure to ethanol in all tissues except the liver (Anderson *et al.*, 1996). Effects were seen in the oesophagus (17-fold increase), colonic mucosa (12-fold), pancreas (sixfold), urinary bladder (11-fold), ovary (ninefold), uterus (eightfold), brain (ninefold), spleen (13-fold) and nasal mucosa (fivefold). In these studies, ethanol treatment was acute, so that enzyme induction was improbable, and the oesophagus was not directly exposed to either ethanol or carcinogen. This indicates that a systemic interaction, most likely inhibition of hepatic carcinogen clearance, was responsible for the observed effects in the oesophagus and other extrahepatic tissues. The 17-fold increase in DNA adducts in the monkey oesophagus is similar to the 18-fold increased risk for human oesophageal cancers in tobacco smokers combined with heavy alcohol drinking (Tuyns *et al.*, 1977).

The relevance of increased genotoxic effects in extrahepatic target sites by ethanol is confirmed by many rodent experiments. Oral dosing of mice with NDMA in ethanol resulted in nasal cavity tumours (olfactory neuroblastoma) that were not seen with NDMA or ethanol alone (Griciute

et al., 1981). Ethanol in the drinking-water led to a ninefold increase in oesophageal tumours in rats induced by NDEA (Aze *et al.*, 1993). Ethanol given by gavage to nursing dams together with NDMA or NNK (Chhabra *et al.*, 2000) increased O^6-methylguanine-DNA adducts in maternal mammary glands, by 10-fold with NDMA and to a lesser extent with NNK. In the suckling infants, DNA adducts were detected in the lungs and kidneys after maternal exposure to NDMA and increased about fourfold after maternal co-treatment with ethanol. In mice, ethanol given with NDMA in the drinking-water resulted in a fourfold increase in lung tumours, but had no significant effect when NDMA was given intragastrically, intraperitoneally, subcutaneously or intravenously (Anderson, 1992). These negative findings support that direct inhibition of hepatic carcinogen clearance by ethanol is the main operative mechanism.

There is indirect evidence that ethanol can inhibit the *in vivo* clearance of the carcinogen NDMA in humans: individuals with chronic renal failure showed detectable blood and urine levels of NDMA, which were increased by consumption of ethanol (Dunn *et al.*, 1990). Other studies that involved sources of NDMA from tobacco smoke, diet or pharmaceuticals are consistent with ethanol reducing its clearance rate in humans (Anderson, 1992).

Other possible modifying effects of ethanol in tobacco-related tumorigenesis are presented in Section 4 of the *Monograph* on Consumption of Alcoholic Beverages in this Volume.

4.5 Synthesis

4.5.1 Mechanisms of tobacco-related carcinogenesis

The pathways by which tobacco products cause cancer essentially recapitulate established mechanisms of carcinogenesis by individual compounds, which were elaborated by landmark studies during the second half of the 20th century. These studies demonstrate that most carcinogens, either directly or after metabolism catalyzed by multiple cytochrome P450 enzymes, react with nucleophilic sites in DNA to form covalent binding products called adducts (a contraction for "addition products"). These DNA adducts, if left unrepaired by cellular DNA repair enzymes, persist and cause mistakes during DNA replication leading to incorporation of the wrong base in a DNA strand and consequent permanent mutations. If these permanent mutations occur in important regions of critical growth control genes such as the oncogene *KRAS* or the tumor suppressor gene *p53*, cellular growth processes can become severely unregulated and cancer can result. Multiple studies of mutations in *KRAS*, *p53*, and other growth control genes in lung tumours from smokers, some of which report thousands of mutations, are fully consistent with this overall concept.

It is the complexity of tobacco carcinogenesis which challenges investigators to identify specific mechanisms that fully explain the ways in which tobacco products cause each type of cancer. There are over 70 established carcinogens in cigarette smoke, and analyses of smokers' urine and blood clearly demonstrate higher uptake of these compounds in smokers than in non-smokers. The urine of smokers is consistently mutagenic. Similar considerations apply to smokeless tobacco users, although there are fewer identified carcinogens. Multiple DNA adducts are present in the lungs and other tissues of smokers, and sister chromatid exchanges as well as other genetic effects are consistently observed. But much less is known about the specifics of the process. Only relatively few DNA adducts in smokers' lungs have been structurally characterized and the relationship between specific adducts and the consequent mutations in critical genes is still somewhat unsettled.

There are other processes which contribute to cancer induction by tobacco products, based on

multiple studies in both laboratory animals and humans. These include inflammation, tumor promotion, oxidative damage, co-carcinogenesis, and direct activation of cellular growth pathways by constituents of smoke. Many studies demonstrate the involvement of these processes in tobacco carcinogenesis but the details by which they interact with the DNA damage pathways and their roles in specific cancers caused by tobacco products are still not fully understood.

4.5.2 Genetic polymorphisms

Multiple studies have been carried out on the role of genetic polymorphisms of xenobiotic metabolism in smoking-related carcinogenesis in humans. These studies have covered various cancer types, with lung cancer representing one of the most intensively studied. The polymorphic genes, their variant forms, and the genotype combinations investigated in these studies have similarly been numerous. In addition to the associations with increased risk of cancer, much data have accumulated on relationships between the polymorphisms and the various biomarkers of tobacco carcinogenesis in non-cancer control populations, whether smokers or non-smokers, in subjects with work-related exposure or in patients with other cancers.

Despite the massive body of research, many observations remain ambiguous. Some associations between genetic polymorphism and increased risk for cancer, such as for the *GSTM1* null genotype, alone or in combination with *CYP1A1* polymorphism, in lung cancer, or the *NAT2* slow acetylator genotype in bladder cancer and breast cancer appear stronger and more consistent, but not without controversies. Similarly, the data on the various biomarkers of tobacco-related carcinogenesis exhibit inconsistencies.

The variability in the data is at least partially likely due to differences between the studies in the genes and gene variants included (many of which are still of unknown functional or regulatory consequence), in the types of cancer studied, in levels and sources of exposure, in ethnic backgrounds, in sex, in histological types and in the features of the genome such as haplotype blocks and copy number variation resulting in linkage disequilibrium. In addition, gene-gene interactions and gene-environment interactions are likely to contribute to the discrepancies in current data. Mechanisms of tobacco-related carcinogenesis also involve genes from numerous other classes, such as those encoding for DNA repair proteins and many other regulators of cell cycle and growth. In addition; there are well described mechanistic pathways of carcinogenesis mediated via epigenetic alterations and genetic instability, to mention a few.

4.5.3 Site-specific mechanisms

The Working Group reviewed the mechanistic evidence relative to specific target sites for which there is sufficient evidence of carcinogenicity in humans, i.e. lung, oral cavity, oesophagus, larynx and nasopharynx, pancreas, stomach, liver, urinary bladder, leukaemia, cervix and ovary. Genotoxic effects have been found in eight organ sites at which tobacco smoke causes cancer in humans (DeMarini, 2004).

Sites with limited evidence of carcinogenicity or evidence suggesting lack of carcinogenicity in humans include the breast and the endothelium and relevant mechanisms are presented below.

Breast — There are several plausible mechanisms by which smoking may increase breast cancer risk, and some data support such an effect, including the increased risk among long-term smokers with *NAT2* slow genotype. Despite the overall lack of clear association in epidemiological studies, and the potential anti-estrogenic effects of smoking, the possibility that smoking increases breast cancer risk is biologically plausible.

Endothelium — The mechanisms by which cigarette smoking reduces the risk for endometrial cancer among current smokers, mainly among postmenopausal, remain unclear.

4.5.4 Interaction of ethanol and tobacco carcinogens

Data in rodents and non-human primates on the relationships between a) inhibition of hepatic clearance of nitrosamines by ethanol, b) the formation of promutagenic DNA adducts and c) tumours in extra-hepatic targets, likely also pertain in humans.

5. Evaluation

There is *sufficient evidence* in humans for the carcinogenicity of tobacco smoking.

Tobacco smoking causes cancers of the lung, oral cavity, naso-, oro- and hypopharynx, nasal cavity and accesory sinuses, larynx, oesophagus, stomach, pancreas, colorectum, liver, kidney (body and pelvis), ureter, urinary bladder, uterine cervix and ovary (mucinous), and myeloid leukaemia. Also, a positive association has been observed between tobacco smoking and cancer of the female breast. For cancers of the endometrium (post-menopausal) and of the thyroid, there is *evidence suggesting lack of carcinogenicity*.

There is *sufficient evidence* in humans for the carcinogenicity of parental smoking. Parental smoking causes hepatoblastoma in children. Also, a positive association has been observed between parental smoking and childhood leukaemia (particularly acute lymphocytic leukaemia).

There is *sufficient evidence* in experimental animals for the carcinogenicity of tobacco smoke and of tobacco smoke condensates.

Tobacco smoking is *carcinogenic to humans (Group 1)*.

References

Abdel-Rahman SZ, Ammenheuser MM, Ward JB Jr (2001). Human sensitivity to 1,3-butadiene: role of microsomal epoxide hydrolase polymorphisms. *Carcinogenesis*, 22: 415–423. doi:10.1093/carcin/22.3.415 PMID:11238181

Abdel-Rahman SZ, El-Zein RA, Ammenheuser MM *et al.* (2003). Variability in human sensitivity to 1,3-butadiene: Influence of the allelic variants of the microsomal epoxide hydrolase gene. *Environ Mol Mutagen*, 41: 140–146. doi:10.1002/em.10142 PMID:12605384

Abou-Alfa GK (2006). Hepatocellular carcinoma: molecular biology and therapy. *Semin Oncol*, 33: Suppl 11S79–S83. doi:10.1053/j.seminoncol.2006.10.015 PMID:17178294

Abrams JA, Terry MB, Neugut AI (2008). Cigarette smoking and the colorectal adenoma-carcinoma sequence. *Gastroenterology*, 134: 617–619. doi:10.1053/j.gastro.2007.12.015 PMID:18242224

Adami HO, Lund E, Bergström R, Meirik O (1988). Cigarette smoking, alcohol consumption and risk of breast cancer in young women. *Br J Cancer*, 58: 832–837. PMID:3224085

Adami J, Nyrén O, Bergström R *et al.* (1998). Smoking and the risk of leukemia, lymphoma, and multiple myeloma (Sweden). *Cancer Causes Control*, 9: 49–56. doi:10.1023/A:1008897203337 PMID:9486463

Agudo A, Sala N, Pera G *et al.* (2006). Polymorphisms in metabolic genes related to tobacco smoke and the risk of gastric cancer in the European prospective investigation into cancer and nutrition. *Cancer Epidemiol Biomarkers Prev*, 15: 2427–2434. doi:10.1158/1055-9965.EPI-06-0072 PMID:17164366

Agundez JA (2004). Cytochrome P450 gene polymorphism and cancer. *Curr Drug Metab*, 5: 211–224. doi:10.2174/1389200043335621 PMID:15180491

Ahern TP, Lash TL, Egan KM, Baron JA (2009). Lifetime tobacco smoke exposure and breast cancer incidence. *Cancer Causes Control*, 20: 1837–1844. doi:10.1007/s10552-009-9376-1 PMID:19533391

Ahrendt SA, Decker PA, Alawi EA *et al.* (2001). Cigarette smoking is strongly associated with mutation of the *K-ras* gene in patients with primary adenocarcinoma of the lung. *Cancer*, 92: 1525–1530. doi:10.1002/1097-0142(20010915)92:6<1525::AID-CNCR1478>3.0.CO;2-H PMID:11745231

Ahrens W, Jöckel KH, Patzak W, Elsner G (1991). Alcohol, smoking, and occupational factors in cancer of the larynx: a case-control study. *Am J Ind Med*, 20: 477–493. doi:10.1002/ajim.4700200404 PMID:1785612

Ahrens W, Timmer A, Vyberg M *et al.* (2007). Risk factors for extrahepatic biliary tract carcinoma in men: medical conditions and lifestyle: results from a European multicentre case-control study. *Eur J Gastroenterol Hepatol*,

19: 623–630. doi:10.1097/01.meg.0000243876.79325.a1 PMID:17625430

Akhmedkhanov A, Zeleniuch-Jacquotte A, Toniolo P (2001). Role of exogenous and endogenous hormones in endometrial cancer: review of the evidence and research perspectives. *Ann N Y Acad Sci*, 943: 296–315. PMID:11594550

Akhter M, Nishino Y, Nakaya N *et al.* (2007). Cigarette smoking and the risk of colorectal cancer among men: a prospective study in Japan. *Eur J Cancer Prev*, 16: 102–107. doi:10.1097/01.cej.0000228412.98847.bc PMID:17297385

Akiba S (1994). Analysis of cancer risk related to longitudinal information on smoking habits. *Environ Health Perspect*, 102: Suppl 815–19. PMID:7851325

Akiba S & Hirayama T (1990). Cigarette smoking and cancer mortality risk in Japanese men and women–results from reanalysis of the six-prefecture cohort study data. *Environ Health Perspect*, 87: 19–26. doi:10.2307/3431001 PMID:2269225

Aksoy M (1989). Hematotoxicity and carcinogenicity of benzene. *Environ Health Perspect*, 82: 193–197. doi:10.2307/3430775 PMID:2676498

Al-Delaimy WK (2002). Hair as a biomarker for exposure to tobacco smoke. *Tob Control*, 11: 176–182. doi:10.1136/tc.11.3.176 PMID:12198265

Al-Delaimy WK, Cho E, Chen WY *et al.* (2004). A prospective study of smoking and risk of breast cancer in young adult women. *Cancer Epidemiol Biomarkers Prev*, 13: 398–404. PMID:15006915

Al-Delaimy WK & Willett WC (2008). Measurement of tobacco smoke exposure: comparison of toenail nicotine biomarkers and self-reports. *Cancer Epidemiol Biomarkers Prev*, 17: 1255–1261. doi:10.1158/1055-9965.EPI-07-2695 PMID:18483348

Al-Zoughool M, Dossus L, Kaaks R *et al.* (2007). Risk of endometrial cancer in relationship to cigarette smoking: results from the EPIC study. *Int J Cancer*, 121: 2741–2747. doi:10.1002/ijc.22990 PMID:17657712

Alberg AJ, Daudt A, Huang HY *et al.* (2004). N-acetyltransferase 2 (*NAT2*) genotypes, cigarette smoking, and the risk of breast cancer. *Cancer Detect Prev*, 28: 187–193. doi:10.1016/j.cdp.2004.04.001 PMID:15225898

Alberg AJ, Kouzis A, Genkinger JM *et al.* (2007). A prospective cohort study of bladder cancer risk in relation to active cigarette smoking and household exposure to secondhand cigarette smoke. *Am J Epidemiol*, 165: 660–666. doi:10.1093/aje/kwk047 PMID:17204516

Alexandrie AK, Warholm M, Carstensen U *et al.* (2000). CYP1A1 and GSTM1 polymorphisms affect urinary 1-hydroxypyrene levels after PAH exposure. *Carcinogenesis*, 21: 669–676. doi:10.1093/carcin/21.4.669 PMID:10753202

Alexandrov K, Cascorbi I, Rojas M *et al.* (2002). CYP1A1 and GSTM1 genotypes affect benzo[a]pyrene DNA adducts in smokers' lung: comparison with aromatic/hydrophobic adduct formation. *Carcinogenesis*, 23: 1969–1977. doi:10.1093/carcin/23.12.1969 PMID:12507920

Alexandrov K, Rojas M, Kadlubar FF *et al.* (1996). Evidence of *anti*-benzo[*a*]pyrene diolepoxide-DNA adduct formation in human colon mucosa. *Carcinogenesis*, 17: 2081–2083. doi:10.1093/carcin/17.9.2081 PMID:8824539

Alfano CM, Klesges RC, Murray DM *et al.* (2004). Physical activity in relation to all-site and lung cancer incidence and mortality in current and former smokers. *Cancer Epidemiology, Biomarkers & Prevention*, 13: 2233–2241.

Alguacil J & Silverman DT (2004). Smokeless and other noncigarette tobacco use and pancreatic cancer: a case-control study based on direct interviews. *Cancer Epidemiol Biomarkers Prev*, 13: 55–58. doi:10.1158/1055-9965.EPI-03-0033 PMID:14744733

Ambrosone CB (2001). Impact of genetics on the relationship between smoking and breast cancer risk. *Journal of Women's Cancer*, 3: 17–22.

Ambrosone CB, Freudenheim JL, Graham S *et al.* (1996). Cigarette smoking, N-acetyltransferase 2 genetic polymorphisms, and breast cancer risk. *JAMA*, 276: 1494–1501. doi:10.1001/jama.276.18.1494 PMID:8903261

Ambrosone CB, Kropp S, Yang J *et al.* (2008). Cigarette smoking, *N-acetyltransferase 2* genotypes, and breast cancer risk: pooled analysis and meta-analysis. *Cancer Epidemiol Biomarkers Prev*, 17: 15–26. doi:10.1158/1055-9965.EPI-07-0598 PMID:18187392

Ambrosone CB, Shields PG (1999). *Smoking as a risk factor for breast cancer.* In: *Breast cancer: molecular genetics, pathogenesis, and therapeutics*. Bowcock AM, editor. Tetowa, NJ: Humana Press, pp. 519–536,.

Amos CI, Wu X, Broderick P *et al.* (2008). Genome-wide association scan of tag SNPs identifies a susceptibility locus for lung cancer at 15q25.1. *Nat Genet*, 40: 616–622. doi:10.1038/ng.109 PMID:18385676

Andersen V, Agerstjerne L, Jensen D *et al.* (2009). The multidrug resistance 1 (MDR1) gene polymorphism G-rs3789243-A is not associated with disease susceptibility in Norwegian patients with colorectal adenoma and colorectal cancer; a case control study. *BMC Med Genet*, 10: 18 doi:10.1186/1471-2350-10-18 PMID:19250544

Anderson JC, Attam R, Alpern Z *et al.* (2003). Prevalence of colorectal neoplasia in smokers. *Am J Gastroenterol*, 98: 2777–2783. doi:10.1111/j.1572-0241.2003.08671.x PMID:14687832

Anderson LM (1992). *Modulation of nitrosamine metabolism by ethanol: implications for cancer risk.* In: *Alcohol and Cancer.* Watson RR, editor. Boca Raton, FL: CRC Press, pp. 17–54.

Anderson LM, Souliotis VL, Chhabra SK *et al.* (1996). *N*-nitrosodimethylamine-derived O^6-methylguanine in DNA of monkey gastrointestinal and urogenital organs

and enhancement by ethanol. *Int J Cancer*, 66: 130–134. doi:10.1002/(SICI)1097-0215(19960328)66:1<130::AID-IJC22>3.0.CO;2-G PMID:8608956

Ansary-Moghaddam A, Huxley R, Barzi F *et al.*Asia Pacific Cohort Studies Collaboration (2006). The effect of modifiable risk factors on pancreatic cancer mortality in populations of the Asia-Pacific region. *Cancer Epidemiol Biomarkers Prev*, 15: 2435–2440. doi:10.1158/1055-9965.EPI-06-0368 PMID:17164367

Applebaum KM, Furniss CS, Zeka A *et al.* (2007). Lack of association of alcohol and tobacco with HPV16-associated head and neck cancer. *J Natl Cancer Inst*, 99: 1801–1810. doi:10.1093/jnci/djm233 PMID:18042931

Appleby P, Beral V, Berrington de González A *et al.* International Collaboration of Epidemiological Studies of Cervical Cancer (2006). Carcinoma of the cervix and tobacco smoking: collaborative reanalysis of individual data on 13,541 women with carcinoma of the cervix and 23,017 women without carcinoma of the cervix from 23 epidemiological studies. *Int J Cancer*, 118: 1481–1495. doi:10.1002/ijc.21493 PMID:16206285

Arif JM, Dresler C, Clapper ML *et al.* (2006). Lung DNA adducts detected in human smokers are unrelated to typical polyaromatic carcinogens. *Chem Res Toxicol*, 19: 295–299. doi:10.1021/tx0502443 PMID:16485906

Armstrong RW, Imrey PB, Lye MS *et al.* (2000). Nasopharyngeal carcinoma in Malaysian Chinese: occupational exposures to particles, formaldehyde and heat. *Int J Epidemiol*, 29: 991–998. doi:10.1093/ije/29.6.991 PMID:11101539

Ashktorab H, Begum R, Akhgar A *et al.* (2007). Folate status and risk of colorectal polyps in African Americans. *Dig Dis Sci*, 52: 1462–1470. doi:10.1007/s10620-006-9236-8 PMID:17372834

Asma S, Bettcher DW, Samet J *et al.* (2009). *Tobacco*. In: *Oxford Textbook Of Public Health*. Detels R, editor. Oxford: Oxford University Press.

Ateş NA, Tamer L, Ateş C *et al.* (2005). Glutathione S-transferase M1, T1, P1 genotypes and risk for development of colorectal cancer. *Biochem Genet*, 43: 149–163. doi:10.1007/s10528-005-1508-z PMID:15932063

Austin H, Drews C, Partridge EE (1993). A case-control study of endometrial cancer in relation to cigarette smoking, serum estrogen levels, and alcohol use. *Am J Obstet Gynecol*, 169: 1086–1091. PMID:8238164

Autrup H (2000). Genetic polymorphisms in human xenobiotica metabolizing enzymes as susceptibility factors in toxic response. *Mutat Res*, 464: 65–76. PMID:10633178

Aze Y, Toyoda K, Furukawa F *et al.* (1993). Enhancing effect of ethanol on esophageal tumor development in rats by initiation of diethylnitrosamine. *Carcinogenesis*, 14: 37–40. doi:10.1093/carcin/14.1.37 PMID:8425269

Bain C, Feskanich D, Speizer FE *et al.* (2004). Lung cancer rates in men and women with comparable histories of smoking. *J Natl Cancer Inst*, 96: 826–834. PMID:15173266

Baker JA, Odunuga OO, Rodabaugh KJ *et al.* (2006). Active and passive smoking and risk of ovarian cancer. *Int J Gynecol Cancer*, 16: Suppl 1211–218. doi:10.1111/j.1525-1438.2006.00473.x PMID:16515593

Balansky R, Ganchev G, Iltcheva M *et al.* (2007). Potent carcinogenicity of cigarette smoke in mice exposed early in life. *Carcinogenesis*, 28: 2236–2243. doi:10.1093/carcin/bgm122 PMID:17522065

Balaram P, Sridhar H, Rajkumar T *et al.* (2002). Oral cancer in southern India: the influence of smoking, drinking, paan-chewing and oral hygiene. *Int J Cancer*, 98: 440–445. doi:10.1002/ijc.10200 PMID:11920597

Bamber DE, Fryer AA, Strange RC *et al.* (2001). Phenol sulphotransferase *SULT1A1*1* genotype is associated with reduced risk of colorectal cancer. *Pharmacogenetics*, 11: 679–685. doi:10.1097/00008571-200111000-00006 PMID:11692076

Band PR, Le ND, Fang R, Deschamps M (2002). Carcinogenic and endocrine disrupting effects of cigarette smoke and risk of breast cancer. *Lancet*, 360: 1044–1049. doi:10.1016/S0140-6736(02)11140-8 PMID:12383984

Baron JA (1984). Smoking and estrogen-related disease. *Am J Epidemiol*, 119: 9–22. PMID:6362403

Baron JA, Byers T, Greenberg ER *et al.* (1986). Cigarette smoking in women with cancers of the breast and reproductive organs. *J Natl Cancer Inst*, 77: 677–680. PMID:3462409

Baron JA, La Vecchia C, Levi F (1990). The antiestrogenic effect of cigarette smoking in women. *Am J Obstet Gynecol*, 162: 502–514. PMID:2178432

Baron JA, Newcomb PA, Longnecker MP *et al.* (1996). Cigarette smoking and breast cancer. *Cancer Epidemiol Biomarkers Prev*, 5: 399–403. PMID:9162307

Barra S, Barón AE, Franceschi S *et al.* (1991). Cancer and non-cancer controls in studies on the effect of tobacco and alcohol consumption. *Int J Epidemiol*, 20: 845–851. doi:10.1093/ije/20.4.845 PMID:1800421

Bartsch H, Nair U, Risch A *et al.* (2000). Genetic polymorphism of *CYP* genes, alone or in combination, as a risk modifier of tobacco-related cancers. *Cancer Epidemiol Biomarkers Prev*, 9: 3–28. PMID:10667460

Batty GD, Kivimaki M, Gray L *et al.* (2008). Cigarette smoking and site-specific cancer mortality: testing uncertain associations using extended follow-up of the original Whitehall study. *Ann Oncol*, 19: 996–1002. doi:10.1093/annonc/mdm578 PMID:18212091

Baumgartner KB, Schlierf TJ, Yang D *et al.* (2009). N-acetyltransferase 2 genotype modification of active cigarette smoking on breast cancer risk among hispanic and non-hispanic white women. *Toxicol Sci*, 112: 211–220. PMID:19692670

Belinsky SA (2004). Gene-promoter hypermethylation as a biomarker in lung cancer. *Nat Rev Cancer*, 4: 707–717. PMID:15343277

Belinsky SA (2005). Silencing of genes by promoter hypermethylation: key event in rodent and human lung cancer. *Carcinogenesis*, 26: 1481–1487. doi:10.1093/carcin/bgi020 PMID:15661809

Belinsky SA, Klinge DM, Dekker JD *et al.* (2005). Gene promoter methylation in plasma and sputum increases with lung cancer risk. *Clin Cancer Res*, 11: 6505–6511. PMID:16166426

Belinsky SA, Liechty KC, Gentry FD *et al.* (2006). Promoter hypermethylation of multiple genes in sputum precedes lung cancer incidence in a high-risk cohort. *Cancer Res*, 66: 3338–3344. PMID:16540689

Benhamou S, Lee WJ, Alexandrie AK *et al.* (2002). Meta- and pooled analyses of the effects of glutathione *S*-transferase M1 polymorphisms and smoking on lung cancer risk. *Carcinogenesis*, 23: 1343–1350. doi:10.1093/carcin/23.8.1343 PMID:12151353

Beral V, Bull D, Reeves GMillion Women Study Collaborators (2005). Endometrial cancer and hormone-replacement therapy in the Million Women Study. *Lancet*, 365: 1543–1551. doi:10.1016/S0140-6736(05)66455-0 PMID:15866308

Bergmark E (1997). Hemoglobin adducts of acrylamide and acrylonitrile in laboratory workers, smokers and nonsmokers. *Chem Res Toxicol*, 10: 78–84. doi:10.1021/tx960113p PMID:9074806

Bernfeld P, Homburger F, Russfield AB (1974). Strain differences in the response of inbred Syrian hamsters to cigarette smoke inhalation. *J Natl Cancer Inst*, 53: 1141–1157. PMID:4279301

Bernfeld P, Homburger F, Soto E, Pai KJ (1979). Cigarette smoke inhalation studies in inbred Syrian golden hamsters. *J Natl Cancer Inst*, 63: 675–689. PMID:288930

Berrettini W, Yuan X, Tozzi F *et al.* (2008). Alpha-5/alpha-3 nicotinic receptor subunit alleles increase risk for heavy smoking. *Mol Psychiatry*, 13: 368–373. doi:10.1038/sj.mp.4002154 PMID:18227835

Berrington de González A, Sweetland S, Green J (2004). Comparison of risk factors for squamous cell and adenocarcinomas of the cervix: a meta-analysis. *Br J Cancer*, 90: 1787–1791. PMID:15150591

Berstein LM, Tsyrlina EV, Kolesnik OS *et al.* (2000). Catecholestrogens excretion in smoking and nonsmoking postmenopausal women receiving estrogen replacement therapy. *J Steroid Biochem Mol Biol*, 72: 143–147. doi:10.1016/S0960-0760(00)00038-8 PMID:10775805

Berta L, Fortunati N, Gennari P *et al.* (1991). Influence of cigarette smoking on pituitary and sex hormone balance in healthy premenopausal women. *Fertil Steril*, 56: 788–789. PMID:1915961

Berta L, Frairia R, Fortunati N *et al.* (1992). Smoking effects on the hormonal balance of fertile women. *Horm Res*, 37: 45–48. doi:10.1159/000182280 PMID:1398476

Besson H, Brennan P, Becker N *et al.* (2006). Tobacco smoking, alcohol drinking and Hodgkin's lymphoma: A European multicenter case-control study (Epilymph). *Br J Cancer*, 95: 378–384.

Bhutani M, Pathak AK, Fan YH *et al.* (2008). Oral epithelium as a surrogate tissue for assessing smoking-induced molecular alterations in the lungs. *Cancer Prev Res (Phila)*, 1: 39–44. PMID:19138934

Bin P, Leng S, Cheng J *et al.* (2008). Association of aryl hydrocarbon receptor gene polymorphisms and urinary 1-hydroxypyrene in polycyclic aromatic hydrocarbon-exposed workers. *Cancer Epidemiol Biomarkers Prev*, 17: 1702–1708. doi:10.1158/1055-9965.EPI-07-2812 PMID:18628420

Bjelke E (1975). Dietary vitamin A and human lung cancer. *Int J Cancer*, 15: 561–565. doi:10.1002/ijc.2910150405 PMID:1140863

Bjerregaard BK, Raaschou-Nielsen O, Sørensen M *et al.* (2006). Tobacco smoke and bladder cancer–in the European Prospective Investigation into Cancer and Nutrition. *Int J Cancer*, 119: 2412–2416. doi:10.1002/ijc.22169 PMID:16894557

Bjørge T, Engeland A, Tretli S, Weiderpass E (2007). Body size in relation to cancer of the uterine corpus in 1 million Norwegian women. *Int J Cancer*, 120: 378–383. doi:10.1002/ijc.22260 PMID:17066451

Bleeker MC, Heideman DA, Snijders PJ *et al.* (2009). Penile cancer: epidemiology, pathogenesis and prevention. *World J Urol*, 27: 141–150. doi:10.1007/s00345-008-0302-z PMID:18607597

Blot WJ, Devesa SS, Kneller RW, Fraumeni JF Jr (1991). Rising incidence of adenocarcinoma of the esophagus and gastric cardia. *JAMA*, 265: 1287–1289. doi:10.1001/jama.265.10.1287 PMID:1995976

Blot WJ, McLaughlin JK, Winn DM *et al.* (1988). Smoking and drinking in relation to oral and pharyngeal cancer. *Cancer Res*, 48: 3282–3287. PMID:3365707

Boccia S, Sayed-Tabatabaei FA, Persiani R *et al.* (2007). Polymorphisms in metabolic genes, their combination and interaction with tobacco smoke and alcohol consumption and risk of gastric cancer: a case-control study in an Italian population. *BMC Cancer*, 7: 206 PMID:17996038

Bode AM & Dong Z (2005). Signal transduction pathways in cancer development and as targets for cancer prevention. *Prog Nucleic Acid Res Mol Biol*, 79: 237–297. PMID:16096030

Boffetta P, Clark S, Shen M *et al.* (2006). Serum cotinine level as predictor of lung cancer risk. *Cancer Epidemiol Biomarkers Prev*, 15: 1184–1188. doi:10.1158/1055-9965.EPI-06-0032 PMID:16775179

Boffetta P, Hecht SS, Gray N *et al.* (2008). Smokeless tobacco and cancer. *Lancet Oncol*, 9: 667–675. doi:10.1016/S1470-2045(08)70173-6 PMID:18598931

Bogen E (1929). The composition of cigarets and cigaret smoke. *Journal of the American Medical Association*, 93: 1110–1114.

Bolt HM & Thier R (2006). Relevance of the deletion polymorphisms of the glutathione *S*-transferases *GSTT1* and *GSTM1* in pharmacology and toxicology. *Curr Drug Metab*, 7: 613–628. doi:10.2174/138920006778017786 PMID:16918316

Bono R, Vincenti M, Saglia U *et al.* (2002). Tobacco smoke and formation of *N*-(2-hydroxyethyl)valine in human hemoglobin. *Arch Environ Health*, 57: 416–421. doi:10.1080/00039890209601430 PMID:12641182

Borgerding M & Klus H (2005). Analysis of complex mixtures–cigarette smoke. *Exp Toxicol Pathol*, 57: Suppl 143–73. doi:10.1016/j.etp.2005.05.010 PMID:16092717

Borlak J & Reamon-Buettner SM (2006). N-acetyltransferase 2 (NAT2) gene polymorphisms in colon and lung cancer patients. *BMC Med Genet*, 7: 58 doi:10.1186/1471-2350-7-58 PMID:16827944

Bosetti C, Gallus S, Peto R *et al.* (2008). Tobacco smoking, smoking cessation, and cumulative risk of upper aerodigestive tract cancers. *Am J Epidemiol*, 167: 468–473. doi:10.1093/aje/kwm318 PMID:18056925

Bosetti C, Garavello W, Gallus S, La Vecchia C (2006). Effects of smoking cessation on the risk of laryngeal cancer: an overview of published studies. *Oral Oncol*, 42: 866–872. doi:10.1016/j.oraloncology.2006.02.008 PMID:16931120

Bosetti C, Negri E, Franceschi S *et al.* (2000a). Risk factors for oral and pharyngeal cancer in women: a study from Italy and Switzerland. *Br J Cancer*, 82: 204–207. PMID:10638990

Botteri E, Iodice S, Bagnardi V *et al.* (2008a). Smoking and colorectal cancer: a meta-analysis. *JAMA*, 300: 2765–2778. doi:10.1001/jama.2008.839 PMID:19088354

Botteri E, Iodice S, Raimondi S *et al.* (2008b). Cigarette smoking and adenomatous polyps: a meta-analysis. *Gastroenterology*, 134: 388–395. doi:10.1053/j.gastro.2007.11.007 PMID:18242207

Boyd AS, Shyr Y, King LE Jr (2002). Basal cell carcinoma in young women: an evaluation of the association of tanning bed use and smoking. *J Am Acad Dermatol*, 46: 706–709. doi:10.1067/mjd.2002.120467 PMID:12004311

Boyle P, Maisonneuve P, Bueno de Mesquita B *et al.* (1996). Cigarette smoking and pancreas cancer: a case control study of the search programme of the IARC. *Int J Cancer*, 67: 63–71. doi:10.1002/(SICI)1097-0215(19960703)67:1<63::AID-IJC12>3.0.CO;2-D PMID:8690527

Boysen G & Hecht SS (2003). Analysis of DNA and protein adducts of benzo[a]pyrene in human tissues using structure-specific methods. *Mutat Res*, 543: 17–30. doi:10.1016/S1383-5742(02)00068-6 PMID:12510015

Bracci PM & Holly EA (2005). Tobacco use and non-Hodgkin lymphoma: results from a population-based case-control study in the San Francisco Bay Area, California. *Cancer Causes Control*, 16: 333–346.

Bradlow HL, Telang NT, Sepkovic DW, Osborne MP (1996). 2-hydroxyestrone: the 'good' estrogen. *J Endocrinol*, 150: SupplS259–S265. PMID:8943806

Breast Cancer Family Registry; Kathleen Cuningham Consortium for Research into Familial Breast Cancer (Australasia) Ontario Cancer Genetics Network (Canada) (2008). Smoking and risk of breast cancer in carriers of mutations in BRCA1 or BRCA2 aged less than 50 years. *Breast Cancer Res Treat*, 109: 67–75. doi:10.1007/s10549-007-9621-9 PMID:17972172

Bremnes Y, Ursin G, Bjurstam N, Gram IT (2007). Different measures of smoking exposure and mammographic density in postmenopausal Norwegian women: a cross-sectional study. *Breast Cancer Res*, 9: R73 doi:10.1186/bcr1782 PMID:17963507

Brennan P, Hsu CC, Moullan N *et al.* (2005). Effect of cruciferous vegetables on lung cancer in patients stratified by genetic status: a mendelian randomisation approach. *Lancet*, 366: 1558–1560. PMID:16257343

Brennan P, van der Hel O, Moore LE *et al.* (2008). Tobacco smoking, body mass index, hypertension, and kidney cancer risk in central and eastern Europe. *Br J Cancer*, 99: 1912–1915. doi:10.1038/sj.bjc.6604761 PMID:19034282

Brenner H, Arndt V, Bode G *et al.* (2002). Risk of gastric cancer among smokers infected with Helicobacter pylori. *Int J Cancer*, 98: 446–449. doi:10.1002/ijc.10201 PMID:11920598

Brinton LA, Barrett RJ, Berman ML *et al.* (1993). Cigarette smoking and the risk of endometrial cancer. *Am J Epidemiol*, 137: 281–291. PMID:8452136

Brinton LA, Blot WJ, Becker JA *et al.* (1984). A case-control study of cancers of the nasal cavity and paranasal sinuses. *Am J Epidemiol*, 119: 896–906. PMID:6731431

Brinton LA, Schairer C, Stanford JL, Hoover RN (1986). Cigarette smoking and breast cancer. *Am J Epidemiol*, 123: 614–622. PMID:3953540

Brooks DR, Palmer JR, Strom BL, Rosenberg L (2003). Menthol cigarettes and risk of lung cancer. *Am J Epidemiol*, 158: 609–616, discussion 617–620. doi:10.1093/aje/kwg182 PMID:14507595

Brown LM, Blot WJ, Schuman SH *et al.* (1988). Environmental factors and high risk of esophageal cancer among men in coastal South Carolina. *J Natl Cancer Inst*, 80: 1620–1625. doi:10.1093/jnci/80.20.1620 PMID:3193480

Brown LM, Hoover R, Silverman D *et al.* (2001). Excess incidence of squamous cell esophageal cancer among US Black men: role of social class and other risk factors.

Am J Epidemiol, 153: 114–122. doi:10.1093/aje/153.2.114 PMID:11159155

Brown LM, Hoover RN, Greenberg RS *et al.* (1994a). Are racial differences in squamous cell esophageal cancer explained by alcohol and tobacco use? *J Natl Cancer Inst*, 86: 1340–1345. doi:10.1093/jnci/86.17.1340 PMID:8064893

Brown LM, Silverman DT, Pottern LM *et al.* (1994b). Adenocarcinoma of the esophagus and esophagogastric junction in white men in the United States: alcohol, tobacco, and socioeconomic factors. *Cancer Causes Control*, 5: 333–340. doi:10.1007/BF01804984 PMID:8080945

Brugere J, Guenel P, Leclerc A, Rodriguez J (1986). Differential effects of tobacco and alcohol in cancer of the larynx, pharynx, and mouth. *Cancer*, 57: 391–395. doi:10.1002/1097-0142(19860115)57:2<391::AID-CNCR2820570235>3.0.CO;2-Q PMID:3942973

Brunet JS, Ghadirian P, Rebbeck TR *et al.* (1998). Effect of smoking on breast cancer in carriers of mutant BRCA1 or BRCA2 genes. *J Natl Cancer Inst*, 90: 761–766. doi:10.1093/jnci/90.10.761 PMID:9605646

Bufalo NE, Leite JL, Guilhen ACT *et al.* (2006). Smoking and susceptibility to thyroid cancer: an inverse association with CYP1A1 allelic variants. *Endocr Relat Cancer*, 13: 1185–1193. doi:10.1677/ERC-06-0002 PMID:17158763

Bundgaard T, Wildt J, Frydenberg M *et al.* (1995). Case-control study of squamous cell cancer of the oral cavity in Denmark. *Cancer Causes Control*, 6: 57–67. doi:10.1007/BF00051681 PMID:7718736

Burchell B (2003). Genetic variation of human UDP-glucuronosyltransferase: implications in disease and drug glucuronidation. *Am J Pharmacogenomics*, 3: 37–52. PMID:12562215

Butler LM, Gold EB, Greendale GA *et al.* (2008). Menstrual and reproductive factors in relation to mammographic density: the Study of Women's Health Across the Nation (SWAN). *Breast Cancer Res Treat*, 112: 165–174. doi:10.1007/s10549-007-9840-0 PMID:18066689

Butler LM, Wang R, Wong AS *et al.* (2009). Cigarette smoking and risk of prostate cancer among Singapore Chinese. *Cancer Causes Control*, 20: 1967–1974.

Calaf G & Russo J (1993). Transformation of human breast epithelial cells by chemical carcinogens. *Carcinogenesis*, 14:483–492. doi:10.1093/carcin/14.3.483 PMID:8453725

California Environmental Protection Agency (2005). *Health effects assessment for ETS: final*. Sacramento, CA: California Environmental Protection Agency, Office of Environmental Health Hazard Assesment.

Calle EE, Miracle-McMahill HL, Thun MJ, Heath CW Jr (1994). Cigarette smoking and risk of fatal breast cancer. *Am J Epidemiol*, 139: 1001–1007. PMID:8178779

Campbell PT, Curtin K, Ulrich CM *et al.* (2009). Mismatch repair polymorphisms and risk of colon cancer, tumour microsatellite instability and interactions with lifestyle factors. *Gut*, 58: 661–667. doi:10.1136/gut.2007.144220 PMID:18523027

Campos F, Carrasquilla G, Koriyama C *et al.* (2006). Risk factors of gastric cancer specific for tumor location and histology in Cali, Colombia. *World J Gastroenterol*, 12: 5772–5779. PMID:17007041

Canova C, Hashibe M, Simonato L *et al.* (2009). Genetic associations of 115 polymorphisms with cancers of the upper aerodigestive tract across 10 European countries: the ARCAGE project. *Cancer Res*, 69: 2956–2965. doi:10.1158/0008-5472.CAN-08-2604 PMID:19339270

Cao SM, Liu Q, Huang TB (2000). Analysis for risk factors of nasopharyngeal carcinoma in Sihui city. [chinese] *Cancer*, 19: 987–989.

Cao W, Cai L, Rao JY *et al.* (2005). Tobacco smoking, GSTP1 polymorphism, and bladder carcinoma. *Cancer*, 104: 2400–2408. doi:10.1002/cncr.21446 PMID:16240451

Caplan LS, Hall HI, Levine RS, Zhu K (2000). Preventable risk factors for nasal cancer. *Ann Epidemiol*, 10: 186–191. doi:10.1016/S1047-2797(99)00049-6 PMID:10813512

Cardoso MA, Maruta LM, Kida AA *et al.* (2002). Micronutrients and the risk of colorectal adenomas: a case-control study in São Paulo, Brazil. *IARC Sci Publ*, 156: 361–363. PMID:12484206

Carlsten C, Sagoo GS, Frodsham AJ *et al.* (2008). Glutathione *S*-transferase M1 (*GSTM1*) polymorphisms and lung cancer: a literature-based systematic HuGE review and meta-analysis. *Am J Epidemiol*, 167: 759–774. doi:10.1093/aje/kwm383 PMID:18270371

Carmella SG, Chen M, Han S *et al.* (2009). Effects of smoking cessation on eight urinary tobacco carcinogen and toxicant biomarkers. *Chem Res Toxicol*, 22: 734–741. doi:10.1021/tx800479s PMID:19317515

Carmella SG, Chen M, Villalta PW *et al.* (2002). Ethylation and methylation of hemoglobin in smokers and nonsmokers. *Carcinogenesis*, 23: 1903–1910. doi:10.1093/carcin/23.11.1903 PMID:12419839

Carpenter CL, Jarvik ME, Morgenstern H *et al.* (1999). Mentholated cigarette smoking and lung-cancer risk. *Ann Epidemiol*, 9: 114–120. doi:10.1016/S1047-2797(98)00042-8 PMID:10037555

Cascorbi I, Brockmöller J, Roots I (1996). A C4887A polymorphism in exon 7 of human *CYP1A1*: population frequency, mutation linkages, and impact on lung cancer susceptibility. *Cancer Res*, 56: 4965–4969. PMID:8895751

Cassidenti DL, Pike MC, Vijod AG *et al.* (1992). A reevaluation of estrogen status in postmenopausal women who smoke. *Am J Obstet Gynecol*, 166: 1444–1448. PMID:1534446

Cassidenti DL, Vijod AG, Vijod MA *et al.* (1990). Short-term effects of smoking on the pharmacokinetic profiles of micronized estradiol in postmenopausal women. *Am J Obstet Gynecol*, 163: 1953–1960. PMID:2256508

Castellsagué X, Muñoz N, De Stefani E *et al.* (1999). Independent and joint effects of tobacco smoking and

alcohol drinking on the risk of esophageal cancer in men and women. *Int J Cancer*, 82: 657–664. doi:10.1002/(SICI)1097-0215(19990827)82:5<657::AID-IJC7>3.0.CO;2-C PMID:10417762

Castellsagué X, Quintana MJ, Martínez MC *et al.* (2004). The role of type of tobacco and type of alcoholic beverage in oral carcinogenesis. *Int J Cancer*, 108: 741–749. doi:10.1002/ijc.11627 PMID:14696101

Cauley JA, Gutai JP, Kuller LH *et al.* (1989). The epidemiology of serum sex hormones in postmenopausal women. *Am J Epidemiol*, 129: 1120–1131. PMID:2729251

Cavalieri E, Frenkel K, Liehr JG *et al.* (2000). Estrogens as endogenous genotoxic agents–DNA adducts and mutations. *J Natl Cancer Inst Monogr*, 2775–93. PMID:10963621

Cederbaum AI (2006). CYP2E1–biochemical and toxicological aspects and role in alcohol-induced liver injury. *Mt Sinai J Med*, 73: 657–672. PMID:16878272

Chameaud J, Perraud R, Chrétien J *et al.* (1982). Lung carcinogenesis during in vivo cigarette smoking and radon daughter exposure in rats. *Recent Results Cancer Res*, 82: 11–20. PMID:7111833

Chan AO, Jim MH, Lam KF *et al.* (2007). Prevalence of colorectal neoplasm among patients with newly diagnosed coronary artery disease. *JAMA*, 298: 1412–1419. PMID:17895457

Chang-Claude J, Kropp S, Jäger B *et al.* (2002). Differential effect of NAT2 on the association between active and passive smoke exposure and breast cancer risk. *Cancer Epidemiol Biomarkers Prev*, 11: 698–704. PMID:12163321

Chao A, Thun MJ, Jacobs EJ *et al.* (2000). Cigarette smoking and colorectal cancer mortality in the cancer prevention study II. *J Natl Cancer Inst*, 92: 1888–1896. doi:10.1093/jnci/92.23.1888 PMID:11106680

Chen B, Hu Y, Jin T *et al.* (2007). The influence of metabolic gene polymorphisms on urinary 1-hydroxypyrene concentrations in Chinese coke oven workers. *Sci Total Environ*, 381: 38–46. doi:10.1016/j.scitotenv.2007.02.021 PMID:17498780

Chen CC, Neugut AI, Rotterdam H (1994). Risk factors for adenocarcinomas and malignant carcinoids of the small intestine: preliminary findings. *Cancer Epidemiol Biomarkers Prev*, 3: 205–207. PMID:8019367

Cheng TJ, Christiani DC, Xu X *et al.* (1995). Glutathione *S*-transferase *mu* genotype, diet, and smoking as determinants of sister chromatid exchange frequency in lymphocytes. *Cancer Epidemiol Biomarkers Prev*, 4: 535–542. PMID:7549811

Cheng YJ, Hildesheim A, Hsu MM *et al.* (1999). Cigarette smoking, alcohol consumption and risk of nasopharyngeal carcinoma in Taiwan. *Cancer Causes Control*, 10: 201–207. doi:10.1023/A:1008893109257 PMID:10454065

Chhabra SK, Anderson LM, Perella C *et al.* (2000). Coexposure to ethanol with N-nitrosodimethylamine or 4-(Methylnitrosamino)-1-(3-pyridyl)-1-butanone during lactation of rats: marked increase in O^6-methylguanine-DNA adducts in maternal mammary gland and in suckling lung and kidney. *Toxicol Appl Pharmacol*, 169: 191–200. doi:10.1006/taap.2000.9068 PMID:11097872

Chia VM, Newcomb PA, Bigler J *et al.* (2006). Risk of microsatellite-unstable colorectal cancer is associated jointly with smoking and nonsteroidal anti-inflammatory drug use. *Cancer Res*, 66: 6877–6883. doi:10.1158/0008-5472.CAN-06-1535 PMID:16818666

Choi SY & Kahyo H (1991). Effect of cigarette smoking and alcohol consumption in the aetiology of cancer of the oral cavity, pharynx and larynx. *Int J Epidemiol*, 20: 878–885. doi:10.1093/ije/20.4.878 PMID:1800426

Chow WH, Hsing AW, McLaughlin JK, Fraumeni JF Jr (1996). Smoking and adrenal cancer mortality among United States veterans. *Cancer Epidemiol Biomarkers Prev*, 5: 79–80. PMID:8850265

Chow WH, Linet MS, McLaughlin JK *et al.* (1993b). Risk factors for small intestine cancer. *Cancer Causes Control*, 4: 163–169. doi:10.1007/BF00053158 PMID:8481495

Chow WH, McLaughlin JK, Hrubec Z *et al.* (1993a). Tobacco use and nasopharyngeal carcinoma in a cohort of US veterans. *Int J Cancer*, 55: 538–540. doi:10.1002/ijc.2910550403 PMID:8406978

Christmann M, Tomicic MT, Roos WP, Kaina B (2003). Mechanisms of human DNA repair: an update. *Toxicology*, 193: 3–34. doi:10.1016/S0300-483X(03)00287-7 PMID:14599765

Chu SY, Stroup NE, Wingo PA *et al.* (1990). Cigarette smoking and the risk of breast cancer. *Am J Epidemiol*, 131: 244–253. PMID:2404408

Church TR, Anderson KE, Caporaso NE *et al.* (2009). A prospectively measured serum biomarker for a tobacco-specific carcinogen and lung cancer in smokers. *Cancer Epidemiol Biomarkers Prev*, 18: 260–266. doi:10.1158/1055-9965.EPI-08-0718 PMID:19124507

Chyou PH, Nomura AM, Stemmermann GN (1995). Diet, alcohol, smoking and cancer of the upper aerodigestive tract: a prospective study among Hawaii Japanese men. *Int J Cancer*, 60: 616–621. doi:10.1002/ijc.2910600508 PMID:7860134

Cocco P, Moore PS, Ennas MG *et al.* (2007). Effect of urban traffic, individual habits, and genetic polymorphisms on background urinary 1-hydroxypyrene excretion. *Ann Epidemiol*, 17: 1–8. doi:10.1016/j.annepidem.2005.11.001 PMID:16406813

Colangelo LA, Gapstur SM, Gann PH, Dyer AR (2004). Cigarette smoking and colorectal carcinoma mortality in a cohort with long-term follow-up. *Cancer*, 100: 288–293. doi:10.1002/cncr.11923 PMID:14716762

Colilla S, Kantoff PW, Neuhausen SL *et al.* (2006). The joint effect of smoking and AIB1 on breast cancer risk in BRCA1 mutation carriers. *Carcinogenesis*, 27: 599–605. doi:10.1093/carcin/bgi246 PMID:16244359

Collishaw NE, Boyd NF, Cantor KP *et al.* (2009). *Canadian expert panel on tobacco smoke and breast cancer risk*. Toronto: Ontario Tobacco Research Unit.

Conway K, Edmiston SN, Cui L *et al.* (2002). Prevalence and spectrum of p53 mutations associated with smoking in breast cancer. *Cancer Res*, 62: 1987–1995. PMID:11929815

Cooper CS, Grover PL, Sims P (1983). *The metabolism and activation of benzo[a]pyrene*. In: *Progress in Drug Metabolism*, Vol. 7. Bridges JW, Chasseaud LF, editors. London: Wiley & Sons, pp. 295–396

Corona R, Dogliotti E, D'Errico M *et al.* (2001). Risk factors for basal cell carcinoma in a Mediterranean population: role of recreational sun exposure early in life. *Arch Dermatol*, 137: 1162–1168. PMID:11559211

Cortez CC & Jones PA (2008). Chromatin, cancer and drug therapies. *Mutat Res*, 647: 44–51. PMID:18691602

Cote ML, Chen W, Smith DW *et al.* (2009). Meta- and pooled analysis of *GSTP1* polymorphism and lung cancer: a HuGE-GSEC review. *Am J Epidemiol*, 169: 802–814. doi:10.1093/aje/kwn417 PMID:19240225

Cote ML, Kardia SL, Wenzlaff AS *et al.* (2005). Combinations of glutathione S-transferase genotypes and risk of early-onset lung cancer in Caucasians and African Americans: a population-based study. *Carcinogenesis*, 26: 811–819. doi:10.1093/carcin/bgi023 PMID:15661806

Couch FJ, Cerhan JR, Vierkant RA *et al.* (2001). Cigarette smoking increases risk for breast cancer in high-risk breast cancer families. *Cancer Epidemiol Biomarkers Prev*, 10: 327–332. PMID:11319172

Coughlin SS, Calle EE, Patel AV, Thun MJ (2000). Predictors of pancreatic cancer mortality among a large cohort of United States adults. *Cancer Causes Control*, 11: 915–923. doi:10.1023/A:1026580131793 PMID:11142526

Cozen W, Hamilton AS, Zhao P *et al.* (2009). A protective role for early oral exposures in the etiology of young adult Hodgkin lymphoma. *Blood*, 114: 4014–4020.

Crofts F, Cosma GN, Currie D *et al.* (1993). A novel CYP1A1 gene polymorphism in African-Americans. *Carcinogenesis*, 14: 1729–1731. doi:10.1093/carcin/14.9.1729 PMID:8104732

Cui Y, Miller AB, Rohan TE (2006). Cigarette smoking and breast cancer risk: update of a prospective cohort study. *Breast Cancer Res Treat*, 100: 293–299. doi:10.1007/s10549-006-9255-3 PMID:16773435

Curtin K, Samowitz WS, Wolff RK *et al.* (2009). Somatic alterations, metabolizing genes and smoking in rectal cancer. *Int J Cancer*, 125: 158–164. doi:10.1002/ijc.24338 PMID:19358278

Cybulski C, Masojc B, Oszutowska D *et al.* (2008). Constitutional CHEK2 mutations are associated with a decreased risk of lung and laryngeal cancers. *Carcinogenesis*, 29: 762–765. doi:10.1093/carcin/bgn044 PMID:18281249

D'Agostini F, Izzotti A, Balansky R *et al.* (2006). Early loss of Fhit in the respiratory tract of rodents exposed to environmental cigarette smoke. *Cancer Res*, 66: 3936–3941. doi:10.1158/0008-5472.CAN-05-3666 PMID:16585223

d'Errico A, Malats N, Vineis P, Boffetta P (1999). Review of studies of selected metabolic polymorphisms and cancer. *IARC Sci Publ*, 148: 323–393. PMID:10493265

Dagle GE, McDonald KE, Smith LG, Stevens DL Jr (1978). Pulmonary carcinogenesis in rats given implants of cigarette smoke condensate in beeswax pellets. *J Natl Cancer Inst*, 61: 905–910. PMID:278868

Dalbey WE, Nettesheim P, Griesemer R *et al.* (1980). Chronic inhalation of cigarette smoke by F344 rats. *J Natl Cancer Inst*, 64: 383–390. PMID:6928229

Daling JR, Madeleine MM, Johnson LG *et al.* (2005). Penile cancer: importance of circumcision, human papillomavirus and smoking in in situ and invasive disease. *Int J Cancer*, 116: 606–616. doi:10.1002/ijc.21009 PMID:15825185

Daling JR, Sherman KJ, Hislop TG *et al.* (1992). Cigarette smoking and the risk of anogenital cancer. *Am J Epidemiol*, 135: 180–189. PMID:1311142

Dally H, Edler L, Jäger B *et al.* (2003). The *CYP3A4*1B* allele increases risk for small cell lung cancer: effect of gender and smoking dose. *Pharmacogenetics*, 13: 607–618. doi:10.1097/00008571-200310000-00004 PMID:14515059

Daniell HW (1988). Increased lymph node metastases at mastectomy for breast cancer associated with host obesity, cigarette smoking, age, and large tumor size. *Cancer*, 62: 429–435. doi:10.1002/1097-0142(19880715)62:2<429::AID-CNCR2820620230>3.0.CO;2-4 PMID:3383142

Daniell HW, Tam E, Filice A (1993). Larger axillary metastases in obese women and smokers with breast cancer–an influence by host factors on early tumor behavior. *Breast Cancer Res Treat*, 25: 193–201. doi:10.1007/BF00689833 PMID:8369520

Darby S, Hill D, Auvinen A *et al.* (2005). Radon in homes and risk of lung cancer: collaborative analysis of individual data from 13 European case-control studies. *BMJ*, 330: 223 doi:10.1136/bmj.38308.477650.63 PMID:15613366

Davies MJ, Turner JG, Vives-Bauza C, Rumsby PC (1999). Investigation of mutant frequency at the *HPRT* locus and changes in microsatellite sequences in healthy young adults. *Mutat Res*, 431: 317–323. PMID:10635997

Davis BR, Whitehead JK, Gill ME *et al.* (1975). Response of rat lung to inhaled tobacco smoke with or without prior exposure to 3,4-benzpyrene (BP) given by intratracheal instillation. *Br J Cancer*, 31: 469–484. PMID:1156528

Day GL, Blot WJ, Austin DF *et al.* (1993). Racial differences in risk of oral and pharyngeal cancer: alcohol, tobacco, and other determinants. *J Natl Cancer Inst*, 85: 465–473. doi:10.1093/jnci/85.6.465 PMID:8445674

de Klerk NH, Musk AW, Eccles JL *et al.* (1996). Exposure to crocidolite and the incidence of different histological types of lung cancer. *Occup Environ Med*, 53: 157–159. doi:10.1136/oem.53.3.157 PMID:8704855

De Stefani E, Boffetta P, Deneo-Pellegrini H *et al.* (2007). The effect of smoking and drinking in oral and pharyngeal cancers: a case-control study in Uruguay. *Cancer Lett*, 246: 282–289. doi:10.1016/j.canlet.2006.03.008 PMID:16624486

De Stefani E, Boffetta P, Oreggia F *et al.* (1998). Smoking patterns and cancer of the oral cavity and pharynx: a case-control study in Uruguay. *Oral Oncol*, 34: 340–346. doi:10.1016/S1368-8375(98)00014-1 PMID:9861338

De Stefani E, Correa P, Oreggia F *et al.* (1987). Risk factors for laryngeal cancer. *Cancer*, 60: 3087–3091. doi:10.1002/1097-0142(19871215)60:12<3087::AID-CNCR2820601238>3.0.CO;2-6 PMID:3677031

De Stefani E, Espasandin J, Ronco A *et al.* (1995). Tobacco smoking and the risk of non-melanoma skin cancer. *Tob Control*, 4(2): 175–179. PMCID: PMC1759430 doi:10.1136/tc.4.2.175

De Stefani E, Muñoz N, Estève J *et al.* (1990). Mate drinking, alcohol, tobacco, diet, and esophageal cancer in Uruguay. *Cancer Res*, 50: 426–431. PMID:2295081

De Stefani E, Oreggia F, Rivero S, Fierro L (1992). Hand-rolled cigarette smoking and risk of cancer of the mouth, pharynx, and larynx. *Cancer*, 70: 679–682. doi:10.1002/1097-0142(19920801)70:3<679::AID-CNCR2820700323>3.0.CO;2-Z PMID:1623483

Delaney JC & Essigmann JM (2008). Biological properties of single chemical-DNA adducts: a twenty year perspective. *Chem Res Toxicol*, 21: 232–252. doi:10.1021/tx700292a PMID:18072751

Delfino RJ, Smith C, West JG *et al.* (2000). Breast cancer, passive and active cigarette smoking and N-acetyltransferase 2 genotype. *Pharmacogenetics*, 10: 461–469. doi:10.1097/00008571-200007000-00009 PMID:10898115

DeMarini DM (2004). Genotoxicity of tobacco smoke and tobacco smoke condensate: a review. *Mutat Res*, 567: 447–474. doi:10.1016/j.mrrev.2004.02.001 PMID:15572290

DeMarini DM, Gudi R, Szkudlinska A *et al.* (2008). Genotoxicity of 10 cigarette smoke condensates in four test systems: comparisons between assays and condensates. *Mutat Res*, 650: 15–29. PMID:18006367

DeMarini DM, Shelton ML, Levine JG (1995). Mutation spectrum of cigarette smoke condensate in Salmonella: comparison to mutations in smoking-associated tumors. *Carcinogenesis*, 16: 2535–2542. doi:10.1093/carcin/16.10.2535 PMID:7586163

Department of Health and Human Services (2001). *Women and Smoking: A Report of the Surgeon General.* Washington: Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health.

Department of Health and Human Services (2004). *The Health Consequences of Smoking: A Report of the Surgeon General.* Atlanta, Georgia: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health.

Devesa SS, Bray F, Vizcaino AP, Parkin DM (2005). International lung cancer trends by histologic type: male:female differences diminishing and adenocarcinoma rates rising. *Int J Cancer*, 117: 294–299. doi:10.1002/ijc.21183 PMID:15900604

Diergaarde B, Braam H, Vasen HF *et al.* (2007). Environmental factors and colorectal tumor risk in individuals with hereditary nonpolyposis colorectal cancer. *Clin Gastroenterol Hepatol*, 5: 736–742. PMID:17544999

Diergaarde B, Vrieling A, van Kraats AA *et al.* (2003). Cigarette smoking and genetic alterations in sporadic colon carcinomas. *Carcinogenesis*, 24: 565–571. doi:10.1093/carcin/24.3.565 PMID:12663519

Dikshit RP & Kanhere S (2000). Tobacco habits and risk of lung, oropharyngeal and oral cavity cancer: a population-based case-control study in Bhopal, India. *Int J Epidemiol*, 29: 609–614. doi:10.1093/ije/29.4.609 PMID:10922335

Dillner J, von Krogh G, Horenblas S, Meijer CJLM (2000). Etiology of squamous cell carcinoma of the penis. *Scand J Urol Nephrol Suppl*, 34: 189–193. doi:10.1080/00365590050509913 PMID:11144896

Ding L, Getz G, Wheeler DA *et al.* (2008). Somatic mutations affect key pathways in lung adenocarcinoma. *Nature*, 455: 1069–1075. doi:10.1038/nature07423 PMID:18948947

Djordjević D, Nikolić J, Stefanović V (1998). Ethanol interactions with other cytochrome P450 substrates including drugs, xenobiotics, and carcinogens. *Pathol Biol (Paris)*, 46: 760–770. PMID:9922992

Doll R, Gray R, Hafner B, Peto R (1980). Mortality in relation to smoking: 22 years' observations on female British doctors. *Br Med J*, 280: 967–971. doi:10.1136/bmj.280.6219.967 PMID:7417764

Doll R & Peto R (1978). Cigarette smoking and bronchial carcinoma: dose and time relationships among regular smokers and lifelong non-smokers. *J Epidemiol Community Health*, 32: 303–313. doi:10.1136/jech.32.4.303 PMID:744822

Doll R, Peto R (1985). *Effects on health of exposure to asbestos*. London: Health and Safety Commission.

Doll R, Peto R, Boreham J, Sutherland I (2005). Mortality from cancer in relation to smoking: 50 years observations on British doctors. *Br J Cancer*, 92: 426–429. PMID:15668706

Doll R, Peto R, Wheatley K *et al.* (1994). Mortality in relation to smoking: 40 years' observations on male British doctors. *BMJ*, 309: 901–911. PMID:7755693

Dong LM, Potter JD, White E *et al.* (2008). Genetic susceptibility to cancer: the role of polymorphisms in candidate genes. *JAMA*, 299: 2423–2436. doi:10.1001/jama.299.20.2423 PMID:18505952

Dontenwill W, Chevalier HJ, Harke HP *et al.* (1973). Investigations on the effects of chronic cigarette-smoke inhalation in Syrian golden hamsters. *J Natl Cancer Inst*, 51: 1781–1832. PMID:4765388

Dontenwill W, Chevalier HJ, Harke HP *et al.* (1977). Untersuchungen über den Effekt der chronischen Zigarettenrauchinhalation beim syrischen Goldhamster und über die Bedeutung des Vitamin A auf die bei Berauchung gefundenen Organveränderungen *Z Krebsforsch Klin Onkol Cancer Res Clin Oncol*, 89: 153–180. doi:10.1007/BF00308516 PMID:143143

Du X, Squier CA, Kremer MJ, Wertz PW (2000). Penetration of N-nitrosonornicotine (NNN) across oral mucosa in the presence of ethanol and nicotine. *J Oral Pathol Med*, 29: 80–85. doi:10.1034/j.1600-0714.2000.290205.x PMID:10718403

Duell EJ, Holly EA, Bracci PM *et al.* (2002). A population-based, case-control study of polymorphisms in carcinogen-metabolizing genes, smoking, and pancreatic adenocarcinoma risk. *J Natl Cancer Inst*, 94: 297–306. PMID:11854392

Dunn SR, Simenhoff ML, Lele PS *et al.* (1990). *N*-nitrosodimethylamine blood levels in patients with chronic renal failure: modulation of levels by ethanol and ascorbic acid. *J Natl Cancer Inst*, 82: 783–787. doi:10.1093/jnci/82.9.783 PMID:2325149

Dusek L, Abrahamova J, Lakomy R *et al.* (2008). Multivariate analysis of risk factors for testicular cancer: a hospital-based case-control study in the Czech Republic. *Neoplasma*, 55: 356–368. PMID:18505349

Dyke GW, Craven JL, Hall R, Garner RC (1992). Smoking-related DNA adducts in human gastric cancers. *Int J Cancer*, 52: 847–850. doi:10.1002/ijc.2910520602 PMID:1459723

Egan KM, Newcomb PA, Titus-Ernstoff L *et al.* (2003). Association of NAT2 and smoking in relation to breast cancer incidence in a population-based case-control study (United States). *Cancer Causes Control*, 14: 43–51. doi:10.1023/A:1022517506689 PMID:12708724

Egan KM, Stampfer MJ, Hunter D *et al.*Nurses' Health Study (2002). Active and passive smoking in breast cancer: prospective results from the Nurses' Health Study.*Epidemiology*,13:138–145.doi:10.1097/00001648-200203000-00007 PMID:11880753

Eichholzer M, Stähelin HB, Lüdin E, Bernasconi F (1999). Smoking, plasma vitamins C, E, retinol, and carotene, and fatal prostate cancer: seventeen-year follow-up of the prospective basel study. *Prostate*, 38: 189–198. doi:10.1002/(SICI)1097-0045(19990215)38:3<189::AID-PROS3>3.0.CO;2-N PMID:10068343

el-Bayoumy K (1992). Environmental carcinogens that may be involved in human breast cancer etiology. *Chem Res Toxicol*, 5: 585–590. doi:10.1021/tx00029a001 PMID:1445997

Eldridge SR, Gould MN, Butterworth BE (1992). Genotoxicity of environmental agents in human mammary epithelial cells. *Cancer Res*, 52: 5617–5621. PMID:1394185

Elliott EA, Matanoski GM, Rosenshein NB *et al.* (1990). Body fat patterning in women with endometrial cancer. *Gynecol Oncol*, 39: 253–258. doi:10.1016/0090-8258(90)90247-I PMID:2258066

Elwood JM, Pearson JC, Skippen DH, Jackson SM (1984). Alcohol, smoking, social and occupational factors in the aetiology of cancer of the oral cavity, pharynx and larynx. *Int J Cancer*, 34: 603–612. doi:10.1002/ijc.2910340504 PMID:6500740

Engeland A, Andersen A, Haldorsen T, Tretli S (1996). Smoking habits and risk of cancers other than lung cancer: 28 years' follow-up of 26,000 Norwegian men and women. *Cancer Causes Control*, 7: 497–506. doi:10.1007/BF00051881 PMID:8877046

Engels EA, Biggar RJ, Hall HI *et al.* (2008). Cancer risk in people infected with human immunodeficiency virus in the United States. *International Journal of Cancer*, 123: 187–194. doi:10.1002/ijc.23487

Epplein M, Schwartz SM, Potter JD, Weiss NS (2005). Smoking-adjusted lung cancer incidence among Asian-Americans (United States). *Cancer Causes Control*, 16: 1085–1090. doi:10.1007/s10552-005-0330-6 PMID:16184474

Erhardt JG, Kreichgauer HP, Meisner C *et al.* (2002). Alcohol, cigarette smoking, dietary factors and the risk of colorectal adenomas and hyperplastic polyps–a case control study. *Eur J Nutr*, 41: 35–43. doi:10.1007/s003940200004 PMID:11990006

Escribano Uzcudun A, Rabanal Retolaza I, García Grande A *et al.* (2002). Pharyngeal cancer prevention: evidence from a case–control study involving 232 consecutive patients. *J Laryngol Otol*, 116: 523–531. PMID:12238672

Ewertz M (1990). Smoking and breast cancer risk in Denmark. *Cancer Causes Control*, 1: 31–37. doi:10.1007/BF00053181 PMID:2102274

Ewertz M, Gillanders S, Meyer L, Zedeler K (1991). Survival of breast cancer patients in relation to factors which affect the risk of developing breast cancer. *Int J Cancer*, 49: 526–530. doi:10.1002/ijc.2910490409 PMID:1917153

Falk RT, Pickle LW, Brown LM *et al.* (1989). Effect of smoking and alcohol consumption on laryngeal cancer risk in coastal Texas. *Cancer Res*, 49: 4024–4029. PMID:2736543

Falk RT, Pickle LW, Fontham ET *et al.* (1992). Epidemiology of bronchioloalveolar carcinoma. *Cancer Epidemiology, Biomarkers & Prevention*, 1: 339–344.

Falter B, Kutzer C, Richter E (1994). Biomonitoring of hemoglobin adducts: aromatic amines and tobacco-specific nitrosamines. *Clin Investig*, 72: 364–371. doi:10.1007/BF00252829 PMID:8086771

Favorito LA, Nardi AC, Ronalsa M *et al.* (2008). Epidemiologic study on penile cancer in Brazil. *Int Braz J Urol*, 34: 587–591, discussion 591–593. doi:10.1590/S1677-55382008000500007 PMID:18986562

Federal Trade Commission (2001). *Cigarette report for 1999*. C. Department. Washington D.C.

Feng BJ, Khyatti M, Ben-Ayoub W *et al.* (2009). Cannabis, tobacco and domestic fumes intake are associated with nasopharyngeal carcinoma in North Africa. *Br J Cancer*, 101: 1207–1212. doi:10.1038/sj.bjc.6605281 PMID:19724280

Fennell TR, MacNeela JP, Morris RW *et al.* (2000). Hemoglobin adducts from acrylonitrile and ethylene oxide in cigarette smokers: effects of *glutathione S-transferase T1*-null and *M1*-null genotypes. *Cancer Epidemiol Biomarkers Prev*, 9: 705–712. PMID:10919741

Fernberg P, Odenbro A, Bellocco R *et al.* (2007). Tobacco use, body mass index, and the risk of leukemia and multiple myeloma: a nationwide cohort study in Sweden. *Cancer Res*, 67: 5983–5986. doi:10.1158/0008-5472.CAN-07-0274 PMID:17575169

Ferson M, Edwards A, Lind A *et al.* (1979). Low natural killer-cell activity and immunoglobulin levels associated with smoking in human subjects. *Int J Cancer*, 23: 603–609. doi:10.1002/ijc.2910230504 PMID:457307

Finch GL, Nikula KJ, Barr EB *et al.* (1995). *Effects of combined exposure of F344 rats to radiation and chronically inhaled cigarette smoke*. In: *Annual Report of the Inhalation Toxicology Research Institute. ITRI-149.* Bice DE, Hahn FF, Hoover R *et al.*,editors

Fine LJ, Philogene GS, Gramling R *et al.* (2004). Prevalence of multiple chronic disease risk factors. 2001 National Health Interview Survey. *Am J Prev Med*, 27: Suppl18–24. doi:10.1016/j.amepre.2004.04.017 PMID:15275670

Fink AK & Lash TL (2003). A null association between smoking during pregnancy and breast cancer using Massachusetts registry data (United States). *Cancer Causes Control*, 14: 497–503. doi:10.1023/A:1024922824237 PMID:12946045

Firozi PF, Bondy ML, Sahin AA *et al.* (2002). Aromatic DNA adducts and polymorphisms of *CYP1A1*, *NAT2*, and *GSTM1* in breast cancer. *Carcinogenesis*, 23: 301–306. doi:10.1093/carcin/23.2.301 PMID:11872636

Flanders WD, Lally CA, Zhu BP *et al.* (2003). Lung cancer mortality in relation to age, duration of smoking, and daily cigarette consumption: results from Cancer Prevention Study II. *Cancer Res*, 63: 6556–6562. PMID:14559851

Folsom AR, Demissie Z, Harnack LIowa Women's Health Study (2003). Glycemic index, glycemic load, and incidence of endometrial cancer: the Iowa women's health study. *Nutr Cancer*, 46: 119–124. doi:10.1207/S15327914NC4602_03 PMID:14690786

Franceschi S, Barra S, La Vecchia C *et al.* (1992). Risk factors for cancer of the tongue and the mouth. A case-control study from northern Italy. *Cancer*, 70: 2227–2233. doi:10.1002/1097-0142(19921101)70:9<2227::AID-CNCR2820700902>3.0.CO;2-Z PMID:1394055

Franceschi S, Levi F, La Vecchia C *et al.* (1999). Comparison of the effect of smoking and alcohol drinking between oral and pharyngeal cancer. *Int J Cancer*, 83: 1–4. doi:10.1002/(SICI)1097-0215(19990924)83:1<1::AID-IJC1>3.0.CO;2-8 PMID:10449598

Franceschi S, Montella M, Polesel J *et al.* (2006). Hepatitis viruses, alcohol, and tobacco in the etiology of hepatocellular carcinoma in Italy. *Cancer Epidemiol Biomarkers Prev*, 15: 683–689. doi:10.1158/1055-9965.EPI-05-0702 PMID:16614109

Franceschi S & Serraino D (1992). Risk factors for adult soft tissue sarcoma in northern Italy. *Ann Oncol*, 3: Suppl 2S85–S88. PMID:1622876

Franceschi S, Talamini R, Barra S *et al.* (1990). Smoking and drinking in relation to cancers of the oral cavity, pharynx, larynx, and esophagus in northern Italy. *Cancer Res*, 50: 6502–6507. PMID:2208109

Franco EL, Villa LL, Sobrinho JP *et al.* (1999). Epidemiology of acquisition and clearance of cervical human papillomavirus infection in women from a high-risk area for cervical cancer. *J Infect Dis*, 180: 1415–1423. doi:10.1086/315086 PMID:10515798

Franks AL, Kendrick JS, Tyler CW Jr (1987). Postmenopausal smoking, estrogen replacement therapy, and the risk of endometrial cancer. *Am J Obstet Gynecol*, 156: 20–23. PMID:3799753

Freedman DM, Sigurdson A, Doody MM *et al.* (2003a). Risk of melanoma in relation to smoking, alcohol intake, and other factors in a large occupational cohort. *Cancer Causes Control*, 14: 847–857. doi:10.1023/B:CACO.0000003839.56954.73 PMID:14682442

Freedman DM, Sigurdson A, Doody MM *et al.* (2003b). Risk of basal cell carcinoma in relation to alcohol intake and smoking. *Cancer Epidemiol Biomarkers Prev*, 12: 1540–1543. PMID:14693751

Freedman ND, Abnet CC, Leitzmann MF *et al.* (2007a). Prospective investigation of the cigarette smoking-head and neck cancer association by sex. *Cancer*, 110: 1593–1601. doi:10.1002/cncr.22957 PMID:17724671

Freedman ND, Abnet CC, Leitzmann MF *et al.* (2007b). A prospective study of tobacco, alcohol, and the risk of esophageal and gastric cancer subtypes. *Am J Epidemiol*, 165: 1424–1433. doi:10.1093/aje/kwm051 PMID:17420181

Freedman ND, Leitzmann MF, Hollenbeck AR *et al.* (2008). Cigarette smoking and subsequent risk of lung

cancer in men and women: analysis of a prospective cohort study. *Lancet Oncol*, 9: 649–656. doi:10.1016/S1470-2045(08)70154-2 PMID:18556244

Friborg JT, Yuan JM, Wang R *et al.* (2007). A prospective study of tobacco and alcohol use as risk factors for pharyngeal carcinomas in Singapore Chinese. *Cancer*, 109: 1183–1191.

Friedman AJ, Ravnikar VA, Barbieri RL (1987). Serum steroid hormone profiles in postmenopausal smokers and nonsmokers. *Fertil Steril*, 47: 398–401. PMID:2951278

Fuchs CS, Colditz GA, Stampfer MJ *et al.* (1996). A prospective study of cigarette smoking and the risk of pancreatic cancer. *Arch Intern Med*, 156: 2255–2260. doi:10.1001/archinte.156.19.2255 PMID:8885826

Fujino Y, Mizoue T, Tokui N *et al.*JACC Study Group (2005). Cigarette smoking and mortality due to stomach cancer: findings from the JACC Study. *J Epidemiol*, 15: Suppl 2S113–S119. doi:10.2188/jea.15.S113 PMID:16127222

Fujita M, Tase T, Kakugawa Y *et al.* (2008). Smoking, earlier menarche and low parity as independent risk factors for gynecologic cancers in Japanese: a case-control study. *Tohoku J Exp Med*, 216: 297–307. doi:10.1620/tjem.216.297 PMID:19060444

Fukuda K & Shibata A (1990). Exposure-response relationships between woodworking, smoking or passive smoking, and squamous cell neoplasms of the maxillary sinus. *Cancer Causes Control*, 1: 165–168. doi:10.1007/BF00053168 PMID:2102286

Furberg AS & Thune I (2003). Metabolic abnormalities (hypertension, hyperglycemia and overweight), lifestyle (high energy intake and physical inactivity) and endometrial cancer risk in a Norwegian cohort. *Int J Cancer*, 104: 669–676. doi:10.1002/ijc.10974 PMID:12640672

Gaikwad NW, Yang L, Muti P *et al.* (2008). The molecular etiology of breast cancer: evidence from biomarkers of risk. *Int J Cancer*, 122: 1949–1957. PMID:18098283

Gajalakshmi CK & Shanta V (1996). Lifestyle and risk of stomach cancer: a hospital-based case-control study. *Int J Epidemiol*, 25: 1146–1153. doi:10.1093/ije/25.6.1146 PMID:9027518

Gajalakshmi V, Hung RJ, Mathew A *et al.* (2003). Tobacco smoking and chewing, alcohol drinking and lung cancer risk among men in southern India. *Int J Cancer*, 107: 441–447. doi:10.1002/ijc.11377 PMID:14506745

Gallagher CJ, Muscat JE, Hicks AN *et al.* (2007). The UDP-glucuronosyltransferase 2B17 gene deletion polymorphism: sex-specific association with urinary 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol glucuronidation phenotype and risk for lung cancer. *Cancer Epidemiol Biomarkers Prev*, 16: 823–828. doi:10.1158/1055-9965.EPI-06-0823 PMID:17416778

Gallicchio L, Boyd K, Matanoski G *et al.* (2008). Carotenoids and the risk of developing lung cancer: a systematic review. *Am J Clin Nutr*, 88: 372–383. PMID:18689373

Gallicchio L, Kouzis A, Genkinger JM *et al.* (2006). Active cigarette smoking, household passive smoke exposure, and the risk of developing pancreatic cancer. *Prev Med*, 42: 200–205. doi:10.1016/j.ypmed.2005.12.014 PMID:16458957

Gallus S, Altieri A, Bosetti C *et al.* (2003a). Cigarette tar yield and risk of upper digestive tract cancers: case-control studies from Italy and Switzerland. *Ann Oncol*, 14: 209–213. doi:10.1093/annonc/mdg074 PMID:12562646

Gallus S, Bosetti C, Franceschi S *et al.* (2003b). Laryngeal cancer in women: tobacco, alcohol, nutritional, and hormonal factors. *Cancer Epidemiol Biomarkers Prev*, 12: 514–517. PMID:12814996

Gambier N, Batt AM, Marie B *et al.* (2005). Association of *CYP2A6*1B* genetic variant with the amount of smoking in French adults from the Stanislas cohort. *Pharmacogenomics J*, 5: 271–275. doi:10.1038/sj.tpj.6500314 PMID:15940289

Gammon MD, Eng SM, Teitelbaum SL *et al.* (2004). Environmental tobacco smoke and breast cancer incidence. *Environ Res*, 96: 176–185. doi:10.1016/j.envres.2003.08.009 PMID:15325878

Gammon MD, Schoenberg JB, Ahsan H *et al.* (1997). Tobacco, alcohol, and socioeconomic status and adenocarcinomas of the esophagus and gastric cardia. *J Natl Cancer Inst*, 89: 1277–1284. doi:10.1093/jnci/89.17.1277 PMID:9293918

Gammon MD, Schoenberg JB, Teitelbaum SL *et al.* (1998). Cigarette smoking and breast cancer risk among young women (United States). *Cancer Causes Control*, 9: 583–590. doi:10.1023/A:1008868922799 PMID:10189043

Gandini S, Botteri E, Iodice S *et al.* (2008). Tobacco smoking and cancer: a meta-analysis. *International Journal of Cancer*, 122: 155–164. doi:10.1002/ijc.23033

Gao CM, Takezaki T, Wu JZ *et al.* (2007). CYP2E1 Rsa I polymorphism impacts on risk of colorectal cancer association with smoking and alcohol drinking. *World J Gastroenterol*, 13: 5725–5730. PMID:17963298

Gao YT, McLaughlin JK, Blot WJ *et al.* (1994). Risk factors for esophageal cancer in Shanghai, China. I. Role of cigarette smoking and alcohol drinking. *Int J Cancer*, 58: 192–196. doi:10.1002/ijc.2910580208 PMID:8026880

Gapstur SM, Gann PH, Lowe W *et al.* (2000). Abnormal glucose metabolism and pancreatic cancer mortality. *JAMA*, 283: 2552–2558. doi:10.1001/jama.283.19.2552 PMID:10815119

García-Closas M, Malats N, Silverman D *et al.* (2005). NAT2 slow acetylation, GSTM1 null genotype, and risk of bladder cancer: results from the Spanish Bladder Cancer Study and meta-analyses. *Lancet*, 366: 649–659. doi:10.1016/S0140-6736(05)67137-1 PMID:16112301

García-González MA, Lanas A, Quintero E *et al.*Spanish Gastroenterological Association AEG (2007). Gastric cancer susceptibility is not linked to pro-and anti-inflammatory cytokine gene polymorphisms in whites: a Nationwide Multicenter Study in Spain. *Am J Gastroenterol*, 102: 1878–1892. doi:10.1111/j.1572-0241.2007.01423.x PMID:17640324

Gargus JL, Powers MB, Habermann RT *et al.* (1976). *Mouse dermal bioassays of cigarette smoke condensates*. In: *Report No. 1. Toward Less Hazardous Cigarettes. The First Set of Experimental Cigarettes (DHEW Publ. No. (NIH)76–905).* Washington DC: Public Health Service, National Institutes of Health, National Cancer Institute, pp. 85–94.

Garrote LF, Herrero R, Reyes RM *et al.* (2001). Risk factors for cancer of the oral cavity and oro-pharynx in Cuba. *Br J Cancer*, 85: 46–54. doi:10.1054/bjoc.2000.1825 PMID:11437401

Garte S (2001). Metabolic susceptibility genes as cancer risk factors: time for a reassessment? *Cancer Epidemiol Biomarkers Prev*, 10: 1233–1237. PMID:11751439

Garte S, Gaspari L, Alexandrie AK *et al.* (2001). Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol Biomarkers Prev*, 10: 1239–1248. PMID:11751440

Garte S, Taioli E, Popov T *et al.* (2008). Genetic susceptibility to benzene toxicity in humans. *J Toxicol Environ Health A*, 71: 1482–1489. PMID:18836923

Gasper AV, Al-Janobi A, Smith JA *et al.* (2005). Glutathione S-transferase M1 polymorphism and metabolism of sulforaphane from standard and high-glucosinolate broccoli. *Am J Clin Nutr*, 82: 1283–1291. PMID:16332662

Ghadirian P, Lubinski J, Lynch H *et al.* (2004). Smoking and the risk of breast cancer among carriers of BRCA mutations. *Int J Cancer*, 110: 413–416. doi:10.1002/ijc.20106 PMID:15095307

Ghadirian P, Maisonneuve P, Perret C *et al.* (1998). Epidemiology of sociodemographic characteristics, lifestyle, medical history, and colon cancer: a case-control study among French Canadians in Montreal. *Cancer Detect Prev*, 22: 396–404. doi:10.1046/j.1525-1500.1998.00058.x PMID:9727620

Ginsburg O, Ghadirian P, Lubinski J *et al.*Hereditary Breast Cancer Clinical Study Group (2009). Smoking and the risk of breast cancer in BRCA1 and BRCA2 carriers: an update. *Breast Cancer Res Treat*, 114: 127–135. doi:10.1007/s10549-008-9977-5 PMID:18483851

Giovannucci E, Rimm EB, Stampfer MJ *et al.* (1994). A prospective study of cigarette smoking and risk of colorectal adenoma and colorectal cancer in U.S. men. *J Natl Cancer Inst*, 86: 183–191. PMID:8283490

Giuliano AR, Sedjo RL, Roe DJ *et al.* (2002). Clearance of oncogenic human papillomavirus (HPV) infection: effect of smoking (United States). *Cancer Causes Control*, 13: 839–846. doi:10.1023/A:1020668232219 PMID:12462549

Glaser SL, Keegan TH, Clarke CA *et al.* (2004). Smoking and Hodgkin lymphoma risk in women United States. *Cancer Causes Control*, 15: 387–397.

Glatt H, Boeing H, Engelke CE *et al.* (2001). Human cytosolic sulphotransferases: genetics, characteristics, toxicological aspects. *Mutat Res*, 482: 27–40. PMID:11535246

Goldman R & Shields PG (1998). Molecular epidemiology of breast cancer. *In Vivo*, 12: 43–48. PMID:9575425

Gong Z, Xie D, Deng Z *et al.* (2005). The PPARgamma Pro12Ala polymorphism and risk for incident sporadic colorectal adenomas. *Carcinogenesis*, 26: 579–585. doi:10.1093/carcin/bgh343 PMID:15564289

González CA, Pera G, Agudo A *et al.* (2003). Smoking and the risk of gastric cancer in the European Prospective Investigation Into Cancer and Nutrition (EPIC). *Int J Cancer*, 107: 629–634. doi:10.1002/ijc.11426 PMID:14520702

Goodman MT, Hankin JH, Wilkens LR *et al.* (1997). Diet, body size, physical activity, and the risk of endometrial cancer. *Cancer Res*, 57: 5077–5085. PMID:9371506

Goodman MT, McDuffie K, Kolonel LN *et al.* (2001). Case-control study of ovarian cancer and polymorphisms in genes involved in catecholestrogen formation and metabolism. *Cancer Epidemiol Biomarkers Prev*, 10: 209–216. PMID:11303589

Goodman MT & Tung KH (2003). Active and passive tobacco smoking and the risk of borderline and invasive ovarian cancer (United States). *Cancer Causes Control*, 14: 569–577. doi:10.1023/A:1024828309874 PMID:12948288

Gori GB (1976). *Report on the second set of experimental cigarettes*. In: *Report No. 2. Toward Less Hazardous Cigarettes. The Second Set of Experimental Cigarettes (DHEW Publ. No. (NIH) 76–1111).* Washington DC: Public Health Service, National Institutes of Health, National Cancer Institute.

Gorlewska-Roberts K, Green B, Fares M *et al.* (2002). Carcinogen-DNA adducts in human breast epithelial cells. *Environ Mol Mutagen*, 39: 184–192. doi:10.1002/em.10060 PMID:11921188

Goy J, Rosenberg MW, King WD (2008). Health risk behaviors: examining social inequalities in bladder and colorectal cancers. *Ann Epidemiol*, 18: 156–162. PMID:18191762

Graham EA, Croninger AB, Wynder EL (1957). Experimental production of carcinoma with cigarette tar. IV. Successful experiments with rabbits. *Cancer Res*, 17: 1058–1066. PMID:13489709

Grainge MJ, West J, Solaymani-Dodaran M *et al.* (2009). The antecedents of biliary cancer: a primary care case-control study in the United Kingdom. *Br J Cancer*, 100: 178–180. doi:10.1038/sj.bjc.6604765 PMID:19018260

Gram IT, Braaten T, Adami HO *et al.* (2008). Cigarette smoking and risk of borderline and invasive epithelial ovarian cancer. *Int J Cancer*, 122: 647–652.

Gram IT, Braaten T, Lund E *et al.* (2009). Cigarette smoking and risk of colorectal cancer among Norwegian women. *Cancer Causes Control*, 20: 895–903. doi:10.1007/s10552-009-9327-x PMID:19274482

Gram IT, Braaten T, Terry PD *et al.* (2005). Breast cancer risk among women who start smoking as teenagers. *Cancer Epidemiol Biomarkers Prev*, 14: 61–66. PMID:15668477

Green A, Purdie D, Bain C *et al.* (2001). Cigarette smoking and risk of epithelial ovarian cancer (Australia). *Cancer Causes Control*, 12: 713–719. doi:10.1023/A:1011297403819 PMID:11562111

Greenland S & Longnecker MP (1992). Methods for trend estimation from summarized dose-response data, with applications to meta-analysis. *Am J Epidemiol*, 135: 1301–1309. PMID:1626547

Griciute L, Castegnaro M, Bereziat JC (1981). Influence of ethyl alcohol on carcinogenesis with *N*-nitrosodimethylamine. *Cancer Lett*, 13: 345–352. doi:10.1016/0304-3835(81)90063-X PMID:7306961

Gronwald J, Byrski T, Huzarski T *et al.* (2006). Influence of selected lifestyle factors on breast and ovarian cancer risk in BRCA1 mutation carriers from Poland. *Breast Cancer Res Treat*, 95: 105–109. doi:10.1007/s10549-005-9051-5 PMID:16261399

Gross CP, Filardo G, Singh HS *et al.* (2006). The relation between projected breast cancer risk, perceived cancer risk, and mammography use. Results from the National Health Interview Survey. *J Gen Intern Med*, 21: 158–164. PMID:16390511

Gudmundsdottir K, Tryggvadottir L, Eyfjord JE (2001). *GSTM1*, *GSTT1*, and *GSTP1* genotypes in relation to breast cancer risk and frequency of mutations in the *p53* gene. *Cancer Epidemiol Biomarkers Prev*, 10: 1169–1173. PMID:11700265

Guengerich FP (2001). Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity. *Chem Res Toxicol*, 14: 611–650. doi:10.1021/tx0002583 PMID:11409933

Guengerich FP & Shimada T (1998). Activation of procarcinogens by human cytochrome P450 enzymes. *Mutat Res*, 400: 201–213. PMID:9685642

Guengerich FP, Shimada T, Yun CH *et al.* (1994). Interactions of ingested food, beverage, and tobacco components involving human cytochrome P4501A2, 2A6, 2E1, and 3A4 enzymes. *Environ Health Perspect*, 102: Suppl 949–53. PMID:7698084

Guillemette C (2003). Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. *Pharmacogenomics J*, 3: 136–158. doi:10.1038/sj.tpj.6500171 PMID:12815363

Gunnell AS, Tran TN, Torrång A *et al.* (2006). Synergy between cigarette smoking and human papillomavirus type 16 in cervical cancer in situ development. *Cancer Epidemiol Biomarkers Prev*, 15: 2141–2147. doi:10.1158/1055-9965.EPI-06-0399 PMID:17057029

Guo X, Johnson RC, Deng H *et al.* (2009). Evaluation of nonviral risk factors for nasopharyngeal carcinoma in a high-risk population of Southern China. *Int J Cancer*, 124: 2942–2947. doi:10.1002/ijc.24293 PMID:19296536

Gupta D, Boffetta P, Gaborieau V, Jindal SK (2001). Risk factors of lung cancer in Chandigarh, India. *Indian J Med Res*, 113: 142–150. PMID:11558323

Gupta MP, Khanduja KL, Koul IB, Sharma RR (1990). Effect of cigarette smoke inhalation on benzo[a]pyrene-induced lung carcinogenesis in vitamin A deficiency in the rat. *Cancer Lett*, 55: 83–88. doi:10.1016/0304-3835(90)90015-P PMID:2265418

Ha M, Mabuchi K, Sigurdson AJ *et al.* (2007). Smoking cigarettes before first childbirth and risk of breast cancer. *Am J Epidemiol*, 166: 55–61. doi:10.1093/aje/kwm045 PMID:17426039

Haiman CA, Stram DO, Wilkens LR *et al.* (2006). Ethnic and racial differences in the smoking-related risk of lung cancer. *N Engl J Med*, 354: 333–342.

Hamajima N, Hirose K, Tajima K *et al.*Collaborative Group on Hormonal Factors in Breast Cancer (2002). Alcohol, tobacco and breast cancer–collaborative reanalysis of individual data from 53 epidemiological studies, including 58,515 women with breast cancer and 95,067 women without the disease. *Br J Cancer*, 87: 1234–1245. doi:10.1038/sj.bjc.6600596 PMID:12439712

Hammond EC & Horn D (1958). Smoking and death rates: report on forty-four months of follow-up of 187,783 men. 2. Death rates by cause. *J Am Med Assoc*, 166: 1294–1308. PMID:12308037

Hammond EC & Seidman H (1980). Smoking and cancer in the United States. *Prev Med*, 9: 169–174. doi:10.1016/0091-7435(80)90071-7 PMID:7383981

Hammond EC, Selikoff IJ, Seidman H (1979). Asbestos exposure, cigarette smoking and death rates. *Ann N Y Acad Sci*, 330: 1 Health Hazard473–490. doi:10.1111/j.1749-6632.1979.tb18749.x PMID:294198

Hanaoka T, Yamamoto S, Sobue T *et al.*Japan Public Health Center-Based Prospective Study on Cancer and Cardiovascular Disease Study Group (2005). Active and passive smoking and breast cancer risk in middle-aged Japanese women. *Int J Cancer*, 114: 317–322. doi:10.1002/ijc.20709 PMID:15540214

Hannan LM, Jacobs EJ, Thun MJ (2009). The association between cigarette smoking and risk of colorectal cancer in a large prospective cohort from the United States. *Cancer Epidemiol Biomarkers Prev*, 18: 3362–3367. doi:10.1158/1055-9965.EPI-09-0661 PMID:19959683

Hansen AM, Mathiesen L, Pedersen M, Knudsen LE (2008). Urinary 1-hydroxypyrene (1-HP) in environmental and occupational studies–a review. *Int J Hyg Environ Health*, 211: 471–503. doi:10.1016/j.ijheh.2007.09.012 PMID:18222724

Hansen RD, Krath BN, Frederiksen K *et al.* (2009). GPX1 Pro(198)Leu polymorphism, erythrocyte GPX activity, interaction with alcohol consumption and smoking,

and risk of colorectal cancer. *Mutat Res*, 664: 13–19. PMID:19428376

Harada T, Kitazawa T, Maita K, Shirasu Y (1985). Effects of vitamin C on tumor induction by diethylnitrosamine in the respiratory tract of hamsters exposed to cigarette smoke. *Cancer Lett*, 26: 163–169. doi:10.1016/0304-3835(85)90022-9 PMID:3978606

Harish K & Ravi R (1995). The role of tobacco in penile carcinoma. *Br J Urol*, 75: 375–377.

Harris JE, Thun MJ, Mondul AM, Calle EE (2004). Cigarette tar yields in relation to mortality from lung cancer in the cancer prevention study II prospective cohort, 1982–1998. *BMJ (Clinical Research Ed.)*, 328: 72–78. doi:10.1136/bmj.37936.585382.44

Harris RJ & Negroni G (1967). Production of lung carcinomas in C57BL mice exposed to a cigarette smoke and air mixture. *Br Med J*, 4: 637–641. doi:10.1136/bmj.4.5580.637 PMID:4293822

Harris RJ, Negroni G, Ludgate S *et al.* (1974). The incidence of lung tumours in c57bl mice exposed to cigarette smoke: air mixtures for prolonged periods. *Int J Cancer*, 14: 130–136. doi:10.1002/ijc.2910140116 PMID:4617705

Hartge P, Schiffman MH, Hoover R *et al.* (1989). A case-control study of epithelial ovarian cancer. *Am J Obstet Gynecol*, 161: 10–16. PMID:2750791

Hashibe M, Boffetta P, Janout V *et al.* (2007a). Esophageal cancer in Central and Eastern Europe: tobacco and alcohol. *Int J Cancer*, 120: 1518–1522. doi:10.1002/ijc.22507 PMID:17205526

Hashibe M, Boffetta P, Zaridze D *et al.* (2007b). Contribution of tobacco and alcohol to the high rates of squamous cell carcinoma of the supraglottis and glottis in Central Europe. *Am J Epidemiol*, 165: 814–820. doi:10.1093/aje/kwk066 PMID:17244634

Hashibe M, Brennan P, Benhamou S *et al.* (2007c). Alcohol drinking in never users of tobacco, cigarette smoking in never drinkers, and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. *J Natl Cancer Inst*, 99: 777–789. doi:10.1093/jnci/djk179 PMID:17505073

Hashibe M, Brennan P, Chuang SC *et al.* (2009). Interaction between tobacco and alcohol use and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. *Cancer Epidemiol Biomarkers Prev*, 18: 541–550. doi:10.1158/1055-9965.EPI-08-0347 PMID:19190158

Hassan MM, Phan A, Li D *et al.* (2008b). Risk factors associated with neuroendocrine tumors: A U.S.-based case-control study. *Int J Cancer*, 123: 867–873. doi:10.1002/ijc.23529 PMID:18491401

Hassan MM, Spitz MR, Thomas MB *et al.* (2008a). Effect of different types of smoking and synergism with hepatitis C virus on risk of hepatocellular carcinoma in American men and women: case-control study. *Int J Cancer*, 123: 1883–1891. doi:10.1002/ijc.23730 PMID:18688864

Hatsukami DK, Benowitz NL, Rennard SI *et al.* (2006a). Biomarkers to assess the utility of potential reduced exposure tobacco products. *Nicotine Tob Res*, 8: 600–622. PMID:16920658

Hatsukami DK, Le CT, Zhang Y *et al.* (2006b). Toxicant exposure in cigarette reducers versus light smokers. *Cancer Epidemiol Biomarkers Prev*, 15: 2355–2358. doi:10.1158/1055-9965.EPI-06-0240 PMID:17164356

Hatsukami DK, Lemmonds C, Zhang Y *et al.* (2004). Evaluation of carcinogen exposure in people who used "reduced exposure" tobacco products. *J Natl Cancer Inst*, 96: 844–852. PMID:15173268

Hawkins NJ & Ward RL (2001). Sporadic colorectal cancers with microsatellite instability and their possible origin in hyperplastic polyps and serrated adenomas. *J Natl Cancer Inst*, 93: 1307–1313. PMID:11535705

Hayes JR, Meckley DR, Stavanja MS *et al.* (2007). Effect of a flue-curing process that reduces tobacco specific nitrosamines on the tumor promotion in SENCAR mice by cigarette smoke condensate. *Food Chem Toxicol*, 45: 419–430. doi:10.1016/j.fct.2006.08.024 PMID:17070977

Hayes RB, Bravo-Otero E, Kleinman DV *et al.* (1999). Tobacco and alcohol use and oral cancer in Puerto Rico. *Cancer Causes Control*, 10: 27–33. doi:10.1023/A:1008876115797 PMID:10334639

Hayes RB, Kardaun JW, de Bruyn A (1987). Tobacco use and sinonasal cancer: a case-control study. *Br J Cancer*, 56: 843–846. PMID:3435710

Hecht SS (1998). Biochemistry, biology, and carcinogenicity of tobacco-specific *N*-nitrosamines. *Chem Res Toxicol*, 11: 559–603. doi:10.1021/tx980005y PMID:9625726

Hecht SS (1999). Tobacco smoke carcinogens and lung cancer. *J Natl Cancer Inst*, 91: 1194–1210. doi:10.1093/jnci/91.14.1194 PMID:10413421

Hecht SS (2000). Inhibition of carcinogenesis by isothiocyanates. *Drug Metab Rev*, 32: 395–411. doi:10.1081/DMR-100102342 PMID:11139137

Hecht SS (2002). Human urinary carcinogen metabolites: biomarkers for investigating tobacco and cancer. *Carcinogenesis*, 23: 907–922. doi:10.1093/carcin/23.6.907 PMID:12082012

Hecht SS (2003). Tobacco carcinogens, their biomarkers and tobacco-induced cancer. *Nat Rev Cancer*, 3: 733–744. doi:10.1038/nrc1190 PMID:14570033

Hecht SS (2008). Progress and challenges in selected areas of tobacco carcinogenesis. *Chem Res Toxicol*, 21: 160–171. doi:10.1021/tx7002068 PMID:18052103

Hecht SS, Carmella SG, Edmonds A *et al.* (2008b). Exposure to nicotine and a tobacco-specific carcinogen increase with duration of use of smokeless tobacco. *Tob Control*, 17: 128–131. doi:10.1136/tc.2007.023242 PMID:18375734

Hecht SS, Carmella SG, Murphy SE *et al.* (2007). Similar exposure to a tobacco-specific carcinogen in smokeless tobacco users and cigarette smokers. *Cancer Epidemiol*

Biomarkers Prev, 16: 1567–1572. doi:10.1158/1055-9965.EPI-07-0227 PMID:17684130

Hecht SS, Carmella SG, Stepanov I *et al.* (2008a). Metabolism of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone to its biomarker total NNAL in smokeless tobacco users. *Cancer Epidemiol Biomarkers Prev*, 17: 732–735. doi:10.1158/1055-9965.EPI-07-2843 PMID:18349296

Hecht SS, Carmella SG, Ye M *et al.* (2002). Quantitation of metabolites of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone after cessation of smokeless tobacco use. *Cancer Res*, 62: 129–134. PMID:11782369

Hecht SS, Carmella SG, Yoder A *et al.* (2006). Comparison of polymorphisms in genes involved in polycyclic aromatic hydrocarbon metabolism with urinary phenanthrene metabolite ratios in smokers. *Cancer Epidemiol Biomarkers Prev*, 15: 1805–1811. doi:10.1158/1055-9965.EPI-06-0173 PMID:17035385

Hecht SS, Chen M, Yoder A *et al.* (2005a). Longitudinal study of urinary phenanthrene metabolite ratios: effect of smoking on the diol epoxide pathway. *Cancer Epidemiol Biomarkers Prev*, 14: 2969–2974. doi:10.1158/1055-9965.EPI-05-0396 PMID:16365018

Hecht SS & Hoffmann D (1988). Tobacco-specific nitrosamines, an important group of carcinogens in tobacco and tobacco smoke. *Carcinogenesis*, 9: 875–884. doi:10.1093/carcin/9.6.875 PMID:3286030

Hecht SS, Murphy SE, Carmella SG *et al.* (2004). Effects of reduced cigarette smoking on the uptake of a tobacco-specific lung carcinogen. *J Natl Cancer Inst*, 96: 107–115. PMID:14734700

Hecht SS, Murphy SE, Carmella SG *et al.* (2005b). Similar uptake of lung carcinogens by smokers of regular, light, and ultralight cigarettes. *Cancer Epidemiol Biomarkers Prev*, 14: 693–698. doi:10.1158/1055-9965.EPI-04-0542 PMID:15767351

Hecht SS, Rivenson A, Braley J *et al.* (1986). Induction of oral cavity tumors in F344 rats by tobacco-specific nitrosamines and snuff. *Cancer Res*, 46: 4162–4166. PMID:3731083

Hedberg K, Vaughan TL, White E *et al.* (1994). Alcoholism and cancer of the larynx: a case-control study in western Washington (United States). *Cancer Causes Control*, 5: 3–8. doi:10.1007/BF01830720 PMID:8123776

Heeschen C, Jang JJ, Weis M *et al.* (2001). Nicotine stimulates angiogenesis and promotes tumor growth and atherosclerosis. *Nat Med*, 7: 833–839. doi:10.1038/89961 PMID:11433349

Hein DW (2002). Molecular genetics and function of NAT1 and NAT2: role in aromatic amine metabolism and carcinogenesis. *Mutat Res*, 506–507: 65–77. PMID:12351146

Hein DW (2006). *N*-acetyltransferase 2 genetic polymorphism: effects of carcinogen and haplotype on urinary bladder cancer risk. *Oncogene*, 25: 1649–1658. doi:10.1038/sj.onc.1209374 PMID:16550165

Heineman EF, Zahm SH, McLaughlin JK, Vaught JB (1994). Increased risk of colorectal cancer among smokers: results of a 26-year follow-up of US veterans and a review. *Int J Cancer*, 59: 728–738. doi:10.1002/ijc.2910590603 PMID:7989109

Heling A-K, Schutte-Borkovec K, Heppel C *et al.* (2008). *Pyridyloxobutylated DNA adducts in oral mucosa of nonsmokers, smokers and snuff dippers in relation to other biomarkers of exposure*. AACR Meeting Abstracts, April 2008, Abstract 1883

Hellberg D & Stendahl U (2005). The biological role of smoking, oral contraceptive use and endogenous sexual steroid hormones in invasive squamous epithelial cervical cancer. *Anticancer Res*, 25: 3041–3046. PMID:16080563

Hellberg D, Valentin J, Eklund T, Nilsson S (1987). Penile cancer: is there an epidemiological role for smoking and sexual behaviour? *Br Med J (Clin Res Ed)*, 295: 1306–1308. doi:10.1136/bmj.295.6609.1306 PMID:3120988

Hemminki K, Koskinen M, Zhao C (2001). DNA adducts as a marker for cancer risk? *Int J Cancer*, 92: 923–925. doi:10.1002/ijc.1273 PMID:11351318

Henderson BE, Louie E, SooHoo Jing J *et al.* (1976). Risk factors associated with nasopharyngeal carcinoma. *N Engl J Med*, 295: 1101–1106. PMID:980005

Henley SJ, Thun MJ, Chao A, Calle EE (2004). Association between exclusive pipe smoking and mortality from cancer and other diseases. *J Natl Cancer Inst*, 96: 853–861. doi:10.1093/jnci/djh144 PMID:15173269

Henry CJ & Kouri RE (1986). Chronic inhalation studies in mice. II. Effects of long-term exposure to 2R1 cigarette smoke on (C57BL/Cum x C3H/AnfCum)F1 mice. *J Natl Cancer Inst*, 77: 203–212. PMID:3459913

Herity B, Moriarty M, Daly L *et al.* (1982). The role of tobacco and alcohol in the aetiology of lung and larynx cancer. *Br J Cancer*, 46: 961–964. PMID:7150489

Hermann S, Rohrmann S, Linseisen J (2009). *Lifestyle factors, obesity and the risk of colorectal adenomas in EPIC-Heidelberg*. Cancer Causes Control.

Herrinton LJ & Friedman GD (1998). Cigarette smoking and risk of non-Hodgkin's lymphoma subtypes. *Cancer Epidemiol Biomarkers Prev*, 7: 25–28. PMID:9456239

Hickey K, Do KA, Green A (2001). Smoking and prostate cancer. *Epidemiol Rev*, 23: 115–125. PMID:11588835

Hill M (1978). Etiology of the adenoma-carcinoma sequence. *Major Probl Pathol*, 10: 153–162. PMID:616868

Hjalgrim H, Ekström-Smedby K, Rostgaard K *et al.* (2007). Cigarette smoking and risk of Hodgkin lymphoma: a population-based case-control study. *Cancer Epidemiol Biomarkers Prev*, 16: 1561–1566.

Ho JW, Lam TH, Tse CW *et al.* (2004). Smoking, drinking and colorectal cancer in Hong Kong Chinese: a case-control study. *Int J Cancer*, 109: 587–597. doi:10.1002/ijc.20018 PMID:14991582

Hoffmann D & Brunnemann KD (1983). Endogenous formation of *N*-nitrosoproline in cigarette smokers. *Cancer Res*, 43: 5570–5574. PMID:6616484

Hoffmann D, Hoffmann I, El-Bayoumy K (2001). The less harmful cigarette: a controversial issue. a tribute to Ernst L. Wynder. *Chem Res Toxicol*, 14: 767–790. doi:10.1021/tx000260u PMID:11453723

Hoffmann D, Rivenson A, Hecht SS *et al.* (1979). Model studies in tobacco carcinogenesis with the Syrian golden hamster. *Prog Exp Tumor Res*, 24: 370–390. PMID:395571

Hoffmann D & Wynder EL (1971). A study of tobacco carcinogenesis. XI. Tumor initiators, tumor accelerators, and tumor promoting activity of condensate fractions. *Cancer*, 27: 848–864. doi:10.1002/1097-0142(197104)27:4<848::AID-CNCR2820270415>3.0.CO;2-4 PMID:5574075

Holmes MD, Murin S, Chen WY *et al.* (2007). Smoking and survival after breast cancer diagnosis. *Int J Cancer*, 120: 2672–2677. doi:10.1002/ijc.22575 PMID:17278091

Homann N, Tillonen J, Meurman JH *et al.* (2000). Increased salivary acetaldehyde levels in heavy drinkers and smokers: a microbiological approach to oral cavity cancer. *Carcinogenesis*, 21: 663–668. doi:10.1093/carcin/21.4.663 PMID:10753201

Horn-Ross PL, Ljung BM, Morrow M (1997). Environmental factors and the risk of salivary gland cancer. *Epidemiology*, 8: 414–419. doi:10.1097/00001648-199707000-00011 PMID:9209856

Hosgood HD 3rd, Zhang L, Shen M *et al.* (2009). Association between genetic variants in *VEGF*, *ERCC3* and occupational benzene haematotoxicity. *Occup Environ Med*, 66: 848–853. PMID:19773279

Hoshiyama Y, Kono S, Sasaba T *et al.* (2000). Relation of Cigarette Smoking, Alcohol Use, and Dietary Habits to Colon Adenomas: A Case-Control Study in Saitama, Japan. *Asian Pac J Cancer Prev*, 1: 139–146. PMID:12718681

Houlston RS (1999). Glutathione *S*-transferase M1 status and lung cancer risk: a meta-analysis. *Cancer Epidemiol Biomarkers Prev*, 8: 675–682. PMID:10744127

Houlston RS (2000). *CYP1A1* polymorphisms and lung cancer risk: a meta-analysis. *Pharmacogenetics*, 10: 105–114. doi:10.1097/00008571-200003000-00002 PMID:10761998

Hsing AW, McLaughlin JK, Chow WH *et al.* (1998). Risk factors for colorectal cancer in a prospective study among U.S. white men. *Int J Cancer*, 77: 549–553. doi:10.1002/(SICI)1097-0215(19980812)77:4<549::AID-IJC13>3.0.CO;2-1 PMID:9679757

Hsing AW, Nam JM, Co Chien HT *et al.* (1996). Risk factors for adrenal cancer: an exploratory study. *Int J Cancer*, 65: 432–436. doi:10.1002/(SICI)1097-0215(19960208)65:4<432::AID-IJC6>3.0.CO;2-Y PMID:8621222

Hsu WL, Chen JY, Chien YC *et al.* (2009). Independent effect of EBV and cigarette smoking on nasopharyngeal carcinoma: a 20-year follow-up study on 9,622 males without family history in Taiwan. *Cancer Epidemiol Biomarkers Prev*, 18: 1218–1226. doi:10.1158/1055-9965.EPI-08-1175 PMID:19336547

Hu J, Morrison H, Mery L *et al.*Canadian Cancer Registries Epidemiology Research Group (2007). Diet and vitamin or mineral supplementation and risk of colon cancer by subsite in Canada. *Eur J Cancer Prev*, 16: 275–291. doi:10.1097/01.cej.0000228411.21719.25 PMID:17554200

Hu J & Ugnat AMCanadian Cancer Registries Epidemiology Research Group (2005). Active and passive smoking and risk of renal cell carcinoma in Canada. *Eur J Cancer*, 41: 770–778. doi:10.1016/j.ejca.2005.01.003 PMID:15763654

Hukkanen J, Jacob P 3rd, Benowitz NL (2005). Metabolism and disposition kinetics of nicotine. *Pharmacol Rev*, 57: 79–115. doi:10.1124/pr.57.1.3 PMID:15734728

Huncharek M, Haddock KS, Reid R, Kupelnick B (2010). Smoking as a risk factor for prostate cancer: a meta-analysis of 24 prospective cohort studies. *Am J Public Health*, 100: 693–701. doi:10.2105/AJPH.2008.150508 PMID:19608952

Hung HC, Chuang J, Chien YC *et al.* (1997). Genetic polymorphisms of CYP2E1, GSTM1, and GSTT1; environmental factors and risk of oral cancer. *Cancer Epidemiol Biomarkers Prev*, 6: 901–905.

Hung RJ, Christiani DC, Risch A *et al.* (2008a). International lung cancer consortium: pooled analysis of sequence variants in DNA repair and cell cycle pathways. *Cancer Epidemiology, Biomarkers & Prevention*, 17: 3081–3089. doi:10.1158/1055-9965.EPI-08-0411

Hung RJ, McKay JD, Gaborieau V *et al.* (2008). A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. *Nature*, 452: 633–637. doi:10.1038/nature06885 PMID:18385738

Hung RJ, McKay JD, Gaborieau V *et al.* (2008b). A susceptibility locus for lung cancer maps to nicotinic acetylcholine receptor subunit genes on 15q25. *Nature*, 452: 633–637. doi:10.1038/nature06885 PMID:18385738

Hunt JD, van der Hel OL, McMillan GP *et al.* (2005). Renal cell carcinoma in relation to cigarette smoking: meta-analysis of 24 studies. *Int J Cancer*, 114: 101–108. doi:10.1002/ijc.20618 PMID:15523697

Hunter DJ, Hankinson SE, Hough H *et al.* (1997). A prospective study of *NAT2* acetylation genotype, cigarette smoking, and risk of breast cancer. *Carcinogenesis*, 18: 2127–2132. doi:10.1093/carcin/18.11.2127 PMID:9395212

Husgafvel-Pursiainen K (2004). Genotoxicity of environmental tobacco smoke: a review. *Mutat Res*, 567: 427–445. doi:10.1016/j.mrrev.2004.06.004 PMID:15572289

Hutt JA, Vuillemenot BR, Barr EB *et al.* (2005). Life-span inhalation exposure to mainstream cigarette smoke

induces lung cancer in B6C3F1 mice through genetic and epigenetic pathways. *Carcinogenesis*, 26: 1999–2009. doi:10.1093/carcin/bgi150 PMID:15944214

Huusom LD, Frederiksen K, Høgdall EV *et al.* (2006). Association of reproductive factors, oral contraceptive use and selected lifestyle factors with the risk of ovarian borderline tumors: a Danish case-control study. *Cancer Causes Control*, 17: 821–829. doi:10.1007/s10552-006-0022-x PMID:16783610

Huxley R; Asia Pacific Cohort Studies Collaboration (2007a). The role of lifestyle risk factors on mortality from colorectal cancer in populations of the Asia-Pacific region. *Asian Pac J Cancer Prev*, 8: 191–198. PMID:17696730

Huxley R, Jamrozik K, Lam TH *et al.*Asia Pacific Cohort Studies Collaboration (2007b). Impact of smoking and smoking cessation on lung cancer mortality in the Asia-Pacific region. *Am J Epidemiol*, 165: 1280–1286. doi:10.1093/aje/kwm002 PMID:17369610

Huxley RR, Ansary-Moghaddam A, Clifton P *et al.* (2009). The impact of dietary and lifestyle risk factors on risk of colorectal cancer: a quantitative overview of the epidemiological evidence. *Int J Cancer*, 125: 171–180. doi:10.1002/ijc.24343 PMID:19350627

Hymowitz N, Corle D, Royce J *et al.* (1995). Smokers' baseline characteristics in the COMMIT trial. *Prev Med*, 24: 503–508. doi:10.1006/pmed.1995.1080 PMID:8524726

IARC (1986). Tobacco smoking. *IARC Monogr Eval Carcinog Risk Chem Hum*, 38: 1–421.

IARC (1987). Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. *IARC Monogr Eval Carcinog Risks Hum Suppl*, 7: 1–440. PMID:3482203

IARC (1995). Human papillomaviruses. *IARC Monogr Eval Carcinog Risks Hum*, 64: 1–378. PMID:16755705

IARC (2002). *IARC handbook of cancer prevention, vol. 6. Weight control and physical activity*. IARC Press, Lyon: International Agency for Research on Cancer

IARC (2004a). Tobacco smoke and involuntary smoking. *IARC Monogr Eval Carcinog Risks Hum*, 83: 1–1438. PMID:15285078

IARC (2004b). Betel-quid and areca-nut chewing and some areca-nut derived nitrosamines. *IARC Monogr Eval Carcinog Risks Hum*, 85: 1–334. PMID:15635762

IARC (2006a). Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol. *IARC Monogr Eval Carcinog Risks Hum*, 88: 1–478. PMID:17366697

IARC (2006b). *TP53 mutation database*. http://www.p53.iarc.fr/index.htlm.

IARC (2007a). Smokeless tobacco and some tobacco-specific N-nitrosamines. *IARC Monogr Eval Carcinog Risks Hum*, 89: 1–592. PMID:18335640

IARC (2007b). Human papillomaviruses. *IARC Monogr Eval Carcinog Risks Hum*, 90: 1–636. PMID:18354839

IARC (2007c). Combined estrogen-progestogen contraceptives and combined estrogen-progestogen menopausal therapy. *IARC Monogr Eval Carcinog Risks Hum*, 91: 1–528. PMID:18756632

IARC (2008). 1,3-Butadiene, ethylene oxide and vinyl halides (vinyl fluoride, vinyl chloride and vinyl bromide). *IARC Monogr Eval Carcinog Risks Hum*, 97: 1–510.

IARC (2010a). Alcohol consumption and ethyl carbamate. *IARC Monogr Eval Carcinog Risks Hum*, 96: 1–1428.

IARC (2010b). Some Aromatic Amines, Organic Dyes, and Related Exposures *IARC Monogr Eval Carcinog Risks Hum*, 99: 1–692.

IARC (2012c). Pharmaceuticals. *IARC Monogr Eval Carcinog Risks Hum*, 100A: 1–437. PMID:18335640

IARC (2012b). Biological agents. *IARC Monogr Eval Carcinog Risks Hum*, 100B: 1–475. PMID:18335640

IARC (2012a). Chemical agents and related occupations. *IARC Monogr Eval Carcinog Risks Hum*, 100F: PMID:18335640

Iarmarcovai G, Bonassi S, Botta A *et al.* (2008). Genetic polymorphisms and micronucleus formation: a review of the literature. *Mutat Res*, 658: 215–233. doi:10.1016/j.mrrev.2007.10.001 PMID:18037339

Ide R, Mizoue T, Fujino Y *et al.*JACC Study Group (2008). Cigarette smoking, alcohol drinking, and oral and pharyngeal cancer mortality in Japan. *Oral Dis*, 14: 314–319. doi:10.1111/j.1601-0825.2007.01378.x PMID:18449960

Iguchi T, Sugita S, Wang CY *et al.* (2009). MnSOD genotype and prostate cancer risk as a function of NAT genotype and smoking status. *In Vivo*, 23: 7–12. PMID:19368118

IIPS; International Institute for Population Sciences (2010).* *Mumbai and Ministry of Health and Family Welfare, Government of India. Global Adult Tobacco Survey India (GATS India)*. New Delhi, India: Ministry of Health and Family Welfare, Government of India

Ingelman-Sundberg M (2004). Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms. *Naunyn Schmiedebergs Arch Pharmacol*, 369: 89–104. doi:10.1007/s00210-003-0819-z PMID:14574440

Ingelman-Sundberg M (2005). Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J*, 5: 6–13. doi:10.1038/sj.tpj.6500285 PMID:15492763

Ingelman-Sundberg M, Ronis MJ, Lindros KO *et al.* (1994). Ethanol-inducible cytochrome P4502E1: regulation, enzymology and molecular biology. *Alcohol Alcohol Suppl*, 2: 131–139. PMID:8974327

Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C (2007). Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. *Pharmacol Ther*, 116: 496–526. doi:10.1016/j.pharmthera.2007.09.004 PMID:18001838

Innes KE & Byers TE (2001). Smoking during pregnancy and breast cancer risk in very young women (United States). *Cancer Causes Control*, 12: 179–185. doi:10.1023/A:1008961512841 PMID:11246847

Inoue H, Kiyohara C, Marugame T *et al.* (2000). Cigarette smoking, CYP1A1 MspI and GSTM1 genotypes, and colorectal adenomas. *Cancer Res*, 60: 3749–3752. PMID:10919645

Inoue M, Tajima K, Takezaki T *et al.* (2003). Epidemiology of pancreatic cancer in Japan: a nested case-control study from the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC). *Int J Epidemiol*, 32: 257–262. doi:10.1093/ije/dyg062 PMID:12714546

Ioannidis JP (2008). Calibration of credibility of agnostic genome-wide associations. *Am J Med Genet B Neuropsychiatr Genet*, 147B: 964–972. PMID:18361430

Iodice S, Gandini S, Maisonneuve P, Lowenfels AB (2008). Tobacco and the risk of pancreatic cancer: a review and meta-analysis. *Langenbecks Arch Surg*, 393: 535–545. doi:10.1007/s00423-007-0266-2 PMID:18193270

Iribarren C, Tekawa IS, Sidney S, Friedman GD (1999). Effect of cigar smoking on the risk of cardiovascular disease, chronic obstructive pulmonary disease, and cancer in men. *N Engl J Med*, 340: 1773–1780. doi:10.1056/NEJM199906103402301 PMID:10362820

Isaksson B, Jonsson F, Pedersen NL *et al.* (2002). Lifestyle factors and pancreatic cancer risk: a cohort study from the Swedish Twin Registry. *Int J Cancer*, 98: 480–482. doi:10.1002/ijc.10256 PMID:11920604

Izzotti A, Calin GA, Arrigo P *et al.* (2009). Downregulation of microRNA expression in the lungs of rats exposed to cigarette smoke. *FASEB J*, 23: 806–812. doi:10.1096/fj.08-121384 PMID:18952709

Jacob P 3rd, Wilson M, Benowitz NL (2007). Determination of phenolic metabolites of polycyclic aromatic hydrocarbons in human urine as their pentafluorobenzyl ether derivatives using liquid chromatography-tandem mass spectrometry. *Anal Chem*, 79: 587–598. doi:10.1021/ac060920l PMID:17222024

Jain MG, Howe GR, Rohan TE (2000). Nutritional factors and endometrial cancer in Ontario, Canada. *Cancer Control*, 7: 288–296. PMID:10832115

Jalas JR, Hecht SS, Murphy SE (2005). Cytochrome P450 enzymes as catalysts of metabolism of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, a tobacco specific carcinogen. *Chem Res Toxicol*, 18: 95–110. doi:10.1021/tx049847p PMID:15720112

Jass JR (2007). Molecular heterogeneity of colorectal cancer: Implications for cancer control. *Surg Oncol*, 16: Suppl 1S7–S9. doi:10.1016/j.suronc.2007.10.039 PMID:18023574

Jass JR, Young J, Leggett BA (2000). Hyperplastic polyps and DNA microsatellite unstable cancers of the colorectum. *Histopathology*, 37: 295–301. PMID:11012735

Jayalekshmy PA, Akiba S, Nair MK *et al.* (2008). Bidi smoking and lung cancer incidence among males in Karunagappally cohort in Kerala, India. *Int J Cancer*, 123: 1390–1397. doi:10.1002/ijc.23618 PMID:18623085

Jean RT, Wilkinson AV, Spitz MR *et al.* (2011). Psychosocial risk and correlates of early menarche in Mexican-American girls. *Am J Epidemiol*, 173: 1203–1210. Epub 2011 Mar 31.

Jee SH, Samet JM, Ohrr H *et al.* (2004). Smoking and cancer risk in Korean men and women. *Cancer Causes Control*, 15:341–348.doi:10.1023/B:CACO.0000027481.48153.97 PMID:15141135

Jeffreys M, Warren R, Gunnell D *et al.* (2004). Life course breast cancer risk factors and adult breast density (United Kingdom). *Cancer Causes Control*, 15: 947–955. PMID:15577297

Jensen J & Christiansen C (1988). Effects of smoking on serum lipoproteins and bone mineral content during postmenopausal hormone replacement therapy. *Am J Obstet Gynecol*, 159: 820–825. PMID:2972209

Jensen J, Christiansen C, Rødbro P (1985). Cigarette smoking, serum estrogens, and bone loss during hormone-replacement therapy early after menopause. *N Engl J Med*, 313: 973–975. doi:10.1056/NEJM198510173131602 PMID:4047104

Ji BT, Dai Q, Gao YT *et al.* (2002). Cigarette and alcohol consumption and the risk of colorectal cancer in Shanghai, China. *Eur J Cancer Prev*, 11: 237–244. doi:10.1097/00008469-200206000-00007 PMID:12131657

Ji BT, Weissfeld JL, Chow WH *et al.* (2006). Tobacco smoking and colorectal hyperplastic and adenomatous polyps. *Cancer Epidemiol Biomarkers Prev*, 15: 897–901. doi:10.1158/1055-9965.EPI-05-0883 PMID:16702367

Jiang J, Suzuki S, Xiang J *et al.* (2005). Plasma carotenoid, alpha-tocopherol and retinol concentrations and risk of colorectal adenomas: A case-control study in Japan. *Cancer Lett*, 226: 133–141. doi:10.1016/j.canlet.2005.03.040 PMID:15885891

Jin MJ, Chen K, Song L *et al.* (2005). The association of the DNA repair gene XRCC3 Thr241Met polymorphism with susceptibility to colorectal cancer in a Chinese population. *Cancer Genet Cytogenet*, 163: 38–43. doi:10.1016/j.cancergencyto.2005.05.001 PMID:16271954

Jin Q, Menter DG, Mao L *et al.* (2008). Survivin expression in normal human bronchial epithelial cells: an early and critical step in tumorigenesis induced by tobacco exposure. *Carcinogenesis*, 29: 1614–1622. doi:10.1093/carcin/bgm234 PMID:18635526

Johnson KC (2005). Accumulating evidence on passive and active smoking and breast cancer risk. *Int J Cancer*, 117: 619–628. doi:10.1002/ijc.21150 PMID:15929073

Johnson KC, Goodman M, Kelsh M *et al.* (2002). Passive and active smoking as possible confounders in studies of breast cancer risk. *Epidemiology*, 13: 610–611, author

reply 610–611. doi:10.1097/00001648-200209000-00029 PMID:12192238

Johnson KC, Hu J, Mao YCanadian Cancer Registries Epidemiology Research Group (2000). Passive and active smoking and breast cancer risk in Canada, 1994–97. *Cancer Causes Control*, 11: 211–221. doi:10.1023/A:1008906105790 PMID:10782655

Johnson L, Mercer K, Greenbaum D *et al.* (2001). Somatic activation of the K-ras oncogene causes early onset lung cancer in mice. *Nature*, 410: 1111–1116. doi:10.1038/35074129 PMID:11323676

Jones PA & Baylin SB (2007). The epigenomics of cancer. *Cell*, 128: 683–692. doi:10.1016/j.cell.2007.01.029 PMID:17320506

Kabat GC, Chang CJ, Wynder EL (1994). The role of tobacco, alcohol use, and body mass index in oral and pharyngeal cancer. *Int J Epidemiol*, 23: 1137–1144. doi:10.1093/ije/23.6.1137 PMID:7721514

Kabat GC & Hebert JR (1994). Use of mentholated cigarettes and oropharyngeal cancer. *Epidemiology*, 5: 183–188. PMID:8172993

Kabat GC, Ng SKC, Wynder EL (1993). Tobacco, alcohol intake, and diet in relation to adenocarcinoma of the esophagus and gastric cardia. *Cancer Causes Control*, 4: 123–132.

Kaczynski AT, Manske SR, Mannell RC, Grewal K (2008). Smoking and physical activity: a systematic review. *Am J Health Behav*, 32: 93–110. PMID:18021037

Kadlubar FF, Beland FA (1985). *Chemical properties of ultimate carcinogenic metabolites of arylamines and arylamides.* In: *Polycyclic Hydrocarbons and Carcinogenesis.* Harvey RG, editor. Washington, D.C.: American Chemical Society.

Kaerlev L, Teglbjaerg PS, Sabroe S *et al.* (2002). The importance of smoking and medical history for development of small bowel carcinoid tumor: a European population-based case-control study. *Cancer Causes Control*, 13: 27–34.

Kamataki T, Fujieda M, Kiyotani K *et al.* (2005). Genetic polymorphism of CYP2A6 as one of the potential determinants of tobacco-related cancer risk. *Biochem Biophys Res Commun*, 338: 306–310. doi:10.1016/j.bbrc.2005.08.268 PMID:16176798

Kanda J, Matsuo K, Kawase T *et al.* (2009). Association of alcohol intake and smoking with malignant lymphoma risk in Japanese: a hospital-based case-control study at Aichi Cancer Center. *Cancer Epidemiol Biomarkers Prev*, 18: 2436–2441. Epub 2009 Sep 1.

Kapeu AS, Luostarinen T, Jellum E *et al.* (2009). Is smoking an independent risk factor for invasive cervical cancer? A nested case-control study within Nordic biobanks. *Am J Epidemiol*, 169: 480–488. doi:10.1093/aje/kwn354 PMID:19074773

Kasahara M, Osawa K, Yoshida K *et al.* (2008). Association of *MUTYH Gln324His* and *APEX1 Asp148Glu* with colorectal cancer and smoking in a Japanese population. *J Exp Clin Cancer Res*, 27: 49 doi:10.1186/1756-9966-27-49 PMID:18823566

Kato M, Loomis D, Brooks LM *et al.* (2004). Urinary biomarkers in charcoal workers exposed to wood smoke in Bahia State, Brazil. *Cancer Epidemiol Biomarkers Prev*, 13: 1005–1012. PMID:15184257

Kawajiri K (1999). CYP1A1. *IARC Sci Publ*, 148: 159–172. PMID:10493257

Kawajiri K, Eguchi H, Nakachi K *et al.* (1996). Association of *CYP1A1* germ line polymorphisms with mutations of the *p53* gene in lung cancer. *Cancer Res*, 56: 72–76. PMID:8548778

Kelsey JL, Gammon MD, John EM (1993). Reproductive factors and breast cancer. *Epidemiol Rev*, 15: 36–47. PMID:8405211

Kelsey JL, LiVolsi VA, Holford TR *et al.* (1982). A case-control study of cancer of the endometrium. *Am J Epidemiol*, 116: 333–342. PMID:7114042

Kenfield SA, Stampfer MJ, Rosner BA, Colditz GA (2008). Smoking and smoking cessation in relation to mortality in women. *JAMA*, 299: 2037–2047. doi:10.1001/jama.299.17.2037 PMID:18460664

Kesarwani P, Singh R, Mittal RD (2009). Association of GSTM3 intron 6 variant with cigarette smoking, tobacco chewing and alcohol as modifier factors for prostate cancer risk. *Arch Toxicol*, 83: 351–356. doi:10.1007/s00204-008-0343-5 PMID:18668224

Ketterer B, Harris JM, Talaska G *et al.* (1992). The human glutathione *S*-transferase supergene family, its polymorphism, and its effects on susceptibility to lung cancer. *Environ Health Perspect*, 98: 87–94. doi:10.2307/3431252 PMID:1486868

Key TJ, Pike MC, Baron JA *et al.* (1991). Cigarette smoking and steroid hormones in women. *J Steroid Biochem Mol Biol*, 39: 4A529–534. doi:10.1016/0960-0760(91)90247-3 PMID:1832941

Key TJ, Pike MC, Brown JB *et al.* (1996). Cigarette smoking and urinary oestrogen excretion in premenopausal and post-menopausal women. *Br J Cancer*, 74: 1313–1316. PMID:8883424

Khaw KT, Tazuke S, Barrett-Connor E (1988). Cigarette smoking and levels of adrenal androgens in post-menopausal women. *N Engl J Med*, 318: 1705–1709. doi:10.1056/NEJM198806303182601 PMID:2967432

Kiemeney LA, Thorlacius S, Sulem P *et al.* (2008). Sequence variant on 8q24 confers susceptibility to urinary bladder cancer. *Nat Genet*, 40: 1307–1312. doi:10.1038/ng.229 PMID:18794855

Kim CS, Kim MC, Cheong HK *et al.* (2005[The association of obesity and left colonic adenomatous polyps in Korean adult men]). *J Prev Med Pub Health*, 38: 415–419. PMID:16358826.

Kim HJ, Lee SM, Choi NK *et al.* (2006). [Smoking and colorectal cancer risk in the Korean elderly][Article in Korean]*J Prev Med Pub Health*, 39: 123–129.

Kim JI, Park YJ, Kim KH *et al.* (2003). hOGG1 Ser326Cys polymorphism modifies the significance of the environmental risk factor for colon cancer. *World J Gastroenterol*, 9: 956–960. PMID:12717837

Kim SG & Novak RF (1990). Role of P450IIE1 in the metabolism of 3-hydroxypyridine, a constituent of tobacco smoke: redox cycling and DNA strand scission by the metabolite 2,5-dihydroxypyridine. *Cancer Res*, 50: 5333–5339. PMID:2167153

Kim Y, Shin A, Gwack J *et al.* (2007). Cigarette smoking and gastric cancer risk in a community-based cohort study in Korea *J Prev Med Public Health*, 40: 467–474. doi:10.3961/jpmph.2007.40.6.467 PMID:18063902

Kiyohara C, Yoshimasu K, Shirakawa T, Hopkin JM (2004). Genetic polymorphisms and environmental risk of lung cancer: a review. *Rev Environ Health*, 19: 15–38. PMID:15186038

Kiyohara C, Yoshimasu K, Takayama K, Nakanishi Y (2005). NQO1, MPO, and the risk of lung cancer: a HuGE review. *Genet Med*, 7: 463–478. doi:10.1097/01.gim.0000177530.55043.c1 PMID:16170238

Kiyohara C, Yoshimasu K, Takayama K, Nakanishi Y (2006). EPHX1 polymorphisms and the risk of lung cancer: a HuGE review. *Epidemiology*, 17: 89–99. PMID:16357600

Kjaerheim K, Gaard M, Andersen A (1998). The role of alcohol, tobacco, and dietary factors in upper aero-gastric tract cancers: a prospective study of 10,900 Norwegian men. *Cancer Causes Control*, 9: 99–108. doi:10.1023/A:1008809706062 PMID:9486469

Klotz U & Ammon E (1998). Clinical and toxicological consequences of the inductive potential of ethanol. *Eur J Clin Pharmacol*, 54: 7–12. doi:10.1007/s002280050412 PMID:9591923

Ko Y, Abel J, Harth V *et al.* (2001). Association of CYP1B1 codon 432 mutant allele in head and neck squamous cell cancer is reflected by somatic mutations of p53 in tumor tissue. *Cancer Res*, 61: 4398–4404. PMID:11389067

Ko YC, Huang YL, Lee CH *et al.* (1995). Betel quid chewing, cigarette smoking and alcohol consumption related to oral cancer in Taiwan. *J Oral Pathol Med*, 24: 450–453. doi:10.1111/j.1600-0714.1995.tb01132.x PMID:8600280

Koizumi Y, Tsubono Y, Nakaya N *et al.* (2004). Cigarette smoking and the risk of gastric cancer: a pooled analysis of two prospective studies in Japan. *Int J Cancer*, 112: 1049–1055. doi:10.1002/ijc.20518 PMID:15386347

Kono S, Ikeda M, Tokudome S *et al.* (1987). Cigarette smoking, alcohol and cancer mortality: a cohort study of male Japanese physicians. *Jpn J Cancer Res*, 78: 1323–1328. PMID:3123436

Kotwall CA (1992). Smoking as an etiologic factor in the development of Warthin's tumor of the parotid gland. *Am J Surg*, 164: 646–647. doi:10.1016/S0002-9610(05)80725-1 PMID:1334381

Koumantaki Y, Tzonou A, Koumantakis E *et al.* (1989). A case-control study of cancer of endometrium in Athens. *Int J Cancer*, 43: 795–799. doi:10.1002/ijc.2910430509 PMID:2714884

Kreuzer M, Boffetta P, Whitley E *et al.* (2000). Gender differences in lung cancer risk by smoking: a multicentre case-control study in Germany and Italy. *Br J Cancer*, 82: 227–233. PMID:10638994

Krewski D, Lubin JH, Zielinski JM *et al.* (2005). Residential radon and risk of lung cancer: a combined analysis of 7 North American case-control studies. *Epidemiology*, 16: 137–145. PMID:15703527

Kropp S & Chang-Claude J (2002). Active and passive smoking and risk of breast cancer by age 50 years among German women. *Am J Epidemiol*, 156: 616–626. doi:10.1093/aje/kwf093 PMID:12244030

Kubík A, Zatloukal P, Boyle P *et al.* (2001). A case-control study of lung cancer among Czech women. *Lung Cancer*, 31: 111–122. PMID:11165390

Kuljukka-Rabb T, Nylund L, Vaaranrinta R *et al.* (2002). The effect of relevant genotypes on PAH exposure-related biomarkers. *J Expo Anal Environ Epidemiol*, 12: 81–91. doi:10.1038/sj.jea.7500204 PMID:11859435

Kuller LH, Ockene JK, Meilahn E *et al.*MRFIT Research Group (1991). Cigarette smoking and mortality. *Prev Med*, 20: 638–654. doi:10.1016/0091-7435(91)90060-H PMID:1758843

Kundu CN, Balusu R, Jaiswal AS *et al.* (2007). Cigarette smoke condensate-induced level of adenomatous polyposis coli blocks long-patch base excision repair in breast epithelial cells. *Oncogene*, 26: 1428–1438. doi:10.1038/sj.onc.1209925 PMID:16924228

Kuper H, Titus-Ernstoff L, Harlow BL, Cramer DW (2000b). Population based study of coffee, alcohol and tobacco use and risk of ovarian cancer. *Int J Cancer*, 88: 313–318. doi:10.1002/1097-0215(20001015)88:2<313::AID-IJC26>3.0.CO;2-5 PMID:11004686

Kuper H, Tzonou A, Kaklamani E *et al.* (2000a). Tobacco smoking, alcohol consumption and their interaction in the causation of hepatocellular carcinoma. *Int J Cancer*, 85: 498–502. doi:10.1002/(SICI)1097-0215(20000215)85:4<498::AID-IJC9>3.0.CO;2-F PMID:10699921

Kurian AW, Balise RR, McGuire V, Whittemore AS (2005). Histologic types of epithelial ovarian cancer: have they different risk factors? *Gynecol Oncol*, 96: 520–530. doi:10.1016/j.ygyno.2004.10.037 PMID:15661246

Kurosawa M, Kikuchi S, Xu J, Inaba Y (2006). Highly salted food and mountain herbs elevate the risk for stomach cancer death in a rural area of Japan. *J Gastroenterol Hepatol*, 21: 1681–1686. doi:10.1111/j.1440-1746.2006.04290.x PMID:16984589

La Torre G, Chiaradia G, Gianfagna F *et al.* (2009). Smoking status and gastric cancer risk: an updated meta-analysis of case-control studies published in the past ten years. *Tumori*, 95: 13–22. PMID:19366050

La Vecchia C, Franceschi S, Bosetti C *et al.* (1999). Time since stopping smoking and the risk of oral and

pharyngeal cancers. *J Natl Cancer Inst*, 91: 726–728. doi:10.1093/jnci/91.8.726a PMID:10218516

Lacey JV Jr, Leitzmann MF, Chang SC *et al.* (2007). Endometrial cancer and menopausal hormone therapy in the National Institutes of Health-AARP Diet and Health Study cohort. *Cancer*, 109: 1303–1311. doi:10.1002/cncr.22525 PMID:17315161

Ladeiras-Lopes R, Pereira AK, Nogueira A *et al.* (2008). Smoking and gastric cancer: systematic review and meta-analysis of cohort studies. *Cancer Causes Control*, 19: 689–701. doi:10.1007/s10552-008-9132-y PMID:18293090

Lagergren J, Bergström R, Lindgren A, Nyrén O (2000). The role of tobacco, snuff and alcohol use in the aetiology of cancer of the oesophagus and gastric cardia. *Int J Cancer*, 85: 340–346. doi:10.1002/(SICI)1097-0215(20000201)85:3<340::AID-IJC8>3.0.CO;2-N PMID:10652424

Lam TK, Gallicchio L, Lindsley K *et al.* (2009). Cruciferous vegetable consumption and lung cancer risk: a systematic review. *Cancer Epidemiol Biomarkers Prev*, 18: 184–195. doi:10.1158/1055-9965.EPI-08-0710 PMID:19124497

Lan Q, Zhang L, Li G *et al.* (2004). Hematotoxicity in workers exposed to low levels of benzene. *Science*, 306: 1774–1776. doi:10.1126/science.1102443 PMID:15576619

Landi MT, Chatterjee N, Yu K *et al.* (2009). A genome-wide association study of lung cancer identifies a region of chromosome 5p15 associated with risk for adenocarcinoma. *Am J Hum Genet*, 85: 679–691. doi:10.1016/j.ajhg.2009.09.012 PMID:19836008

Lang M & Pelkonen O (1999). Metabolism of xenobiotics and chemical carcinogenesis. *IARC Sci Publ*, 14813–22. PMID:10493245

Lanier A, Bender T, Talbot M *et al.* (1980). Nasopharyngeal carcinoma in Alaskan Eskimos Indians, and Aleuts: a review of cases and study of Epstein-Barr virus, HLA, and environmental risk factors. *Cancer*, 46: 2100–2106. doi:10.1002/1097-0142(19801101)46:9<2100::AID-CNCR2820460932>3.0.CO;2-G PMID:6253051

Lao Y, Yu N, Kassie F *et al.* (2007). Analysis of pyridyloxobutyl DNA adducts in F344 rats chronically treated with (*R*)- and (*S*)-*N*'-nitrosonornicotine. *Chem Res Toxicol*, 20: 246–256. doi:10.1021/tx060208j PMID:17305408

Lapidus L, Lindstedt G, Lundberg PA *et al.* (1986). Concentrations of sex-hormone binding globulin and corticosteroid binding globulin in serum in relation to cardiovascular risk factors and to 12-year incidence of cardiovascular disease and overall mortality in postmenopausal women. *Clin Chem*, 32: 146–152. PMID:3940696

Larsen IK, Grotmol T, Almendingen K, Hoff G (2006). Lifestyle as a predictor for colonic neoplasia in asymptomatic individuals. *BMC Gastroenterol*, 6: 5 doi:10.1186/1471-230X-6-5 PMID:16412216

Larsson SC, Permert J, Håkansson N *et al.* (2005). Overall obesity, abdominal adiposity, diabetes and cigarette smoking in relation to the risk of pancreatic cancer in two Swedish population-based cohorts. *Br J Cancer*, 93: 1310–1315. doi:10.1038/sj.bjc.6602868 PMID:16288300

Lash TL & Aschengrau A (1999). Active and passive cigarette smoking and the occurrence of breast cancer. *Am J Epidemiol*, 149: 5–12. PMID:9883788

Lash TL & Aschengrau A (2002). A null association between active or passive cigarette smoking and breast cancer risk. *Breast Cancer Res Treat*, 75: 181–184. doi:10.1023/A:1019625102365 PMID:12243511

Lau A, Belanger CL, Winn LM (2009). *In utero* and acute exposure to benzene: investigation of DNA double-strand breaks and DNA recombination in mice. *Mutat Res*, 676: 74–82. PMID:19486867

Launoy G, Milan C, Faivre J *et al.* (2000). Tobacco type and risk of squamous cell cancer of the oesophagus in males: a French multicentre case-control study. *Int J Epidemiol*, 29: 36–42. doi:10.1093/ije/29.1.36 PMID:10750601

Law MR, Cheng R, Hackshaw AK *et al.* (1997). Cigarette smoking, sex hormones and bone density in women. *Eur J Epidemiol*, 13: 553–558. doi:10.1023/A:1007389712487 PMID:9258568

Lawlor DA, Ebrahim S, Smith GD (2004). Smoking before the birth of a first child is not associated with increased risk of breast cancer: findings from the British Women's Heart and Health Cohort Study and a meta-analysis. *Br J Cancer*, 91: 512–518. doi:10.1038/sj.bjc.6601916 PMID:15226777

Lawrence C, Tessaro I, Durgerian S *et al.* (1987). Smoking, body weight, and early-stage endometrial cancer. *Cancer*, 59: 1665–1669. doi:10.1002/1097-0142(19870501)59:9<1665::AID-CNCR2820590924>3.0.CO;2-2 PMID:3828966

Lawrence C, Tessaro I, Durgerian S *et al.* (1989). Advanced-stage endometrial cancer: contributions of estrogen use, smoking, and other risk factors. *Gynecol Oncol*, 32: 41–45. doi:10.1016/0090-8258(89)90847-0 PMID:2909447

Le Calvez F, Mukeria A, Hunt JD *et al.* (2005). *TP53* and *KRAS* mutation load and types in lung cancers in relation to tobacco smoke: distinct patterns in never, former, and current smokers. *Cancer Res*, 65: 5076–5083. doi:10.1158/0008-5472.CAN-05-0551 PMID:15958551

Le Marchand L, Derby KS, Murphy SE *et al.* (2008). Smokers with the *CHRNA* lung cancer-associated variants are exposed to higher levels of nicotine equivalents and a carcinogenic tobacco-specific nitrosamine. *Cancer Res*, 68: 9137–9140. doi:10.1158/0008-5472.CAN-08-2271 PMID:19010884

Le Marchand L, Guo C, Benhamou S *et al.* (2003). Pooled analysis of the *CYP1A1* exon 7 polymorphism

and lung cancer (United States). *Cancer Causes Control*, 14: 339–346. doi:10.1023/A:1023956201228 PMID:12846365

Le Marchand L, Sivaraman L, Pierce L *et al.* (1998). Associations of *CYP1A1*, GSTM1, and *CYP2E1* polymorphisms with lung cancer suggest cell type specificities to tobacco carcinogens. *Cancer Res*, 58: 4858–4863. PMID:9809991

Le Marchand L, Wilkens LR, Kolonel LN *et al.* (1997). Associations of sedentary lifestyle, obesity, smoking, alcohol use, and diabetes with the risk of colorectal cancer. *Cancer Res*, 57: 4787–4794. PMID:9354440

Lea IA, Jackson MA, Li X *et al.* (2007). Genetic pathways and mutation profiles of human cancers: site- and exposure-specific patterns. *Carcinogenesis*, 28: 1851–1858. doi:10.1093/carcin/bgm176 PMID:17693665

Lee CH, Wu DC, Lee JM *et al.* (2007a). Carcinogenetic impact of alcohol intake on squamous cell carcinoma risk of the oesophagus in relation to tobacco smoking. *Eur J Cancer*, 43: 1188–1199. doi:10.1016/j.ejca.2007.01.039 PMID:17383866

Lee CH, Wu DC, Lee JM *et al.* (2007b). Anatomical subsite discrepancy in relation to the impact of the consumption of alcohol, tobacco and betel quid on esophageal cancer. *Int J Cancer*, 120: 1755–1762. doi:10.1002/ijc.22324 PMID:17230518

Lee CY, Lee JY, Kang JW, Kim H (2001). Effects of genetic polymorphisms of CYP1A1, CYP2E1, GSTM1, and GSTT1 on the urinary levels of 1-hydroxypyrene and 2-naphthol in aircraft maintenance workers. *Toxicol Lett*, 123: 115–124. doi:10.1016/S0378-4274(01)00374-5 PMID:11641039

Lee JM, Yanagawa J, Peebles KA *et al.* (2008b). Inflammation in lung carcinogenesis: new targets for lung cancer chemoprevention and treatment. *Crit Rev Oncol Hematol*, 66: 208–217. doi:10.1016/j.critrevonc.2008.01.004 PMID:18304833

Lee K, Cáceres D, Varela N *et al.* (2006). Allelic variants of cytochrome P4501A1 (CYP1A1), glutathione S transferase M1 (GSTM1) polymorphisms and their association with smoking and alcohol consumption as gastric cancer susceptibility biomarkers *Rev Med Chil*, 134: 1107–1115. PMID:17171211

Lee KM, Kang D, Clapper ML *et al.* (2008a). *CYP1A1*, *GSTM1*, and *GSTT1* polymorphisms, smoking, and lung cancer risk in a pooled analysis among Asian populations. *Cancer Epidemiol Biomarkers Prev*, 17: 1120–1126. doi:10.1158/1055-9965.EPI-07-2786 PMID:18463401

Lee KW, Kuo WR, Tsai SM *et al.* (2005a). Different impact from betel quid, alcohol and cigarette: risk factors for pharyngeal and laryngeal cancer. *Int J Cancer*, 117: 831–836. doi:10.1002/ijc.21237 PMID:15957167

Lee PN (1999). *Uses and abuses of cotinine as a marker of tobacco smoke exposure*. In: *Analytical Determination of Nicotine and Related Compounds and Their Metabolites*. Gorrod JW, Jacob P III, editors. Amsterdam: Elsevier, pp. 669–719

Lee PN (2001). Relation between exposure to asbestos and smoking jointly and the risk of lung cancer. *Occup Environ Med*, 58: 145–153. doi:10.1136/oem.58.3.145 PMID:11171926

Lee YCA, Cohet C, Yang YC *et al.* (2009). Meta-analysis of epidemiologic studies on cigarette smoking and liver cancer. *Int J Epidemiol*, 38: 1497–1511. doi:10.1093/ije/dyp280 PMID:19720726

Lesko SM, Rosenberg L, Kaufman DW *et al.* (1985). Cigarette smoking and the risk of endometrial cancer. *N Engl J Med*, 313: 593–596. doi:10.1056/NEJM198509053131001 PMID:4022045

Leuchtenberger C, Leuchtenberger R (1970). *Effects of chronic inhalation of whole fresh cigarette smoke and of its gas phase on pulmonary tumorigenesis in Snell's mice*. In: *Morphology of Experimental Respiratory Carcinogenesis. Proceedings of a Biology Division*. Nettesheim P, Hanna MGJ, Deathevage JWJ, editors. Oak Ridge National Laboratory Conference, US Atomic Energy Commission, pp. 329–346.

Levi F & La Vecchia C (2001). Tobacco smoking and prostate cancer: time for an appraisal. *Ann Oncol*, 12: 733–738. doi:10.1023/A:1011124523984 PMID:11484946

Levi F, la Vecchia C, Decarli A (1987). Cigarette smoking and the risk of endometrial cancer. *Eur J Cancer Clin Oncol*, 23: 1025–1029. doi:10.1016/0277-5379(87)90353-1 PMID:3665988

Levi F, Ollyo JB, La Vecchia C *et al.* (1990). The consumption of tobacco, alcohol and the risk of adenocarcinoma in Barrett's oesophagus. *Int J Cancer*, 45: 852–854. doi:10.1002/ijc.2910450511 PMID:2335388

Lewin F, Norell SE, Johansson H *et al.* (1998). Smoking tobacco, oral snuff, and alcohol in the etiology of squamous cell carcinoma of the head and neck: a population-based case-referent study in Sweden. *Cancer*, 82: 1367–1375.

Li CI, Malone KE, Daling JR (2005). The relationship between various measures of cigarette smoking and risk of breast cancer among older women 65–79 years of age (United States). *Cancer Causes Control*, 16: 975–985. doi:10.1007/s10552-005-2906-6 PMID:16132806

Li JY, Ershow AG, Chen ZJ *et al.* (1989). A case-control study of cancer of the esophagus and gastric cardia in Linxian. *Int J Cancer*, 43: 755–761. doi:10.1002/ijc.2910430502 PMID:2714880

Li W, Ray RM, Gao DL *et al.* (2006). Occupational risk factors for pancreatic cancer among female textile workers in Shanghai, China. *Occup Environ Med*, 63: 788–793. doi:10.1136/oem.2005.026229 PMID:16847032

Li X & Hemminki K (2004). Inherited predisposition to early onset lung cancer according to histological type. *International Journal of Cancer*, 112: 451–457. doi:10.1002/ijc.20436

Li Y, Jiang Y, Zhang M *et al.* (2011). Drinking behaviour among men and women in China: the 2007 China Chronic Disease and Risk Factor Surveillance. *Addiction*, 106: 1946–1956.

Li Y, Millikan RC, Bell DA *et al.* (2004). Cigarette smoking, cytochrome P4501A1 polymorphisms, and breast cancer among African-American and white women. *Breast Cancer Res*, 6: R460–R473. doi:10.1186/bcr814 PMID:15217514

Liang PS, Chen TY, Giovannucci E (2009). Cigarette smoking and colorectal cancer incidence and mortality: systematic review and meta-analysis. *Int J Cancer*, 124: 2406–2415. doi:10.1002/ijc.24191 PMID:19142968

Liaw KM & Chen CJ (1998). Mortality attributable to cigarette smoking in Taiwan: a 12-year follow-up study. *Tob Control*, 7: 141–148. doi:10.1136/tc.7.2.141 PMID:9789932

Liddell FD (2001). The interaction of asbestos and smoking in lung cancer. *Ann Occup Hyg*, 45: 341–356. PMID:11418084

Lieber CS (1999). Microsomal ethanol-oxidizing system (MEOS): the first 30 years (1968–1998) – a review. *Alcohol Clin Exp Res*, 23: 991–1007. doi:10.1111/j.1530-0277.1999.tb04217.x PMID:10397283

Lieber CS (2004). The discovery of the microsomal ethanol oxidizing system and its physiologic and pathologic role. *Drug Metab Rev*, 36: 511–529. doi:10.1081/DMR-200033441 PMID:15554233

Lijinsky W (1992). *Chemistry and biology of N-Nitroso compounds*. Monographs on Cancer Research. Cambridge University Press

Lilla C, Risch A, Kropp S, Chang-Claude J (2005). SULT1A1 genotype, active and passive smoking, and breast cancer risk by age 50 years in a German case-control study. *Breast Cancer Res*, 7: R229–R237. doi:10.1186/bcr976 PMID:15743503

Lilla C, Verla-Tebit E, Risch A *et al.* (2006). Effect of NAT1 and NAT2 genetic polymorphisms on colorectal cancer risk associated with exposure to tobacco smoke and meat consumption. *Cancer Epidemiol Biomarkers Prev*, 15: 99–107. PMID:16434594

Lim U, Morton LM, Subar AF *et al.* (2007). Alcohol, smoking, and body size in relation to incident Hodgkin's and non-Hodgkin's lymphoma risk. *Am J Epidemiol*, 166: 697–708.

Limburg PJ, Vierkant RA, Cerhan JR *et al.* (2003). Cigarette smoking and colorectal cancer: long-term, subsite-specific risks in a cohort study of postmenopausal women. *Clin Gastroenterol Hepatol*, 1: 202–210. PMID:15017492

Lin TM, Chen KP, Lin CC *et al.* (1973). Retrospective study on nasopharyngeal carcinoma. *J Natl Cancer Inst*, 51: 1403–1408. PMID:4762926

Lin Y, Tamakoshi A, Kawamura T *et al.*JACC Study Group. Japan Collaborative Cohort (2002a). A prospective cohort study of cigarette smoking and pancreatic cancer in Japan. *Cancer Causes Control*, 13: 249–254. doi:10.1023/A:1015052710213 PMID:12020106

Lindblad M, Rodríguez LA, Lagergren J (2005). Body mass, tobacco and alcohol and risk of esophageal, gastric cardia, and gastric non-cardia adenocarcinoma among men and women in a nested case-control study. *Cancer Causes Control*, 16: 285–294. doi:10.1007/s10552-004-3485-7 PMID:15947880

Lindemann K, Vatten LJ, Ellstrøm-Engh M, Eskild A (2008). Body mass, diabetes and smoking, and endometrial cancer risk: a follow-up study. *Br J Cancer*, 98: 1582–1585. doi:10.1038/sj.bjc.6604313 PMID:18362938

Linet MS, McLaughlin JK, Hsing AW *et al.* (1992). Is cigarette smoking a risk factor for non-Hodgkin's lymphoma or multiple myeloma? Results from the Lutheran Brotherhood Cohort Study. *Leuk Res*, 16: 621–624. doi:10.1016/0145-2126(92)90011-U PMID:1635380

Linseisen J, Rohrmann S, Miller AB *et al.* (2007). Fruit and vegetable consumption and lung cancer risk: updated information from the European Prospective Investigation into Cancer and Nutrition (EPIC). *International Journal of Cancer*, 121: 1103–1114. doi:10.1002/ijc.22807

Lissowska J, Brinton LA, Garcia-Closas M (2007). Re: more data regarding the effects of passive smoking on breast cancer risk among younger women. *Int J Cancer*, 120: 2517–2518. doi:10.1002/ijc.22610 PMID:17290385

Lissowska J, Brinton LA, Zatonski W *et al.* (2006). Tobacco smoking, NAT2 acetylation genotype and breast cancer risk. *Int J Cancer*, 119: 1961–1969. doi:10.1002/ijc.22044 PMID:16721725

Lissowska J, Pilarska A, Pilarski P *et al.* (2003). Smoking, alcohol, diet, dentition and sexual practices in the epidemiology of oral cancer in Poland. *Eur J Cancer Prev*, 12: 25–33. doi:10.1097/00008469-200302000-00005 PMID:12548107

Loerbroks A, Schouten LJ, Goldbohm RA, van den Brandt PA (2007). Alcohol consumption, cigarette smoking, and endometrial cancer risk: results from the Netherlands Cohort Study. *Cancer Causes Control*, 18: 551–560. doi:10.1007/s10552-007-0127-x PMID:17437180

London SJ, Colditz GA, Stampfer MJ *et al.* (1989a). Prospective study of relative weight, height, and risk of breast cancer. *JAMA*, 262: 2853–2858. doi:10.1001/jama.262.20.2853 PMID:2810620

London SJ, Colditz GA, Stampfer MJ *et al.* (1989b). Prospective study of smoking and the risk of breast cancer. *J Natl Cancer Inst*, 81: 1625–1631. doi:10.1093/jnci/81.21.1625 PMID:2795691

London SJ, Smart J, Daly AK (2000b). Lung cancer risk in relation to genetic polymorphisms of microsomal epoxide hydrolase among African-Americans and Caucasians in Los Angeles County. *Lung Cancer*, 28: 147–155. doi:10.1016/S0169-5002(99)00130-0 PMID:10717332

London SJ, Yuan JM, Chung FL *et al.* (2000a). Isothiocyanates, glutathione S-transferase M1 and T1 polymorphisms, and lung-cancer risk: a prospective study of men in Shanghai, China. *Lancet*, 356: 724–729. doi:10.1016/S0140-6736(00)02631-3 PMID:11085692

Longcope C, Baker R, Johnston CC Jr (1986). Androgen and estrogen metabolism: relationship to obesity. *Metabolism*, 35: 235–237. doi:10.1016/0026-0495(86)90206-4 PMID:3456482

Longcope C & Johnston CC Jr (1988). Androgen and estrogen dynamics in pre- and postmenopausal women: a comparison between smokers and nonsmokers. *J Clin Endocrinol Metab*, 67: 379–383. doi:10.1210/jcem-67-2-379 PMID:3392163

Lopez AD, Collishaw N, Piha T (1994). A descriptive model of the cigarette epidemic in developed countries. *Tobacco Control*, 3: 242–247. doi:10.1136/tc.3.3.242

López-Abente G, Pollán M, Monge V, Martínez-Vidal A (1992). Tobacco smoking, alcohol consumption, and laryngeal cancer in Madrid. *Cancer Detect Prev*, 16: 265–271. PMID:1473116

Lu J, Lian S, Sun X *et al.* (2000). A case-control study on the risk factors of esophageal cancer in Linzhou *Zhonghua Liu Xing Bing Xue Za Zhi*, 21: 434–436. PMID:11860829

Lubet RA, Zhang Z, Wiseman RW, You M (2000). Use of p53 transgenic mice in the development of cancer models for multiple purposes. *Exp Lung Res*, 26: 581–593. doi:10.1080/01902140150216684 PMID:11195457

Lubin JH, Alavanja MC, Caporaso N *et al.* (2007a). Cigarette smoking and cancer risk: modeling total exposure and intensity. *Am J Epidemiol*, 166: 479–489. doi:10.1093/aje/kwm089 PMID:17548786

Lubin JH, Caporaso N, Hatsukami DK *et al.* (2007b). The association of a tobacco-specific biomarker and cigarette consumption and its dependence on host characteristics. *Cancer Epidemiology, Biomarkers & Prevention*, 16: 1852–1857. doi:10.1158/1055-9965.EPI-07-0018

Lubin JH & Caporaso NE (2006). Cigarette smoking and lung cancer: modeling total exposure and intensity. *Cancer Epidemiology, Biomarkers & Prevention*, 15: 517–523. doi:10.1158/1055-9965.EPI-05-0863

Lubin JH, Kogevinas M, Silverman D *et al.* (2007). Evidence for an intensity-dependent interaction of NAT2 acetylation genotype and cigarette smoking in the Spanish Bladder Cancer Study. *Int J Epidemiol*, 36: 236–241. doi:10.1093/ije/dym043 PMID:17510079

Lubin JH, Virtamo J, Weinstein SJ, Albanes D (2008). Cigarette smoking and cancer: intensity patterns in the alpha-tocopherol, beta-carotene cancer prevention study in Finnish men. *Am J Epidemiol*, 167: 970–975. doi:10.1093/aje/kwm392 PMID:18250081

Lüchtenborg M, Weijenberg MP, Kampman E *et al.* (2005a). Cigarette smoking and colorectal cancer: APC mutations, hMLH1 expression, and GSTM1 and GSTT1 polymorphisms. *Am J Epidemiol*, 161: 806–815. doi:10.1093/aje/kwi114 PMID:15840612

Lüchtenborg M, White KK, Wilkens L *et al.* (2007). Smoking and colorectal cancer: different effects by type of cigarettes? *Cancer Epidemiol Biomarkers Prev*, 16: 1341–1347. doi:10.1158/1055-9965.EPI-06-0519 PMID:17626999

Lurie G, Wilkens LR, Thompson PJ *et al.* (2008). Genetic polymorphisms in the Paraoxonase 1 gene and risk of ovarian epithelial carcinoma. *Cancer Epidemiol Biomarkers Prev*, 17: 2070–2077. doi:10.1158/1055-9965.EPI-08-0145 PMID:18708400

Lynch SM, Vrieling A, Lubin JH *et al.* (2009). Cigarette smoking and pancreatic cancer: a pooled analysis from the pancreatic cancer cohort consortium. *Am J Epidemiol*, 170: 403–413. doi:10.1093/aje/kwp134 PMID:19561064

Mabuchi K, Bross DS, Kessler II (1985). Cigarette smoking and nasopharyngeal carcinoma. *Cancer*, 55: 2874–2876. doi:10.1002/1097-0142(19850615)55:12<2874::AID-CNCR2820551228>3.0.CO;2-4 PMID:3995493

Macfarlane GJ, Zheng T, Marshall JR *et al.* (1995). Alcohol, tobacco, diet and the risk of oral cancer: a pooled analysis of three case-control studies. *Eur J Cancer B Oral Oncol*, 31B: 181–187. doi:10.1016/0964-1955(95)00005-3 PMID:7549758

Mack WJ, Preston-Martin S, Dal Maso L *et al.* (2003). A pooled analysis of case-control studies of thyroid cancer: cigarette smoking and consumption of alcohol, coffee, and tea. *Cancer Causes Control*, 14: 773–785. doi:10.1023/A:1026349702909 PMID:14674742

Mackenzie PI, Owens IS, Burchell B *et al.* (1997). The UDP glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence. *Pharmacogenetics*, 7: 255–269. PMID:9295054

MacMahon B, Trichopoulos D, Cole P, Brown J (1982). Cigarette smoking and urinary estrogens. *N Engl J Med*, 307: 1062–1065. PMID:7121516

MacNicoll AD, Easty GC, Neville AM *et al.* (1980). Metabolism and activation of carcinogenic polycyclic hydrocarbons by human mammary cells. *Biochem Biophys Res Commun*, 95: 1599–1606. doi:10.1016/S0006-291X(80)80081-7 PMID:6774726

Maden C, Sherman KJ, Beckmann AM *et al.* (1993). History of circumcision, medical conditions, and sexual activity and risk of penile cancer. *J Natl Cancer Inst*, 85: 19–24. doi:10.1093/jnci/85.1.19 PMID:8380060

Magnusson C, Wedrén S, Rosenberg LU (2007). Cigarette smoking and breast cancer risk: a population-based study in Sweden. *Br J Cancer*, 97: 1287–1290. doi:10.1038/sj.bjc.6604007 PMID:17912245

Maier H, Dietz A, Gewelke U *et al.* (1992a). Tobacco and alcohol and the risk of head and neck cancer. *Clin Investig*, 70: 320–327. doi:10.1007/BF00184668 PMID:1521046

Maier H, Gewelke U, Dietz A *et al.* (1992b[). Laryngeal cancer and occupation — results of the Heidelberg laryngeal cancer study]*HNO*, 40: 44–51.

Maier H, Sennewald E, Heller GF, Weidauer H (1994). Chronic alcohol consumption–the key risk factor for pharyngeal cancer. *Otolaryngol Head Neck Surg*, 110: 168–173. PMID:7906410

Maier H & Tisch M (1997). Epidemiology of laryngeal cancer: results of the Heidelberg case-control study. *Acta Otolaryngol Suppl*, 527: 160–164. doi:10.3109/00016489709124063 PMID:9197510

Malaiyandi V, Sellers EM, Tyndale RF (2005). Implications of *CYP2A6* genetic variation for smoking behaviors and nicotine dependence. *Clin Pharmacol Ther*, 77: 145–158. doi:10.1016/j.clpt.2004.10.011 PMID:15735609

Malats N (2008). Genetic epidemiology of bladder cancer: scaling up in the identification of low-penetrance genetic markers of bladder cancer risk and progression. *Scand J Urol Nephrol Suppl*, 42: 131–140. doi:10.1080/03008880802285172 PMID:18815927

Mandai M, Konishi I, Kuroda H *et al.* (1998). Heterogeneous distribution of K-ras-mutated epithelia in mucinous ovarian tumors with special reference to histopathology. *Hum Pathol*, 29: 34–40. doi:10.1016/S0046-8177(98)90387-2 PMID:9445131

Mandelzweig L, Novikov I, Sadetzki S (2009). *Smoking and risk of glioma: a meta-analysis*. Cancer Causes Control.

Mane SS, Purnell DM, Hsu IC (1990). Genotoxic effects of five polycyclic aromatic hydrocarbons in human and rat mammary epithelial cells. *Environ Mol Mutagen*, 15: 78–82. doi:10.1002/em.2850150204 PMID:2307152

Manjer J, Andersson I, Berglund G *et al.* (2000). Survival of women with breast cancer in relation to smoking. *Eur J Surg*, 166: 852–858. doi:10.1080/110241500447227 PMID:11097150

Manjer J, Malina J, Berglund G *et al.* (2001). Smoking associated with hormone receptor negative breast cancer. *Int J Cancer*, 91: 580–584. doi:10.1002/1097-0215(200002)9999:9999<::AID-IJC1091>3.0.CO;2-V PMID:11251985

Manuguerra M, Saletta F, Karagas MR *et al.* (2006). XRCC3 and XPD/ERCC2 single nucleotide polymorphisms and the risk of cancer: a HuGE review. *Am J Epidemiol*, 164: 297–302. doi:10.1093/aje/kwj189 PMID:16707649

Mao GE, Morris G, Lu QY *et al.* (2004). Glutathione S-transferase P1 Ile105Val polymorphism, cigarette smoking and prostate cancer. *Cancer Detect Prev*, 28: 368–374. doi:10.1016/j.cdp.2004.07.003 PMID:15542263

Marchbanks PA, Wilson H, Bastos E *et al.* (2000). Cigarette smoking and epithelial ovarian cancer by histologic type. *Obstet Gynecol*, 95: 255–260. doi:10.1016/S0029-7844(99)00531-1 PMID:10674590

Marsh GM, Youk AO, Buchanich JM *et al.* (2007). Work in the metal industry and nasopharyngeal cancer mortality among formaldehyde-exposed workers. *Regul Toxicol Pharmacol*, 48: 308–319. doi:10.1016/j.yrtph.2007.04.006 PMID:17544557

Marshall JR, Hastrup JL, Ross JS (1999). Mismeasurement and the resonance of strong confounders: correlated errors. *Am J Epidemiol*, 150: 88–96. PMID:10400558

Marsit CJ, Karagas MR, Schned A, Kelsey KT (2006). Carcinogen exposure and epigenetic silencing in bladder cancer. *Ann N Y Acad Sci*, 1076: 810–821. doi:10.1196/annals.1371.031 PMID:17119258

Mashberg A, Boffetta P, Winkelman R, Garfinkel L (1993). Tobacco smoking, alcohol drinking, and cancer of the oral cavity and oropharynx among U.S. veterans. *Cancer*, 72: 1369–1375. doi:10.1002/1097-0142(19930815)72:4<1369::AID-CNCR2820720436>3.0.CO;2-L PMID:8339227

Masson LF, Sharp L, Cotton SC, Little J (2005). Cytochrome P-450 1A1 gene polymorphisms and risk of breast cancer: a HuGE review. *Am J Epidemiol*, 161: 901–915. doi:10.1093/aje/kwi121 PMID:15870154

Matthews CE, Xu WH, Zheng W *et al.* (2005). Physical activity and risk of endometrial cancer: a report from the Shanghai endometrial cancer study. *Cancer Epidemiol Biomarkers Prev*, 14: 779–785. doi:10.1158/1055-9965.EPI-04-0665 PMID:15824143

Mauderly JL, Gigliotti AP, Barr EB *et al.* (2004). Chronic inhalation exposure to mainstream cigarette smoke increases lung and nasal tumor incidence in rats. *Toxicol Sci*, 81: 280–292. doi:10.1093/toxsci/kfh203 PMID:15213336

McCann SE, Freudenheim JL, Marshall JR *et al.* (2000). Diet in the epidemiology of endometrial cancer in western New York (United States). *Cancer Causes Control*, 11: 965–974. doi:10.1023/A:1026551309873 PMID:11142531

McCormack VA & dos Santos Silva I (2006). Breast density and parenchymal patterns as markers of breast cancer risk: a meta-analysis. *Cancer Epidemiol Biomarkers Prev*, 15: 1159–1169. doi:10.1158/1055-9965.EPI-06-0034 PMID:16775176

McCoy GD, Hecht SS, Katayama S, Wynder EL (1981). Differential effect of chronic ethanol consumption on the carcinogenicity of *N*-nitrosopyrrolidine and *N*'-nitrosonornicotine in male Syrian golden hamsters. *Cancer Res*, 41: 2849–2854. PMID:7248945

McDivit AM, Greendale GA, Stanczyk FZ, Huang M-H (2008). Effects of alcohol and cigarette smoking on change in serum estrone levels in postmenopausal women randomly assigned to fixed doses of conjugated equine estrogens with or without a progestin. *Menopause*, 15: 382–385. doi:10.1097/gme.0b013e3181407cb2 PMID:18000469

McGrath M, Hankinson SE, De Vivo I (2007). Cytochrome P450 1A1, cigarette smoking, and risk of endometrial cancer (United States). *Cancer Causes Control*, 18: 1123–1130. doi:10.1007/s10552-007-9051-3 PMID:17717632

McIlwain CC, Townsend DM, Tew KD (2006). Glutathione *S*-transferase polymorphisms: cancer incidence and therapy. *Oncogene*, 25: 1639–1648. doi:10.1038/sj.onc.1209373 PMID:16550164

McIntyre-Seltman K, Castle PE, Guido R *et al.* ALTS Group (2005). Smoking is a risk factor for cervical intraepithelial neoplasia grade 3 among oncogenic human papillomavirus DNA-positive women with equivocal or mildly abnormal cytology. *Cancer Epidemiol Biomarkers Prev*, 14: 1165–1170. doi:10.1158/1055-9965.EPI-04-0918 PMID:15894667

McKay JD, Hashibe M, Hung RJ *et al.* (2008). Sequence variants of *NAT1* and *NAT2* and other xenometabolic genes and risk of lung and aerodigestive tract cancers in Central Europe. *Cancer Epidemiol Biomarkers Prev*, 17: 141–147. doi:10.1158/1055-9965.EPI-07-0553 PMID:18199719

McKinlay SM (1996). The normal menopause transition: an overview. *Maturitas*, 23: 137–145. doi:10.1016/0378-5122(95)00985-X PMID:8735352

McLaughlin JK, Hrubec Z, Blot WJ, Fraumeni JF Jr (1995). Smoking and cancer mortality among U.S. veterans: a 26-year follow-up. *Int J Cancer*, 60: 190–193. doi:10.1002/ijc.2910600210 PMID:7829214

Mechanic LE, Bowman ED, Welsh JA *et al.* (2007). Common genetic variation in TP53 is associated with lung cancer risk and prognosis in African Americans and somatic mutations in lung tumors. *Cancer Epidemiology, Biomarkers & Prevention*, 16: 214–222. doi:10.1158/1055-9965.EPI-06-0790

Mechanic LE, Millikan RC, Player J *et al.* (2006). Polymorphisms in nucleotide excision repair genes, smoking and breast cancer in African Americans and whites: a population-based case-control study. *Carcinogenesis*, 27: 1377–1385. doi:10.1093/carcin/bgi330 PMID:16399771

Meckley D, Hayes JR, Van Kampen KR *et al.* (2004a). A responsive, sensitive, and reproducible dermal tumor promotion assay for the comparative evaluation of cigarette smoke condensates. *Regul Toxicol Pharmacol*, 39: 135–149. doi:10.1016/j.yrtph.2003.12.003 PMID:15041145

Meckley DR, Hayes JR, Van Kampen KR *et al.* (2004b). Comparative study of smoke condensates from 1R4F cigarettes that burn tobacco versus ECLIPSE cigarettes that primarily heat tobacco in the SENCAR mouse dermal tumor promotion assay. *Food Chem Toxicol*, 42: 851–863. doi:10.1016/j.fct.2004.01.009 PMID:15046832

Menke-Pluymers MB, Hop WC, Dees J *et al.* The Rotterdam Esophageal Tumor Study Group (1993). Risk factors for the development of an adenocarcinoma in columnar-lined (Barrett) esophagus. *Cancer*, 72: 1155–1158. doi:10.1002/1097-0142(19930815)72:4<1155::AID-CNCR2820720404>3.0.CO;2-C PMID:8339208

Menvielle G, Luce D, Goldberg P *et al.* (2004a). Smoking, alcohol drinking and cancer risk for various sites of the larynx and hypopharynx. A case-control study in France. *Eur J Cancer Prev*, 13: 165–172. doi:10.1097/01.cej.0000130017.93310.76 PMID:15167214

Menvielle G, Luce D, Goldberg P, Leclerc A (2004b). Smoking, alcohol drinking, occupational exposures and social inequalities in hypopharyngeal and laryngeal cancer. *Int J Epidemiol*, 33: 799–806. doi:10.1093/ije/dyh090 PMID:15155704

Merletti F, Boffetta P, Ciccone G *et al.* (1989). Role of tobacco and alcoholic beverages in the etiology of cancer of the oral cavity/oropharynx in Torino, Italy. *Cancer Res*, 49: 4919–4924. PMID:2758421

Meskar A, Plee-Gautier E, Amet Y *et al.* (2001). Alcohol-xenobiotic interactions. Role of cytochrome P450 2E1 *Pathol Biol (Paris)*, 49: 696–702. doi:10.1016/S0369-8114(01)00235-8 PMID:11762131

Michnovicz JJ, Hershcopf RJ, Naganuma H *et al.* (1986). Increased 2-hydroxylation of estradiol as a possible mechanism for the anti-estrogenic effect of cigarette smoking. *N Engl J Med*, 315: 1305–1309. PMID:3773953

Michnovicz JJ, Naganuma H, Hershcopf RJ *et al.* (1988). Increased urinary catechol estrogen excretion in female smokers. *Steroids*, 52: 69–83. doi:10.1016/0039-128X(88)90218-8 PMID:2854668

Miller MD, Marty MA, Broadwin R *et al.* California Environmental Protection Agency (2007). The association between exposure to environmental tobacco smoke and breast cancer: a review by the California Environmental Protection Agency. *Prev Med*, 44: 93–106. doi:10.1016/j.ypmed.2006.08.015 PMID:17027075

Millikan RC, Pittman GS, Newman B *et al.* (1998). Cigarette smoking, N-acetyltransferases 1 and 2, and breast cancer risk. *Cancer Epidemiol Biomarkers Prev*, 7: 371–378. PMID:9610785

Minami Y & Tateno H (2003). Associations between cigarette smoking and the risk of four leading cancers in Miyagi Prefecture, Japan: a multi-site case-control study. *Cancer Sci*, 94: 540–547. doi:10.1111/j.1349-7006.2003.tb01480.x PMID:14529588

Minna JD (2003). Nicotine exposure and bronchial epithelial cell nicotinic acetylcholine receptor expression in the pathogenesis of lung cancer. *J Clin Invest*, 111: 31–33. PMID:12511585

Mitrou PN, Watson MA, Loktionov AS *et al.* (2006). MTHFR (C677T and A1298C) polymorphisms and risk of sporadic distal colorectal adenoma in the UK Flexible Sigmoidoscopy Screening Trial (United Kingdom). *Cancer Causes Control*, 17: 793–801. doi:10.1007/s10552-006-0016-8 PMID:16783607

Modugno F, Ness RB, Cottreau CM (2002). Cigarette smoking and the risk of mucinous and nonmucinous epithelial ovarian cancer. *Epidemiology*, 13: 467–471. doi:10.1097/00001648-200207000-00016 PMID:12094103

Modugno F, Ngo DL, Allen GO *et al.* (2006). Breast cancer risk factors and mammographic breast density in women over age 70. *Breast Cancer Res Treat*, 97: 157–166. doi:10.1007/s10549-005-9105-8 PMID:16362132

Molano M, Van den Brule A, Plummer M *et al.* (2003). Determinants of clearance of human papillomavirus infections in Colombian women with normal cytology: a population-based, 5-year follow-up study. *Am J Epidemiol*, 158: 486–494.

Møllersen L, Vikse R, Andreassen A *et al.* (2004). Adenomatous polyposis coli truncation mutations in 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP)-induced intestinal tumours of multiple intestinal neoplasia mice. *Mutat Res*, 557: 29–40. PMID:14706516

Monnereau A, Orsi L, Troussard X *et al.* (2008). Cigarette smoking, alcohol drinking, and risk of lymphoid neoplasms: results of a French case-control study. *Cancer Causes Control*, 19: 1147–1160.

Montelongo A, Lasunción MA, Pallardo LF, Herrera E (1992). Longitudinal study of plasma lipoproteins and hormones during pregnancy in normal and diabetic women. *Diabetes*, 41: 1651–1659. doi:10.2337/diabetes.41.12.1651 PMID:1446807

Morabia A, Bernstein M, Héritier S, Khatchatrian N (1996). Relation of breast cancer with passive and active exposure to tobacco smoke. *Am J Epidemiol*, 143: 918–928. PMID:8610705

Morabia A, Bernstein M, Ruiz J *et al.* (1998). Relation of smoking to breast cancer by estrogen receptor status. *Int J Cancer*, 75: 339–342. doi:10.1002/(SICI)1097-0215(19980130)75:3<339::AID-IJC2>3.0.CO;2-3 PMID:9455790

Morabia A, Bernstein MS, Bouchardy I *et al.* (2000). Breast cancer and active and passive smoking: the role of the N-acetyltransferase 2 genotype. *Am J Epidemiol*, 152: 226–232. doi:10.1093/aje/152.3.226 PMID:10933269

Morabia A & Wynder EL (1992). Relation of bronchioloalveolar carcinoma to tobacco. *BMJ*, 304: 541–543. doi:10.1136/bmj.304.6826.541 PMID:1313719

Moreno V, Glatt H, Guino E *et al.*Bellvitge Colorectal Cancer Study Group (2005). Polymorphisms in sulfotransferases *SULT1A1* and *SULT1A2* are not related to colorectal cancer. *Int J Cancer*, 113: 683–686. doi:10.1002/ijc.20613 PMID:15455379

Morris JJ & Seifter E (1992). The role of aromatic hydrocarbons in the genesis of breast cancer. *Med Hypotheses*, 38: 177–184. doi:10.1016/0306-9877(92)90090-Y PMID:1513270

Morton LM, Hartge P, Holford TR *et al.* (2005). Cigarette smoking and risk of non-Hodgkin lymphoma: a pooled analysis from the International Lymphoma Epidemiology Consortium (interlymph). *Cancer Epidemiol Biomarkers Prev*, 14: 925–933.

Mounawar M, Mukeria A, Le Calvez F *et al.* (2007). Patterns of *EGFR*, *HER2*, *TP53*, and *KRAS* mutations of *p14^{arf}* expression in non-small cell lung cancers in relation to smoking history. *Cancer Res*, 67: 5667–5672. doi:10.1158/0008-5472.CAN-06-4229 PMID:17575133

Mountzios G, Fouret P, Soria J-C (2008). Mechanisms of Disease: signal transduction in lung carcinogenesis – a comparison of smokers and never-smokers. *Nat Clin Pract Oncol*, 5: 610–618. doi:10.1038/ncponc1181 PMID:18628738

Murata M, Takayama K, Choi BCK, Pak AWP (1996). A nested case-control study on alcohol drinking, tobacco smoking, and cancer. *Cancer Detect Prev*, 20: 557–565. PMID:8939341

Murayama N, Soyama A, Saito Y *et al.* (2004). Six novel nonsynonymous *CYP1A2* gene polymorphisms: catalytic activities of the naturally occurring variant enzymes. *J Pharmacol Exp Ther*, 308: 300–306. doi:10.1124/jpet.103.055798 PMID:14563787

Murin S & Inciardi J (2001). Cigarette smoking and the risk of pulmonary metastasis from breast cancer. *Chest*, 119: 1635–1640. doi:10.1378/chest.119.6.1635 PMID:11399684

Murphy G, Shu XO, Gao YT *et al.* (2009). *Family cancer history affecting risk of colorectal cancer in a prospective cohort of Chinese women*. Cancer Causes Control.

Muscat JE, Richie JP Jr, Thompson S, Wynder EL (1996). Gender differences in smoking and risk for oral cancer. *Cancer Res*, 56: 5192–5197. PMID:8912856

Muscat JE & Wynder EL (1992). Tobacco, alcohol, asbestos, and occupational risk factors for laryngeal cancer. *Cancer*, 69: 2244–2251. doi:10.1002/1097-0142(19920501)69:9<2244::AID-CNCR2820690906>3.0.CO;2-O PMID:1562970

Muscat JE & Wynder EL (1998). A case/control study of risk factors for major salivary gland cancer. *Otolaryngol Head Neck Surg*, 118: 195–198. doi:10.1016/S0194-5998(98)80013-2 PMID:9482552

Mutch MG, Schoen RE, Fleshman JW *et al.* (2009). A multicenter study of prevalence and risk factors for aberrant crypt foci. *Clin Gastroenterol Hepatol*, 7: 568–574. doi:10.1016/j.cgh.2009.01.016 PMID:19418605

Muti P, Bradlow HL, Micheli A *et al.* (2000). Estrogen metabolism and risk of breast cancer: a prospective study of the 2:16alpha-hydroxyestrone ratio in premenopausal and postmenopausal women. *Epidemiology*, 11: 635–640. doi:10.1097/00001648-200011000-00004 PMID:11055622

Muwonge R, Ramadas K, Sankila R *et al.* (2008). Role of tobacco smoking, chewing and alcohol drinking in the risk of oral cancer in Trivandrum, India: a nested case-control design using incident cancer cases. *Oral Oncol*, 44: 446–454. doi:10.1016/j.oraloncology.2007.06.002 PMID:17933578

Mwenifumbo JC & Tyndale RF (2007). Genetic variability in CYP2A6 and the pharmacokinetics of nicotine. *Pharmacogenomics*, 8: 1385–1402. doi:10.2217/14622416.8.10.1385 PMID:17979512

Nagano J, Mabuchi K, Yoshimoto Y *et al.* (2007). A case-control study in Hiroshima and Nagasaki examining non-radiation risk factors for thyroid cancer. *J Epidemiol*, 17: 76–85. doi:10.2188/jea.17.76 PMID:17545694

Nagar S & Remmel RP (2006). Uridine diphosphoglucuronosyltransferase pharmacogenetics and cancer. *Oncogene*, 25: 1659–1672. doi:10.1038/sj.onc.1209375 PMID:16550166

Nagle CM, Olsen CM, Webb PM *et al.*Australian Cancer Study Group; Australian Ovarian Cancer Study Group (2008). Endometrioid and clear cell ovarian cancers: a comparative analysis of risk factors. *Eur J Cancer*, 44: 2477–2484. doi:10.1016/j.ejca.2008.07.009 PMID:18707869

Nakajima M (2007). Smoking behavior and related cancers: the role of *CYP2A6* polymorphisms. *Curr Opin Mol Ther*, 9: 538–544. PMID:18041664

Nakajima M, Yokoi T, Mizutani M *et al.* (1999). Genetic polymorphism in the 5′ -flanking region of human *CYP1A2* gene: effect on the CYP1A2 inducibility in humans. *J Biochem*, 125: 803–808. PMID:10101295

Nam JM, McLaughlin JK, Blot WJ (1992). Cigarette smoking, alcohol, and nasopharyngeal carcinoma: a case-control study among U.S. whites. *J Natl Cancer Inst*, 84: 619–622. doi:10.1093/jnci/84.8.619 PMID:1556772

Nandakumar A, Anantha N, Pattabhiraman V *et al.* (1996). Importance of anatomical subsite in correlating risk factors in cancer of the oesophagus–report of a case–control study. *Br J Cancer*, 73: 1306–1311. PMID:8630297

Nandakumar A, Thimmasetty KT, Sreeramareddy NM *et al.* (1990). A population-based case-control investigation on cancers of the oral cavity in Bangalore, India. *Br J Cancer*, 62: 847–851. PMID:2245179

Narayan S, Jaiswal AS, Kang D *et al.* (2004). Cigarette smoke condensate-induced transformation of normal human breast epithelial cells in vitro. *Oncogene*, 23: 5880–5889. doi:10.1038/sj.onc.1207792 PMID:15208684

Navarro Silvera SA, Miller AB, Rohan TE (2005). Risk factors for thyroid cancer: a prospective cohort study. *Int J Cancer*, 116: 433–438. doi:10.1002/ijc.21079 PMID:15818623

Nayar D, Kapil U, Joshi YK *et al.* (2000). Nutritional risk factors in esophageal cancer. *J Assoc Physicians India*, 48: 781–787. PMID:11273469

Nebert DW & Dalton TP (2006). The role of cytochrome P450 enzymes in endogenous signalling pathways and environmental carcinogenesis. *Nat Rev Cancer*, 6: 947–960. doi:10.1038/nrc2015 PMID:17128211

Nebert DW, Dalton TP, Okey AB, Gonzalez FJ (2004). Role of aryl hydrocarbon receptor-mediated induction of the CYP1 enzymes in environmental toxicity and cancer. *J Biol Chem*, 279: 23847–23850. doi:10.1074/jbc.R400004200 PMID:15028720

Nebert DW, Roe AL, Vandale SE *et al.* (2002). NAD(P)H:quinone oxidoreductase (*NQO1*) polymorphism, exposure to benzene, and predisposition to disease: a HuGEreview. *Genet Med*, 4: 62–70. doi:10.1097/00125817-200203000-00003 PMID:11882782

Negri E, Bosetti C, La Vecchia C *et al.* (1999). Risk factors for adenocarcinoma of the small intestine. *Int J Cancer*, 82: 171–174. doi:10.1002/(SICI)1097-0215(19990719)82:2<171::AID-IJC3>3.0.CO;2-T PMID:10389747

Newcomb PA, Storer BE, Marcus PM (1995). Cigarette smoking in relation to risk of large bowel cancer in women. *Cancer Res*, 55: 4906–4909. PMID:7585528

Newcomb PA & Trentham-Dietz A (2003). Patterns of postmenopausal progestin use with estrogen in relation to endometrial cancer (United States). *Cancer Causes Control*, 14: 195–201. doi:10.1023/A:1023066304473 PMID:12749724

Newcomer LM, Newcomb PA, Trentham-Dietz A, Storer BE (2001). Hormonal risk factors for endometrial cancer: modification by cigarette smoking (United States). *Cancer Causes Control*, 12: 829–835. doi:10.1023/A:1012297905601 PMID:11714111

Newhouse ML, Pearson RM, Fullerton JM *et al.* (1977). A case control study of carcinoma of the ovary. *Br J Prev Soc Med*, 31: 148–153. PMID:588853

Ng TP (1986). A case-referent study of cancer of the nasal cavity and sinuses in Hong Kong. *Int J Epidemiol*, 15: 171–175. doi:10.1093/ije/15.2.171 PMID:3721678

Nieters A, Deeg E, Becker N (2006). Tobacco and alcohol consumption and risk of lymphoma: results of a population-based case-control study in Germany. *Int J Cancer*, 118: 422–430.

Nieters A, Rohrmann S, Becker N *et al.* (2008). Smoking and lymphoma risk in the European prospective investigation into cancer and nutrition. *Am J Epidemiol*, 167: 1081–1089.

Nilsen TI & Vatten LJ (2000). A prospective study of lifestyle factors and the risk of pancreatic cancer in Nord-Trøndelag, Norway. *Cancer Causes Control*, 11: 645–652. doi:10.1023/A:1008916123357 PMID:10977109

Nilsson S, Carstensen JM, Pershagen G (2001). Mortality among male and female smokers in Sweden: a 33 year follow up. *J Epidemiol Community Health*, 55: 825–830. doi:10.1136/jech.55.11.825 PMID:11604439

Ning JP, Yu MC, Wang QS, Henderson BE (1990). Consumption of salted fish and other risk factors for nasopharyngeal carcinoma (NPC) in Tianjin, a low-risk region for NPC in the People's Republic of China. *J Natl Cancer Inst*, 82: 291–296. doi:10.1093/jnci/82.4.291 PMID:2299678

Nishino K, Sekine M, Kodama S *et al.* (2008). Cigarette smoking and glutathione S-transferase M1 polymorphism associated with risk for uterine cervical cancer. *J Obstet Gynaecol Res*, 34: 994–1001.

Nishino Y, Inoue M, Tsuji I *et al.*Research Group for the Development and Evaluation of Cancer Prevention Strategies in Japan (2006). Tobacco smoking and gastric cancer risk: an evaluation based on a systematic review of epidemiologic evidence among the Japanese population. *Jpn J Clin Oncol*, 36: 800–807. doi:10.1093/jjco/hyl112 PMID:17210611

Niwa Y, Wakai K, Suzuki S *et al.*JACC Study Group (2005). Cigarette smoking and the risk of ovarian cancer in the Japanese population: findings from the Japanese Collaborate Cohort study. *J Obstet Gynaecol Res*, 31: 144–151. doi:10.1111/j.1447-0756.2005.00261.x PMID:15771641

Nkondjock A & Ghadirian P (2004). Dietary carotenoids and risk of colon cancer: case-control study. *Int J Cancer*, 110: 110–116. doi:10.1002/ijc.20066 PMID:15054875

Nkondjock A, Robidoux A, Paredes Y *et al.* (2006). Diet, lifestyle and BRCA-related breast cancer risk among French-Canadians. *Breast Cancer Res Treat*, 98: 285–294. doi:10.1007/s10549-006-9161-8 PMID:16541324

Nock NL, Liu X, Cicek MS *et al.* (2006). Polymorphisms in polycyclic aromatic hydrocarbon metabolism and conjugation genes, interactions with smoking and prostate cancer risk. *Cancer Epidemiol Biomarkers Prev*, 15: 756–761. doi:10.1158/1055-9965.EPI-05-0826 PMID:16614120

Noda N, Matsuzoe D, Konno T *et al.* (2004). Risk for K-ras gene mutations in smoking-induced lung cancer is associated with cytochrome P4501A1 and glutathione S-transferase micro1 polymorphisms. *Oncol Rep*, 12: 773–779. PMID:15375499

Nordlund LA, Carstensen JM, Pershagen G (1997). Cancer incidence in female smokers: a 26-year follow-up. *Int J Cancer*, 73: 625–628. doi:10.1002/(SICI)1097-0215(19971127)73:5<625::AID-IJC2>3.0.CO;2-Z PMID:9398036

Norppa H (2001). Genetic polymorphisms and chromosome damage. *Int J Hyg Environ Health*, 204: 31–38. doi:10.1078/1438-4639-00069 PMID:11725342

Norppa H (2003). Genetic susceptibility, biomarker respones, and cancer. *Mutat Res*, 544: 339–348. doi:10.1016/j.mrrev.2003.09.006 PMID:14644336

Norppa H (2004). Cytogenetic biomarkers and genetic polymorphisms. *Toxicol Lett*, 149: 309–334. doi:10.1016/j.toxlet.2003.12.042 PMID:15093278

Novak RF & Woodcroft KJ (2000). The alcohol-inducible form of cytochrome P450 (CYP 2E1): role in toxicology and regulation of expression. *Arch Pharm Res*, 23: 267–282. doi:10.1007/BF02975435 PMID:10976571

O'Brien MJ, Yang S, Mack C *et al.* (2006). Comparison of microsatellite instability, CpG island methylation phenotype, BRAF and KRAS status in serrated polyps and traditional adenomas indicates separate pathways to distinct colorectal carcinoma end points. *Am J Surg Pathol*, 30: 1491–1501.

Obana H, Hori S, Kashimoto T, Kunita N (1981). Polycyclic aromatic hydrocarbons in human fat and liver. *Bull Environ Contam Toxicol*, 27: 23–27. doi:10.1007/BF01610981 PMID:7296033

Ochs-Balcom HM, Wiesner G, Elston RC (2007). A meta-analysis of the association of *N*-acetyltransferase 2 gene (*NAT2*) variants with breast cancer. *Am J Epidemiol*, 166: 246–254. PMID:17535831

Odenbro A, Gillgren P, Bellocco R *et al.* (2007). The risk for cutaneous malignant melanoma, melanoma in situ and intraocular malignant melanoma in relation to tobacco use and body mass index. *Br J Dermatol*, 156: 99–105. doi:10.1111/j.1365-2133.2006.07537.x PMID:17199574

Okamura C, Tsubono Y, Ito K *et al.* (2006). Lactation and risk of endometrial cancer in Japan: a case-control study. *Tohoku J Exp Med*, 208: 109–115. doi:10.1620/tjem.208.109 PMID:16434833

Olivier M & Hainaut P (2001). TP53 mutation patterns in breast cancers: searching for clues of environmental carcinogenesis. *Semin Cancer Biol*, 11: 353–360. doi:10.1006/scbi.2001.0390 PMID:11562177

Olsen J, Sabreo S, Fasting U (1985a). Interaction of alcohol and tobacco as risk factors in cancer of the laryngeal region. *J Epidemiol Community Health*, 39: 165–168. doi:10.1136/jech.39.2.165 PMID:4009100

Olsen J, Sabroe S, Ipsen J (1985b). Effect of combined alcohol and tobacco exposure on risk of cancer of the hypopharynx. *J Epidemiol Community Health*, 39: 304–307. doi:10.1136/jech.39.4.304 PMID:4086960

Olson JE, Vachon CM, Vierkant RA *et al.* (2005). Prepregnancy exposure to cigarette smoking and subsequent risk of postmenopausal breast cancer. *Mayo Clin Proc*, 80: 1423–1428. doi:10.4065/80.11.1423 PMID:16295021

Omata F, Brown WR, Tokuda Y *et al.* (2009). Modifiable risk factors for colorectal neoplasms and hyperplastic polyps. *Intern Med*, 48: 123–128. doi:10.2169/internalmedicine.48.1562 PMID:19182421

Oreggia F, De Stefani E, Correa P, Fierro L (1991). Risk factors for cancer of the tongue in Uruguay. *Cancer*, 67: 180–183. doi:10.1002/1097-0142(19910101)67:1<180::AID-CNCR2820670130>3.0.CO;2-R PMID:1985715

Otani T, Iwasaki M, Ikeda S *et al.* (2006). Serum triglycerides and colorectal adenoma in a case-control study among cancer screening examinees (Japan). *Cancer Causes Control*, 17: 1245–1252. doi:10.1007/s10552-006-0065-z PMID:17111255

Otani T, Iwasaki M, Yamamoto S *et al.*Japan Public Health Center-based Prospective Study Group (2003). Alcohol consumption, smoking, and subsequent risk of colorectal cancer in middle-aged and elderly Japanese men and women: Japan Public Health Center-based prospective study. *Cancer Epidemiol Biomarkers Prev*, 12: 1492–1500. PMID:14693743

Otto H & Elmenhorst H (1967). Experimental studies on tumour induction with the gas phase of cigarette smoke.] *Z Krebsforsch*, 70: 45–47. doi:10.1007/BF00524450

Pacella-Norman R, Urban MI, Sitas F *et al.* (2002). Risk factors for oesophageal, lung, oral and laryngeal cancers in black South Africans. *Br J Cancer*, 86: 1751–1756. doi:10.1038/sj.bjc.6600338 PMID:12087462

Pakhale SS, Sarkar S, Jayant K, Bhide SV (1988). Carcinogenicity of Indian bidi and cigarette smoke condensate in Swiss albino mice. *J Cancer Res Clin Oncol*, 114: 647–649. doi:10.1007/BF00398193 PMID:3204112

Palmer JR & Rosenberg L (1993). Cigarette smoking and the risk of breast cancer. *Epidemiol Rev*, 15: 145–156. PMID:8405197

Palmer JR, Rosenberg L, Clarke EA *et al.* (1991). Breast cancer and cigarette smoking: a hypothesis. *Am J Epidemiol*, 134: 1–13. PMID:1853854

Pan SY, Ugnat AM, Mao Y *et al.*Canadian Cancer Registries Epidemiology Research Group (2004). Association of cigarette smoking with the risk of ovarian cancer. *Int J Cancer*, 111: 124–130. doi:10.1002/ijc.20242 PMID:15185353

Pandey M & Shukla VK (2003). Lifestyle, parity, menstrual and reproductive factors and risk of gallbladder cancer. *Eur J Cancer Prev*, 12: 269–272. doi:10.1097/00008469-200308000-00005 PMID:12883378

Pandeya N, Williams GM, Sadhegi S *et al.* (2008). Associations of duration, intensity, and quantity of smoking with adenocarcinoma and squamous cell carcinoma of the esophagus. *Am J Epidemiol*, 168: 105–114. doi:10.1093/aje/kwn091 PMID:18483122

Parente RC, Faerstein E, Celeste RK, Werneck GL (2008). The relationship between smoking and age at the menopause: A systematic review. *Maturitas*, 61: 287–298. doi:10.1016/j.maturitas.2008.09.021 PMID:19019585

Park NH, Sapp JP, Herbosa EG (1986). Oral cancer induced in hamsters with herpes simplex infection and simulated snuff dipping. *Oral Surg Oral Med Oral Pathol*, 62: 164–168. doi:10.1016/0030-4220(86)90039-3 PMID:3462612

Park SL, Chang S-C, Cai L *et al.* (2008). Associations between variants of the 8q24 chromosome and nine smoking-related cancer sites. *Cancer Epidemiol Biomarkers Prev*, 17: 3193–3202. doi:10.1158/1055-9965.EPI-08-0523 PMID:18990762

Parker AS, Cerhan JR, Dick F *et al.* (2000). Smoking and risk of non-Hodgkin lymphoma subtypes in a cohort of older women. *Leuk Lymphoma*, 37: 341–349. PMID:10752985

Parker AS, Cerhan JR, Janney CA *et al.* (2003). Smoking cessation and renal cell carcinoma. *Ann Epidemiol*, 13: 245–251. doi:10.1016/S1047-2797(02)00271-5 PMID:12684190

Paskett ED, Reeves KW, Rohan TE *et al.* (2007). Association between cigarette smoking and colorectal cancer in the Women's Health Initiative. *J Natl Cancer Inst*, 99: 1729–1735. doi:10.1093/jnci/djm176 PMID:18000222

Pavanello S & Clonfero E (2000). Biological indicators of genotoxic risk and metabolic polymorphisms. *Mutat Res*, 463: 285–308. doi:10.1016/S1383-5742(00)00051-X PMID:11018745

Pavanello S, Pulliero A, Lupi S *et al.* (2005). Influence of the genetic polymorphism in the 5′-noncoding region of the *CYP1A2* gene on *CYP1A2* phenotype and urinary mutagenicity in smokers. *Mutat Res*, 587: 59–66. PMID:16188490

Pavanello S, Simioli P, Lupi S *et al.* (2002). Exposure levels and cytochrome P450 1A2 activity, but not *N*-acetyltransferase, glutathione *S*-transferase (GST) *M1* and *T1*, influence urinary mutagen excretion in smokers. *Cancer Epidemiol Biomarkers Prev*, 11: 998–1003. PMID:12376499

Peach HG & Barnett NE (2001). Critical review of epidemiological studies of the association between smoking and non-Hodgkin's lymphoma. *Hematol Oncol*, 19: 67–80. doi:10.1002/hon.677 PMID:11438976

Penning TM & Drury JE (2007). Human aldo-keto reductases: Function, gene regulation, and single nucleotide polymorphisms. *Arch Biochem Biophys*, 464: 241–250. doi:10.1016/j.abb.2007.04.024 PMID:17537398

Peterson GM, Schmitz K *et al.* (2001). *Physical activity among smokers is inversely associated with lung cancer incidence in Iowa Women's Health Study*. 25th Annual Meeting of the American Society of Preventive Oncology

Peto R, Chen ZM, Boreham J (1999). Tobacco–the growing epidemic. *Nat Med*, 5: 15–17. doi:10.1038/4691 PMID:9883828

Petrakis NL, Gruenke LD, Beelen TC *et al.* (1978). Nicotine in breast fluid of nonlactating women. *Science*, 199: 303–305. doi:10.1126/science.619458 PMID:619458

Petridou E, Koukoulomatis P, Dessypris N *et al.* (2002). Why is endometrial cancer less common in Greece than in other European Union countries? *Eur J Cancer Prev*, 11: 427–432. doi:10.1097/00008469-200210000-00004 PMID:12394239

Pfeifer GP, Denissenko MF, Olivier M *et al.* (2002). Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. *Oncogene*, 21: 7435–7451. doi:10.1038/sj.onc.1205803 PMID:12379884

Phillips DH (1983). Fifty years of benzo(a)pyrene. *Nature*, 303: 468–472. doi:10.1038/303468a0 PMID:6304528

Phillips DH (2002). Smoking-related DNA and protein adducts in human tissues. *Carcinogenesis*, 23: 1979–2004. doi:10.1093/carcin/23.12.1979 PMID:12507921

Phillips LE, Longstreth WT Jr, Koepsell T *et al.* (2005). Active and passive cigarette smoking and risk of intracranial meningioma. *Neuroepidemiology*, 24: 117–122. Epub 2004 Dec 30.

Phukan RK, Zomawia E, Narain K *et al.* (2005). Tobacco use and stomach cancer in Mizoram, India. *Cancer Epidemiol Biomarkers Prev*, 14: 1892–1896.

Pinkston JA & Cole P (1996). Cigarette smoking and Warthin's tumor. *Am J Epidemiol*, 144: 183–187. PMID:8678050

Pinsky PF (2006). Racial and ethnic differences in lung cancer incidence: how much is explained by differences in smoking patterns? (United States). *Cancer Causes Control*, 17: 1017–1024. doi:10.1007/s10552-006-0038-2 PMID:16933052

Pirkle JL, Bernert JT, Caudill SP *et al.* (2006). Trends in the exposure of nonsmokers in the U.S. population to secondhand smoke: 1988–2002. *Environ Health Perspect*, 114: 853–858. PMID:16759984

Plant AL, Benson DM, Smith LC (1985). Cellular uptake and intracellular localization of benzo(a)pyrene by digital fluorescence imaging microscopy. *J Cell Biol*, 100: 1295–1308. doi:10.1083/jcb.100.4.1295 PMID:3980583

Plummer M, Herrero R, Franceschi S *et al.*IARC Multicentre Cervical Cancer Study Group (2003). Smoking and cervical cancer: pooled analysis of the IARC multicentric case–control study. *Cancer Causes Control*, 14: 805–814. doi:10.1023/B:CACO.0000003811.98261.3e PMID:14682438

Polychronopoulou A, Tzonou A, Hsieh CC *et al.* (1993). Reproductive variables, tobacco, ethanol, coffee and somatometry as risk factors for ovarian cancer. *Int J Cancer*, 55: 402–407. doi:10.1002/ijc.2910550312 PMID:8375923

Poplawski T, Tomaszewska K, Galicki M *et al.* (2008). Promoter methylation of cancer-related genes in gastric carcinoma. *Exp Oncol*, 30: 112–116. PMID:18566573

Powell J & McConkey CC (1990). Increasing incidence of adenocarcinoma of the gastric cardia and adjacent sites. *Br J Cancer*, 62: 440–443. PMID:2206952

Prescott J, Ma H, Bernstein L, Ursin G (2007). Cigarette smoking is not associated with breast cancer risk in young women. *Cancer Epidemiol Biomarkers Prev*, 16: 620–622. doi:10.1158/1055-9965.EPI-06-0873 PMID:17372262

Preussmann R, Stewart BW (1984). *N-Nitroso Carcinogens.* In: *Chemical Carcinogens, Second Edition, ACS Monograph 182.* Searle CE, editor. Washington, DC: American Chemical Society.

Proia NK, Paszkiewicz GM, Nasca MA *et al.* (2006). Smoking and smokeless tobacco-associated human buccal cell mutations and their association with oral cancer–a review. *Cancer Epidemiol Biomarkers Prev*, 15: 1061–1077. doi:10.1158/1055-9965.EPI-05-0983 PMID:16775162

Prokopczyk B, Hoffmann D, Bologna M *et al.* (2002). Identification of tobacco-derived compounds in human pancreatic juice. *Chem Res Toxicol*, 15: 677–685. doi:10.1021/tx0101088 PMID:12018989

Prokopczyk B, Leder G, Trushin N *et al.* (2005). 4-Hydroxy-1-(3-pyridyl)-1-butanone, an indicator for 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced DNA damage, is not detected in human pancreatic tissue. *Cancer Epidemiol Biomarkers Prev*, 14: 540–541. doi:10.1158/1055-9965.EPI-04-0626 PMID:15734986

Quinn AM, Harvey RG, Penning TM (2008). Oxidation of PAH *trans*-dihydrodiols by human aldo-keto reductase AKR1B10. *Chem Res Toxicol*, 21: 2207–2215. doi:10.1021/tx8002005 PMID:18788756

Quiñones LA, Irarrázabal CE, Rojas CR *et al.* (2006). Joint effect among p53, CYP1A1, GSTM1 polymorphism combinations and smoking on prostate cancer risk: an exploratory genotype-environment interaction study. *Asian J Androl*, 8: 349–355. doi:10.1111/j.1745-7262.2006.00135.x PMID:16625286

Rahman M, Sakamoto J, Fukui T (2003). Bidi smoking and oral cancer: a meta-analysis. *Int J Cancer*, 106: 600–604. doi:10.1002/ijc.11265 PMID:12845659

Raimondi S, Paracchini V, Autrup H *et al.* (2006). Meta- and pooled analysis of GSTT1 and lung cancer: a HuGE-GSEC review. *Am J Epidemiol*, 164: 1027–1042. doi:10.1093/aje/kwj321 PMID:17000715

Raitiola HS & Pukander JS (1997). Etiological factors of laryngeal cancer. *Acta Otolaryngol Suppl*, 529: 215–217. doi:10.3109/00016489709124126 PMID:9288314

Rao AR (1984). Modifying influences of betel quid ingredients on B(a)P-induced carcinogenesis in the buccal pouch of hamster. *Int J Cancer*, 33: 581–586. doi:10.1002/ijc.2910330506 PMID:6327538

Rao DN & Desai PB (1998). Risk assessment of tobacco, alcohol and diet in cancers of base tongue and oral tongue–a case control study. *Indian J Cancer*, 35: 65–72. PMID:9849026

Rao DN, Desai PB, Ganesh B (1999). Alcohol as an additional risk factor in laryngopharyngeal cancer in Mumbai–a case-control study. *Cancer Detect Prev*, 23: 37–44. doi:10.1046/j.1525-1500.1999.09906.x PMID:9892989

Rao DN, Ganesh B, Dinshaw KA, Mohandas KM (2002). A case-control study of stomach cancer in Mumbai, India. *Int J Cancer*, 99: 727–731. doi:10.1002/ijc.10339 PMID:12115507

Rao DN, Ganesh B, Rao RS, Desai PB (1994). Risk assessment of tobacco, alcohol and diet in oral cancer–a case-control study. *Int J Cancer*, 58: 469–473. doi:10.1002/ijc.2910580402 PMID:8056441

Rappaport SM, Kim S, Lan Q *et al.* (2009). Evidence that humans metabolize benzene via two pathways. *Environ Health Perspect*, 117: 946–952. PMID:19590688

Raucy JL & Carpenter SJ (1993). The expression of xenobiotic-metabolizing cytochromes P450 in fetal tissues. *J Pharmacol Toxicol Methods*, 29: 121–128. doi:10.1016/1056-8719(93)90062-J PMID:8364226

Ray CS & Gupta P (2009). Bidis and Smokeless Tobacco. *Current Science*, 96: 1324–1334.

Rebbeck TR (1997). Molecular epidemiology of the human glutathione *S*-transferase genotypes *GSTM1* and *GSTT1* in cancer susceptibility. *Cancer Epidemiol Biomarkers Prev*, 6: 733–743. PMID:9298582

Reid A, de Klerk NH, Ambrosini GL *et al.* (2006a). The risk of lung cancer with increasing time since ceasing exposure to asbestos and quitting smoking. *Occup Environ Med*, 63: 509–512. doi:10.1136/oem.2005.025379 PMID:16849527

Reid ME, Duffield-Lillico AJ, Sunga A *et al.* (2006b). Selenium supplementation and colorectal adenomas: an analysis of the nutritional prevention of cancer trial. *Int J Cancer*, 118: 1777–1781. doi:10.1002/ijc.21529 PMID:16217756

Reynolds P, Hurley S, Goldberg DE *et al.* (2004a). Active smoking, household passive smoking, and breast cancer: evidence from the California Teachers Study. *J Natl Cancer Inst*, 96: 29–37. doi:10.1093/jnci/djh002 PMID:14709736

Reynolds P, Hurley SE, Hoggatt K *et al.* (2004b). Correlates of active and passive smoking in the California Teachers Study cohort. *J Womens Health (Larchmt)*, 13: 778–790. doi:10.1089/jwh.2004.13.778 PMID:15385072

Richardson H, Abrahamowicz M, Tellier PP *et al.* (2005). Modifiable risk factors associated with clearance of type-specific cervical human papillomavirus infections in a cohort of university students. *Cancer Epidemiol Biomarkers Prev*, 14: 1149–1156. doi:10.1158/1055-9965.EPI-04-0230 PMID:15894665

Riman T, Dickman PW, Nilsson S *et al.* (2004). Some lifestyle factors and the risk of invasive epithelial ovarian cancer in Swedish women. *Eur J Epidemiol*, 19: 1011–1019. doi:10.1007/s10654-004-1633-8 PMID:15648594

Riopel MA, Ronnett BM, Kurman RJ (1999). Evaluation of diagnostic criteria and behavior of ovarian intestinal-type mucinous tumors: atypical proliferative (borderline) tumors and intraepithelial, microinvasive, invasive, and metastatic carcinomas. *Am J Surg Pathol*, 23: 617–635. doi:10.1097/00000478-199906000-00001 PMID:10366144

Risch A & Plass C (2008). Lung cancer epigenetics and genetics. *Int J Cancer*, 123: 1–7. PMID:18425819

Risch HA, Howe GR, Jain M *et al.* (1993). Are female smokers at higher risk for lung cancer than male smokers? A case-control analysis by histologic type. *Am J Epidemiol*, 138: 281–293. PMID:8395141

Risch HA, Marrett LD, Jain M, Howe GR (1996). Differences in risk factors for epithelial ovarian cancer by histologic type. Results of a case-control study. *Am J Epidemiol*, 144: 363–372. PMID:8712193

Roddam AW, Pirie K, Pike MC *et al.* (2007). Active and passive smoking and the risk of breast cancer in women aged 36–45 years: a population based case-control study in the UK. *Br J Cancer*, 97: 434–439. doi:10.1038/sj.bjc.6603859 PMID:17579618

Rodgman A & Perfetti TA (2006). The composition of cigarette smoke: a catalogue of the polycyclic aromatic hydrocarbons. *Beitr. Tabakforschung Inti.*, 22: 13–69.

Rodgman A, Perfetti TA (2009). *Alphabetical Component Index*. In: *The Chemical Components of Tobacco and Tobacco Smoke*. Rodgman A, Perfetti TA, editors. Boca Raton, FL: CRC Press, pp. 1483–1784.

Rodin SN & Rodin AS (2005). Origins and selection of p53 mutations in lung carcinogenesis. *Semin Cancer Biol*, 15: 103–112. doi:10.1016/j.semcancer.2004.08.005 PMID:15652455

Rodriguez T, Altieri A, Chatenoud L *et al.* (2004). Risk factors for oral and pharyngeal cancer in young adults. *Oral Oncol*, 40: 207–213. doi:10.1016/j.oraloncology.2003.08.014 PMID:14693246

Rodriguez-Antona C & Ingelman-Sundberg M (2006). Cytochrome *P450* pharmacogenetics and cancer. *Oncogene*, 25: 1679–1691. doi:10.1038/sj.onc.1209377 PMID:16550168

Rohan TE & Baron JA (1989). Cigarette smoking and breast cancer. *Am J Epidemiol*, 129: 36–42. PMID:2910070

Rohrmann S, Genkinger JM, Burke A *et al.* (2007). Smoking and risk of fatal prostate cancer in a prospective U.S. study. *Urology*, 69: 721–725. doi:10.1016/j.urology.2006.12.020 PMID:17445658

Rollison DE, Brownson RC, Hathcock HL, Newschaffer CJ (2008). Case-control study of tobacco smoke exposure and breast cancer risk in Delaware. *BMC Cancer*, 8: 157 doi:10.1186/1471-2407-8-157 PMID:18518960

Rollison DE, Helzlsouer KJ, Lee JH *et al.* (2009). Prospective study of JC virus seroreactivity and the development of colorectal cancers and adenomas. *Cancer Epidemiol Biomarkers Prev*, 18: 1515–1523. doi:10.1158/1055-9965.EPI-08-1119 PMID:19383887

Rolón PA, Castellsagué X, Benz M, Muñoz N (1995). Hot and cold mate drinking and esophageal cancer in Paraguay. *Cancer Epidemiol Biomarkers Prev*, 4: 595–605. PMID:8547825

Rossing MA, Cushing-Haugen KL, Wicklund KG, Weiss NS (2008). Cigarette smoking and risk of epithelial ovarian cancer. *Cancer Causes Control*, 19: 413–420. doi:10.1007/s10552-007-9103-8 PMID:18080774

Rothman KJ, Greenland S (1998). *Modern Epidemiology*, 2nd ed. Philadelphia, USA: Lippincott-Raven Publishers.

Roubidoux MA, Kaur JS, Griffith KA *et al.* (2003). Relationship of mammographic parenchymal patterns to breast cancer risk factors and smoking in Alaska Native women. *Cancer Epidemiol Biomarkers Prev*, 12: 1081–1086. PMID:14578146

Royce JM, Hymowitz N, Corbett K *et al.*COMMIT Research Group (1993). Smoking cessation factors among African Americans and whites. *Am J Public Health*, 83: 220–226. doi:10.2105/AJPH.83.2.220 PMID:8427327

Ruano-Ravina A, Figueiras A, Freire-Garabal M, Barros-Dios JM (2006). Antioxidant vitamins and risk of lung cancer. *Curr Pharm Des*, 12: 599–613. doi:10.2174/138161206775474396 PMID:16472151

Rubin GL, Peterson HB, Lee NC *et al.* (1990). Estrogen replacement therapy and the risk of endometrial cancer: remaining controversies. *Am J Obstet Gynecol*, 162: 148–154. PMID:2301483

Ryk C, Berggren P, Kumar R *et al.* (2005). Influence of *GSTM1*, *GSTT1*, *GSTP1* and *NAT2* genotypes on the *p53* mutational spectrum in bladder tumours. *Int J Cancer*, 113: 761–768. doi:10.1002/ijc.20650 PMID:15499621

Saccone SF, Hinrichs AL, Saccone NL *et al.* (2007). Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. *Hum Mol Genet*, 16: 36–49. doi:10.1093/hmg/ddl438 PMID:17135278

Saintot M, Malaveille C, Hautefeuille A, Gerber M (2003). Interactions between genetic polymorphism of cytochrome P450–1B1, sulfotransferase 1A1, catechol-o-methyltransferase and tobacco exposure in breast cancer risk. *Int J Cancer*, 107: 652–657. doi:10.1002/ijc.11432 PMID:14520706

Sala E, Warren R, McCann J *et al.* (2000). Smoking and high-risk mammographic parenchymal patterns: a case-control study. *Breast Cancer Res*, 2: 59–63. doi:10.1186/bcr29 PMID:11056684

Salama SA, Abdel-Rahman SZ, Sierra-Torres CH *et al.* (1999). Role of polymorphic *GSTM1* and *GSTT1* genotypes on NNK-induced genotoxicity. *Pharmacogenetics*, 9: 735–743. doi:10.1097/00008571-199912000-00008 PMID:10634136

Salaspuro V & Salaspuro M (2004). Synergistic effect of alcohol drinking and smoking on *in vivo* acetaldehyde concentration in saliva. *Int J Cancer*, 111: 480–483. doi:10.1002/ijc.20293 PMID:15239123

Samanic C, Kogevinas M, Dosemeci M *et al.* (2006). Smoking and bladder cancer in Spain: effects of tobacco type, timing, environmental tobacco smoke, and gender. *Cancer Epidemiol Biomarkers Prev*, 15: 1348–1354. doi:10.1158/1055-9965.EPI-06-0021 PMID:16835335

Samowitz WS (2008). Genetic and epigenetic changes in colon cancer. *Exp Mol Pathol*, 85: 64–67. doi:10.1016/j.yexmp.2008.03.008 PMID:18482722

Samowitz WS, Albertsen H, Sweeney C *et al.* (2006). Association of smoking, CpG island methylator phenotype, and V600E BRAF mutations in colon cancer. *J Natl Cancer Inst*, 98: 1731–1738. doi:10.1093/jnci/djj468 PMID:17148775

Samowitz WS, Slattery ML, Sweeney C *et al.* (2007). *APC* mutations and other genetic and epigenetic changes in colon cancer. *Mol Cancer Res*, 5: 165–170. doi:10.1158/1541-7786.MCR-06-0398 PMID:17293392

Sanderson S, Salanti G, Higgins J (2007). Joint effects of the *N*-acetyltransferase 1 and 2 (*NAT1* and *NAT2*) genes and smoking on bladder carcinogenesis: a literature-based systematic HuGE review and evidence synthesis. *Am J Epidemiol*, 166: 741–751. doi:10.1093/aje/kwm167 PMID:17675654

Sanjoaquin MA, Appleby PN, Thorogood M *et al.* (2004). Nutrition, lifestyle and colorectal cancer incidence: a prospective investigation of 10998 vegetarians and non-vegetarians in the United Kingdom. *Br J Cancer*, 90: 118–121. doi:10.1038/sj.bjc.6601441 PMID:14710217

Sankaranarayanan R, Duffy SW, Day NE *et al.* (1989b). A case-control investigation of cancer of the oral tongue and the floor of the mouth in southern India. *Int J Cancer*, 44: 617–621. doi:10.1002/ijc.2910440410 PMID:2793234

Sankaranarayanan R, Duffy SW, Nair MK *et al.* (1990b). Tobacco and alcohol as risk factors in cancer of the larynx in Kerala, India. *Int J Cancer*, 45: 879–882. doi:10.1002/ijc.2910450517 PMID:2335391

Sankaranarayanan R, Duffy SW, Padmakumary G *et al.* (1989a). Tobacco chewing, alcohol and nasal snuff in cancer of the gingiva in Kerala, India. *Br J Cancer*, 60: 638–643. PMID:2803939

Sankaranarayanan R, Duffy SW, Padmakumary G *et al.* (1990a). Risk factors for cancer of the buccal and labial mucosa in Kerala, southern India. *J Epidemiol Community Health*, 44: 286–292. doi:10.1136/jech.44.4.286 PMID:2277249

Sankaranarayanan R, Duffy SW, Padmakumary G *et al.* (1991). Risk factors for cancer of the oesophagus in Kerala, India. *Int J Cancer*, 49: 485–489. doi:10.1002/ijc.2910490402 PMID:1917146

Sapkota A, Gajalakshmi V, Jetly DH *et al.* (2007). Smokeless tobacco and increased risk of hypopharyngeal and laryngeal cancers: a multicentric case-control study from India. *Int J Cancer*, 121: 1793–1798. doi:10.1002/ijc.22832 PMID:17583577

Sasazuki S, Sasaki S, Tsugane SJapan Public Health Center Study Group (2002). Cigarette smoking, alcohol consumption and subsequent gastric cancer risk by subsite and histologic type. *Int J Cancer*, 101: 560–566. doi:10.1002/ijc.10649 PMID:12237898

Sauvaget C, Lagarde F, Nagano J *et al.* (2005). Lifestyle factors, radiation and gastric cancer in atomic-bomb survivors (Japan). *Cancer Causes Control*, 16: 773–780. doi:10.1007/s10552-005-5385-x PMID:16132787

Scanlon EF, Suh O, Murthy SM *et al.* (1995). Influence of smoking on the development of lung metastases from breast cancer. *Cancer*, 75: 2693–2699. doi:10.1002/1097-0142(19950601)75:11<2693::AID-CNCR2820751109>3.0.CO;2-E PMID:7743472

Schechter MT, Miller AB, Howe GR (1985). Cigarette smoking and breast cancer: a case-control study of screening program participants. *Am J Epidemiol*, 121: 479–487. PMID:4014139

Schildt EB, Eriksson M, Hardell L, Magnuson A (1998). Oral snuff, smoking habits and alcohol consumption in

relation to oral cancer in a Swedish case-control study. *Int J Cancer*, 77: 341–346. doi:10.1002/(SICI)1097-0215(19980729)77:3<341::AID-IJC6>3.0.CO;2-O PMID:9663593

Schlecht NF, Franco EL, Pintos J *et al.* (1999b). Interaction between tobacco and alcohol consumption and the risk of cancers of the upper aero-digestive tract in Brazil. *Am J Epidemiol*, 150: 1129–1137. PMID:10588073

Schlecht NF, Franco EL, Pintos J, Kowalski LP (1999a). Effect of smoking cessation and tobacco type on the risk of cancers of the upper aero-digestive tract in Brazil. *Epidemiology*, 10: 412–418. doi:10.1097/00001648-199907000-00012 PMID:10401876

Schlemmer A, Jensen J, Riis BJ, Christiansen C (1990). Smoking induces increased androgen levels in early post-menopausal women. *Maturitas*, 12: 99–104. doi:10.1016/0378-5122(90)90087-M PMID:2123959

Schlicht KE, Michno N, Smith BD *et al.* (2007). Functional characterization of CYP2A13 polymorphisms. *Xenobiotica*, 37: 1439–1449. PMID:17922361

Schlöbe D, Hölzle D, Hatz D *et al.* (2008). 4-Hydroxy-1-(3-pyridyl)-1-butanone-releasing DNA adducts in lung, lower esophagus and cardia of sudden death victims. *Toxicology*, 245: 154–161. doi:10.1016/j.tox.2007.12.021 PMID:18243467

Schöllkopf C, Smedby KE, Hjalgrim H *et al.* (2005). Cigarette smoking and risk of non-Hodgkin's lymphoma — a population-based case-control study. *Cancer Epidemiol Biomarkers Prev*, 14: 1791–1796.

Schuller HM (2002). Mechanisms of smoking-related lung and pancreatic adenocarcinoma development. *Nat Rev Cancer*, 2: 455–463. PMID:12189387

Schuller HM (2009). Is cancer triggered by altered signalling of nicotinic acetylcholine receptors? *Nat Rev Cancer*, 9: 195–205. doi:10.1038/nrc2590 PMID:19194381

Schütze N, Vollmer G, Knuppen R (1994). Catecholestrogens are agonists of estrogen receptor dependent gene expression in MCF-7 cells. *J Steroid Biochem Mol Biol*, 48: 453–461. doi:10.1016/0960-0760(94)90193-7 PMID:8180106

Schütze N, Vollmer G, Tiemann I *et al.* (1993). Catecholestrogens are MCF-7 cell estrogen receptor agonists. *J Steroid Biochem Mol Biol*, 46: 781–789. doi:10.1016/0960-0760(93)90319-R PMID:8274412

Schwartz AG & Swanson GM (1997). Lung carcinoma in African Americans and whites. A population-based study in metropolitan Detroit, Michigan. *Cancer*, 79: 45–52. doi:10.1002/(SICI)1097-0142(19970101)79:1<45::AID-CNCR7>3.0.CO;2-L PMID:8988725

SEER (2004). *Surveillance research Program, Cancer Statistics Branch*. http://www.seer.cancer.gov/publications/csr.html

Seidegård J, Pero RW, Markowitz MM *et al.* (1990). Isoenzyme(s) of glutathione transferase (class Mu) as a marker for the susceptibility to lung cancer: a follow up study. *Carcinogenesis*, 11: 33–36. doi:10.1093/carcin/11.1.33 PMID:2295125

Seidegård J, Pero RW, Miller DG, Beattie EJ (1986). A glutathione transferase in human leukocytes as a marker for the susceptibility to lung cancer. *Carcinogenesis*, 7: 751–753. doi:10.1093/carcin/7.5.751 PMID:3698203

Sen B, Mahadevan B, DeMarini DM (2007). Transcriptional responses to complex mixtures: a review. *Mutat Res*, 636: 144–177. doi:10.1016/j.mrrev.2007.08.002 PMID:17888717

Seow A, Vainio H, Yu MC (2005). Effect of glutathione-S-transferase polymorphisms on the cancer preventive potential of isothiocyanates: an epidemiological perspective. *Mutat Res*, 592: 58–67. doi:10.1016/j.mrfmmm.2005.06.004 PMID:16019037

Setiawan VW, Pike MC, Kolonel LN *et al.* (2007). Racial/ethnic differences in endometrial cancer risk: the multiethnic cohort study. *Am J Epidemiol*, 165: 262–270. doi:10.1093/aje/kwk010 PMID:17090617

Shaib YH, El-Serag HB, Nooka AK *et al.* (2007). Risk factors for intrahepatic and extrahepatic cholangiocarcinoma: a hospital-based case-control study. *Am J Gastroenterol*, 102: 1016–1021. doi:10.1111/j.1572-0241.2007.01104.x PMID:17324130

Shapiro JA, Jacobs EJ, Thun MJ (2000). Cigar smoking in men and risk of death from tobacco-related cancers. *J Natl Cancer Inst*, 92: 333–337. doi:10.1093/jnci/92.4.333 PMID:10675383

Sharpe CR & Siemiatycki J (2001). Joint effects of smoking and body mass index on prostate cancer risk. *Epidemiology*, 12: 546–551. doi:10.1097/00001648-200109000-00014 PMID:11505174

Sharpe CR, Siemiatycki JA, Rachet BP (2002). The effects of smoking on the risk of colorectal cancer. *Dis Colon Rectum*, 45: 1041–1050. PMID:12195188

Shi X, Zhou S, Wang Z *et al.* (2008). *CYP1A1* and *GSTM1* polymorphisms and lung cancer risk in Chinese populations: a meta-analysis. *Lung Cancer*, 59: 155–163. doi:10.1016/j.lungcan.2007.08.004 PMID:17900751

Shields TS, Weiss NS, Voigt LF, Beresford SA (1999). The additional risk of endometrial cancer associated with unopposed estrogen use in women with other risk factors. *Epidemiology*, 10: 733–738. doi:10.1097/00001648-199911000-00014 PMID:10535788

Shikata K, Doi Y, Yonemoto K *et al.* (2008). Population-based prospective study of the combined influence of cigarette smoking and Helicobacter pylori infection on gastric cancer incidence: the Hisayama Study. *Am J Epidemiol*, 168: 1409–1415. doi:10.1093/aje/kwn276 PMID:18945691

Shimizu M, Lasker JM, Tsutsumi M, Lieber CS (1990). Immunohistochemical localization of ethanol-inducible P450IIE1 in the rat alimentary tract. *Gastroenterology*, 99: 1044–1053. PMID:2203661

Shimizu N, Nagata C, Shimizu H *et al.* (2003). Height, weight, and alcohol consumption in relation to the

risk of colorectal cancer in Japan: a prospective study. *Br J Cancer*, 88: 1038–1043. doi:10.1038/sj.bjc.6600845 PMID:12671701

Shklar G (1972). Experimental oral pathology in the Syrian hamster. *Prog Exp Tumor Res*, 16: 518–538. PMID:4557243

Shrubsole MJ, Wu H, Ness RM *et al.* (2008). Alcohol drinking, cigarette smoking, and risk of colorectal adenomatous and hyperplastic polyps. *Am J Epidemiol*, 167: 1050–1058. doi:10.1093/aje/kwm400 PMID:18304959

Shu HP & Bymun EN (1983). Systemic excretion of benzo(a)pyrene in the control and microsomally induced rat: the influence of plasma lipoproteins and albumin as carrier molecules. *Cancer Res*, 43: 485–490. PMID:6293698

Sikström B, Hellberg D, Nilsson S, Mårdh PA (1995). Smoking, alcohol, sexual behaviour and drug use in women with cervical human papillomavirus infection. *Arch Gynecol Obstet*, 256: 131–137. doi:10.1007/BF01314641 PMID:7574905

Sillanpää P, Heikinheimo L, Kataja V *et al.* (2007). CYP1A1 and CYP1B1 genetic polymorphisms, smoking and breast cancer risk in a Finnish Caucasian population. *Breast Cancer Res Treat*, 104: 287–297. doi:10.1007/s10549-006-9414-6 PMID:17063266

Simán JH, Forsgren A, Berglund G, Florén C-H (2001). Tobacco smoking increases the risk for gastric adenocarcinoma among Helicobacter pylori-infected individuals. *Scand J Gastroenterol*, 36: 208–213. doi:10.1080/003655201750065988 PMID:11252415

Simarak S, de Jong UW, Breslow N *et al.* (1977). Cancer of the oral cavity, pharynx/larynx and lung in North Thailand: case-control study and analysis of cigar smoke. *Br J Cancer*, 36: 130–140. PMID:19028

Singh R & Farmer PB (2006). Liquid chromatography-electrospray ionization-mass spectrometry: the future of DNA adduct detection. *Carcinogenesis*, 27: 178–196. doi:10.1093/carcin/bgi260 PMID:16272169

Singh R, Kaur B, Farmer PB (2005). Detection of DNA damage derived from a direct acting ethylating agent present in cigarette smoke by use of liquid chromatography-tandem mass spectrometry. *Chem Res Toxicol*, 18: 249–256. doi:10.1021/tx049793j PMID:15720129

Sjödahl K, Lu Y, Nilsen TI *et al.* (2007). Smoking and alcohol drinking in relation to risk of gastric cancer: a population-based, prospective cohort study. *Int J Cancer*, 120: 128–132. doi:10.1002/ijc.22157 PMID:17036324

Skipper PL & Tannenbaum SR (1990). Protein adducts in the molecular dosimetry of chemical carcinogens. *Carcinogenesis*, 11: 507–518. doi:10.1093/carcin/11.4.507 PMID:2182215

Skjelbred CF, Saebø M, Wallin H *et al.* (2006). Polymorphisms of the XRCC1, XRCC3 and XPD genes and risk of colorectal adenoma and carcinoma, in a Norwegian cohort: a case control study. *BMC Cancer*, 6: 67 doi:10.1186/1471-2407-6-67 PMID:16542436

Slattery ML, Curtin K, Anderson K *et al.* (2000). Associations between cigarette smoking, lifestyle factors, and microsatellite instability in colon tumors. *J Natl Cancer Inst*, 92: 1831–1836. doi:10.1093/jnci/92.22.1831 PMID:11078760

Slattery ML, Curtin K, Giuliano AR *et al.* (2008). Active and passive smoking, IL6, ESR1, and breast cancer risk. *Breast Cancer Res Treat*, 109: 101–111. doi:10.1007/s10549-007-9629-1 PMID:17594514

Slattery ML, Curtin K, Ma K *et al.* (2002). *GSTM*-1 and *NAT2* and genetic alterations in colon tumors. *Cancer Causes Control*, 13: 527–534. doi:10.1023/A:1016376016716 PMID:12195642

Slattery ML, Edwards S, Curtin K *et al.* (2003). Associations between smoking, passive smoking, GSTM-1, NAT2, and rectal cancer. *Cancer Epidemiol Biomarkers Prev*, 12: 882–889. PMID:14504199

Slattery ML, Samowtiz W, Ma K *et al.* (2004). CYP1A1, cigarette smoking, and colon and rectal cancer. *Am J Epidemiol*, 160: 842–852. doi:10.1093/aje/kwh298 PMID:15496536

Slemenda CW, Hui SL, Longcope C, Johnston CC Jr (1989). Cigarette smoking, obesity, and bone mass. *J Bone Miner Res*, 4: 737–741. PMID:2816518

Smith CJ, Perfetti TA, King JA (2006). Perspectives on pulmonary inflammation and lung cancer risk in cigarette smokers. *Inhal Toxicol*, 18: 667–677. doi:10.1080/08958370600742821 PMID:16864557

Smith EM, Sowers MF, Burns TL (1984). Effects of smoking on the development of female reproductive cancers. *J Natl Cancer Inst*, 73: 371–376. PMID:6589429

Smith JB & Randle HW (2001). Giant basal cell carcinoma and cigarette smoking. *Cutis*, 67: 73–76. PMID:11204609

Smits KM, Gaspari L, Weijenberg MP *et al.* (2003). Interaction between smoking, GSTM1 deletion and colorectal cancer: results from the GSEC study. *Biomarkers*, 8: 299–310. doi:10.1080/1354750031000121467 PMID:12944179

Sokić SI, Adanja BJ, Marinković JP, Vlajinac HD (1994). Case-control study of risk factors in laryngeal cancer. *Neoplasma*, 41: 43–47. PMID:8202195

Song DK, Xing DL, Zhang LR *et al.* (2009). Association of *NAT2*, *GSTM1*, *GSTT1*, *CYP2A6*, and *CYP2A13* gene polymorphisms with susceptibility and clinicopathologic characteristics of bladder cancer in Central China. *Cancer Detect Prev*, 32: 416–423. PMID:19303722

Sparks R, Bigler J, Sibert JG *et al.* (2004). TGFbeta1 polymorphism (L10P) and risk of colorectal adenomatous and hyperplastic polyps. *Int J Epidemiol*, 33: 955–961. doi:10.1093/ije/dyh102 PMID:15020570

Spira A, Beane J, Shah V *et al.* (2004). Effects of cigarette smoke on the human airway epithelial cell transcriptome. *Proc Natl Acad Sci U S A*, 101: 10143–10148. doi:10.1073/pnas.0401422101 PMID:15210990

Spitz MR, Amos CI, Dong Q *et al.* (2008). The CHRNA5–A3 region on chromosome 15q24–25.1 is a risk factor both for nicotine dependence and for lung cancer. *J Natl Cancer Inst*, 100: 1552–1556. doi:10.1093/jnci/djn363 PMID:18957677

Spitz MR, Duphorne CM, Detry MA *et al.* (2000). Dietary intake of isothiocyanates: evidence of a joint effect with glutathione *S*-transferase polymorphisms in lung cancer risk. *Cancer Epidemiol Biomarkers Prev*, 9: 1017–1020. PMID:11045782

Spitz MR, Fueger JJ, Goepfert H, Newell GR (1990). Salivary gland cancer. A case-control investigation of risk factors. *Arch Otolaryngol Head Neck Surg*, 116: 1163–1166. PMID:2206501

Spitz MR, Wu X, Wilkinson A, Wei D (2006). *Cancer of the Lung*. In: *Cancer Epidemiology and Prevention*. Schottenfeld D, Fraumeni JF, editors. New York: Oxford University Press, Chap 33, pp. 638–658.

Sriamporn S, Vatanasapt V, Pisani P *et al.* (1992). Environmental risk factors for nasopharyngeal carcinoma: a case-control study in northeastern Thailand. *Cancer Epidemiol Biomarkers Prev*, 1: 345–348. PMID:1305465

Srivastava A & Kreiger N (2004). Cigarette smoking and testicular cancer. *Cancer Epidemiol Biomarkers Prev*, 13: 49–54. doi:10.1158/1055-9965.EPI-03-0133 PMID:14744732

Stagnaro E, Ramazzotti V, Crosignani P *et al.* (2001). Smoking and hematolymphopoietic malignancies. *Cancer Causes Control*, 12: 325–334. doi:10.1023/A:1011216102871 PMID:11456228

Stagnaro E, Tumino R, Parodi S *et al.* (2004). Non-Hodgkin's lymphoma and type of tobacco smoke. *Cancer Epidemiol Biomarkers Prev*, 13: 431–437.

Stanton MF, Miller E, Wrench C, Blackwell R (1972). Experimental induction of epidermoid carcinoma in the lungs of rats by cigarette smoke condensate. *J Natl Cancer Inst*, 49: 867–877. PMID:4647499

Stavrides JC (2006). Lung carcinogenesis: pivotal role of metals in tobacco smoke. *Free Radic Biol Med*, 41: 1017–1030. doi:10.1016/j.freeradbiomed.2006.06.024 PMID:16962926

Steinmetz J, Spyckerelle Y, Guéguen R, Dupré C (2007). Alcohol, tobacco and colorectal adenomas and cancer. Case-control study in a population with positive fecal occult blood tests *Presse Med*, 36: 1174–1182. PMID:17350789

Stellman SD, Chen Y, Muscat JE *et al.* (2003). Lung cancer risk in white and black Americans. *Ann Epidemiol*, 13: 294–302. doi:10.1016/S1047-2797(02)00420-9 PMID:12684197

Stellman SD, Takezaki T, Wang L *et al.* (2001). Smoking and lung cancer risk in American and Japanese men: an international case-control study. *Cancer Epidemiol Biomarkers Prev*, 10: 1193–1199. PMID:11700268

Stepanov I, Carmella SG, Briggs A *et al.* (2009a). Presence of the carcinogen *N*'-nitrosonornicotine in the urine of some users of oral nicotine replacement therapy products. *Cancer Res*, 69: 8236–8240. PMID:19843845

Stepanov I, Carmella SG, Han S *et al.* (2009b). Evidence for endogenous formation of *N*'-nitrosonornicotine in some long-term nicotine patch users. *Nicotine Tob Res*, 11: 99–105. PMID:19246447

Stepanov I & Hecht SS (2005). Tobacco-specific nitrosamines and their pyridine-*N*-glucuronides in the urine of smokers and smokeless tobacco users. *Cancer Epidemiol Biomarkers Prev*, 14: 885–891. doi:10.1158/1055-9965.EPI-04-0753 PMID:15824160

Stepanov I, Hecht SS, Lindgren B *et al.* (2007). Relationship of human toenail nicotine, cotinine, and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol to levels of these biomarkers in plasma and urine. *Cancer Epidemiol Biomarkers Prev*, 16: 1382–1386. doi:10.1158/1055-9965.EPI-07-0145 PMID:17627002

Stepanov I, Upadhyaya P, Carmella SG *et al.* (2008). Extensive metabolic activation of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone in smokers. *Cancer Epidemiol Biomarkers Prev*, 17: 1764–1773. doi:10.1158/1055-9965.EPI-07-2844 PMID:18628430

Stern MC, Conti DV, Siegmund KD *et al.* (2007). DNA repair single-nucleotide polymorphisms in colorectal cancer and their role as modifiers of the effect of cigarette smoking and alcohol in the Singapore Chinese Health Study. *Cancer Epidemiol Biomarkers Prev*, 16: 2363–2372. doi:10.1158/1055-9965.EPI-07-0268 PMID:18006925

Stern MC, Siegmund KD, Conti DV *et al.* (2006). XRCC1, XRCC3, and XPD polymorphisms as modifiers of the effect of smoking and alcohol on colorectal adenoma risk. *Cancer Epidemiol Biomarkers Prev*, 15: 2384–2390. doi:10.1158/1055-9965.EPI-06-0381 PMID:17164360

Stockwell HG & Lyman GH (1987). Cigarette smoking and the risk of female reproductive cancer. *Am J Obstet Gynecol*, 157: 35–40. PMID:3605266

Strader CH, Vaughan TL, Stergachis A (1988). Use of nasal preparations and the incidence of sinonasal cancer. *J Epidemiol Community Health*, 42: 243–248. doi:10.1136/jech.42.3.243 PMID:2471766

Strange RC, Spiteri MA, Ramachandran S, Fryer AA (2001). Glutathione-*S*-transferase family of enzymes. *Mutat Res*, 482: 21–26. PMID:11535245

Strom BL, Schinnar R, Weber AL *et al.* (2006). Case-control study of postmenopausal hormone replacement therapy and endometrial cancer. *Am J Epidemiol*, 164: 775–786. doi:10.1093/aje/kwj316 PMID:16997897

Stürmer T, Glynn RJ, Lee IM *et al.* (2000). Lifetime cigarette smoking and colorectal cancer incidence in the Physicians' Health Study I. *J Natl Cancer Inst*, 92: 1178–1181. doi:10.1093/jnci/92.14.1178 PMID:10904092

Subapriya R, Thangavelu A, Mathavan B *et al.* (2007). Assessment of risk factors for oral squamous cell carcinoma in Chidambaram, Southern India: a case-control study. *Eur J Cancer Prev*, 16: 251–256. doi:10.1097/01.cej.0000228402.53106.9e PMID:17415096

Subramanian J & Govindan R (2008). Molecular genetics of lung cancer in people who have never smoked. *Lancet Oncol*, 9: 676–682. doi:10.1016/S1470-2045(08)70174-8 PMID:18598932

Sudo H, Li-Sucholeiki X-C, Marcelino LA *et al.* (2008). Fetal-juvenile origins of point mutations in the adult human tracheal-bronchial epithelium: absence of detectable effects of age, gender or smoking status. *Mutat Res*, 646: 25–40. PMID:18824180

Sung NY, Choi KS, Park EC *et al.* (2007). Smoking, alcohol and gastric cancer risk in Korean men: the National Health Insurance Corporation Study. *Br J Cancer*, 97: 700–704. doi:10.1038/sj.bjc.6603893 PMID:17637680

Suwan-ampai P, Navas-Acien A, Strickland PT, Agnew J (2009). Involuntary tobacco smoke exposure and urinary levels of polycyclic aromatic hydrocarbons in the United States, 1999 to 2002. *Cancer Epidemiol Biomarkers Prev*, 18: 884–893. doi:10.1158/1055-9965.EPI-08-0939 PMID:19258471

Suwanrungruang K, Sriamporn S, Wiangnon S *et al.* (2008). Lifestyle-related risk factors for stomach cancer in northeast Thailand. *Asian Pac J Cancer Prev*, 9: 71–75. PMID:18439078

Suzuki T, Matsuo K, Wakai K *et al.* (2007). Effect of familial history and smoking on common cancer risks in Japan. *Cancer*, 109: 2116–2123. doi:10.1002/cncr.22685 PMID:17410537

Swann PF (1984). Effect of ethanol on nitrosamine metabolism and distribution. Implications for the role of nitrosamines in human cancer and for the influence of alcohol consumption on cancer incidence. *IARC Sci Publ*, 57501–512. PMID:6533040

Swanson GM & Burns PB (1997). Cancers of the salivary gland: workplace risks among women and men. *Ann Epidemiol*, 7: 369–374. doi:10.1016/S1047-2797(97)00041-0 PMID:9279445

Sweeney C, Boucher KM, Samowitz WS *et al.* (2009). Oncogenetic tree model of somatic mutations and DNA methylation in colon tumors. *Genes Chromosomes Cancer*, 48: 1–9. doi:10.1002/gcc.20614 PMID:18767147

Syrjänen K, Shabalova I, Petrovichev N *et al.* (2007). Smoking is an independent risk factor for oncogenic human papillomavirus (HPV) infections but not for high-grade CIN. *Eur J Epidemiol*, 22: 723–735. doi:10.1007/s10654-007-9180-8 PMID:17828436

Szeliga J & Dipple A (1998). DNA adduct formation by polycyclic aromatic hydrocarbon dihydrodiol epoxides. *Chem Res Toxicol*, 11: 1–11. doi:10.1021/tx970142f PMID:9477220

Szyfter K, Szmeja Z, Szyfter W *et al.* (1999). Molecular and cellular alterations in tobacco smoke-associated larynx cancer. *Mutat Res*, 445: 259–274. PMID:10575435

't Mannetje A, Kogevinas M, Luce D *et al.* (1999). Sinonasal cancer, occupation, and tobacco smoking in European women and men. *Am J Ind Med*, 36: 101–107. doi:10.1002/(SICI)1097-0274(199907)36:1<101::AID-AJIM14>3.0.CO;2-A PMID:10361593

Takahashi I, Matsuzaka M, Umeda T *et al.* (2008). Differences in the influence of tobacco smoking on lung cancer between Japan and the USA: possible explanations for the 'smoking paradox' in Japan. *Public Health*, 122: 891–896. doi:10.1016/j.puhe.2007.10.004 PMID:18420238

Takahashi M, Imaida K, Mitsumori K *et al.* (1992). Promoting effects of cigarette smoke on the respiratory tract carcinogenesis of Syrian golden hamsters treated with diethylnitrosamine. *Carcinogenesis*, 13: 569–572. doi:10.1093/carcin/13.4.569 PMID:1576708

Talamini R, Bosetti C, La Vecchia C *et al.* (2002). Combined effect of tobacco and alcohol on laryngeal cancer risk: a case-control study. *Cancer Causes Control*, 13: 957–964. doi:10.1023/A:1021944123914 PMID:12588092

Tan W, Chen GF, Xing DY *et al.* (2001). Frequency of *CYP2A6* gene deletion and its relation to risk of lung and esophageal cancer in the Chinese population. *Int J Cancer*, 95: 96–101. doi:10.1002/1097-0215(20010320)95:2<96::AID-IJC1017>3.0.CO;2-2 PMID:11241319

Tang Y, Simoneau AR, Liao WX *et al.* (2009). WIF1, a Wnt pathway inhibitor, regulates SKP2 and c-myc expression leading to G1 arrest and growth inhibition of human invasive urinary bladder cancer cells. *Mol Cancer Ther*, 8: 458–468. doi:10.1158/1535-7163.MCT-08-0885 PMID:19174556

Tavani A, Negri E, Franceschi S *et al.* (1994). Attributable risk for laryngeal cancer in northern Italy. *Cancer Epidemiol Biomarkers Prev*, 3: 121–125. PMID:8049633

Terry MB & Neugut AI (1998). Cigarette smoking and the colorectal adenoma-carcinoma sequence: a hypothesis to explain the paradox. *Am J Epidemiol*, 147: 903–910. PMID:9596467

Terry P, Baron JA, Weiderpass E *et al.* (1999). Lifestyle and endometrial cancer risk: a cohort study from the Swedish Twin Registry. *Int J Cancer*, 82: 38–42. doi:10.1002/(SICI)1097-0215(19990702)82:1<38::AID-IJC8>3.0.CO;2-Q PMID:10360818

Terry P, Rohan TE, Franceschi S *et al.* (2004a). *Endometrial cancer*. In: *Tobacco: Science, Policy and Public Health*. Boyle P, Gray N, Henningfield J *et al.*, editors. Oxford University Press.

Terry PD & Goodman M (2006). Is the association between cigarette smoking and breast cancer modified by genotype? A review of epidemiologic studies and meta-analysis. *Cancer Epidemiol Biomarkers*

Prev, 15: 602–611. doi:10.1158/1055-9965.EPI-05-0853 PMID:16614098

Terry PD, Miller AB, Rohan TE (2002a). Prospective cohort study of cigarette smoking and colorectal cancer risk in women. *Int J Cancer*, 99: 480–483. doi:10.1002/ijc.10364 PMID:11992421

Terry PD, Miller AB, Rohan TE (2002b). A prospective cohort study of cigarette smoking and the risk of endometrial cancer. *Br J Cancer*, 86: 1430–1435. PMID:11986776

Terry PD & Rohan TE (2002). Cigarette smoking and the risk of breast cancer in women: a review of the literature. *Cancer Epidemiol Biomarkers Prev*, 11: 953–971. PMID:12376493

Terry PD, Rohan TE, Franceschi S, Weiderpass E (2002c). Cigarette smoking and the risk of endometrial cancer. *Lancet Oncol*, 3: 470–480. doi:10.1016/S1470-2045(02)00816-1 PMID:12147433

Theis RP, Dolwick Grieb SM, Burr D *et al.* (2008). Smoking, environmental tobacco smoke, and risk of renal cell cancer: a population-based case-control study. *BMC Cancer*, 8: 387 doi:10.1186/1471-2407-8-387 PMID:19108730

Thier R, Brüning T, Roos PH *et al.* (2003). Markers of genetic susceptibility in human environmental hygiene and toxicology: the role of selected CYP, NAT and GST genes. *Int J Hyg Environ Health*, 206: 149–171. doi:10.1078/1438-4639-00209 PMID:12872524

Thompson PA, DeMarini DM, Kadlubar FF *et al.* (2002). Evidence for the presence of mutagenic arylamines in human breast milk and DNA adducts in exfoliated breast ductal epithelial cells. *Environ Mol Mutagen*, 39: 134–142. doi:10.1002/em.10067 PMID:11921181

Thorgeirsson TE, Geller F, Sulem P *et al.* (2008). A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. *Nature*, 452: 638–642. doi:10.1038/nature06846 PMID:18385739

Thun MJ & Heath CW Jr (1997). Changes in mortality from smoking in two American Cancer Society prospective studies since 1959. *Prev Med*, 26: 422–426. doi:10.1006/pmed.1997.0182 PMID:9245660

Thun MJ, Henley SJ, Burns D *et al.* (2006). Lung cancer death rates in lifelong nonsmokers. *J Natl Cancer Inst*, 98: 691–699. PMID:16705123

Tiemersma EW, Bunschoten A, Kok FJ *et al.* (2004). Effect of SULT1A1 and NAT2 genetic polymorphism on the association between cigarette smoking and colorectal adenomas. *Int J Cancer*, 108: 97–103. doi:10.1002/ijc.11533 PMID:14618622

Tiemersma EW, Kampman E, Bueno de Mesquita HB *et al.* (2002a). Meat consumption, cigarette smoking, and genetic susceptibility in the etiology of colorectal cancer: results from a Dutch prospective study. *Cancer Causes Control*, 13: 383–393. doi:10.1023/A:1015236701054 PMID:12074508

Tiemersma EW, Kloosterman J, Bunschoten A *et al.* (2002b). Role of EPHX genotype in the associations of smoking and diet with colorectal adenomas. *IARC Sci Publ*, 156: 491–493. PMID:12484240

Tijhuis MJ, Visker MH, Aarts JM *et al.* (2008). NQO1 and NFE2L2 polymorphisms, fruit and vegetable intake and smoking and the risk of colorectal adenomas in an endoscopy-based population. *Int J Cancer*, 122: 1842–1848. doi:10.1002/ijc.23246 PMID:18074351

Timofeeva MN, Kropp S, Sauter W *et al.*LUCY-Consortium (2009). CYP450 polymorphisms as risk factors for early-onset lung cancer: gender-specific differences. *Carcinogenesis*, 30: 1161–1169. doi:10.1093/carcin/bgp102 PMID:19414505

To-Figueras J, Gené M, Gómez-Catalán J *et al.* (2001). Lung cancer susceptibility in relation to combined polymorphisms of microsomal epoxide hydrolase and glutathione *S*-transferase P1. *Cancer Lett*, 173: 155–162. doi:10.1016/S0304-3835(01)00626-7 PMID:11597790

Tokunaga M, Land CE, Yamamoto T *et al.* (1987). Incidence of female breast cancer among atomic bomb survivors, Hiroshima and Nagasaki, 1950–1980. *Radiat Res*, 112: 243–272. doi:10.2307/3577254 PMID:3685255

Tolstrup J, Munk C, Thomsen BL *et al.* (2006). The role of smoking and alcohol intake in the development of high-grade squamous intraepithelial lesions among high-risk HPV-positive women. *Acta Obstet Gynecol Scand*, 85: 1114–1119. doi:10.1080/00016340600677027 PMID:16929418

Tomita M, Odaka M, Matsumoto M *et al.* (1991). Cigarette smoking and mortality among Japanese males in a prospective cohort study *Nippon Koshu Eisei Zasshi*, 38: 492–497. PMID:1747539

Toyomura K, Yamaguchi K, Kawamoto H *et al.* (2004). Relation of cigarette smoking and alcohol use to colorectal adenomas by subsite: the self-defense forces health study. *Cancer Sci*, 95: 72–76. doi:10.1111/j.1349-7006.2004.tb03173.x PMID:14720330

Toyooka S, Tokumo M, Shigematsu H *et al.* (2006). Mutational and epigenetic evidence for independent pathways for lung adenocarcinomas arising in smokers and never smokers. *Cancer Res*, 66: 1371–1375. PMID:16452191

Tran GD, Sun XD, Abnet CC *et al.* (2005). Prospective study of risk factors for esophageal and gastric cancers in the Linxian general population trial cohort in China. *Int J Cancer*, 113: 456–463. doi:10.1002/ijc.20616 PMID:15455378

Tranah GJ, Giovannucci E, Ma J *et al.* (2004). Epoxide hydrolase polymorphisms, cigarette smoking and risk of colorectal adenoma in the Nurses' Health Study and the Health Professionals Follow-up Study. *Carcinogenesis*, 25: 1211–1218. doi:10.1093/carcin/bgh126 PMID:14988221

Tranah GJ, Giovannucci E, Ma J *et al.* (2005). APC Asp1822Val and Gly2502Ser polymorphisms and risk

of colorectal cancer and adenoma. *Cancer Epidemiol Biomarkers Prev*, 14: 863–870. doi:10.1158/1055-9965.EPI-04-0687 PMID:15824157

Trentham-Dietz A, Nichols HB, Hampton JM, Newcomb PA (2006). Weight change and risk of endometrial cancer. *Int J Epidemiol*, 35: 151–158. doi:10.1093/ije/dyi226 PMID:16278243

Trichopoulos D, Brown J, MacMahon B (1987). Urine estrogens and breast cancer risk factors among post-menopausal women. *Int J Cancer*, 40: 721–725. doi:10.1002/ijc.2910400602 PMID:3692620

Trost SG, Owen N, Bauman AE *et al.* (2002). Correlates of adults' participation in physical activity: review and update. *Med Sci Sports Exerc*, 34: 1996–2001. doi:10.1097/00005768-200212000-00020 PMID:12471307

Tsai HT, Tsai YM, Yang SF *et al.* (2007). Lifetime cigarette smoke and second-hand smoke and cervical intraepithelial neoplasm — a community-based case-control study. *Gynecol Oncol*, 105: 181–188. Epub 2007 Jan 3.

Tsoi KK, Pau CY, Wu WK *et al.* (2009). Cigarette smoking and the risk of colorectal cancer: a meta-analysis of prospective cohort studies. *Clin Gastroenterol Hepatol*, 7: 682–688, e1–e5. PMID:19245853

Tsong WH, Koh WP, Yuan JM *et al.* (2007). Cigarettes and alcohol in relation to colorectal cancer: the Singapore Chinese Health Study. *Br J Cancer*, 96: 821–827. PMID:17311023

Tu JT, Gao RN, Zhang DH, Gu BC (1985). Hepatitis B virus and primary liver cancer on Chongming Island, People's Republic of China. *Natl Cancer Inst Monogr*, 69: 213–215. PMID:3834335

Turner MC, Chen Y, Krewski D *et al.* (2007). Chronic obstructive pulmonary disease is associated with lung cancer mortality in a prospective study of never smokers. *Am J Respir Crit Care Med*, 176: 285–290. doi:10.1164/rccm.200612-1792OC PMID:17478615

Tuyns AJ, Estève J, Raymond L *et al.* (1988). Cancer of the larynx/hypopharynx, tobacco and alcohol: IARC international case-control study in Turin and Varese (Italy), Zaragoza and Navarra (Spain), Geneva (Switzerland) and Calvados (France). *Int J Cancer*, 41: 483–491. doi:10.1002/ijc.2910410403 PMID:3356483

Tuyns AJ, Péquignot G, Jensen OM (1977). [Esophageal cancer in Ille-et-Vilaine in relation to levels of alcohol and tobacco consumption. Risks are multiplying] *Bull Cancer*, 64: 45–60. PMID:861389

Tverdal A, Thelle D, Stensvold I *et al.* (1993). Mortality in relation to smoking history: 13 years' follow-up of 68,000 Norwegian men and women 35–49 years. *J Clin Epidemiol*, 46: 475–487. doi:10.1016/0895-4356(93)90025-V PMID:8501474

Tworoger SS, Gertig DM, Gates MA *et al.* (2008). Caffeine, alcohol, smoking, and the risk of incident epithelial ovarian cancer. *Cancer*, 112: 1169–1177. doi:10.1002/cncr.23275 PMID:18213613

Tyler CW Jr, Webster LA, Ory HW, Rubin GL (1985). Endometrial cancer: how does cigarette smoking influence the risk of women under age 55 years having this tumor? *Am J Obstet Gynecol*, 151: 899–905. PMID:3985056

Tzonou A, Day NE, Trichopoulos D *et al.* (1984). The epidemiology of ovarian cancer in Greece: a case-control study. *Eur J Cancer Clin Oncol*, 20: 1045–1052. doi:10.1016/0277-5379(84)90107-X PMID:6540687

Ulrich CM, Bigler J, Whitton JA *et al.* (2001). Epoxide hydrolase Tyr113His polymorphism is associated with elevated risk of colorectal polyps in the presence of smoking and high meat intake. *Cancer Epidemiol Biomarkers Prev*, 10: 875–882. PMID:11489754

Ursin G, London S, Stanczyk FZ *et al.* (1999). Urinary 2-hydroxyestrone/16alpha-hydroxyestrone ratio and risk of breast cancer in postmenopausal women. *J Natl Cancer Inst*, 91: 1067–1072. doi:10.1093/jnci/91.12.1067 PMID:10379970

Vaccarella S, Herrero R, Snijders PJ *et al.*IARC HPV Prevalence Surveys (IHPS) Study Group (2008). Smoking and human papillomavirus infection: pooled analysis of the International Agency for Research on Cancer HPV Prevalence Surveys. *Int J Epidemiol*, 37: 536–546. doi:10.1093/ije/dyn033 PMID:18316350

Vachon CM, Kuni CC, Anderson K *et al.* (2000). Association of mammographically defined percent breast density with epidemiologic risk factors for breast cancer (United States). *Cancer Causes Control*, 11: 653–662. doi:10.1023/A:1008926607428 PMID:10977110

Vairaktaris E, Spyridonidou S, Papakosta V *et al.* (2008). The hamster model of sequential oral oncogenesis. *Oral Oncol*, 44: 315–324. doi:10.1016/j.oraloncology.2007.08.015 PMID:18061531

van Dam RM, Huang Z, Rimm EB *et al.* (1999). Risk factors for basal cell carcinoma of the skin in men: results from the health professionals follow-up study. *Am J Epidemiol*, 150: 459–468. PMID:10472945

van den Donk M, Buijsse B, van den Berg SW *et al.* (2005). Dietary intake of folate and riboflavin, MTHFR C677T genotype, and colorectal adenoma risk: a Dutch case-control study. *Cancer Epidemiol Biomarkers Prev*, 14: 1562–1566. doi:10.1158/1055-9965.EPI-04-0419 PMID:15941973

van der Hel OL, Bueno de Mesquita HB, Sandkuijl L *et al.* (2003a). Rapid N-acetyltransferase 2 imputed phenotype and smoking may increase risk of colorectal cancer in women (Netherlands). *Cancer Causes Control*, 14: 293–298. doi:10.1023/A:1023601922106 PMID:12814209

van der Hel OL, Bueno-de-Mesquita HB, van Gils CH *et al.* (2005). Cumulative genetic defects in carcinogen metabolism may increase breast cancer risk (The Netherlands). *Cancer Causes Control*, 16: 675–681. doi:10.1007/s10552-005-1227-0 PMID:16049806

Van Emburgh BO, Hu JJ, Levine EA *et al.* (2008). Polymorphisms in drug metabolism genes, smoking, and *p53* mutations in breast cancer. *Mol Carcinog*, 47: 88–99. doi:10.1002/mc.20365 PMID:17683074

van Poppel G, de Vogel N, van Balderen PJ, Kok FJ (1992). Increased cytogenetic damage in smokers deficient in glutathione S-transferase isozyme *mu*. *Carcinogenesis*, 13:303–305.doi:10.1093/carcin/13.2.303 PMID:1740022

Vaughan TL, Davis S, Kristal A, Thomas DB (1995). Obesity, alcohol, and tobacco as risk factors for cancers of the esophagus and gastric cardia: adenocarcinoma versus squamous cell carcinoma. *Cancer Epidemiol Biomarkers Prev*, 4: 85–92. PMID:7742727

Vaughan TL, Shapiro JA, Burt RD *et al.* (1996). Nasopharyngeal cancer in a low-risk population: defining risk factors by histological type. *Cancer Epidemiol Biomarkers Prev*, 5: 587–593. PMID:8824359

Veglia F, Loft S, Matullo G *et al.*Genair-EPIC Investigators (2008). DNA adducts and cancer risk in prospective studies: a pooled analysis and a meta-analysis. *Carcinogenesis*, 29: 932–936. doi:10.1093/carcin/bgm286 PMID:18343884

Veglia F, Matullo G, Vineis P (2003). Bulky DNA adducts and risk of cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev*, 12: 157–160. PMID:12582026

Verla-Tebit E, Lilla C, Hoffmeister M *et al.* (2006). Cigarette smoking and colorectal cancer risk in Germany: a population-based case-control study. *Int J Cancer*, 119: 630–635. doi:10.1002/ijc.21875 PMID:16496385

Viezzer C, Norppa H, Clonfero E *et al.* (1999). Influence of *GSTM1*, *GSTT1*, *GSTP1*, and *EPHX* gene polymorphisms on DNA adduct level and *HPRT* mutant frequency in coke-oven workers. *Mutat Res*, 431: 259–269. PMID:10635992

Vineis P, Anttila S, Benhamou S *et al.* (2007). Evidence of gene gene interactions in lung carcinogenesis in a large pooled analysis. *Carcinogenesis*, 28: 1902–1905. doi:10.1093/carcin/bgm039 PMID:17307802

Vineis P & Malats N (1999). Strategic issues in the design and interpretation of studies on metabolic polymorphisms and cancer. *IARC Sci Publ*, 148: 51–61. PMID:10493248

Vineis P, Manuguerra M, Kavvoura FK *et al.* (2009). A field synopsis on low-penetrance variants in DNA repair genes and cancer susceptibility. *J Natl Cancer Inst*, 101: 24–36. PMID:19116388

Vineis P, Marinelli D, Autrup H *et al.* (2001). Current smoking, occupation, *N*-acetyltransferase-2 and bladder cancer: a pooled analysis of genotype-based studies. *Cancer Epidemiol Biomarkers Prev*, 10: 1249–1252. PMID:11751441

Vineis P, Veglia F, Anttila S *et al.* (2004). CYP1A1, GSTM1 and GSTT1 polymorphisms and lung cancer: a pooled analysis of gene-gene interactions. *Biomarkers*, 9: 298–305. doi:10.1080/13547500400011070 PMID:15764294

Vineis P, Veglia F, Benhamou S *et al.* (2003). CYP1A1 $T^{3801}C$ polymorphism and lung cancer: a pooled analysis of 2451 cases and 3358 controls. *Int J Cancer*, 104: 650–657. doi:10.1002/ijc.10995 PMID:12594823

Vioque J, Barber X, Bolumar F *et al.*PANESOES Study Group (2008). Esophageal cancer risk by type of alcohol drinking and smoking: a case-control study in Spain. *BMC Cancer*, 8: 221 doi:10.1186/1471-2407-8-221 PMID:18673563

Viswanathan AN, Feskanich D, De Vivo I *et al.* (2005). Smoking and the risk of endometrial cancer: results from the Nurses' Health Study. *Int J Cancer*, 114: 996–1001. doi:10.1002/ijc.20821 PMID:15645490

Vlajinac HD, Marinkovic JM, Sipetic SB *et al.* (2006). Case-control study of oropharyngeal cancer. *Cancer Detect Prev*, 30: 152–157. doi:10.1016/j.cdp.2006.02.001 PMID:16647226

Vlajinac HD, Pekmezović TD, Adanja BJ *et al.* (2003). Case-control study of multiple myeloma with special reference to diet as risk factor. *Neoplasma*, 50: 79–83.

Vories AA & Ramirez SG (1997). Warthin's tumor and cigarette smoking. *South Med J*, 90: 416–418. PMID:9114834

Voskuil DW, Kampman E, Grubben MJ *et al.* (2002). Meat consumption and meat preparation in relation to colorectal adenomas among sporadic and HNPCC family patients in The Netherlands. *Eur J Cancer*, 38: 2300–2308. doi:10.1016/S0959-8049(02)00455-0 PMID:12441267

Vrieling A, Bueno-De-Mesquita B, Boshuizen HC *et al.* (2009). Fruit and vegetable consumption and pancreatic cancer risk in the European Prospective Investigation into Cancer and Nutrition. *Int J Cancer*, 124: 1926–1934. PMID:19107929

Wakai K, Hayakawa N, Kojima M *et al.*JACC Study Group (2003). Smoking and colorectal cancer in a non-Western population: a prospective cohort study in Japan. *J Epidemiol*, 13: 323–332. PMID:14674660

Wakai K, Inoue M, Mizoue T *et al.*Research Group for the Development and Evaluation of Cancer Prevention Strategies in Japan (2006). Tobacco smoking and lung cancer risk: an evaluation based on a systematic review of epidemiological evidence among the Japanese population. *Jpn J Clin Oncol*, 36: 309–324. doi:10.1093/jjco/hyl025 PMID:16735374

Walser T, Cui X, Yanagawa J *et al.* (2008). Smoking and lung cancer: the role of inflammation. *Proc Am Thorac Soc*, 5: 811–815. doi:10.1513/pats.200809-100TH PMID:19017734

Wang H, Tan W, Hao B *et al.* (2003). Substantial reduction in risk of lung adenocarcinoma associated with genetic polymorphism in CYP2A13, the most active cytochrome P450 for the metabolic activation of tobacco-specific carcinogen NNK. *Cancer Res*, 63: 8057–8061. PMID:14633739

Wang M, Cheng G, Balbo S *et al.* (2009). Clear differences in levels of a formaldehyde-DNA adduct in leukocytes of smokers and non-smokers. *Cancer Research*, 69:7170–7174.

Wang SS, Schiffman M, Herrero R *et al.* (2004). Determinants of human papillomavirus 16 serological conversion and persistence in a population-based cohort of 10 000 women in Costa Rica. *Br J Cancer*, 91: 1269–1274. doi:10.1038/sj.bjc.6602088 PMID:15292929

Wang Y, Pereira EF, Maus AD *et al.* (2001). Human bronchial epithelial and endothelial cells express alpha7 nicotinic acetylcholine receptors. *Mol Pharmacol*, 60: 1201–1209. PMID:11723227

Warnakulasuriya KA & Ralhan R (2007). Clinical, pathological, cellular and molecular lesions caused by oral smokeless tobacco–a review. *J Oral Pathol Med*, 36: 63–77. PMID:17238967

Warren CW, Jones NR, Eriksen MP, Asma SGlobal Tobacco Surveillance System (GTSS) collaborative group (2006). Patterns of global tobacco use in young people and implications for future chronic disease burden in adults. *Lancet*, 367: 749–753. doi:10.1016/S0140-6736(06)68192-0 PMID:16517275

Warwick J, Pinney E, Warren RM *et al.* (2003). Breast density and breast cancer risk factors in a high-risk population. *Breast*, 12: 10–16. doi:10.1016/S0960-9776(02)00212-6 PMID:14659350

Wasnik KS, Ughade SN, Zodpey SP, Ingole DL (1998). Tobacco consumption practices and risk of oropharyngeal cancer: a case-control study in Central India. *Southeast Asian J Trop Med Public Health*, 29: 827–834. PMID:10772572

Watson MA, Stewart RK, Smith GB *et al.* (1998). Human glutathione *S*-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. *Carcinogenesis*, 19: 275–280. doi:10.1093/carcin/19.2.275 PMID:9498276

Watson P, Ashwathnarayan R, Lynch HT, Roy HK (2004). Tobacco use and increased colorectal cancer risk in patients with hereditary nonpolyposis colorectal cancer (Lynch syndrome). *Arch Intern Med*, 164: 2429–2431. PMID:15596632

Wei YS, Lu JC, Wang L *et al.* (2009). Risk factors for sporadic colorectal cancer in southern Chinese. *World J Gastroenterol*, 15: 2526–2530. PMID:19469004

Weiderpass E & Baron JA (2001). Cigarette smoking, alcohol consumption, and endometrial cancer risk: a population-based study in Sweden. *Cancer Causes Control*, 12: 239–247. doi:10.1023/A:1011201911664 PMID:11405329

Weiss JM, Saltzman BS, Doherty JA *et al.* (2006a). Risk factors for the incidence of endometrial cancer according to the aggressiveness of disease. *Am J Epidemiol*, 164: 56–62. doi:10.1093/aje/kwj152 PMID:16675538

Weiss JM, Weiss NS, Ulrich CM *et al.* (2005). Interindividual variation in nucleotide excision repair genes and risk of endometrial cancer. *Cancer Epidemiol Biomarkers Prev*, 14: 2524–2530. doi:10.1158/1055-9965.EPI-05-0414 PMID:16284373

Weiss JM, Weiss NS, Ulrich CM *et al.* (2006b). Nucleotide excision repair genotype and the incidence of endometrial cancer: effect of other risk factors on the association. *Gynecol Oncol*, 103: 891–896. doi:10.1016/j.ygyno.2006.05.020 PMID:16806437

Weiss NS, Farewall VT, Szekely DR *et al.* (1980). Oestrogens and endometrial cancer: effect of other risk factors on the association. *Maturitas*, 2: 185–190. doi:10.1016/0378-5122(80)90003-1 PMID:7442552

Wells PG, Mackenzie PI, Chowdhury JR *et al.* (2004). Glucuronidation and the UDP-glucuronosyltransferases in health and disease. *Drug Metab Dispos*, 32: 281–290. doi:10.1124/dmd.32.3.281 PMID:14977861

Welzel TM, Graubard BI, El-Serag HB *et al.* (2007). Risk factors for intrahepatic and extrahepatic cholangiocarcinoma in the United States: a population-based case-control study. *Clin Gastroenterol Hepatol*, 5: 1221–1228. doi:10.1016/j.cgh.2007.05.020 PMID:17689296

Wen CP, Tsai SP, Chen CJ, Cheng TY (2004). The mortality risks of smokers in Taiwan: Part I: cause-specific mortality. *Prev Med*, 39: 528–535. doi:10.1016/j.ypmed.2004.02.010 PMID:15313092

Wenzlaff AS, Cote ML, Bock CH *et al.* (2005). CYP1A1 and CYP1B1 polymorphisms and risk of lung cancer among never smokers: a population-based study. *Carcinogenesis*, 26: 2207–2212. doi:10.1093/carcin/bgi191 PMID:16051642

West KA, Brognard J, Clark AS *et al.* (2003). Rapid Akt activation by nicotine and a tobacco carcinogen modulates the phenotype of normal human airway epithelial cells. *J Clin Invest*, 111: 81–90. PMID:12511591

West S, Hildesheim A, Dosemeci M (1993). Non-viral risk factors for nasopharyngeal carcinoma in the Philippines: results from a case-control study. *Int J Cancer*, 55: 722–727. doi:10.1002/ijc.2910550504 PMID:7503957

Whittemore AS, Wu ML, Paffenbarger RS Jr *et al.* (1988). Personal and environmental characteristics related to epithelial ovarian cancer. II. Exposures to talcum powder, tobacco, alcohol, and coffee. *Am J Epidemiol*, 128: 1228–1240. PMID:3195564

WHO (2011).* *Third WHO Report on the Global Tobacco Epidemic*. World Health Organization. http://www.who.int/tobacco/global_report/2011/en/index.html

Wiencke JK (2002). DNA adduct burden and tobacco carcinogenesis. *Oncogene*, 21: 7376–7391. doi:10.1038/sj.onc.1205799 PMID:12379880

Willett EV, O'Connor S, Smith AG, Roman E (2007). Does smoking and alcohol modify the risk of Epstein-Barr virus-positive or -negative Hodgkin lymphoma. *Epidemiology*, 18: 130–136.

Wojno TH (1999). The association between cigarette smoking and basal cell carcinoma of the eyelids in women. *Ophthal Plast Reconstr Surg*, 15: 390–392. doi:10.1097/00002341-199911000-00004 PMID:10588245

Wojnowski L & Kamdem LK (2006). Clinical implications of *CYP3A* polymorphisms. *Expert Opin Drug Metab Toxicol*, 2: 171–182. doi:10.1517/17425255.2.2.171 PMID:16866606

Wolf CR & Smith G (1999). Cytochrome P450 CYP2D6. *IARC Sci Publ*, 148: 209–229. PMID:10493260

Wolff H, Saukkonen K, Anttila S *et al.* (1998). Expression of cyclooxygenase-2 in human lung carcinoma. *Cancer Res*, 58: 4997–5001. PMID:9823297

Wong HL, Murphy SE, Hecht SS (2005). Cytochrome P450 2A-catalyzed metabolic activation of structurally similar carcinogenic nitrosamines: *N*'-nitrosonornicotine enantiomers, *N*-nitrosopiperidine, and *N*-nitrosopyrrolidine. *Chem Res Toxicol*, 18: 61–69. doi:10.1021/tx0497696 PMID:15651850

Wright ME, Park Y, Subar AF *et al.* (2008). Intakes of fruit, vegetables, and specific botanical groups in relation to lung cancer risk in the NIH-AARP Diet and Health Study. *Am J Epidemiol*, 168: 1024–1034. doi:10.1093/aje/kwn212 PMID:18791192

Wu AH, Crabtree JE, Bernstein L *et al.* (2003). Role of Helicobacter pylori CagA+ strains and risk of adenocarcinoma of the stomach and esophagus. *Int J Cancer*, 103: 815–821. doi:10.1002/ijc.10887 PMID:12516104

Wu AH, Wan P, Bernstein L (2001). A multiethnic population-based study of smoking, alcohol and body size and risk of adenocarcinomas of the stomach and esophagus (United States). *Cancer Causes Control*, 12: 721–732. doi:10.1023/A:1011290704728 PMID:11562112

Wu AH, Yu MC, Mack TM (1997). Smoking, alcohol use, dietary factors and risk of small intestinal adenocarcinoma. *Int J Cancer*, 70: 512–517. doi:10.1002/(SICI)1097-0215(19970304)70:5<512::AID-IJC4>3.0.CO;2-0 PMID:9052748

Wu C, Hu Z, Yu D *et al.* (2009a). Genetic variants on chromosome 15q25 associated with lung cancer risk in Chinese populations. *Cancer Res*, 69: 5065–5072. doi:10.1158/0008-5472.CAN-09-0081 PMID:19491260

Wu IC, Lee CH, Kuo CH *et al.* (2009b). Consumption of cigarettes but not betel quid or alcohol increases colorectal cancer risk. *J Formos Med Assoc*, 108: 155–163. doi:10.1016/S0929-6646(09)60046-2 PMID:19251551

Wynder EL, Gottlieb S, Wright G (1957). A study of tobacco carcinogenesis. IV. Different tobacco types. *Cancer*, 10: 1206–1209. doi:10.1002/1097-0142(195711/12)10:6<1206::AID-CNCR2820100618>3.0.CO;2-P PMID:13489672

Wynder EL, Graham EA, Croninger AB (1953). Experimental production of carcinoma with cigarette tar. *Cancer Res*, 13: 855–864. PMID:13116124

Wynder EL & Hoffmann D (1961). A study of tobacco carcinogenesis. VIII. The role of the acidic fractions as promoters. *Cancer*, 14: 1306–1315. doi:10.1002/1097-0142(196111/12)14:6<1306::AID-CNCR2820140621>3.0.CO;2-T PMID:14008628

Wynder EL & Stellman SD (1979). Impact of long-term filter cigarette usage on lung and larynx cancer risk: a case-control study. *J Natl Cancer Inst*, 62: 471–477. PMID:283277

Yagyu K, Kikuchi S, Obata Y *et al.*JACC Study Group (2008). Cigarette smoking, alcohol drinking and the risk of gallbladder cancer death: a prospective cohort study in Japan. *Int J Cancer*, 122: 924–929. doi:10.1002/ijc.23159 PMID:17955487

Yamasaki E & Ames BN (1977). Concentration of mutagens from urine by absorption with the nonpolar resin XAD-2: cigarette smokers have mutagenic urine. *Proc Natl Acad Sci U S A*, 74: 3555–3559. doi:10.1073/pnas.74.8.3555 PMID:333441

Yang J, Qian LX, Wu HF *et al.* (2006). Genetic polymorphisms in the cytochrome P450 1A1 and 2E1 genes, smoking, drinking and prostate cancer susceptibility: a case-control study in a Han nationality population in Southern China. *Int J Urol*, 13: 773–780. doi:10.1111/j.1442-2042.2006.01401.x PMID:16834659

Yang M, Kim S, Lee E *et al.* (2003). Sources of polycyclic aromatic hydrocarbon exposure in non-occupationally exposed Koreans. *Environ Mol Mutagen*, 42: 250–257. doi:10.1002/em.10196 PMID:14673870

Yang M, Koga M, Katoh T, Kawamoto T (1999). A study for the proper application of urinary naphthols, new biomarkers for airborne polycyclic aromatic hydrocarbons. *Arch Environ Contam Toxicol*, 36: 99–108. doi:10.1007/s002449900447 PMID:9828267

Yang P, Cunningham JM, Halling KC *et al.* (2000). Higher risk of mismatch repair-deficient colorectal cancer in alpha(1)-antitrypsin deficiency carriers and cigarette smokers. *Mol Genet Metab*, 71: 639–645. doi:10.1006/mgme.2000.3089 PMID:11136557

Yang P, Sun Z, Krowka MJ *et al.* (2008). Alpha1-antitrypsin deficiency carriers, tobacco smoke, chronic obstructive pulmonary disease, and lung cancer risk. *Arch Intern Med*, 168: 1097–1103. doi:10.1001/archinte.168.10.1097 PMID:18504338

Yauk CL, Berndt ML, Williams A *et al.* (2007). Mainstream tobacco smoke causes paternal germ-line DNA mutation. *Cancer Res*, 67: 5103–5106. doi:10.1158/0008-5472.CAN-07-0279 PMID:17545587

Ye WM, Ye YN, Lin RD *et al.* (1995). Case control study on nasopharyngeal cancer in southern region of Fujian province. *Chin J Prev Control Chronic Dis*, 3: 158–161.

Ye Z, Song H, Higgins JP *et al.* (2006). Five glutathione s-transferase gene variants in 23,452 cases of lung cancer and 30,397 controls: meta-analysis of 130 studies. *PLoS Med*, 3: 524–534 doi:10.1371/journal.pmed.0030091 PMID:16509765

Yen TT, Lin WD, Wang CP *et al.* (2008). The association of smoking, alcoholic consumption, betel quid chewing and oral cavity cancer: a cohort study. *Eur Arch Otorhinolaryngol*, 265: 1403–1407. doi:10.1007/s00405-008-0659-z PMID:18389268

Yoo KY, Tajima K, Miura S *et al.* (1997). Breast cancer risk factors according to combined estrogen and progesterone receptor status: a case-control analysis. *Am J Epidemiol*, 146: 307–314. PMID:9270409

Young E, Leatherdale S, Sloan M *et al.* (2009). Age of smoking initiation and risk of breast cancer in a sample of Ontario women. *Tob Induc Dis*, 5: 4 doi:10.1186/1617-9625-5-4 PMID:19222858

Yu GP, Ostroff JS, Zhang ZF *et al.* (1997). Smoking history and cancer patient survival: a hospital cancer registry study. *Cancer Detect Prev*, 21: 497–509. PMID:9398990

Yu MC, Garabrant DH, Huang TB, Henderson BE (1990). Occupational and other non-dietary risk factors for nasopharyngeal carcinoma in Guangzhou, China. *Int J Cancer*, 45: 1033–1039. doi:10.1002/ijc.2910450609 PMID:2351484

Yu MC, Ho JH, Lai SH, Henderson BE (1986). Cantonese-style salted fish as a cause of nasopharyngeal carcinoma: report of a case-control study in Hong Kong. *Cancer Res*, 46: 956–961. PMID:3940655

Yuan JM, Koh WP, Murphy SE *et al.* (2009). Urinary levels of tobacco-specific nitrosamine metabolites in relation to lung cancer development in two prospective cohorts of cigarette smokers. *Cancer Res*, 69: 2990–2995. doi:10.1158/0008-5472.CAN-08-4330 PMID:19318550

Yuan JM, Ross RK, Wang XL *et al.* (1996). Morbidity and mortality in relation to cigarette smoking in Shanghai, China. A prospective male cohort study. *JAMA*, 275: 1646–1650. doi:10.1001/jama.275.21.1646 PMID:8637137

Yuan JM, Wang XL, Xiang YB *et al.* (2000). Non-dietary risk factors for nasopharyngeal carcinoma in Shanghai, China. *Int J Cancer*, 85: 364–369. doi:10.1002/(SICI)1097-0215(20000201)85:3<364::AID-IJC12>3.0.CO;2-C PMID:10652428

Yun YH, Jung KW, Bae JM *et al.* (2005). Cigarette smoking and cancer incidence risk in adult men: National Health Insurance Corporation Study. *Cancer Detect Prev*, 29: 15–24. doi:10.1016/j.cdp.2004.08.006 PMID:15734213

Zahm SH, Heineman EF, Vaught JB (1992). Soft tissue sarcoma and tobacco use: data from a prospective cohort study of United States veterans. *Cancer Causes Control*, 3: 371–376. doi:10.1007/BF00146891 PMID:1617125

Zak-Prelich M, Narbutt J, Sysa-Jedrzejowska A (2004). Environmental risk factors predisposing to the development of basal cell carcinoma. *Dermatol Surg*, 30: s2248–252. doi:10.1111/j.1524-4725.2004.30089.x PMID:14871217

Zang EA & Wynder EL (1996). Differences in lung cancer risk between men and women: examination of the evidence. *J Natl Cancer Inst*, 88: 183–192. doi:10.1093/jnci/88.3-4.183 PMID:8632492

Zanieri L, Galvan P, Checchini L *et al.* (2007). Polycyclic aromatic hydrocarbons (PAHs) in human milk from Italian women: influence of cigarette smoking and residential area. *Chemosphere*, 67: 1265–1274. doi:10.1016/j.chemosphere.2006.12.011 PMID:17258279

Zaridze D, Borisova E, Maximovitch D, Chkhikvadze V (2000). Alcohol consumption, smoking and risk of gastric cancer: case-control study from Moscow, Russia. *Cancer Causes Control*, 11: 363–371. doi:10.1023/A:1008907924938 PMID:10843447

Zatonski W, Becher H, Lissowska J, Wahrendorf J (1991). Tobacco, alcohol, and diet in the etiology of laryngeal cancer: a population-based case-control study. *Cancer Causes Control*, 2: 3–10. doi:10.1007/BF00052355 PMID:1873431

Zendehdel K, Nyrén O, Luo J *et al.* (2008). Risk of gastroesophageal cancer among smokers and users of Scandinavian moist snuff. *Int J Cancer*, 122: 1095–1099. doi:10.1002/ijc.23076 PMID:17973262

Zhang J, Ichiba M, Hara K *et al.* (2001). Urinary 1-hydroxypyrene in coke oven workers relative to exposure, alcohol consumption, and metabolic enzymes. *Occup Environ Med*, 58: 716–721. doi:10.1136/oem.58.11.716 PMID:11600727

Zhang L, Rothman N, Li G *et al.* (2007). Aberrations in chromosomes associated with lymphoma and therapy-related leukemia in benzene-exposed workers. *Environ Mol Mutagen*, 48: 467–474. doi:10.1002/em.20306 PMID:17584886

Zhang L, Steinmaus C, Eastmond DA *et al.* (2009c). Formaldehyde exposure and leukemia: a new meta-analysis and potential mechanisms. *Mutat Res*, 681: 150–168. doi:10.1016/j.mrrev.2008.07.002 PMID:18674636

Zhang S, Wang M, Villalta PW *et al.* (2009a). Quantitation of pyridyloxobutyl DNA adducts in nasal and oral mucosa of rats treated chronically with enantiomers of *N*'-nitrosonornicotine. *Chem Res Toxicol*, 22: 949–956. doi:10.1021/tx900040j PMID:19405515

Zhang S, Wang M, Villalta PW *et al.* (2009b). Analysis of pyridyloxobutyl and pyridylhydroxybutyl DNA adducts in extrahepatic tissues of F344 rats treated chronically with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone and enantiomers of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol. *Chem Res Toxicol*, 22: 926–936. doi:10.1021/tx900015d PMID:19358518

Zhang X, Su T, Zhang QY *et al.* (2002). Genetic polymorphisms of the human *CYP2A13* gene: identification of single-nucleotide polymorphisms and functional characterization of an Arg257Cys variant. *J Pharmacol Exp Ther*, 302: 416–423. PMID:12130698

Zhang Y, Coogan PF, Palmer JR *et al.* (2004). Cigarette smoking and increased risk of mucinous

epithelial ovarian cancer. *Am J Epidemiol*, 159: 133–139. doi:10.1093/aje/kwh015 PMID:14718214

Zhang ZF, Kurtz RC, Sun M *et al.* (1996). Adenocarcinomas of the esophagus and gastric cardia: medical conditions, tobacco, alcohol, and socioeconomic factors. *Cancer Epidemiol Biomarkers Prev*, 5: 761–768. PMID:8896886

Zhao B, Seow A, Lee EJ *et al.* (2001). Dietary isothiocyanates, glutathione *S*-transferase -M1, -T1 polymorphisms and lung cancer risk among Chinese women in Singapore. *Cancer Epidemiol Biomarkers Prev*, 10: 1063–1067. PMID:11588132

Zhao C, Tyndyk M, Eide I, Hemminki K (1999). Endogenous and background DNA adducts by methylating and 2-hydroxyethylating agents. *Mutat Res*, 424: 117–125. PMID:10064855

Zheng T, Holford T, Chen Y *et al.* (1997). Risk of tongue cancer associated with tobacco smoking and alcohol consumption: a case-control study. *Oral Oncol*, 33: 82–85. doi:10.1016/S0964-1955(96)00056-5 PMID:9231164

Zheng T, Holford TR, Zahm SH *et al.* (2002). Cigarette smoking, glutathione-s-transferase M1 and t1 genetic polymorphisms, and breast cancer risk (United States). *Cancer Causes Control*, 13: 637–645. doi:10.1023/A:1019500109267 PMID:12296511

Zheng TZ, Boyle P, Hu HF *et al.* (1990). Tobacco smoking, alcohol consumption, and risk of oral cancer: a case-control study in Beijing, People's Republic of China. *Cancer Causes Control*, 1: 173–179. doi:10.1007/BF00053170 PMID:2102288

Zheng W, Blot WJ, Shu XO *et al.* (1992). Diet and other risk factors for laryngeal cancer in Shanghai, China. *Am J Epidemiol*, 136: 178–191. PMID:1415140

Zheng W, Blot WJ, Shu XO *et al.* (1992b). Diet and other risk factors for laryngeal cancer in Shanghai, China. *Am J Epidemiol*, 136: 178–191. PMID:1415140

Zheng W, Blot WJ, Shu XO *et al.* (1992c). A population-based case-control study of cancers of the nasal cavity and paranasal sinuses in Shanghai. *Int J Cancer*, 52: 557–561. doi:10.1002/ijc.2910520410 PMID:1399136

Zheng W, McLaughlin JK, Chow WH *et al.* (1993a). Risk factors for cancers of the nasal cavity and paranasal sinuses among white men in the United States. *Am J Epidemiol*, 138: 965–972. PMID:8256781

Zheng W, McLaughlin JK, Gridley G *et al.* (1993). A cohort study of smoking, alcohol consumption, and dietary factors for pancreatic cancer (United States). *Cancer Causes Control*, 4: 477–482. doi:10.1007/BF00050867 PMID:8218880

Zheng YM, Tuppin P, Hubert A *et al.* (1994). Environmental and dietary risk factors for nasopharyngeal carcinoma: a case-control study in Zangwu County, Guangxi, China. *Br J Cancer*, 69: 508–514. PMID:8123482

Zhu BT & Conney AH (1998). Functional role of estrogen metabolism in target cells: review and perspectives. *Carcinogenesis*, 19: 1–27. doi:10.1093/carcin/19.1.1 PMID:9472688

Zhu K, Levine RS, Brann EA *et al.* (1995). A population-based case-control study of the relationship between cigarette smoking and nasopharyngeal cancer (United States). *Cancer Causes Control*, 6: 507–512. doi:10.1007/BF00054158 PMID:8580298

Zienolddiny S, Campa D, Lind H *et al.* (2008). A comprehensive analysis of phase I and phase II metabolism gene polymorphisms and risk of non-small cell lung cancer in smokers. *Carcinogenesis*, 29: 1164–1169. doi:10.1093/carcin/bgn020 PMID:18258609

Zivaljevic V, Vlajinac H, Marinkovic J *et al.* (2004). Cigarette smoking as a risk factor for cancer of the thyroid in women. *Tumori*, 90: 273–275. PMID:15315303

Znaor A, Brennan P, Gajalakshmi V *et al.* (2003). Independent and combined effects of tobacco smoking, chewing and alcohol drinking on the risk of oral, pharyngeal and esophageal cancers in Indian men. *Int J Cancer*, 105: 681–686. doi:10.1002/ijc.11114 PMID:12740918

Zu K & Giovannucci E (2009). Smoking and aggressive prostate cancer: a review of the epidemiologic evidence. *Cancer Causes Control*, Epub ahead of printPMID: 19562492.