

Inorganic mercury/ elemental mercury

Toxicological overview

Key Points

Kinetics and metabolism

- The main route of exposure to elemental mercury and inorganic mercury is inhalation and ingestion, respectively
- Following inhalation of elemental mercury or ingestion of inorganic mercury compounds, they are distributed to all tissues but mainly accumulate in the kidney. Elemental mercury may readily cross the blood-brain barrier
- Elimination of elemental mercury and inorganic mercury compounds predominantly occurs via the urine and faeces.

Health effects of acute exposure

- Inhalation of elemental mercury may cause respiratory effects (cough, dyspnoea), central nervous system effects (tremor, irritability), renal damage (proteinuria, haematuria, acute renal failure), gastrointestinal disturbances (stomatitis, nausea, diarrhoea) and cardiovascular effects (hypertension and tachycardia)
- Ingestion of inorganic mercury compounds may affect the digestive tract (metallic taste, vomiting, swollen gums, salivation, abdominal pain, diarrhoea), renal damage (oliguria, anuria, acute renal failure), cardiovascular effects (tachycardia, hypertension) and skin/eye effects (acrodynia, burning eyes and conjunctivitis)

Health effects of chronic exposure

- Inhalation of elemental mercury vapour may cause neurotoxicity (fatigue, tremor, headaches, depression, hallucinations), nephrotoxicity (proteinuria and enzymuria) and effects on the oral cavity (stomatitis, sore gums and oral mucosa ulcers)
- Ingestion of inorganic mercury compounds may cause neurotoxicity (irritability, weakness, photophobia, muscle twitching, confusion or dementia), digestive tract effects (swollen gums, salivation, diarrhoea or abdominal pain) or renal failure
- IARC classified elemental mercury and mercury compounds as category 3 carcinogens i.e. not classifiable as to the carcinogenicity to humans

Toxicological Overview

Summary of Health Effects

Following an acute exposure to elemental mercury vapour via inhalation, respiratory effects such as cough, dyspnoea, chest tightness, bronchitis and decreased pulmonary function may occur. Cognitive, personality, sensory or motor disturbances may also arise, including tremor, irritability, hallucinations, muscle weakness and headaches. Due to the accumulation of mercury in the kidneys, acute renal failure indicated by proteinuria, haematuria and oliguria is commonly reported. Acute inhalation of elemental mercury may also cause GI effects such as stomatitis, abdominal pain, vomiting, diarrhoea and ulceration of the oral mucosa, as well as cardiovascular effects such as hypertension and tachycardia.

Inorganic mercury compounds are highly irritating to the GI tract and an acute ingestion may cause a metallic taste, abdominal pain, vomiting, diarrhoea and necrosis of the intestinal mucosa, possibly leading to circulatory collapse and death. Ulceration of the mouth, lips, tongue and GI tract may also occur. If patients survive damage to the GI tract, acute renal failure may occur within 24 hours of ingestion. Hypertension and tachycardia have also been reported following ingestion of inorganic mercury compounds.

Acute dermal exposure to elemental mercury vapour can cause erythematous and pruritic skin rashes, reddening and peeling of skin on palms of feet and hands associated with acrodynia, and contact with soluble inorganic mercury compounds may cause irritation, vesiculation and contact dermatitis.

Chronic exposure to elemental mercury vapour via inhalation may cause neurotoxicity such as decreased psychomotor skills and neuropsychological symptoms including fatigue, tremor, headaches, depression, irritability, and hallucinations. Nephrotoxicity including proteinuria and increase urinary enzyme excretion was observed following occupational exposure to elemental mercury, as well as stomatitis, sore gums and ulceration of the oral mucosa.

Following chronic ingestion of inorganic mercury compounds irritability weakness, insomnia, muscle twitching, swollen gums, excess salivation, anorexia and abdominal pain may occur.

There is little convincing evidence that exposure to mercury causes chromosomal damage or other mutagenic effects.

IARC have classified elemental mercury and inorganic mercury compounds as category 3 carcinogens i.e. not classifiable as to carcinogenicity to humans.

Conflicting evidence regarding the incidence of spontaneous abortion following inorganic mercury exposure has been presented. Some studies have reported a higher incidence of reproductive failures (spontaneous abortions, still births, congenital malformations) and irregular, painful and haemorrhagic menstrual disorders in occupationally exposed women compared to unexposed women.

Kinetics and metabolism

The two forms of mercury, namely elemental and inorganic mercury have different biological properties hence are discussed separately.

Elemental mercury

The absorption of mercury is largely dependent on the form. The predominant route of exposure for elemental mercury is inhalation. After inhalation, approximately 80 % of mercury vapour crosses the alveolar membrane and is rapidly absorbed into the blood. Liquid elemental mercury is poorly absorbed (approximately 0.01 %) from the gastrointestinal tract probably due to its conversion to divalent mercury and binding to sulfhydryl groups [1-3]. Dermal exposure to elemental mercury vapour may also occur to some extent, contributing to approximately 2.6 % of the absorbed dose. Absorption via the olfactory nerves has also been proposed although quantitatively this is a minor pathway [3].

Absorbed elemental mercury is rapidly distributed to all tissues, although it accumulates to the greatest extent in the kidney, reaching between 50 and 90 % of the body burden [1, 3]. It reaches peak levels in all tissues within 24 hours, apart from the brain where peak levels are only reached after 23 days. Due to the high lipophilicity, elemental mercury readily passes the blood-brain barrier and the placenta. [3].

Following inhalation and absorption into the blood, elemental mercury vapour undergoes oxidation in the red blood cells to form divalent mercury, which predominantly exists as a non-diffusible form and binds to albumin and globulins. Oxidation may also occur in the liver and lungs, although it may occur in most other tissues to a lesser extent. In the brain and fetus, elemental mercury may be oxidised and thereby trapped as the divalent form does not cross the blood-brain barrier or the placenta as readily [3].

Elimination of elemental mercury predominantly occurs through the urine and faeces, although some may be excreted in sweat, expired air or saliva, with the half-life being approximately 1-2 months. After an acute exposure, urinary excretion accounts for approximately 13 % of the total body burden, this increasing to approximately 58 % after chronic exposure. Elemental mercury may be exhaled as mercury vapour or excreted in breast milk [2, 3].

Inorganic mercury

For inorganic mercury, the predominant route of exposure is ingestion [3]. In humans, approximately 5 – 10 % of inorganic mercury in food is absorbed after ingestion [2]. Following inhalation, the rate of absorption of inorganic mercury aerosols is dependent on particle size but on average, approximately 10 % is absorbed, as most particles will be deposited in the upper respiratory tract [1, 3].

The extent to which inorganic mercury is transported across the intestinal tract is largely dependent on its solubility and its dissociation in the lumen. Mercuric compounds are more readily absorbed than mercurous forms due to their solubility [3].

Inorganic mercury is distributed to all tissues following absorption, but due to the poor lipid solubility only a small fraction crosses the blood-brain barrier and the placenta. As is the case for elemental mercury, the largest systemic deposition of inorganic mercury occurs in the kidney [2].

The main pathway of excretion of inorganic mercury is via the urine and faeces, with the half life of approximately 1-2 months [1, 3]. Elimination of inorganic mercury from the blood and brain is a biphasic process encompassing an initial rapid elimination phase followed by a

slower phase [3]. Inorganic mercury may also be reduced to form elemental mercury which is exhaled as elemental mercury vapour or excreted in the breast milk [2, 3].

Sources and route of exposure

Mercury occurs naturally and is widely distributed in the environment owing to natural and anthropogenic processes. The major natural sources of mercury in the environment are degassing from the earth's crust, emissions from volcanoes and evaporation from water bodies [1]. Elemental mercury is also released into the air following man-made activities such as mining ore containing mercury, burning fossil fuels and incinerating waste. Mercury also enters the environment from fertilizers, fungicides and from solid waste i.e. thermometers or electrical switches [2].

Mercury may be detected in ground water in the range of 5 – 100 ng L⁻¹. Naturally occurring elemental mercury in both ground and surface water is less than 0.5 µg L⁻¹. Mercury in drinking water is not considered a major source of exposure except when significant pollution occurs [4].

The environmental fate of mercury in the soil is largely dependent on its form. The most common forms are mercury compounds which bind to soil organic matter, adsorb to mineral surfaces or precipitate as compounds of sulphur under reducing conditions [5]. However, some mercury salts are almost insoluble in water and therefore are unlikely to have significant mobility in soil [2]. Elemental mercury occurs in soil due to anthropogenic activities, although it may also form naturally as a by-product from microbial activity. The release of elemental mercury and other volatile mercury compounds from soil may be important in the cycling of mercury and may explain increased concentrations of mercury near to mercury-containing ore bodies [5].

Elevated concentrations of mercury in soil may lead to an increase in the mercury content in plants such as carrots, lettuce, mushrooms and apples, which, if grown in contaminated soil, may accumulate mercury. However, few data are available regarding the relationship between mercury concentration in the soil and the concentration in fruit and vegetables [5].

The general public is predominantly exposed to elemental mercury via inhalation of its vapour from amalgam used in dental fillings or from accidental spillages following breakages of thermometers, barometers or electrical switches [1, 6].

People undergo exposure to inorganic mercury by ingestion due to the use of mercury salts in herbal remedies [3]. Dermal exposure may also occur, as mercury salts were commonly used for their antiseptic, fungicidal and bactericidal properties [3]. In addition, the use of skin-lighteners can result in significant exposure due to dermal absorption [1].

Occupational exposure to mercury may be a major source of exposure. Individuals working in the production of electrical equipment, thermometers or barometers, those working in chemical processing plants or construction may all be exposed to elemental mercury vapour via inhalation or inorganic mercury [6]. Dentists and dental assistants involved with dental amalgam may also be exposed to elemental mercury due to inhalation and to a lesser extent by skin contact [3, 6]. Workplace exposure limits (WELs) for mercury and inorganic mercury compounds have been derived in the UK. The long-term exposure limit (LTTEL) is 0.025 mg m⁻³ (8 hour time weighted exposure (TWA) reference period). No data were available for the short-term exposure limits (STEL) (15 minute reference period) [7].

Health Effects of Acute / Single Exposure

Human Data

General toxicity

The major target organs of elemental mercury-induced toxicity are the central nervous system and the kidneys. The cardiovascular and respiratory system, GI tract and the skin are also affected at higher concentrations. Similarly, the target organs following ingestion of inorganic mercury are the kidneys and the central nervous system [1, 8].

Inhalation

Most data on the toxicity of mercury following inhalation refer to elemental mercury, as other forms, such as inorganic mercury, do not pose a significant risk via this route of exposure [6]. Following inhalation, the major target organs are the central and peripheral nervous system, and the kidneys, although at high concentrations respiratory, cardiovascular and gastrointestinal effects may also occur [6, 8].

Following an acute exposure to elemental mercury vapour, respiratory effects such as cough, dyspnoea and chest tightness have been reported, as well as bronchitis and bronchiolitis with interstitial pneumonitis, airway obstruction, and decreased pulmonary function. In severe cases pulmonary oedema, respiratory distress, and fibrosis may occur. Patients commonly develop respiratory insufficiency [1, 3, 6, 8, 9]. Such effects have been reported following exposure to 1.1 – 44 mg m⁻³ elemental mercury [9]. Inorganic mercury compounds have also been reported to cause respiratory effects such as shortness of breath or pulmonary oedema [3].

The central nervous system is one of the most sensitive targets following exposure to elemental mercury vapour, which may cause cognitive, personality, sensory or motor disturbances [3]. The effects may include tremor, irritability, nervousness, memory loss, hallucinations and neuromuscular changes such as muscle atrophy and muscle weakness, headaches and decreases in cognitive function [1, 3].

The kidneys are a major target organ following exposure to elemental mercury vapour due to the relatively high accumulation of mercury in the kidneys. High concentrations (not stated) have been reported to result in mild transient proteinuria, haematuria, oliguria, acute renal failure and degeneration of the proximal convoluted tubules [1, 3, 6].

Gastrointestinal effects have been reported in humans following acute inhalation of elemental mercury vapour. A classic symptom of mercury toxicity is inflammation of the oral mucosa, known as stomatitis, sometimes accompanied with excessive salivation and difficulty in swallowing. Other gastrointestinal effects including abdominal pain, nausea, diarrhoea, sore gums and ulceration of the oral mucosa may also occur following inhalation of elemental mercury vapour, although few studies report the concentration of mercury at which such symptoms arise [1, 3, 6].

Hypertension and tachycardia have both been reported following inhalation of high concentrations of elemental mercury, as well as hepatocellular effects, hepatomegaly and

central lobular vacuolation. Hypertension and tachycardia may also arise following exposure to inorganic mercury [3, 6].

Elemental mercury vapour has been reported to cause erythematous and pruritic skin rashes, reddening and peeling of skin on palms of feet and hands associated with acrodynia, burning eyes and conjunctivitis [3].

Acute exposure to elemental mercury vapour may produce 'metal fume fever', which is characterised by fatigue, fever and elevated leukocyte count [6].

Ingestion

Most data on the toxicity of mercury following ingestion refer to inorganic mercury compounds, as elemental mercury via ingestion does not, in general, cause serious effects [8].

Following ingestion, the major target organs are the GI tract, kidneys, cardiovascular system and the skin [3, 8, 9].

Ingestion of inorganic mercury salts such as mercuric chloride is highly irritating to the GI tract. One of the earliest symptoms is a metallic taste, followed by gastric pain and vomiting. As the compound passes into the lower GI tract abdominal pain, diarrhoea and necrosis of the intestinal mucosa may occur, possibly leading to circulatory collapse and death [9]. Ingestion of mercuric chloride may also lead to blistering and ulceration of the lips and tongue, oropharyngeal pain and ulceration of the GI tract [3, 6]. In contrast, ingestion of mercurous chloride appears to cause less severe GI effects, although individual case studies have reported nausea, vomiting, swollen gums, excess salivation, diarrhoea, anorexia and abdominal pain following the ingestion of unknown concentrations of mercurous chloride [3, 6]. Ingestion of elemental mercury results in negligible absorption and therefore exerts little effect on the GI tract [6].

The kidney is a critical target organ following the ingestion of inorganic mercury compounds. If patients survive GI tract damage following exposure to mercury salts, oliguria, anuria, necrosis of the proximal tubule epithelium and acute renal failure may occur within 24 hours of the ingestion of mercuric chloride prior to death [1, 9].

Tachycardia and hypertension have been reported following the ingestion of mercuric chloride and mercurous chloride [6]. In addition, tachycardia has been reported secondary to severe pneumonitis after acute exposure to mercury vapour [9].

Limited data are available regarding the respiratory effects following ingestion of inorganic mercury. Pulmonary oedema and shortness of breath have been reported following ingestion of mercuric chloride (dose not stated) [6].

Ingestion of elemental mercury may cause erythematous and pruritic skin rashes, reddening and peeling of skin on palms of feet and hands associated with acrodynia, burning eyes and conjunctivitis [3].

Ingestion of mercuric chloride may cause jaundice and elevation of liver enzymes [3, 6].

Dermal / ocular exposure

Dermal exposure to elemental mercury vapour may cause erythematous and pruritic skin rashes, reddening and peeling of skin on palms of feet and hands associated with acrodynia, burning eyes and conjunctivitis [3].

Soluble inorganic mercury compounds, in particular mercuric chloride, are irritating to the skin and mucous membranes. Exposure to 1 – 5 % may cause irritation, vesiculation, contact dermatitis and corrosion of the skin [3, 9]. Insoluble compounds are not immediately irritating but irritation may slowly develop as the compound is absorbed and ionised in tissues [9].

Amalgam filings have occasionally been shown to cause dermatitis on the face. In addition, dermatitis caused by allergy to elemental mercury has been described in dental personnel [1].

Neurological effects have been reported following dermal exposure to inorganic mercury [8].

Animal and In-Vitro Data

Inhalation

Inhalation of elemental mercury vapour ($1 - 1.1 \text{ mg m}^{-3}$ for 1 – 30 hours resulted in death of rabbits [8]. Mice, guinea pigs and rats inhaling elemental mercury vapour died of pulmonary oedema (concentration unknown) [6, 8].

Cardiovascular effects were also noted in animals. Cellular degeneration with necrosis of the heart tissue was observed in rabbits following exposure to 28.8 mg m^{-3} elemental mercury vapour for 4 – 30 hours. In the same study, gastrointestinal effects were noted, ranging from mild pathological changes to significant cellular degeneration and necrosis of the colon, as well as hepatic effects ranging from moderate pathological changes (unstated) to severe liver necrosis [6]. Rabbits also showed signs of renal effects ranging from cellular degeneration to tissue destruction and necrosis following inhalation of 28.8 mg m^{-3} elemental mercury vapour for 2 – 30 hours [6].

Ingestion

Ingestion of elemental mercury results in negligible absorption and therefore exerts little toxicological effect [6].

Few data are available regarding the toxicity of inorganic mercury following oral exposure.

Rats administered a single gavage dose of mercuric chloride (7.4 or 9.2 mg kg^{-1}) showed no differences in body weight or liver weight compared to controls although LDH activity was significantly decreased at both doses [6].

Renal toxicity was observed in rats and mice following acute exposure to mercuric chloride. Male and female rats were exposed to mercuric chloride ($0.93 - 14.8 \text{ mg kg}^{-1} \text{ day}^{-1}$) by gavage for five days a week. There was a significant increase in the kidney weights in groups exposed to $1.9 \text{ mg kg}^{-1} \text{ day}^{-1}$ and higher. Tubular necrosis occurred in rats exposed to 3.7 mg

kg⁻¹ day⁻¹ and higher, the severity increasing in a dose-dependent manner. An increase in urinary levels of alkaline phosphatase, AST and LDH was also observed in such groups [6].

Mice given a single dose of 10 mg kg⁻¹ day⁻¹ mercuric chloride by gavage showed minor renal tubular damage and rapid regeneration of the tubular epithelium [6].

Neurotoxicity was observed in rats following a single exposure to mercuric chloride (0.74 mg kg⁻¹) given by gavage and subcutaneously. Leakage of dye into the brain tissue, 12 hours after the single dose, demonstrated that the blood-brain barrier had been breached [6].

Health Effects of Chronic / Repeated Exposure

Human Data

General toxicity

Chronic exposure to mercury may affect the central nervous system, GI tract, kidneys, oral cavity, lungs, eyes, reproductive tract and skin.

Inhalation

Long-term exposure to elemental mercury vapour may cause neurotoxicity. Decreased psychomotor skills and neuropsychological symptoms, such as fatigue, tremor and headaches have been reported [3, 8]. As the exposure increases, the frequency and magnitude of muscle tremor increase and there are behavioural changes such as depression, memory loss, irritability and hallucinations [1, 8]. The peripheral nervous system may also be affected following chronic exposure to mercury vapour, resulting in reduced sensory and motor nerve function. No concentrations were reported [1, 8].

Effects on the central nervous system are generally considered to be the most sensitive indicator of toxicity of metallic mercury vapour [1, 2]. Studies on those occupationally exposed have shown a fairly consistent pattern of effects such as irritability, excitability, insomnia, tremor, decreased nerve conduction velocity, fatigue, short term memory deficits and depression. These studies, involving chlor-alkali workers, suggest a lowest observed adverse effect level (LOAEL) of 15-30 $\mu\text{g m}^{-3}$ metallic mercury expressed as an 8 hour concentration [2].

Occupational exposure to elemental mercury vapour may also result in kidney damage, indicated by proteinuria, increased urinary excretion of β -galactosidase, transferrin, β 2-microglobulin or albumin, and proximal tubular and glomerular changes [1, 6]. Slight changes in blood enzymes indicative of renal tubular effects were seen in workers with an estimated exposure to mercury vapour (based on the levels of mercury excreted in the urine) of 15 $\mu\text{g m}^{-3}$ [2].

Effects of chronic inhalation of elemental mercury on the cardiovascular system are equivocal as studies have reported differing results. Two studies reported that after exposure to mercury (0 – 0.27 and 0.075 mg m^{-3}) for more than 6 or 7 years no effects were observed on blood pressure. In contrast, workers exposed to 0.03 mg m^{-3} for at least 5 years showed signs of palpitations and cardiovascular reflex responses. Exposure to elemental mercury vapour has also been reported to cause hypertension and tachycardia, although concentrations were not stated [6].

Ingestion

Few data are available regarding the toxicity of inorganic mercury following chronic exposure. Dementia, colitis and renal failure has been reported following ingestion of mercurous chloride-containing laxative for between 6 and 25 years [8].

Children treated with products containing mercurous chloride (such as teething powders) many years ago exhibited signs of irritability, weakness, insomnia, photophobia, muscle

twitching or confusion. Such treatments no longer exist [3, 8]. Gastrointestinal effects such as swollen gums, excess salivation, anorexia, diarrhoea or abdominal pain were also seen in children treated with mercurous chloride [6]

Genotoxicity

Data from 14 studies of cytogenetic effects, such as sister chromatid exchange, micronucleus formation, chromosomal aberrations, aneuploidy and polyploidy in peripheral blood lymphocytes of individuals exposed to elemental mercury or mercury compounds were inconclusive. Overall, such monitoring studies have provided little convincing evidence that exposure to mercury causes chromosomal damage [3, 10].

Carcinogenicity

There was inadequate evidence in humans for the carcinogenicity of mercury and mercury compounds hence IARC have classified elemental mercury and inorganic mercury compounds as category 3 carcinogens. i.e. not classifiable as to carcinogenicity to humans. [10].

Reproductive and developmental toxicity

Several studies showed that chronic inhalation of elemental mercury had no effect on female fertility. Menstrual cycle disorders were reported to be more frequent in women occupationally exposed to elemental mercury [1, 3].

Data regarding the incidence of spontaneous abortion following inorganic mercury exposure are conflicting. Several studies reported a slightly elevated incidence of spontaneous abortions in women working in smelting plants or dental practices, whereas others did not find a correlation [1, 6]. One study carried out in female dentists and dental assistants reported a higher incidence of reproductive abnormalities (spontaneous abortions, still births, congenital malformations) and irregular, painful and haemorrhagic menstrual disorders than in unexposed women. This study, however, was criticised for erroneous interpretation of data [6]. The Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT) concluded that there was no evidence that occupational exposure to mercury during pregnancy in modern dental practices was harmful [11].

Some studies have reported that various forms of mercury reach the fetus via the placenta. Mercury vapour penetrates the placental barrier easier than inorganic mercury although whether the placenta concentrates mercury is unclear. Following inhalation of mercury, comparable levels of mercury were found in mother, foetus and placenta [12].

It has been reported that dentists or dental assistants who were occupationally exposed to metallic mercury below the threshold limit value had higher concentrations of metallic mercury in the placenta and fetal membranes compared with unexposed women. However, even in non-exposed women, the concentration of mercury in maternal blood increased by 46 % during pregnancy [12].

No information was available regarding the developmental/reproductive toxicity of inorganic mercury following oral exposure [8].

Males exposed to elemental mercury vapour in an occupational setting showed no association between mercury exposure and decreased fertility, or with increased rates of major malformations or serious childhood disease in their offspring [1].

Animal and In-Vitro Data

Inhalation

Respiratory effects were seen following chronic exposure to elemental mercury vapour. Rats exposed to 1 mg m⁻³ mercury vapour for 100 hours per week for six weeks showed signs of lung congestion, whereas rats exposed to 3 mg m⁻³ for 3 hours a day, five days a week for 12 – 42 weeks showed no significant changes [6].

Cardiovascular effects were also noted in animals. Mild to moderate pathological changes in the hearts of rabbits was observed following exposure to 0.86 - 6 mg m⁻³ elemental mercury vapour for 2 – 12 weeks [6].

No gastrointestinal changes were observed in rabbits exposed to 6 mg m⁻³ for seven hours a day, five days a week for up to 11 weeks, although hepatic changes were reported ranging from moderate pathological changes to marked cellular degeneration and necrosis [6].

In rats, slight degenerative changes were seen in the renal tubular epithelium following inhalation of 3 mg m⁻³ mercury vapour for three hours a day five days a week for 12-42 weeks [6].

Ingestion

Studies in animals have indicated that nephrotoxicity is the most sensitive endpoint following repeated exposure to inorganic mercury compounds [2]. Sub-acute studies (6 months) in rats given mercuric chloride orally, indicated a NOAEL of 0.23 mg kg⁻¹ day⁻¹. There was evidence of nephrotoxicity at 0.46 mg kg⁻¹ day⁻¹ and higher dose levels. Studies have also been reported in the Brown Norway rat, a species prone to the development of mercuric chloride-induced glomerulonephritis. Some evidence of effects were seen following oral doses of 3 mg mercuric chloride once a week for 60 days; this was considered to be the LOAEL (the daily dose was estimated to be around 0.3 mg kg⁻¹ day⁻¹ [2].

Respiratory effects such as forceful and laboured breathing, nose bleeds and other unspecified breathing difficulties were observed in rats following dietary exposure to 2.2 mg kg⁻¹ day⁻¹ mercuric chloride for three months [6].

Several studies reported cardiovascular effects following oral exposure to inorganic mercury. Exposure of rats to 28 mg kg⁻¹ day⁻¹ mercuric chloride for 180 days in drinking water resulted in hypertension and a decrease in cardiac contractility but did not affect heart rate. In contrast, a different strain of rat exposed to 7 mg kg⁻¹ day⁻¹ mercuric chloride in drinking water for 360 days as hypertension and increased cardiac contractility were observed, as well as decreased baroreceptor reflex sensitivity [6].

Genotoxicity

No experimental data were available on the genotoxicity of elemental mercury.

Studies with mercuric chloride gave conflicting results [10]. Single-strand DNA breaks have been reported following exposure of cultured mice embryo cells and Chinese hamster ovary cells to mercuric chloride. Other studies reported the induction of gene mutations in mouse lymphoma cells and DNA damage in rat and mouse fibroblasts. In contrast, mercuric chloride did not induce chromosome aberrations in human lymphocytes *in vitro* [1]. No *in-vivo* data are available. However, the data from the carcinogenicity studies do not suggest that inorganic mercury compounds have significant mutagenic potential.

Carcinogenicity

There is inadequate evidence in experimental animals for the carcinogenicity of metallic mercury and limited evidence for the carcinogenicity of mercuric chloride [10]. Following oral exposure to mercuric chloride (1.9 or $3.7 \text{ mg kg}^{-1} \text{ day}^{-1}$ for two years) male rats showed an increased incidence of forestomach hyperplasia compared to controls [6]. Other studies reported that rats given oral mercuric chloride (up to $5 \text{ mg kg}^{-1} \text{ day}^{-1}$) showed a significant increase in squamous cell papillomas of the forestomach and thyroid follicular cell adenomas and carcinomas compared with controls. However, the forestomach tumours did not progress to malignancy and were thought to arise from direct irritation of the tissue [2]. Similar studies on mice treated with up to $10 \text{ mg kg}^{-1} \text{ day}^{-1}$ showed a significant dose-related trend in renal tubular adenomas and adenocarcinomas [8]. However, such kidney tumours occurred at doses that were also nephrotoxic and would be expected to arise by a non-genotoxic mechanism [2].

Reproductive and developmental toxicity

Limited data are available regarding the reproductive toxicity of inorganic mercury. Male mice injected with single doses of mercuric chloride (1 mg kg^{-1}) showed decreased fertility with normal fertility resuming after approximately 2 months. Rats treated with intraperitoneal doses of $0.05 - 0.1 \text{ mg kg}^{-1}$ mercuric chloride over 90 days showed a gradual alteration in testicular tissue, such as a decrease in seminiferous tubular diameter, spermatogenic cell counts and Leydig cell nuclear diameter [1].

An increase in fetal resorptions in hamsters occurred followed a single oral exposure to mercuric chloride ($31.4 \text{ mg Hg kg}^{-1}$) [8]. Several studies have reported the occurrence of spontaneous abortions following exposure to elemental mercury vapour or inorganic mercury compounds. Decreased fetal weight and increased number of malformations i.e. cardiac abnormalities also occurred [1].

Several studies have investigated effects on ovulation in female hamsters. Animals were injected with mercuric chloride ($3 - 12.8 \text{ mg kg}^{-1}$), resulting in the inhibition of ovulation or follicular maturation [1].

References

- [1] International Programme on Chemical Safety (IPCS) (1991). Inorganic mercury. Environmental Health Criteria 118. World Health Organisation. Geneva.
- [2] Department for Environment Food and Rural Affairs (DEFRA) and Environment Agency (EA) (2002). Contaminants in soil: Collation of toxicological data and intake values for humans. Mercury. R&D Publications TOX 7.
- [3] World Health Organisation (WHO) (2003). Elemental mercury and inorganic mercury compounds: Human health aspects. Concise International Chemical Assessment Document. CICAD 50. Geneva.
- [4] World Health Organisation (WHO) (2005). Mercury in drinking water. Background document for development of WHO Guidelines for Drinking-water Quality.
- [5] Department for Environment Food and Rural Affairs (DEFRA) and Environment Agency (EA) (2002). Soil guideline values for inorganic mercury contamination.
- [6] Agency for Toxic Substances and Disease Registry (ATSDR) (1999). Toxicological Profile for Mercury. US Department of Health and Human Services. Atlanta, US.
- [7] Health & Safety Executive (2005). EH40/2005 Workplace Exposure Limits. The Stationery Office. London.
- [8] Risk Assessment Information System (RAIS). Toxicity summary for mercury. Chemical Hazard Evaluation and Communication Group, Biomedical and Environmental Information Analysis Section, Health and Safety Research Division.
- [9] International programme on Chemical Safety (IPCS) - INTOX databank. Mercury. UK PID Monograph. London, UK.
- [10] International Agency for the Research on Cancer (IARC) (1993). Beryllium, cadmium, mercury and exposures in the glass manufacturing industry. IARC monographs on the evaluation of carcinogenic risk to humans, vol 58. Lyon.
- [11] Committee on Toxicity of Chemicals in Food Consumer Products and the Environment (COT) (1997). Statement on dental amalgam.
- [12] National Teratology Information Service (NTIS) (2005). Metallic (elemental) and inorganic mercury exposure in pregnancy. NTIS, Regional drug and therapeutic centre. Newcastle-upon-tyne.

This document will be reviewed not later than 3 years or sooner if substantive evidence becomes available.

MERCURY AND MERCURY COMPOUNDS

1. Exposure Data

1.1 Chemical and physical data and analysis

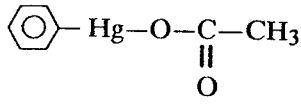
1.1.1 *Synonyms, trade names and molecular formulae*

Synonyms, trade names and molecular formulae for mercury and certain mercury compounds are presented in Table 1. The list of mercury compounds is not exhaustive, nor are those compounds necessarily the most commercially important mercury-containing substances; it includes the mercury compounds for which data on carcinogenicity are considered in this volume.

Table 1. Synonyms (Chemical Abstracts Service [CAS] names are in italics), trade names and atomic or molecular formulae of mercury and mercury compounds

Chemical name	CAS Reg. No. ^a	Synonyms and trade names	Formula
<i>Mercury metal</i>	7439-97-6 (8030-64-6; 51887-47-9; 92355-34-5; 92786-62-4; 123720-03-6)	Colloidal mercury; hydrargyrum; liquid silver; quecksilber; quick- silver	Hg
Mercuric acetate	1600-27-7 (6129-23-3; 7619-62-7; 19701-15-6)	<i>Acetic acid, mercury (2+) salt</i> ; bis(acetyloxy)mercury; diacet- oxymercury; mercuri, diacetic acid; mercury acetate; mercuric diacetate; mercury diacetate	$\begin{array}{c} \text{O} \\ \parallel \\ \text{Hg}(\text{O}-\text{C}-\text{CH}_3)_2 \end{array}$
Mercuric chloride	7487-94-7	Abavit B; bichloride of mercury; Calochlor; corrosive sublimate; corrosive mercury chloride; CRC; dichloromercury; mercuric bi- chloride; mercuric chloride; mer- curic dichloride; mercury bichloride; <i>mercury chloride</i> ; mercury(2+) chloride; mercury(II) chloride; mercury dichloride; mercury perchloride; Sublimate; Sulem	HgCl ₂
Mercuric oxide	21908-53-2 (1344-45-2; 8028-34-0)	Mercuric oxide (HgO); mercury monoxide; mercury oxide; <i>mercury oxide (HgO)</i> ; mercury(II) oxide; mercury(2+) oxide; red mercuric oxide; santar; yellow mercuric oxide	HgO

Table 1 (contd)

Chemical name	CAS Reg. No. ^a	Synonyms and trade names	Formula
<i>Dimethylmercury</i>	593-74-8	Methyl mercury	(CH ₃) ₂ Hg
Methylmercury chloride	115-09-3	Caspan; <i>chloromethylmercury</i> ; mercury methyl chloride; methylmercuric chloride; methylmercury monochloride; monomethyl mercury chloride	CH ₃ ClHg
Phenylmercury acetate	62-38-4 (1337-06-0; 8013-47-4; 61840-45-7; 64684-45-3)	<i>Acetato-O-phenylmercury</i> ; acetato-phenylmercury; acetatophenylmercury; acetic acid, phenyl mercury derivative; (acetoxymercuro)benzene; acetoxypheylmercury; mercuriphenyl acetate; phenylmercuric acetate; phenylmercury(II) acetate	

^aReplaced CAS Registry numbers are shown in parentheses

1.1.2 Chemical and physical properties of the pure substances

Selected chemical and physical properties of mercury and of the mercury compounds covered in this monograph are presented in Table 2.

Mercury (also called quicksilver because of its liquid state at room temperature) was known as early as 1000 BC. The discovery in 1938 of 1 kg of the metal in 2500-year-old sand layers on the eastern coast of Greece indicates that mercury was used in the extraction of gold at an early date. Mercury was mentioned about 200 BC in India as well as in China (Han dynasty). As early as 1556 AD, five different methods for extracting mercury from its ores were reported (Simon *et al.*, 1990).

Inorganic mercury exists in three oxidation states: 0 (metallic), +1 (mercurous) and +2 (mercuric); mercurous ions usually occur as dimers (Hg²⁺). The mercurous and mercuric states form numerous inorganic and organic chemical compounds. Organomercury compounds are those in which mercury is attached covalently to at least one carbon atom (Aylett, 1973; Simon *et al.*, 1990; WHO, 1990, 1991).

In its elemental form, mercury is a dense, silvery-white, shiny metal, which is liquid at room temperature and boils at 357 °C. At 20 °C, the vapour pressure of the metal is 0.17 Pa (0.0013 mm Hg). A saturated atmosphere at 20 °C contains 14 mg/m³ (Simon *et al.*, 1990).

Mercury compounds differ greatly in solubility: for example, in water, the solubility of metallic mercury is 60 µg/L at 25 °C, 250 µg/L at 50 °C and 1100 µg/L at 90 °C (Simon *et al.*, 1990); the solubility of mercurous chloride is 2 mg/L at 25 °C and that of mercuric chloride is 69 g/L at 20 °C (Lide, 1991). Methylmercury chloride is more soluble in water than mercurous chloride by about three orders of magnitude, owing to the very high solubility of the methylmercury cation in water. Certain species of mercury, including metallic mercury and the halide compounds of alkylmercury compounds, are soluble in non-polar solvents

Table 2. Chemical and physical properties of mercury and mercury compounds

Chemical name	Relative atomic/ molecular mass	Melting-point (°C)	Typical physical description	Density	Solubility
Mercury metal	200.59	- 38.87	Silvery-white, heavy, mobile, liquid metal	13.546 (20 °C)	Soluble in nitric acid, sulfuric acid upon heavy boiling, lipids, pentane; insoluble in dilute hydrochloric, hydrobromic and hydroiodic acids, water (2 µg/L at 30 °C), ethanol, diethyl ether, cold sulfuric acid
Mercuric acetate	318.7	178–180 (decomposes)	White crystals or crystalline powder	3.27	Soluble in water (250 g/L at 10 °C), ethanol, acetic acid
Mercuric chloride	271.50	276	Colourless, rhombic, odourless, crystal or white powder	5.44 (25 °C)	Soluble in water (69 g/L at 20 °C), methanol, ethanol, amyl alcohol, acetone, formic acid, acetic acid, the lower acetate esters, diethyl ether, benzene, glycerol; slightly soluble in carbon disulfide and pyridine
Mercuric oxide	216.6	500 (decomposes)	Yellow or red, <i>ortho</i> -rhombic, odourless crystalline powder	11.14	Insoluble in water (53 mg/L at 25 °C), soluble in acids; insoluble in ethanol, diethyl ether, acetone, alkali, ammoniac
Dimethylmercury	230.66	NR	Colourless liquid with a sweet odour	3.19 (20 °C)	Soluble in ethanol and diethyl ether; insoluble in water
Methylmercury chloride	251.10	167–168	White crystalline solid with a disagreeable odour	4.06	Slightly soluble in water
Phenylmercury acetate	336.75	150	White to cream-coloured, small, odourless, lustrous crystalline solid (prism, powder, leaflet)	2.4	Soluble in ethanol, benzene, glacial acetic acid, acetone, ammonium acetate, chloroform, diethyl ether; slightly soluble in water (4.37 g/L at 25 °C)

From Aylett (1973); Lide (1991); Alfa Products (1990); Budavari (1989); Sax & Lewis (1987); Drake (1981); Singer & Nowak (1981); Worthing (1987); Strem Chemicals (1992). NR, not reported

(WHO, 1991). Mercury vapour is more soluble in plasma, whole blood and haemoglobin than in distilled-water or isotonic saline (Hursh, 1985).

Mercury forms monovalent and divalent compounds with the halogens fluorine, chlorine, bromine and iodine. It also forms monovalent and divalent compounds with sulfur. From the biochemical point of view, the most important chemical property of mercuric mercury and alkylmercury compounds may be their high affinity for sulphhydryl groups (Simon *et al.*, 1990; WHO, 1991).

The main volatile mercury species in air is metallic mercury, but dimethylmercury may also occur. Mercury compounds such as mercuric chloride and methylmercury hydroxide are also relatively stable in fresh water, including snow, rain and standing and flowing water. HgCl_4^{2-} is the dominant form of mercury in seawater (WHO, 1991).

1.1.3 *Technical products and impurities*

Metallic mercury—purities: triple-distilled grade, $\geq 99.99\%$ (4N); ACS reagent grade, 99.995–99.9995%; electronic grade, 99.9998%; ultra-high purity grade, 99.99999–99.999999% (Alfa Products, 1990; CERAC, Inc., 1991; Aldrich Chemical Co., 1992; Strem Chemicals, 1992; Atomergic Chemetals Corp., undated; D.F. Goldsmith Chemical & Metal Corp., undated); impurities (%): Ag, 0.0001; Fe, 0.00005; Pb, 0.00001; Cu, 0.00001; Cd, 0.00001; Zn, 0.00005 (Janssen Chimica, 1990).

Mercuric acetate—purities: 97–99.9%; ACS reagent grade, $\geq 98\%$ (Janssen Chimica, 1990; CERAC, Inc., 1991; Aldrich Chemical Co., 1992; Strem Chemicals, 1992).

Mercuric chloride—purities: ACS reagent grade, 99%; 99.9–99.9995%; impurities (%): Fe, 0.002; Pb, 0.002; Cu, 0.002; Ca, max. 0.002 (Janssen Chimica, 1990; CERAC, Inc., 1991; Aldrich Chemical Co., 1992; Strem Chemicals, 1992).

Mercuric oxide—purities: high-purity, 99.999%; ACS grade (yellow or red), 99% (CERAC, Inc., 1991; Aldrich Chemical Co., 1992).

Dimethylmercury—purities, 95–98% (Aldrich Chemical Co., 1992; Strem Chemicals, 1992)

Methylmercury chloride—purity: $\geq 95\%$ (Alfa Products, 1990)

Phenylmercury acetate—purities: 97–97.5%; practical, US Pharmacopeia and National Formulary grades (Janssen Chimica, 1990; Aldrich Chemical Co., 1992; Strem Chemicals, 1992; D.F. Goldsmith Chemical & Metal Corp., undated). Some of the trade names associated with phenylmercuric acetate include: Agrosan D; Agrosan GN5; Algimycin; Aligimycin 200; Anticon; Antimucin WBR; Antimucin WDR; Bufen; Bufen 30; Caswell No. 656; Cekusil; Celmer; Ceresan; Ceresol; Contra Creme; Dyanacide; Femma; FMA; Fungicide R; Fungitox OR; Gallotox; Hexasan; HL-331; Hostaquick; Intercide 60; Intercide PMA 18; Kwixsan; Lerophyn; Leytosan; Liquiphene; Lorophyn; Meracen; Mercron; Mercuron; Mergal A 25; Mersolite; Mersolite 8; Mersolite D; Metasol 30; Neantina; Norforms; Nuodex PMA 18; Nylmerate; Pamisan; Panomatic; Phenmad; Phix; PMA; PMA 220; PMAC; PMAcetate; PMAL; PMAS; Programin; Purasan-SC-10; Puraturf 10; Quicksan; Quicksan 20; Riogen; Ruberon; Samtol; Sanitized SPG; Sanitol; Sanmicron; Scutl; SC-110; Seed Dressing R; Seedtox; Setrete; Shimmerex; Spor-KI; Spruce Seal; Tag; Tag 331; Tag Fungicide; Tag HL-331; Trigosan; Troysan 30; Troysan PMA 30; Verdasan; Volpar; Zaprawa Nasienna R; Ziarnik

Impurities of mercury compounds that are the subjects of other monographs are lead (IARC, 1987a) and cadmium (this volume, p. 119).

1.1.4 Analysis

Selected methods for the determination of mercury in various media are presented in Table 3. Other methods have been reviewed (WHO, 1990, 1991).

Table 3. Methods for the analysis of mercury in various media

Sample matrix	Sample preparation	Assay procedure	Limit of detection	Reference
Air	Collect on Hydrar sorbent; desorb with nitric then hydrochloric acids	CVAA	0.03 µg/sample	Eller (1989)
Drugs	Digest in water-hydrochloric acid-nitric acid; heat; cool; add potassium dichromate	AAS	NR	Helrich (1990a)
Liquid waste, ground-water	Digest with sulfuric and nitric acids; add potassium permanganate and potassium persulfate solutions; heat; cool; reduce with sodium chloride-hydroxylamine sulfate; add stannous sulfate and aerate	CVAA	0.2 µg/L	US Environmental Protection Agency (1986a) (Method 7470); Helrich (1990b)
		AAS	NR	
Soil, sediment, solid and semisolid waste	Digest with distilled water and aqua-regia; heat; cool; add potassium permanganate and heat; cool; add sodium chloride-hydroxylamine sulfate; or digest as above	CVAA	0.2 µg/L	US Environmental Protection Agency (1986b) (Method 7471)
Blood, urine	Reduce inorganic and organic mercury to Hg ⁰ with reducing agents (e.g., SnCl ₂); estimate organic mercury as difference between total and inorganic	CVAA	0.5 µg/L	Magos & Clarkson (1972)
	Reduce total mercury with sodium borohydride; enrich with an amalgamation device (Au/Pt gauze)	CVAA	0.3 µg/L urine or blood	Angerer & Schaller (1988)
Blood, urine, hair, tissues	Automated form of the method of Magos and Clarkson (1972)	CVAA	2.5 µg/kg	Farant <i>et al.</i> (1981)

Abbreviations: CVAA, flameless cold vapour atomic absorption spectroscopy; AAS, flame or flameless atomic absorption spectroscopy; NR, not reported

The original 'dithizone' method has been replaced by atomic absorption spectrometry, neutron activation analysis, atomic fluorescence spectrometry, inductively coupled plasma emission spectrometry and spark source spectrometry. Cold vapour atomic absorption is the most popular and reliable technique. Metallic mercury and inorganic mercury compounds and organomercury compounds in biological and environmental specimens are converted by

reducing agents (tin chloride, cadmium chloride–tin chloride, sodium borohydride) to metallic mercury and released as mercury vapour, which is either pumped directly through the quartz cell of the atomic absorption spectrophotometer or analysed after amalgamation on a silver–platinum gauze. The organic mercury content of the sample is given by the difference between total and inorganic compounds. For routine analysis, especially for blood and urine samples, the total mercury content is determined using sodium borohydride as the reducing agent, avoiding time-consuming decomposition of the samples (Angerer & Schaller, 1988).

The neutron activation procedure for analysis in urine is regarded as the most accurate and sensitive procedure and is usually used as the reference method (WHO, 1991).

Helrich (1990a) described several methods (atomic absorption spectrometry, gravimetry, titrimetry) for the determination of mercury and mercury compounds in various forms of drugs (solutions of organomercury compounds, ointments, calomel tablets, tablets containing purgative drugs). Helrich (1990c) described methods (flameless atomic absorption spectrometry, colorimetric dithizone) for the determination of mercury in food and fish and gas chromatographic methods for the determination of methylmercury compounds in fish and shellfish. Helrich (1990d) described methods (volatilization, precipitation, titrimetry, gravimetry) for the determination of mercury in organomercury seed disinfectants.

Pre-analytical and analytical procedures involve the risk of losing mercury from the sample, or contamination. Owing to the small amounts of mercury (in nanogram or even sub-nanogram ranges) in specimens, especially of biological materials, careful quality control must be undertaken. Control materials (blood and urine) are commercially available for intralaboratory quality control, and national and international intercomparison programmes are offered for external quality control. Reference materials covering the range of samples obtained for monitoring are commercially available for both environmental and biological samples (see WHO, 1991); however, the available control materials for daily use and reference materials do not cover the demand for different mercury species.

(a) *Metallic mercury*

Analytical methods for mercury in air can be divided into instant reading methods and methods with separate sampling and analysis stages. One direct ('instant') reading method is based on the 'cold vapour atomic absorption' technique, which measures the absorption of mercury vapour by ultraviolet light at a wavelength of 253.7 nm. Most of the atomic absorption spectroscopy procedures have a detection limit in the range of 2–5 µg/m³ mercury (WHO, 1991).

Another direct reading method employed increasingly is a special gold amalgamation technique, which has been used in a number of studies to evaluate the release of metallic mercury vapour into the oral cavity from amalgam fillings (WHO, 1991). The method is based on an increase in the electrical resistance of a thin gold film after absorption of mercury vapour. The detection limit is 0.05 ng mercury (McNerney *et al.*, 1972).

In an analytical method based on separate sampling and analysis, air is sampled in two bubblers in series containing sulfuric acid and potassium permanganate. The mercury is subsequently determined by cold vapour atomic absorption. With this method, the total mercury in the air, and not just mercury vapour, can be measured. Another sampling

technique involves solid absorbents. Amalgamation techniques using gold have been shown to collect mercury vapour efficiently (WHO, 1991).

Air can be sampled for the analysis of mercury by static samplers or by personal monitoring (WHO, 1991). In a comparison of results obtained using static samplers and personal samplers, the latter yielded higher time-weighted average concentrations than the former in most work places (Roels *et al.*, 1987).

(b) *Mercuric chloride and mercuric acetate*

A dual-stage differential atomization atomic absorption technique was developed to allow speciation of 10 mercury-containing compounds, including mercuric chloride and mercuric acetate, in aqueous solution and biological fluids (Robinson & Skelly, 1982).

(c) *Methylmercury compounds*

Gas chromatography is usually used for selective measurement of methylmercury compounds and other organomercury compounds, particularly in fish tissues. An alternative approach is to separate methylmercury compounds from inorganic mercury compounds by volatilization, ion exchange or distillation and to estimate them by nonselective methods (e.g. atomic absorption) (WHO, 1990).

(d) *Phenylmercury acetate*

Phenylmercury acetate was determined in pharmaceutical products by reverse-phase high-performance liquid chromatography of a morpholinedithiocarbamate derivative. The method is specific and sensitive and has been used to determine a number of phenylmercury salts in pharmaceutical products (Parkin, 1987).

1.2 Production and use

1.2.1 Production

Worldwide production data for mercury are presented in Table 4. Over the last 10 years, production figures have changed only slightly. Current production in the USA is approximately 53% of the potential capacity: Because of reduced demand, many mines and smelting plants are no longer operating or have greatly cut back production. A large proportion of Mexican production has been exported to Brazil and Argentina. China claims to have the largest mercury resources in the world; most of the Chinese production is exported to the USA. Italy, once a large producer of mercury, now imports it from Algeria and Yugoslavia. The Almadén mercury mine in Spain accounted for 90% of the total output of the European Economic Community for many years, and most of the production has been exported to Belgium, France, Luxembourg and the USA. Whereas in 1986 the former USSR exported most of its mercury, almost the entire production is now reserved for domestic use (Simon *et al.*, 1990; WHO, 1991).

(a) *Metallic mercury*

All mercury ores are relatively low-grade, the average mercury content being about 1%. Mercury ores lie close to the Earth's surface, so that the required mining depth is about 800 m

Table 4. Worldwide production of mercury (tonnes)

Country	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991
Algeria	1049	1055	506	841	877	386	828	587	795	690	773	690	586	637	431
China ^a	700	600	700	800	800	800	850	800	800	850	900	940	880	800	700
Czechoslovakia	183	196	171	159	153	151	144	152	158	168	164	168	131	126	120
Dominican Republic	18	17	21	6	3	2	4	2	1	NR	< 0.5	< 0.5	< 0.5	NR	NR
Finland	22	39	46	75	67	71	65	80	130	147	147	130	159	141	125
Germany	99	84	91	56	76	53	NR	NR	NR	NR	NR	NR	NR	NR	NR
Italy	14	3	NR	3	252	159	NR	NR	NR	NR	NR	NR	NR	NR	NR
Mexico	333	76	68	145	240	295	221	384	264	345	124	345	651	735	720
Russia ^a	2200	2000	2000	1800	1700	1700	1700	1600	1600	1500	1650	2300	2300	2100	1900
Spain	926	1020	1116	1721	1560	1540	1416	1520	1539	1471	1553	1716	1380 ^a	425 ^a	450
Turkey	162	173	163	154	204	246	162	182	226	262	202	97	197	60	60
USA	974	834	1018	1058	962	888	864	657	570	470	34	379	414	NR	NR
Former Yugoslavia	108	NR	NR	NR	NR	NR	52	72	88	75	67	70	51	37	30
Total ^b	6788	6097	5900	6818	6894	6291	6306	6036	6171	5978	5906	6835	6749	5061	4536

From Simon *et al.* (1990); Reese (1992a). NR, not reported

^aEstimated values

^bTotals may not add up because some values are estimates.

at most. The most important ore for mercury extraction is α -mercuric sulfide (red) (cinnabar, cinnabarite). The ore is heated with lime in retorts or furnaces to liberate the metal as vapour, which is cooled in a condensing system to form metallic mercury. Other methods include leaching of ores and concentrates with sodium sulfide and sodium hydroxide and subsequent precipitation with aluminium or by electrolysis; alternatively, mercury in ore is dissolved in a sodium hypochlorite solution, the mercury-laden solution is then passed through activated carbon to absorb the mercury, and the activated carbon is heated to produce metallic mercury. The latter methods are, however, no longer used (Drake, 1981; Simon *et al.*, 1990).

Industrial waste containing mercury also contributes to its production. The majority of plants using chloralkali electrolysis employ liquid mercury cathodes, resulting in residues containing 10% mercury or more. In addition to this major secondary source, mercury batteries, mercury fluorescent tubes, electrical switches, thermometer breakage and obsolete rectifiers should be regarded as sources of mercury. Scrap material and industrial and municipal wastes and sludges containing mercury are treated in much the same manner as ores to recover mercury. Scrap products are first broken down to liberate metallic mercury or its compounds. Heating in retorts vaporizes the mercury which, upon cooling, condenses to high-purity metallic mercury. Industrial and municipal sludges and wastes may be treated chemically before roasting (Drake, 1981; Simon *et al.*, 1990). Although the overall production of mercury has decreased over the last 20 years, sufficient potential uses, and therefore secondary sources, remain for the foreseeable future owing to the unique properties of the metal (Simon *et al.*, 1990).

Most of the metallic mercury on the market is 4N material (99.99% mercury). The most common purification methods include: *Dry oxidation*—with this method, readily oxidizable constituents such as magnesium, zinc, copper, aluminium, calcium, silicon and sodium can be removed by passing air or oxygen through the liquid metal; the oxides that form have a lower density than mercury and float on its surface, where they can be removed by filtration, scooping or by removing the mercury from the bottom. *Wet oxidation*—in an aqueous medium, mercury is dissolved by adding nitric, hydrochloric or sulfuric acid (see IARC, 1992) with dichromate, permanganate or peroxide to oxidize impurities; the aqueous solution can be separated from the mercury by decanting, and traces of water can be removed with calcium oxide. *Electrolytic refining*—perchloric acid containing mercuric oxide serves as the electrolyte. *Distillation*—mercury can be evaporated under atmospheric pressure or *in vacuo*; elements with a lower vapour pressure than mercury can be separated in this way. In many cases, mercury must be distilled repeatedly to achieve the desired purity (Simon *et al.*, 1990).

(b) *Mercuric acetate*

Mercuric acetate is produced by dissolving mercuric oxide in dilute acetic acid and concentrating the resulting solution (Simon *et al.*, 1990).

(c) *Mercuric chloride*

Mercuric chloride is prepared by the direct oxidation of mercury with chlorine gas, the same method (chamber method) that is used to prepare mercurous chloride, except that, for

mercuric chloride, an excess of chlorine gas is used to ensure complete reaction to the higher oxidation state; the reaction is carried out at temperatures $> 300^{\circ}\text{C}$. The escaping sublimate vapour is condensed in cooled receivers, where it settles as fine crystals. Excess chlorine is absorbed by sodium hydroxide in a tower; a very pure product results from use of this method (Singer & Nowak, 1981; Simon *et al.*, 1990).

Mercuric chloride can also be prepared from other mercury compounds. For example, if mercuric sulfate is heated in the dry state with sodium chloride, the evolving mercuric chloride vapour can be condensed to a solid in receivers (Simon *et al.*, 1990).

(d) *Mercuric oxide*

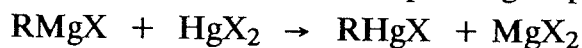
Mercuric oxide can be prepared *via* the anhydrous route by reaction of mercury and oxygen at $350\text{--}420^{\circ}\text{C}$ under oxygen pressure or by thermal decomposition of mercury nitrates at about 320°C . Production *via* the wet route, by precipitation, is more important commercially: The oxide is precipitated from solutions of mercuric salts by addition of caustic alkali (usually mercuric chloride solutions with sodium hydroxide). Whether the yellow or the red form is obtained depends on the reaction conditions: Slow crystal growth during heating of mercury with oxygen or during thermal decomposition of mercurous nitrate leads to relatively large crystals (i.e. the red form); rapid precipitation from solution gives finer particles (i.e. the yellow form) (Simon *et al.*, 1990).

(e) *Dimethylmercury*

The reaction of methyl iodide with mercury–sodium amalgam gives dimethylmercury (Drake, 1981).

(f) *Methylmercury chloride*

Organomercury compounds can be synthesized by reaction of Grignard reagents with mercury halides. In order to obtain pure products, the mercury salt and the Grignard reagent must contain the same anion (R is an aromatic or aliphatic group and X is a halogen):



Organic mercury compounds can also be produced by the reaction of sulfinic acids (RSO_2H) or their sodium salts with mercury halides (Simon *et al.*, 1990).

(g) *Phenylmercury acetate*

Phenylmercury acetate is prepared by refluxing a mixture of mercuric acetate and acetic acid in a large excess of benzene (see IARC, 1987b), in what is generally referred to as a 'mercuration reaction'. The large excess of benzene is necessary because more than one hydrogen on the benzene ring can be replaced. The technical grade of phenylmercury acetate contains about 85% pure compound; the remaining 15% is di- and tri-mercurated products, which are less soluble than phenylmercury acetate and are removed by recrystallization. The product is isolated after distillation of excess benzene and acetic acid (Singer & Nowak, 1981).

1.2.2 Use

(a) *Metallic mercury*

The patterns of use of mercury in Germany and in the USA in different periods are presented in Tables 5 and 6. A major use of mercury is as a cathode in the electrolysis of sodium chloride solution to produce caustic soda and chlorine gas (chloralkali industry). About 50 tonnes of liquid metal are used in each of these plants. In most industrialized countries, stringent procedures have been taken to reduce losses of mercury. Mercury is used widely in the electrical industry (in lamps, arc rectifiers and mercury battery cells), in domestic and industrial control instruments (in switches, thermostats, barometers) and in other laboratory and medical instruments. Another use of liquid metallic mercury is in the extraction of gold from ore concentrates or from recycled gold articles (Kaiser & Tölg, 1984; Sax & Lewis, 1987; Budavari, 1989; Agency for Toxic Substances and Disease Registry, 1989; Simon *et al.*, 1990; WHO, 1991).

Table 5. Use patterns for mercury in Germany (%)

Use category	1973	1976	1979	1982	1985
Chloralkali industry	37	32	28	18	23
Catalysis	13	3	8	7	2
Paints, dyes	6	4	3	1	< 1
Pesticides	9	9	11	2	5
Electrical engineering	8	13	14	21	36
Control instruments and apparatus construction	4	3	4	7	4
Chemicals and reagents	7	14	14	21	None
Medicine	7	8	8	9	13
Miscellaneous	9	14	10	14	17
Total (tonnes)	346	325	313	257	182

From Simon *et al.* (1990)

Table 6. Use patterns for mercury in the USA (%)

Use category	1985	1987	1990	1991	1992
Electrical	64	56	35	33	29
Chloralkali industry	14	12	33	33	34
Paint	9	10	15	} 34	37
Industrial and control instruments	6	6	7		
Other	7	16	10		

From Carrico (1985, 1987); Reese (1990, 1991, 1992b)

WHO (1991) estimated that, in industrialized countries, about 3% of the total consumption of mercury is in dental amalgams. Dental amalgam is a mixture of mercury with a silver-tin alloy. Most conventional amalgams consist of approximately 45–50% mercury, 25–35% silver, 2–30% copper and 15–30% tin. In industrialized countries, the alloy with mercury is now mixed in sealed capsules and applied in the prepared tooth cavity, where excess amalgam (< 5%) is removed immediately before or during condensation of the plastic mix. The amalgam begins to set within minutes of insertion and must therefore be carved to a satisfactory anatomical form within that period of time. Polishing with rotating instruments can take place after 24 h. Amalgam has been used extensively as a tooth-filling material for more than 150 years and accounts for 75–80% of all single tooth restorations. It has been estimated that each US dentist in private practice uses an average of 0.9–1.4 kg of amalgam per year (Sax & Lewis, 1987).

(b) *Mercuric acetate*

Mercuric acetate is used in the synthesis of organomercury compounds, as a catalyst in organic polymerization reactions and as a reagent in analytical chemistry (Singer & Nowak, 1981; Simon *et al.*, 1990).

(c) *Mercuric chloride*

Mercuric chloride is an important intermediate in the production of other mercury compounds, e.g. mercurous chloride, mercuric oxide, mercuric iodide, mercuric ammonium chloride and organomercury compounds. It is also used as a catalyst in the synthesis of vinyl chloride, as a depolarizer in dry batteries and as a reagent in analytical chemistry. It has a minor importance as a wood preservative and retains some importance as a fungicide. Other uses (e.g. as a pesticide or in seed treatment) have declined considerably (Simon *et al.*, 1990).

(d) *Mercuric oxide*

Red mercuric oxide in particular has become increasingly important commercially in the production of galvanic cells with mercuric oxide anodes in combination with zinc or cadmium cathodes. These cells are distinguished from other systems in that their voltage remains constant during discharge: they are used mainly as small, button-shaped batteries, e.g. for hearing devices, digital watches, exposure meters, pocket calculators and security installations. Additional uses of mercuric oxide are in the production of mercury[II] salts, by treatment with the corresponding acids, and as a reagent in analytical chemistry. Its importance as an additive to antifouling paint for ships and in medicine (e.g. for eye ointment) has decreased (Simon *et al.*, 1990).

(e) *Dimethylmercury*

Dimethylmercury is an environmental contaminant that finds limited use as a laboratory reagent (Budavari, 1989; WHO, 1990).

(f) *Phenylmercury acetate*

The primary use for phenylmercury acetate has been in latex paint; it is used at low levels as a preservative and at higher levels to protect the dry film from fungal attack or mildew. It

can be used for these purposes in other aqueous systems, such as inks, adhesives and caulking compounds. Phenylmercury acetate is also used as the starting material in the preparation of many other phenylmercury compounds, which are generally prepared by double-decomposition reactions with the sodium salts of the desired acid groups in aqueous solution. It is also used as a slimicide in paper mills, as a catalyst for the manufacture of certain polyurethanes, as a research chemical (Singer & Nowak, 1981; Sax & Lewis, 1987; Budavari, 1989; Campbell *et al.*, 1992), in contraceptive gels and foams, as a preservative (including in shampoos: see IARC, 1993), as a disinfectant and as a denaturant in ethanol.

(g) *Other mercury-containing compounds*

A number of mercury-containing compounds have been used as topical antiseptics (mercuric iodide, mercuric cyanide, ammoniated mercuric chloride, merbromin [mercuriochrome] and merthiolate) and as fungicides, mildewcides, insecticides and germicides (mercurous chloride, phenylmercury oleate, phenylmercury propionate, phenylmercury naphthenate, phenylmercury lactate, phenylmercury benzoate and phenylmercury borate) (Singer & Nowak, 1981; Sax & Lewis, 1987; Budavari, 1989; Simon *et al.*, 1990). A number of alkylmercury compounds are also used as fungicides in the treatment of seed grains (ethylmercury chloride, ethylmercury *para*-toluenesulfonanilide, ethylmercury acetate, ethylmercury 2,3-dihydroxypropyl mercaptide, bis[methylmercury]sulfate, methylmercury dicyandiamide and methoxyethylmercury acetate or chloride) (Greenwood, 1985; Sax & Lewis, 1987). Mercuric fulminate is used as a detonator in explosives (Singer & Nowak, 1981).

Mercury-containing creams and soaps have long been used by dark-skinned people in some regions to obtain a lighter skin tone. The soaps contain up to 3% mercuric iodide, and the creams contain up to 10% ammoniated mercury. Both the soap and the cream are applied to the skin, allowed to dry and left overnight (WHO, 1991).

1.3 Occurrence

1.3.1 *Natural occurrence*

Metallic mercury occurs as a part of the Earth's natural geochemistry, comprising 50 µg/kg of the Earth's crust. It is 62nd in order of abundance (Aylett, 1973). It is found in the form of the sulfide, as cinnabar ore, which has an average mercury content of 0.1–4%; it is also present in the form of geodes of liquid mercury and as impregnated schist or slate. The major source of atmospheric mercury is suggested to be degassing of the Earth's crust and the oceans (Lauwerys, 1983; Berlin, 1986; WHO, 1990).

Methylmercury compounds are formed in aquatic and terrestrial environments from the methylation of metallic mercury and mercuric mercury. Methylation is likely to occur in bacteria in sediments of sea- or lakebeds. The methylmercury compounds formed are accumulated by aquatic organisms, and dimethylmercury gases are formed by degradation and released into the air. Dimethylmercury can be decomposed in the atmosphere by acidic rainwater to monomethylmercury compounds and thus re-enter the aquatic environment (Berlin, 1986). Little is known about the quantitative aspects of these cycles, and the local load of methylmercury compounds can be increased considerably by anthropogenic sources (Clarkson *et al.*, 1988a; WHO, 1990).

1.3.2 Occupational exposures

Approximately 70 000 workers in the USA are regularly exposed to mercury (Campbell *et al.*, 1992). Table 7 lists some potential occupational exposures to the various forms of mercury. Mercury vapour is the commonest form to which workers are exposed in industries such as mining and processing of cinnabar ore and the chloralkali industry, where brine is electrolysed in mercury cells in which the cathode is a flowing sheet of liquid mercury. The manufacture and use of liquid mercury-containing instruments constitute another source of occupational exposure to mercury vapour through breakage, spillage or careless handling. Dental personnel are exposed to mercury vapours through the preparation of dental amalgams (Stokinger, 1981; Clarkson *et al.*, 1988b).

Table 7. Products, industries and jobs in which there is potential occupational exposure to mercury

Metallic mercury	Inorganic mercury compounds	Organomercury compounds
Dental medicine	Disinfectants	Bactericides
Batteries	Paints and dyes	Embalming preparations
Barometers	Explosives	Paper manufacture
Boiler makers	Fireworks manufacture	Farmers
Calibration instruments	Fur processing	Laundry and diaper services
Caustic soda production	Ink manufacture	External antiseptics
Carbon bush production	Chemical laboratory workers	Fungicides
Ceramics	Percussion caps and detonators	Insecticides manufacture
Chloralkali production	Spermicidal jellies	Seed handling
Ultrasonic amplifiers	Tannery workers	Wood preservatives
Direct current meters	Wood preservatives	Germicides
Infrared detectors	Tattooing materials	
Electrical apparatus	Taxidermists	
Electroplating	Vinyl chloride production	
Fingerprint detectors	Embalming preparations	
Silver and gold extraction	Mercury vapour lamps	
Jewellery	Antisymphilitic agents	
Fluorescent, neon, mercury arc lamps	Thermoscopy	
Manometers	Silvering of mirrors	
Paints	Photography	
Paper pulp manufacture	Perfumery and cosmetics	
Photography	Acetaldehyde production	
Pressure gauges		
Thermometers		
Semiconductor solar cells		

From Campbell *et al.* (1992)

Mixed exposure to aerosols of organic or inorganic mercury compounds also occurs: Chlorine in combination with mercury vapour, produced in chloralkali industries, forms mercuric chloride aerosols. Another source of occupational exposure is in pathology labo-

ratories, where mercuric chloride is used with formalin as a histological fixative. Exposure to aerosols of methyl- and ethylmercury compounds has been described in connection with the manufacture and use of mercuric salts and during seed treatment (Berlin, 1986). Disinfectant manufacturers, fungicide manufacturers, seed handlers, farmers, lumberjacks, pharmaceutical industry workers and wood preservers may be exposed to organomercury compounds (Campbell *et al.*, 1992).

Data on exposure to mercury in air and the results of biological monitoring in various industries and occupations are described below and summarized in Tables 8 and 9 (pp. 258–260). It should be noted that the concentrations of mercury detectable in the general working environment are generally lower than those to which individual workers are exposed, as detected by personal air sampling. This is due to the fact that mercury can accumulate on the clothes, hair and skin of workers, creating a situation which has been called 'micro-environmental exposure'. In a Belgian manufacturing plant, mercury concentrations in the general work environment were between 8 and 88 $\mu\text{g}/\text{m}^3$, while personal samples from the workers showed concentrations ranging from 16 to 680 $\mu\text{g}/\text{m}^3$ (see Ehrenberg *et al.*, 1991).

Biological monitoring of people occupationally exposed to mercury vapours and inorganic mercury compounds, by measuring mercury in urine and blood mercury, reflects recent exposure. Occupational and environmental exposure to methylmercury compounds can be estimated from blood mercury levels. Mercury in hair can be used as an indicator of environmental exposure to methylmercury compounds but not for monitoring exposure to metallic mercury and inorganic mercury compounds (Elinder *et al.*, 1988).

(a) Chloralkali plants

Exposures in chloralkali plants have been reviewed (WHO, 1976). In recent studies, covering mainly Swedish plants, average urinary mercury concentrations of 50–100 $\mu\text{g}/\text{L}$ were reported (WHO, 1991).

In a study in the USA and Canada of 567 male workers in 21 chloralkali plants, the mean atmospheric concentration of mercury was 65 $\mu\text{g}/\text{m}^3$ (SD, 85); in 12 plants, the time-weighted average concentration was 100 $\mu\text{g}/\text{m}^3$ or less, while in the remainder some employees were exposed to higher concentrations. At an ambient air concentration of 100 $\mu\text{g}/\text{m}^3$, the concentration in blood was about 60 $\mu\text{g}/\text{L}$ and that in urine about 200 $\mu\text{g}/\text{L}$. In 117 control subjects, blood mercury concentrations were lower than 50 $\mu\text{g}/\text{L}$; in 138 controls, urinary mercury concentrations were generally less than 10 $\mu\text{g}/\text{L}$ (corrected to specific gravity) (Smith *et al.*, 1970).

The airborne concentrations of mercury in a chloralkali plant in Italy were between 60 and 300 $\mu\text{g}/\text{m}^3$; the mean urinary concentration in 55 workers exposed for 11.5 ± 8.8 years in cell preparation rooms was 158 $\mu\text{g}/\text{L}$ (range, 0–762 $\mu\text{g}/\text{L}$) and that in 17 workers exposed to mercury irregularly for 15.2 ± 10.7 years was 40.3 $\mu\text{g}/\text{L}$ (range, 0–96 $\mu\text{g}/\text{L}$) (Foà *et al.*, 1976).

The atmospheric concentrations of mercury in a chloralkali plant in Sweden in 1975 were 64 $\mu\text{g}/\text{m}^3$ (range, 36–112 $\mu\text{g}/\text{m}^3$); the mean blood mercury concentration in 13 workers employed for 0.5–5.5 years was 238 nmol/L (47.6 $\mu\text{g}/\text{L}$), and the mean urinary concentration in the same subjects was 808 nmol/L (range, 369–1530 nmol/L) [161 $\mu\text{g}/\text{L}$; range, 74–306 $\mu\text{g}/\text{L}$]. Two years later, after improvement of the ventilation systems in the plant, the mean concentrations of mercury were 22.6 (range, 15–43) $\mu\text{g}/\text{m}^3$ in air, 92 nmol/L

(18.4 µg/L) in blood and 196 (range, 117–327) nmol/L [39.2 µg/L; range, 23–65 µg/L] in urine in a group of 16 workers who had been employed for one to seven years (Lindstedt *et al.*, 1979).

Exposure to mercury in a chloralkali plant in Sweden was studied during ordinary maintenance work and in workers hired for a special repair task during a temporary production shutdown. A group of 14 normal maintenance workers were exposed to mean air concentrations of mercury of 65 µg/m³ (range, 24–123 µg/m³) and had a mean blood mercury concentration of 73 nmol/L, ranging from 45 to 150 nmol/L [14.6 µg/L; range, 9–30 µg/L], and a mean urinary concentration of 32 nmol/mmol (57.2 µg/g) creatinine (range, 16–43 nmol/mmol; 28.6–76.9 µg/g). The 16 special repair workers were exposed to a mean air concentration of 131 µg/m³ (range, 38–437 µg/m³) and had a mean blood mercury concentration of 148 nmol/L, ranging from 85 to 240 nmol/L [29.6 µg/L; range, 17–48 µg/L], and a mean urinary mercury concentration of 6.1 nmol/mmol (10.9 µg/g) creatinine (range, 4.7–8.7 nmol/mmol; 8.4–15.5 µg/g) (Sällsten *et al.*, 1992).

In an epidemiological study of 1190 workers in eight Swedish chloralkali plants (described in detail on p. 271), biological monitoring data indicated a substantial reduction in exposure to mercury with time, from about 200 µg/L in urine during the 1950s to 150 µg/L in the 1960s and less than 50 µg/L in 1990 (Barregård *et al.*, 1990). In another Swedish chloralkali plant, the average levels of mercury in air were 25–50 µg/m³ throughout the 1980s. The mean concentrations of mercury in 26 male workers were 252 nmol/L (50.4 µg/L) in urine, 48 nmol/L (9.6 µg/L) in plasma and 78 nmol/L (15.6 µg/L) in erythrocytes, and those in 26 unexposed workers were 19 nmol/L (3.8 µg/L) in urine, 7.5 nmol/L (1.5 µg/L) in plasma and 33 nmol/L (6.6 µg/L) in erythrocytes (Barregård *et al.*, 1991). The mean concentrations of mercury in 1985–86 in another group of 89 chloralkali workers in Sweden, who had been exposed for 1–45 years, were 55 nmol/L (11 µg/L) in blood, 45 nmol/L (9 µg/L) in serum and 14.3 nmol/mmol (25.5 µg/g) creatinine in urine. The concentrations in a control group of 75 non-occupationally exposed workers were 15 nmol/L (3 µg/L) in blood, 4 nmol/L (0.8 µg/L) in serum and 1.1 nmol/mmol (1.95 µg/g) creatinine in urine (Langworth *et al.*, 1991).

In chloralkali plants, exposure to asbestos can occur during various maintenance operations (Barregård *et al.*, 1990; Ellingsen *et al.*, 1993).

(b) *Thermometer production*

In 1979, exposure to metallic mercury vapour was studied in a small thermometer factory in Israel with generally inadequate engineering and hygiene arrangements. The mean mercury concentrations in five workers exposed to 50–99 µg/m³ were 299 nmol/L (59.8 µg/L) in urine and 105 nmol/L (21 µg/L) in blood; those in three workers exposed to 100–149 µg/m³ were 449 nmol/L (89.8 µg/L) in urine and 122 nmol/L (24.4 µg/L) in blood; and those in seven workers exposed to 150–200 µg/m³ were 628 nmol/L (125.6 µg/L) in urine and 143 nmol/L (28.6 µg/L) in blood (Richter *et al.*, 1982).

Concentrations of mercury were measured in four thermometer plants in Japan: The air concentrations ranged from 25 to 226 µg/m³; those of inorganic mercury compounds in blood ranged from 80 to 1150 nmol/L (16–230 µg/L); those of metallic mercury in blood ranged from not detected to 1.10 nmol/L (not detected–0.22 µg/L); those of inorganic

mercury compounds in urine ranged from 96 to 1560 nmol/L (19.2–312 µg/L); and those of metallic mercury in urine ranged from 0.05 to 1.22 nmol/L (0.01–0.24 µg/L) (Yoshida, 1985).

In a thermometer factory in the USA, 17 personal samples showed mean air concentrations of mercury of 75.6 µg/m³ (range, 25.6–270.6); 11 area samples showed a mean of 56.7 µg/m³ (range, 23.7–118.5). The mean urinary mercury concentration in 79 workers employed for 65 ± 48.9 months was 73.2 ± 69.7 µg/g creatinine (range, 1.3–344.5) (Ehrenberg *et al.*, 1991).

In a thermometer factory in Sweden, where filling with mercury was done inside a ventilated hood but with spillage of mercury during temperature conditioning and testing, the mean concentration of mercury in the air was 39 µg/m³ (range, 15–58). In seven workers, the median blood mercury concentration was 57 nmol/L (11.4 µg/L), and in six workers, the median urinary concentration was 21 nmol/mmol (37.5 µg/g) creatinine (Sällsten *et al.*, 1992).

(c) Hospitals

In Belgium, a group of 40 chemical and biological laboratory technicians employed for < 1–15 years were exposed to an average airborne mercury concentration of 28 µg/m³ (range, 2–124). The mean mercury concentration in urine was 10.72 ± 1.49 µg/g creatinine, and that in whole blood was 10.0 ± 0.9 µg/L. The mean mercury concentrations in a group of 23 unexposed technicians were 2.30 ± 1.49 µg/g creatinine in urine and 6.5 ± 1.1 µg/L in blood (Lauwerys & Buchet, 1973).

In a study in Scotland, use of mercuric chloride as a histological fixative was associated with high atmospheric concentrations of mercury vapour (up to 100 µg/m³) and of all mercury compounds (200 µg/m³). Twenty-one technicians exposed to this environment had a median urinary mercury output of 265 nmol (53 µg)/24 h. The median urinary output among a control group of 21 subjects was 72 nmol (14.4 µg)/24 h (Stewart *et al.*, 1977).

Hospital employees who repair sphygmomanometers or work in areas in which such machines are repaired are potentially exposed to mercury. In 13 hospitals in the USA, in which most employees had worked for less than 10 years, the airborne concentrations of mercury in repair rooms ranged from 1 to 514 µg/m³, and 86 employees tested had a mean urinary mercury concentration of 12.4 µg/L (range, 1–200) (Goldberg *et al.*, 1990).

(d) Dental personnel

Special interest has focused on occupational exposure to mercury in dentistry. Several studies conducted during 1960–80 reported average concentrations of mercury vapour in dental clinics ranging between 20 and 30 µg/m³ air; in certain clinics concentrations of 150–170 µg/m³ were measured (WHO, 1991). In some of these studies, urinary mercury concentrations of dental personnel were also reported.

An average urinary mercury concentration of 40 µg/L was found among 50 dentists in the USA, with some values exceeding 100 µg/L (Joselow *et al.*, 1968). In a nationwide US study, the average mercury concentration in the urine of 4272 dentists sampled between 1975 and 1983 was 14.2 µg/L (range, 0–556 µg/L). In 4.9% of the samples, the concentrations were ≥ 50 µg/L, and in 1.3% they were > 100 µg/L. The wide range of values was probably due to variations in occupational exposure to amalgams with time, in addition to variations in

sampling techniques and other methodological problems (Naleway *et al.*, 1985). At the annual sessions of the American Dental Association, on-site screening for exposure to mercury showed mean urinary concentrations of 5.8 µg/L in 1042 dentists in 1985 and 7.6 µg/L in 772 dentists in 1986; 10% contained concentrations above 20 µg/L (Naleway *et al.*, 1991).

Blood samples from a group of 130 dentists in Denmark in 1986 contained a median mercury concentration of 4.0 µg/L (range, 1.2–19.2); 2.0 µg/L (1.1–4.6) were found in controls. Practice characteristics, as stated on questionnaires, were not significantly related to blood mercury concentration, but 49 dentists who ate one or more fish meals per week had a median concentration 47% higher than that of dentists who seldom consumed fish (Möller-Madsen *et al.*, 1988).

In 82 dental clinics in northern Sweden, the median concentration of mercury vapour in air was 1.5 µg/m³ in public surgeries and 3.6 µg/m³ in private ones. The urinary mercury concentrations in 505 occupationally exposed subjects ranged from 1.4 to 2.9 nmol/mmol (2.5–5.13 µg/g) creatinine, which are of the same order of magnitude as those of the Swedish population as a whole. The load derived from the amalgam fillings of the exposed subjects was estimated to be of the same order of magnitude as that from the working environment (Nilsson *et al.*, 1990). In the offices of six dentists in Sweden, the mean concentration of mercury in air was 4.5 µg/m³ (range, 1.7–24); the mean concentrations in 12 subjects were 17 nmol/L (range, 6–29) (3.4 µg/L; range, 1.2–5.8 µg/L) in blood and 2.6 nmol/mmol (4.6 µg/g) creatinine (range, 1.1–5.4 nmol/mmol; 2.00–9.65 µg/g) (Sällsten *et al.*, 1992). In 224 dental personnel in Sweden, the levels of mercury in urine (1.8 nmol/mmol [3.19 µg/g] creatinine) were not significantly higher than those of 81 referents (1.1 nmol/mmol [1.95 µg/g] creatinine), and no difference was seen for the plasma or blood levels. When adjustment was made, however, for amalgam fillings in the mouths of the personnel, significant differences in urinary, plasma and blood mercury concentrations were seen (Akesson *et al.*, 1991).

Urinary excretion of inorganic mercury compounds was determined in 50 individuals attached to Madras Dental College, India. The lowest concentration observed was 3 µg/L and the highest, 136.6 µg/L. Of those subjects who handled mercury, 70% had urinary concentrations > 20 µg/L (Karthikeyan *et al.*, 1986).

(e) *Others*

The airborne concentrations of mercury in Idrija, Slovenia, in 1950 were reported to be 0.05–5.9 mg/m³ in a mine and 0.17–1.1 mg/m³ in a smelter (Vouk *et al.*, 1950). Similar values were reported during a survey conducted in 1963: 0.1–2.0 mg/m³ in both the mine and the smelter. The average concentration of mercury in blood from 57 asymptomatic miners in Idrija was 77 µg/L (range, 0–450); the corresponding value in 16 workers with symptoms of intoxication was 110 µg/L (range, 0–510). The concentrations in urine were 276 µg/L (range, 0–1275) in the asymptomatic miners and 255 µg/L (range, 2.0–601) in those with symptoms (Ladd *et al.*, 1966).

Concentrations > 2.0 mg/m³ were detected in 1964 in a mine and smelter on Palawan Island, the Philippines (Ladd *et al.*, 1966).

The average concentrations of mercury in the air in various departments in the Italian hat manufacturing industry in 1942–52 were 0.09–2.21 mg/m³. The concentrations were > 0.2 mg/m³ in 13 of the 17 departments studied, and concentrations as high as 4 mg/m³ were measured in specific locations (Baldi *et al.*, 1953).

In a mercury distillation plant in Italy, airborne mercury concentrations ranged from 0.005 to 0.278 mg/m³; the mean urinary concentration in 19 workers was 108.26 ± 55.61 µg/L and the mean blood concentration, 77 ± 28 µg/L. In 13 subjects in a control group, the urinary mercury concentration was < 10 µg/L, while in 11 other subjects, the mean value was 15.27 µg/L (range, 11–21) (Angotzi *et al.*, 1980).

In a recycling distillation plant in Germany, the concentration of mercury in air in February 1984 ranged from 115 to 379 µg/m³; in 12 workers in the plant, mercury was found at 28–153 µg/L in blood and 128–609 µg/g creatinine in urine. In previous years, the levels of both biological indicators (determined since 1978) were decidedly higher: 44–255 µg/L in blood and 143–1508 µg/g creatinine in urine. The authors cited the 'normal' values for mercury as 0.2–7.2 µg/L (mean, 0.6) in blood and 0.2–5.0 µg/g creatinine (mean, 0.8) in urine (Schaller *et al.*, 1991).

Individual external exposure in a dry alkaline battery plant in Belgium was to 40 µg/m³ mercury, ranging from 10 to 106 µg/m³. Urinary mercury concentrations were usually < 50 µg/g creatinine in some parts of the plant and between 50 and 100 µg/g creatinine in others (Roels *et al.*, 1987).

In a plant for the manufacture of fluorescent lamps in Italy, the mercury concentrations in air in maintenance areas in 1984–85 were between 2 and 5 µg/m³; 27 workers employed for 10.96 ± 1.14 years in those areas had mean urinary concentrations of 5.15 ± 2.2 µg/L (range, 2–11). In the same plant, the concentrations in the air of production areas varied between 6 and 44 µg/m³, and 22 workers employed for 10.34 ± 1.43 years in those areas showed mean urinary concentrations of 4.94 ± 1.62 µg/L (range, 1.9–8) (Assennato *et al.*, 1989).

In a study of reproductive function among women employed at a mercury vapour lamp factory (described in detail on pp. 296–297), De Rosis *et al.* (1985) reported that time-weighted average concentrations exceeded 50 µg/m³ in 1972–76; after modification of the ventilation system, the concentrations dropped to < 10 µg/m³.

1.3.3 Air

The most important sources of mercury in the atmosphere are degassing of the Earth's crust, emissions from volcanoes and evaporation of mercury vapours from natural bodies of water. Recent estimates indicate that these natural emissions amount to 2700–6000 tonnes per year; however, it is difficult to determine the relative contributions of natural and anthropogenous sources to the general emission of mercury in the biosphere, since some may have been deposited in water from the atmosphere and produced by human activities (WHO, 1990). Traditional municipal solid-waste incinerators may have a significant impact on the ambient air concentration as well as on the deposition rates of mercury. Rates of emission of mercury from traditional incinerators in Europe, Canada and the USA range from 100 to 2200 µg/m³ and those from advanced incinerators, 30–200 µg/m³. Such emissions could result in deposition rates of 0.2–4.0 and 0.02–1.0 µg/m² per day, respectively (WHO, 1988).

Table 8. Occupational exposure to mercury in various industries and occupations

Industry and activity (country) [year, when available]	No. of workers	Air ($\mu\text{g}/\text{m}^3$)		Urine ($\mu\text{g}/\text{L}$, except where noted)		Blood ($\mu\text{g}/\text{L}$)		Reference
		Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	
Chloralkali plants (USA and Canada; 21 plants) Cell room	567	65 $\pm$ 85	< 10–270 (TWA) < 1–2640					Smith <i>et al.</i> (1970)
Chloralkali plant (Italy) Cell rooms	72 55		60–300	157.79 $\pm$ 120.94 40.29 $\pm$ 26.16	0–762 0–96			Foà <i>et al.</i> (1976)
Miscellaneous	17							
Chloralkali plant [1975] (Sweden) [1977]	13 16	64 $\pm$ 21.8 22.6 $\pm$ 7	36–112 15–43	161.6 $\pm$ 62.8 39.2 $\pm$ 14.4	74–306 23–65	47.6 $\pm$ 23.8 18.4 $\pm$ 6.8		Lindstedt <i>et al.</i> (1979)
Chloralkali plant (Sweden)	26	NR	25–50	50.4	5–186	NR		Barregård <i>et al.</i> (1991)
Chloralkali plant (Sweden) [1985–86]	89	NR	NR	25.5 ^a	0.5–84 ^a	11		Langworth <i>et al.</i> (1991)
Chloralkali plant (Sweden) Normal maintenance	NR	65 (14 samples)	24–123	57 ^a (8 samples)	29–77 ^a	14.6 (8 samples)	9–30	Sällsten <i>et al.</i> (1992)
Special maintenance	NR	131 (16 samples)	38–437	10.9 ^a (5 samples)	8.4–15 ^a	29.6 (7 samples)	17–48	
Thermometer factory (Israel) [1979]	5 3 7	NR	50–99 100–149 150–200	59.8 89.8 125.6		21 24.4 28.6		Richter <i>et al.</i> (1982)
Thermometer factories (Japan; 4 factories)	27	NR	25–226	NR	19–312	NR	16–230	Yoshida (1985)
Thermometer factory (USA)	84	75.6 (17 samples)	25.6–271	73.2 $\pm$ 69.7 ^a (79 samples)	1.3–344.5 ^a			Ehrenberg <i>et al.</i> (1991)
Thermometer factory (Sweden)	NR	39 (13 samples)	15–58	37.5 ^a (6 samples)	1.96–91 ^a	11.4 (7 samples)	6–20	Sällsten <i>et al.</i> (1992)
Pathology laboratory (Belgium)	40	28	2–124	10.72 $\pm$ 1.49 ^a	NR	10.0 $\pm$ 0.9		Lauwerys & Buchet (1973)

Table 8 (contd)

Industry and activity (country) [year, when available]	No. of workers	Air ($\mu\text{g}/\text{m}^3$)		Urine ($\mu\text{g}/\text{L}$, except where noted)		Blood ($\mu\text{g}/\text{L}$)		Reference
		Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range	
Pathology laboratory (United Kingdom)	21	200		26.5		NR		Stewart <i>et al.</i> (1977)
Sphygmomanometer repair (USA; 13 facilities)	93	86	1-514	12.4 $\pm$ 22.2 (86 samples)	1-200	NR		Goldberg <i>et al.</i> (1990)
Dental staff (USA) [1975-83]	4272	NR	NR	14.2 $\pm$ 25.4	0-556	NR		Naleway <i>et al.</i> (1985)
Dental staff (India)	50	NR	NR	NR	3-136.6	NR		Karthikeyan <i>et al.</i> (1986)
Dental staff (Sweden; 82 clinics) [1983]	505	NR	1.5-3.6	NG	2.5-5.13	NG		Nilsson <i>et al.</i> (1990)
Dental staff (USA) [1985] [1986]	1042 772	NR	NR	5.8 $\pm$ 8.5 7.6 $\pm$ 11.8	max, 84 max, 115	NR		Naleway <i>et al.</i> (1991)
Dental staff (Denmark) [1986]	130	NR	NR	NR	NR	4.0	1.2-19.2	Möller-Madsen <i>et al.</i> (1988)
Dental staff (Sweden; 6 offices)	NR	4.5 (36 samples)	1.7-24	4.6	1.96-9.65	3.4	1.2-5.8	Sällsten <i>et al.</i> (1992)
Mercury distillation (Italy) [1976-78]			5-279					Angotzi <i>et al.</i> (1980)
Distillation	19	NR		108.26 $\pm$ 55.61	NR	77 $\pm$ 28		
Maintenance	19	NR		84.11 $\pm$ 45.54	NR	53 $\pm$ 16		
Recycling plant (Germany) [1984]	12		115-379		128-609 ^a	28-153		Schaller <i>et al.</i> (1991)
Dry alkaline battery plant (Belgium) [1984]	10	40 (46 samples)	10-106	< 100 ^a (10 samples)				Roels <i>et al.</i> (1987)
Fluorescent lamps (Italy) [1984-85]								Assennato <i>et al.</i> (1989)
Maintenance	27	NR	2-5	5.15 $\pm$ 2.2	2-11	NR		
Production	22	NR	6-44	4.94 $\pm$ 1.62	1.9-8	NR		

NR, not reported

^a μg creatinine

Table 9. Concentration of mercury in the air of work places in Finland during 1977–88 and in blood in 1987

Industrial code or work	Air concentrations ($\mu\text{g}/\text{m}^3$)			Concentration in blood ($\mu\text{g}/\text{L}$)			
	No. of measurements	Mean	Range	No. of workplaces	No. of measurements	Mean	Range
Seed dressing and packing	11	4	1–13	10	27	3.6	1–9
Pesticide manufacture	8	58	29–105	1	24	10.4	3–46
Mercury production	NR	32	13–157	NR	NR	NR	NR
Welding	24	88	2–150	NR	NR	NR	NR
Manufacture of light bulbs, fluorescent tubes and batteries	133	30	1–250	5	17	5.8	2–8
Laboratory work	26	15	1–120	NR	NR	NR	NR
Dentistry	136	10	1–100	21	42	4.4	1–9
Chlorine industry	NR	NR	NR	3	518	13.6	1–69

From Anttila *et al.* (1992). NR, not reported

Mercury concentrations in the atmosphere range from a few nanograms per cubic metre over remote, uncontaminated areas to about 20 ng/m³ in urbanized areas. Concentrations have been estimated to be 2 ng/m³ in the northern hemisphere and about 1 ng/m³ in the southern hemisphere. Concentrations of mercury up to 18 ng/m³ have been reported in the atmosphere close to active volcanoes (Berlin, 1986; Clarkson *et al.*, 1988a).

Mercury vapour is believed to be the predominant form in the atmosphere. There is evidence that some of the mercury in ambient air is in the form of alkylmercury, and the presence of methylmercury compounds has been reported. The particulate fraction of mercury in air (as a percentage of total mercury) is usually 4% or less (WHO, 1990).

Another source of mercury in the atmosphere is the release of metallic mercury vapour during the cremation of cadavers, when all the mercury from amalgam fillings vaporizes as the temperature reaches above 800 °C. It is difficult to estimate the global release of mercury from cremation because of the uncertainties about dental status at the time of death and about the frequency of cremation (WHO, 1991).

1.3.4 Water

Mercury is removed from the atmosphere mainly by precipitation. The chemical species of mercury in water is mainly ionic mercury[II]. Concentrations of mercury in surface water are very low, and accurate analysis is still a problem. Total mercury concentrations range from 0.5 to 3 ng/L in open oceans, from 2 to 15 ng/L in coastal seawater and from 1 to 3 ng/L on average in freshwater rivers and lakes (WHO, 1990). The bottom sediment of lakes and oceans may contain 20–250 µg/kg mercury (Berlin, 1986). Concentrations in drinking-water are generally less than 25 ng/L (WHO, 1990).

Concentrations of mercury in inland waters of gold-mining areas in Rondônia, Brazil, were between < 0.1 and 8.6 µg/L (Pfeiffer *et al.*, 1989). A study of water from the Madeira River and its tributaries, in the centre of the gold rush area in Brazil, showed an average mercury level of 24.6 ng/L (Nriagu *et al.*, 1992).

1.3.5 Soil and plants

The commonest form of mercury in soil is the bivalent ion. Concentrations measured in soils are generally less than 1 ppm (mg/kg). Methylation of mercury has been demonstrated in soil and is influenced by humidity, temperature and the mercury concentration of the soil (Sequi, 1980; Simon *et al.*, 1990).

The accumulation of mercury in plants increases with increasing soil concentration. Soil type has a considerable influence on this process: a high content of organic matter decreases the uptake. Generally, the highest concentrations of mercury are found at the roots, but translocation to other organs (e.g. leaves) occurs. In contrast to higher plants, mosses take up mercury from the atmosphere (WHO, 1989a).

Mercury concentrations in bottom sediments of Brazilian polluted rivers ranged between 50 and 19 800 µg/kg (Pfeiffer *et al.*, 1989).

1.3.6 Food

Environmental contamination with mercury leads to a critical concentration effect in animals that occupy higher positions in the food chain (large fish and fish-eating sea fowl)

(Simon *et al.*, 1990). The factors that determine the methylmercury concentration in fish are the mercury content of the water and bottom sediments, the pH and redox potential of the water and the species, age and size of fish (Berlin, 1986).

The concentrations of mercury in most foods are generally below the reported limit of detection, which is usually 20 µg/kg fresh weight. A large proportion of the mercury in food—at least in animal products—is likely to be in the form of methylmercury compounds. Most of the mercury in fish is as methylmercury compounds, which are formed in the bottom sediment of the ocean and in freshwater systems and are enriched to a high degree in the aquatic food chain, with the highest levels occurring in the predatory fish: The concentrations of total mercury in edible tissues of shark and swordfish are > 1200 µg/kg, whereas anchovies and smelt have average values of < 85 µg/kg (Berlin, 1986; WHO, 1990).

In a survey sponsored by the Ministry of Food, Agriculture and Forestry of Germany, the average mercury contamination of 759 specimens of fish from German fishing grounds was < 100 µg/kg (Jacobs, 1977). The mercury concentrations in edible parts of fish from polluted rivers in Brazil were between 70 and 2700 µg/kg wet wt (Pfeiffer *et al.*, 1989).

The average daily intake of mercury can be estimated by assuming that intake from non-fish food sources is negligible in comparison with that from fish. FAO estimated an average worldwide fish intake of 16 g per person per day but an average daily intake of 300 g in populations that are largely dependent on fish; therefore, the average daily intake of total mercury will result in 3 µg, of which 80% is methylmercury compounds and 20% inorganic mercury. The average intake of methylmercury compounds can thus be calculated as 2.4 µg per day, with 2.16 µg retained (90% absorption), and the average daily intake of inorganic mercury is 0.6 µg per day, with 60 ng retained (10% absorption) (Clarkson *et al.*, 1988a; Table 10). Daily intake from the consumption of fish from polluted water, however, can rise to toxic levels, as occurred in Minamata and Niigata in Japan around 1953–66: Concentrations of 1–20 mg/kg in fish resulted in daily intake, in people with frequent fish consumption (200–500 g per day), of 5 mg per day (Berlin, 1986).

Table 10. Estimated average daily intake and retention of various forms of mercury in populations not occupationally exposed to mercury

Source	Estimated daily intake and retention (ng mercury/day)					
	Mercury vapour		Inorganic mercury compounds		Methylmercury compounds	
	Intake	Absorbed	Intake	Absorbed	Intake	Absorbed
Atmosphere	40	32				
Water			50	5		
Food			600	60	2400	2160
Total intake	40		650		2400	2160
Absorbed		32		65		

From Clarkson *et al.* (1988a)

Toxic levels have also been reached following consumption of bread prepared from wheat treated with methylmercury dicyandiamide fungicide, as occurred in Iraq in the winter of 1971–72 (Bakir *et al.*, 1973; Greenwood, 1985).

1.3.7 Dental amalgam

Dental amalgams are a potential source of exposure to mercury vapour not only for dental staff but also for the general population. Hardening of the amalgam continues over many months, so that stress on the amalgam surface, produced by chewing or grinding of the teeth, causes breakdown of a surface barrier and release of mercury vapour into the mouth. This results in the deposition of mercury in body tissues like kidney and brain and increased urinary excretion. The release of mercury from amalgams makes a significant contribution to human exposure to inorganic mercury, including mercury vapour (Clarkson *et al.*, 1988b; WHO, 1991; US Department of Health and Human Services, 1993).

Different concentrations of mercury are released from unstimulated amalgams (3.3–7.4 ng/min) and stimulated amalgams (16.3–163.2 ng/min) (Clarkson *et al.*, 1988b). Average daily intake of metallic mercury vapour can thus range from 3.8 to 21 µg/day, with corresponding retentions of 3–17 µg/day (WHO, 1990, 1991).

In 147 individuals in an urban Norwegian population, correlations were found between the concentrations of mercury in urine (mean, 17.5 nmol/L [3.5 µg/L]) and in exhaled air (mean, 0.8 µg/m³) and between both urinary and air concentrations and the number of amalgam restorations, the number of amalgam-restored surfaces and the number of amalgam-restored occlusal surfaces. The results suggested that individuals with more than 36 restored surfaces absorb 10–12 µg of mercury per day (Jokstad *et al.*, 1992).

1.3.8 Mercury-containing creams and soaps

The mean concentration of mercury in the urine of 60 African women who used skin-lightening creams, containing 5–10% ammoniated mercury, was 109 µg/L (range, 0–220). Those in the urine of six women who had used skin-lightening creams containing 1–3% ammoniated mercury for two years ranged from 28 to 600 µg/L (WHO, 1991).

Mercury was found in the blood (91.1 µg/L) and urine (784 µg/g creatinine) of a woman who had been using soap containing 1% mercuric iodide for about 15 years. Mercury was also present in the blood (19 µg/L) and urine (274 µg/g creatinine) of her three-month-old child, who was not directly exposed to mercury (Lauwerys *et al.*, 1987).

1.3.9 Mercury-containing paint

Air samples from 19 homes recently painted with an interior latex paint with a median mercury concentration of 754 mg/L contained a median of 2 µg/m³ mercury, while concentrations in 10 uncoated houses were below the detection limit of 0.1 µg/m³. The median concentration in urine was higher for 65 exposed inhabitants (8.4 µg/g creatinine) than for 28 unexposed people (1.9 µg/g creatinine) (Agocs *et al.*, 1990; WHO, 1991).

1.3.10 Human tissues and secretions

In order to establish reference values for mercury concentrations in whole blood, blood cells and plasma, 98 publications in the international scientific literature presenting

biological data on individuals not occupationally exposed to mercury were reviewed critically and graded for quality (Brune *et al.*, 1991). The mean levels of mercury in non-fish eaters were 2.0 µg/L (10th-90th percentiles, 0-4.3) in whole blood, 3.8 (2.8-4.8) in blood cells and 1.3 (0.3-2.3) µg/L in plasma. Although the authors recognized the importance of retrieving information on the number of amalgam restorations, few data were available.

In 380 Italian subjects non-occupationally exposed to mercury, the mean urinary concentration of mercury was 3.5 µg/L (range, 0.1-6.9) (Minoia *et al.*, 1990). Average urinary mercury concentrations in 50 male and 54 female residents of the Monte Amiata mercury mine area in Italy were greater than those in 104 controls from other regions of the country: men, 2.3 µg/g creatinine (95% CI, 1.7-3.0); women, 3.9 µg/g creatinine (95% CI, 2.2-5.6); men and women combined, 3.1 µg/g creatinine (95% CI, 2.2-4.1) (Cicchella *et al.*, 1968).

Mercury levels in the hair of unexposed populations are generally between 0.5 and 4 mg/kg. Hair mercury is indicative of blood mercury concentration at the point of growth, so that sequential analysis of hair segments provides information on past exposure to mercury and particularly to organomercury compounds (Bakir *et al.*, 1973; Kazantzis *et al.*, 1976).

In Sweden, increased concentrations of mercury were found in samples from former dental staff (seven dentists and one dental assistant) of the pituitary gland (average, 9.8 µmol [1.96 mg]/kg wet weight; range, 0.7-28 [0.14-5.6]), occipital cortices (average, 0.33 µmol [0.07 mg]/kg wet weight; range, 0.07-1.43 [0.014-0.3]), renal cortices (average, 8.6 µmol [1.7 mg]/kg wet weight; range, 4.7-11.3 [0.9-2.3]), and thyroid gland (range, 0.32-140 µmol [0.06-28 mg]/kg wet weight). Mercury was found together with selenium at a rough stoichiometric ratio of 1:1. In the general population, the average concentrations were 0.12 (0.03-5.83) µmol/kg wet weight in pituitary gland, 0.053 (0.012-0.114) in occipital cortices, 1.4 (0.11-4.04) in renal cortices and 0.019 (0.004-0.047) in abdominal muscles (Nylander & Weiner, 1991).

1.4 Regulations and guidelines

Occupational exposure limits and guidelines established in different parts of the world are given in Table 11. The recommended health-based occupational exposure limit is 0.05 mg/m³ (WHO, 1980; Simon *et al.*, 1990). The recommended health-based limit for long-term occupational exposure to mercury vapours is 50 µg/g creatinine in urine (WHO, 1980).

The American Conference of Governmental Industrial Hygienists (1992) gave notice of their intent to establish biological exposure indices for mercury in blood and urine. The values proposed are 35 µg/g creatinine for total inorganic mercury in urine in pre-shift samples and 15 µg/L for total inorganic mercury in blood at the end of a working week. The German biological tolerance values for metallic mercury and inorganic mercury compounds are 50 µg/L in blood and 200 µg/L in urine; that for organomercury compounds is 100 µg/L in blood (Deutsche Forschungsgemeinschaft, 1992). The Finnish guideline values for biological measurements are 10 µg/L in blood and 25 µg/L in urine (Anttila *et al.*, 1992).

The WHO recommended guideline for all forms of mercury in drinking-water is 1 µg/L (WHO, 1992). The maximum contaminant level of mercury in drinking-water and the

permissible level in bottled water in the USA is 2 µg/L (US Environmental Protection Agency, 1991; US Food and Drug Administration, 1992).

Table 11. Occupational exposure limits and guidelines for mercury and mercury compounds

Country or region	Year	Concentration (mg/m ³)	Substances affected	Interpretation ^a
Australia	1990	0.01	Alkyl mercury compounds (as Hg)	TWA, S
		0.03	Alkyl mercury compounds (as Hg)	STEL, S
		0.05	Mercury and mercury vapour	TWA, S
		0.1	Aryl mercury compounds, inorganic mercury compounds (as Hg)	TWA, S
Austria	1982	0.1	Mercury and mercury vapour	TWA
		0.01	Organic mercury compounds (as Hg)	TWA, S
Belgium	1990	0.01	Alkyl mercury compounds (as Hg)	TWA, S
		0.03	Alkyl mercury compounds (as Hg)	STEL, S
		0.05	Mercury and mercury vapour, mercury compounds except alkyls (as Hg)	TWA, S
		0.1	Aryl mercury compounds, inorganic mercury compounds (as Hg)	TWA, S
Brazil	1978	0.04	Inorganic mercury compounds (as Hg)	TWA
Bulgaria	1984	0.01	Mercury and mercury vapour, inorganic mercury compounds (as Hg)	TWA
Chile	1983	0.008	Alkyl mercury compounds (as Hg)	TWA, S
		0.04	Mercury and mercury vapour	TWA
China	1979	0.01	Mercury and mercury vapour	TWA
		0.005	Organic mercury compounds (as Hg)	TWA, S
Former Czechoslovakia	1991	0.05	Mercury and mercury vapour, mercury compounds except mono- and dialkyls (as Hg)	TWA
		0.15	Mercury and mercury vapour, mercury compounds except mono- and dialkyls (as Hg)	Ceiling
Denmark	1990	0.01	Alkyl mercury compounds (as Hg)	TWA, S
		0.05	Mercury and mercury vapour, mercury compounds except alkyls (as hg)	TWA
Egypt	1967	0.1	Mercury and mercury vapour	TWA
Finland	1992	0.01	Alkyl mercury compounds (as Hg)	TWA, S
		0.05	Mercury and mercury vapour, inorganic mercury compounds (as Hg)	TWA
France	1990	0.01	Alkyl mercury compounds (as Hg)	TWA, S
		0.05	Mercury and mercury vapour	TWA, S
		0.1	Aryl mercury compounds, inorganic mercury compounds (as Hg)	TWA, S
Germany	1992	0.1	Mercury and mercury vapour	TWA, S
		0.01	Organic mercury compounds except methylmercury (as Hg) (total dust)	TWA, S, sensitizer
		0.01	Methylmercury (total dust)	TWA, PR1, S, sensitizer

Table 11 (contd)

Country or region	Year	Concentration (mg/m ³)	Substances affected	Interpretation ^a
Hungary	1990	0.02	Mercury and mercury vapour, inorganic mercury compounds (as Hg)	TWA, sensitizer
		0.04	Mercury and mercury vapour, inorganic mercury compounds (as Hg)	STEL
		0.01	Inorganic mercury compounds (as Hg)	STEL
		0.01	Organic mercury compounds except mono- and dialkyl compounds (as Hg)	TWA, STEL
India	1983	0.01	Alkyl mercury compounds (as Hg)	TWA, S
		0.03	Alkyl mercury compounds (as Hg)	STEL, S
		0.05	Mercury and mercury vapour	TWA
		0.15	Mercury and mercury vapour	STEL
Indonesia	1978	0.01	Organic mercury compounds (as Hg)	TWA, S
		0.1	Alkyl mercury compounds (as Hg)	TWA, S
Italy	1978	0.01	Organic mercury compounds (as Hg)	TWA, S
		0.05	Inorganic mercury compounds (as Hg)	TWA, S
Japan	1991	0.05	Mercury and mercury vapour, mercury compounds except alkyl compounds (as Hg)	TWA
Mexico	1991	0.05	Mercury compounds except alkyl compounds (Hg)	TWA
		0.01	Alkyl mercury compounds (as Hg)	TWA
		0.03	Alkyl mercury compounds (as Hg)	15-min, 4 ×/day, 1-h interval
Netherlands	1986	0.05	Inorganic mercury compounds (as Hg)	TWA
		0.01	Alkyl mercury compounds (as Hg)	TWA, S
Poland	1990	0.01	Mercury and mercury vapour, organic mercury compounds (as Hg)	TWA
		0.05	Inorganic mercury compounds (as Hg)	TWA
Republic of Korea	1983	0.05	Mercury and mercury vapours	TWA
		0.03	Alkyl mercury compounds (as Hg)	TWA
Romania	1975	0.05	Mercury and mercury vapour	TWA, S
		0.15	Mercury and mercury vapour	STEL, S
		0.01	Organic mercury compounds (as Hg)	STEL, S
Sweden	1992	0.01	Alkyl mercury compounds (as Hg)	TWA, S
		0.05	Mercury and mercury vapour, mercury compounds except alkyl compounds (as Hg)	TWA, S
Switzerland	1990	0.05	Mercury and mercury vapour	TWA, S
		0.01	Organic mercury compounds (as Hg)	TWA, S, sensitizer
		0.1	Inorganic mercury compounds (as Hg)	TWA, PR1, S, sensitizer
Taiwan	1981	0.01	Organic mercury compounds (as Hg)	TWA, S
		0.05	Inorganic mercury compounds (as Hg)	TWA, S

Table 11 (contd)

Country or region	Year	Concentration (mg/m ³)	Substances affected	Interpretation ^a
United Kingdom	1992	0.01	Alkyl mercury compounds (as Hg)	TWA, S
		0.03	Alkyl mercury compounds (as Hg)	STEL, S
		0.05	Mercury and mercury vapour, mercury compounds except alkyls (as Hg)	TWA
		0.15	Mercury and mercury vapour, mercury compounds except alkyls (as Hg)	STEL (10 min)
USA	OSHA	1992	0.01 Alkyl mercury compounds (as Hg), organic mercury compounds (as Hg)	TWA, PEL, S
			0.03 Alkyl mercury compounds (as Hg), organic mercury compounds (as Hg)	STEL, PEL, S
		0.05	Mercury and mercury vapour	TWA, PEL, S
			0.1 Aryl mercury compounds, inorganic mercury compounds (as Hg)	Ceiling, PEL, S
	NIOSH	1990	0.01 Alkyl mercury compounds (as Hg), organic mercury compounds (as Hg)	TWA, REL, S
			0.03 Alkyl mercury compounds (as Hg), organic mercury compounds (as Hg)	STEL, REL, S
		0.05	Mercury and mercury vapour	TWA, REL, S
			0.01 Alkyl mercury compounds (as Hg)	TWA, TLV, S
	ACGIH	1992	0.03 Alkyl mercury compounds (as Hg)	STEL, TLV, S
			0.05 Methylmercury, all forms except alkyl vapours	TWA, TLV, S
		0.1	Aryl mercury compounds, inorganic mercury compounds (as Hg)	TWA, TLV, S
			0.005 Mercury and mercury vapour	TWA
Former USSR	1990	0.05	Inorganic mercury compounds (as Hg)	TWA
		0.2	Inorganic mercury compounds (as Hg)	STEL
Venezuela	1978	0.01	Alkyl mercury compounds (as Hg)	TWA, S
		0.03	Alkyl mercury compounds (as Hg)	Ceiling, S
		0.05	Inorganic mercury compounds (as Hg)	TWA
		0.15	Inorganic mercury compounds (as Hg)	Ceiling
Former Yugoslavia	1971	0.1	Mercury and mercury vapour	TWA
		0.01	Alkyl mercury compounds (as Hg)	TWA, S

From Arbeidsinspectie (1986); Cook (1987); US Occupational Safety and Health Administration (OSHA) (1992); US National Institute for Occupational Safety and Health (1990); International Labour Office (1991); American Conference of Governmental Industrial Hygienists (ACGIH) (1992); Arbejdstilsynet (1992); Deutsche Forschungsgemeinschaft (1992); Health & Safety Executive (1992); UNEP (1993)

^aThe concentrations given may or may not have regulatory or legal status in the various countries; for interpretation of the values, the original references or other authoritative sources should be consulted. PR1, a risk of damage to the developing embryo or fetus has been demonstrated unequivocally, even when exposure limits have been adhered to; S, absorption through the skin may be a significant source of exposure; TWA, time-weighted average; STEL, short-term exposure limit; PEL, permissible exposure limit; REL, recommended exposure limit; TLV, threshold limit value.

The Joint FAO/WHO Expert Committee on Food Additives set a provisional tolerable weekly intake of 300 µg total mercury per person, of which no more than 200 µg (3.33 µg/kg bw for a 60-kg individual) should be present as methylmercury compounds (WHO, 1989b). In Japan, a provisional tolerable weekly intake of 250 µg mercury per week, with no more than 170 µg as methylmercury, was calculated from the WHO values on the basis of 50 kg body weight. This weekly intake is considered to be one-tenth of the minimum toxic dose of adults and is therefore expected to give protection against fetal damage (WHO, 1990).

Stationary sources in the USA where mercury ore is processed to recover mercury, where mercury chloralkali cells are used to produce chlorine gas and alkali metal hydroxide and where wastewater treatment plant sludge is incinerated or dried are subject to the US national emission standard for mercury. Thus, atmospheric emissions from mercury ore processing facilities and mercury-cell chloralkali plants cannot exceed 2300 g of mercury per 24-h period. Atmospheric emissions from sludge incineration plants, sludge drying plants, or a combination of these, where wastewater treatment plant sludges are processed cannot exceed 3200 g of mercury per 24-h period (US Environmental Protection Agency, 1992).

In the countries of the European Communities, no detectable quantity of mercury is allowed in colouring matter authorized for use in food intended for human consumption (Commission of the European Communities, 1981). The threshold value for mercury in tuna fish in Denmark is 0.5 mg/kg (Rasmussen, 1984). In Sweden, it was recommended that the consumption of fish caught in areas of high contamination (but below 1.0 mg/kg) be restricted to one meal per week (Swedish Expert Group, 1970).

Use of mercury compounds as cosmetic ingredients in the USA is limited to eye-area cosmetics, at concentrations not exceeding 65 ppm (0.0065%) of mercury calculated as the metal (about 100 ppm or 0.01% phenylmercury acetate or nitrate) (US Department of Health and Human Services, 1992). In the European Communities, mercury and its compounds must not be used in cosmetic products, except that thiomerosal (mercurothiolate) and phenylmercury salts (including borate) can be used for eye make-up or eye make-up remover, with a maximum concentration of 0.007% mercury (Commission of the European Communities, 1990, 1991).

2. Studies of Cancer in Humans

Many populations have low-grade or infrequent exposure to metallic mercury or mercury compounds. The Working Group restricted their review to studies specific to metallic mercury or mercury compounds and to groups who are known to have considerable exposure.

2.1 Inorganic mercury compounds

2.1.1 Descriptive studies

In a study in Poland, mercury was determined in the hair of leukaemia patients and in healthy relatives and unrelated healthy subjects (Janicki *et al.*, 1987). The mean content of total mercury was 1.24 ± 1.93 mg/kg hair from 23 cases of acute leukaemia and 0.49

± 0.41 mg/kg hair from 79 healthy control subjects. In 47 cases of acute leukaemia (chronic granulocytic as well as chronic lymphocytic), the mercury content was 0.92 ± 1.44 mg/kg hair. For 19 leukaemia cases of all groups and their 52 relatives, the corresponding figures were 0.69 ± 0.75 mg/kg and 0.43 ± 0.24 mg/kg, respectively. These differences between cases and control subjects were significant. [The Working Group noted that comparisons of means are inappropriate, as the distributions were highly skewed, and that the distribution of mercury may have been affected by the disease.]

In Washington State, USA, occupational mortality was studied for the period 1950–71 on the basis of death certificates (Milham, 1976). For male dentists, the proportionate mortality ratio (PMR) for all malignant neoplasms was 1.05 (127 cases [95% confidence interval (CI), 0.88–1.25]). When sites with more than five cases were considered, the PMR was 1.53 for pancreatic cancer based on 12 cases [95% CI, 0.79–2.69], 1.32 for prostatic cancer based on 20 cases [95% CI, 0.80–2.03] and 1.45 for neoplasms of the lymphatic and haematopoietic tissues based on 17 cases [95% CI, 0.84–2.33).

Occupational mortality was studied in British Columbia, Canada, by the proportionate mortality method and based on 320 423 deaths for which valid records were available among men over 20 years of age (Gallagher *et al.*, 1985). The occupational codes were those used in conjunction with the censuses of 1951 and 1961. Among dentists, there were four cases of kidney cancer (PMR, 1.94; 95% CI, 0.52–4.96) and five tumours of the brain and central nervous system (PMR, 2.36; 95% CI, 0.76–5.52). There were even fewer cases at other sites, or no more than slightly elevated PMRs.

2.1.2 Cohort studies (see Table 12, p. 272)

(a) Nuclear weapons industry workers

A cohort of 2133 white men from Oak Ridge, TN, USA, who were exposed to metallic mercury and an unexposed cohort of 3260 workers from the same plant were studied with regard to mortality in comparison with national rates for white men (Cragle *et al.*, 1984). Exposure to mercury occurred in the context of lithium production in a nuclear weapons plant, which earlier had also produced a fissionable isotope of uranium; anyone in whom mercury had ever been found in the urine, regardless of the concentration, was considered to have been exposed. A mercury monitoring programme was started in mid-1953 and became effective in late 1954. The cohorts were followed-up from 1 January 1953 until 1 January 1979, when vital status was assessable for at least 95.5% of the cohort and death certificates were available for 98% or more. Total mortality was lower than expected for both groups, and there was no excess of any non-cancer death possibly related to mercury exposure (target organs were thought to be liver, lung, brain and other central nervous system, and kidney). The cancer mortality rate was lower than expected for the exposed cohort (standardized mortality ratio [SMR], 0.94 [95% CI, 0.75–1.16]; based on 85 cases) but not for the unexposed (SMR, 1.10 [0.94–1.28]; based on 175 cases). An excess of lung cancer was seen in both cohorts (SMR, 1.34 [0.97–1.81], based on 42 cases among exposed; and 1.34 [1.05–1.69], based on 71 cases among unexposed). For cancers of the brain and central nervous system, the corresponding figures were 1.22 ([0.33–3.12]; based on 4 cases) for the exposed cohort and 2.30 ([1.22–3.94]; based on 13 cases) for the unexposed; for kidney cancer, the SMRs were reported to be 1.65 ([0.45–4.23]; based on 4 cases) for the exposed

cohort and 0.72 ([0.15–2.10]; based on 3 cases) for the unexposed. In subgroups with mercury levels in urine exceeding 0.3 mg/L at least once or with more than one year of exposure, there was also no clear increase in cancer mortality rates. No definite explanation could be given for the excess of lung cancer observed in both cohorts, but life-style factors or some factor other than mercury present in the plant were mentioned.

(b) *Dentists*

Cohorts of 3454 male and 1125 female dentists and 4662 dental nurses identified from the Swedish census in 1960 were followed for cancer development in the period 1961–79 by linkage with cancer register data (Ahlbom *et al.*, 1986). The overall standardized incidence ratio (SIR) was 2.1 (95% CI, 1.3–3.4) for glioblastoma (astrocytoma III–IV) in comparison with national incidence rates, based on 18 cases. The SIRs for the various cohorts were 2.0 for male dentists, 2.5 for female dentists and 2.2 for dental nurses. In the combined cohorts, there were also four gliomas (astrocytoma I–II) (SIR, 1.8; 95% CI, 0.5–4.7) and six meningiomas (SIR, 1.3; 95% CI, 0.5–2.8). There was no excess of all tumours in these cohorts. For comparison, physicians and female nurses were also studied; no indication was found of an excess of glioblastomas. Exposures to amalgam, chloroform and X-radiation were mentioned as possible occupational factors.

In another analysis of this population, occupational risks for intracranial gliomas in Sweden were studied by linking cancer incidence data from the national cancer registry during 1961–79 with census data on occupation from 1960 (McLaughlin *et al.*, 1987). The expected number of cases for each occupational category was calculated on the basis of the general population in the study period, and regional adjustment was applied. There were 3394 gliomas in men and 1035 in women who had been employed in 1960. An excess risk was found for male dentists, with an SIR of 2.1 ($p < 0.05$) based on 12 cases; for female dental assistants, nine cases (SIR, 2.1; $p = 0.09$) were reported. For comparison, it may be noted also that among male physicians there were 14 cases (SIR, 1.4; nonsignificant) and among female physicians, four cases (SIR, 3.7; $p < 0.05$). Male chemists, physicists, veterinary surgeons, agricultural research scientists and pharmacists also had SIRs greater than 2.0. [The Working Group noted that no distinction was made between the various subtypes of glioma.]

Mortality risks by occupation have been studied among veterans who served in the US Armed Forces between 1917 and 1940 (Hrubec *et al.*, 1992). Occupation and smoking status were assessed through questionnaires in 1954 and 1957. Follow-up to 1980 was done using insurance and pension systems (96% complete for First World War veterans). The smoking-adjusted relative risk (RR) for each occupation was estimated by using all other occupations as the standard, and Poisson regression modelling was applied. In a subcohort of 2498 dentists with a total of 1740 deaths, there were 299 cancer deaths (RR, 0.9; 90% CI, 0.80–0.97). The risk for pancreatic cancer was 1.4 (90% CI, 0.98–1.86; 27 deaths). No excess of brain or kidney tumours was detected (RR, 0.9; 90% CI, 0.45–1.74; 6 cases; and RR, 0.8; 90% CI, 0.39–1.50; 6 cases, respectively). For a group of 267 medical and dental technicians, there was an elevated risk for all cancers among 40 nonsmokers (RR, 2.5; 90% CI, 1.36–4.73; 7 deaths). For nonsmokers and smokers in this group, the risk for all cancers was only slightly

elevated (RR, 1.2; 90% CI, 0.87–1.54; 34 deaths), but there was an excess of colon cancer (RR, 1.9; 90% CI, 1.01–3.53; 7 deaths).

(c) *Chloralkali workers*

Mortality and cancer incidence were reported for a group of 1190 male Swedish chloralkali workers in whom mercury had been measured in the blood or urine for at least one year between 1946 and 1984 (Barregård *et al.*, 1990). Their mortality and cancer incidence were compared with those of the general male population for the periods 1958–84 and 1958–82, respectively, and the follow-up was complete. The mean level of mercury excreted in the urine had been about 200 µg/L in the 1950s, 150 µg/L in the 1960s and less than 50 µg/L in the 1980s. On the basis of crude estimates, 26% of the cohort was estimated to have had an accumulated urinary mercury dose of 1000 years·µg/L or more, 457 subjects also had some (mostly low-grade) asbestos exposure; exposure to static magnetic fields was reported to have occurred. Mortality from all causes was not significantly increased, the observed to expected mortality being 1.1 (95% CI, 0.9–1.3) based on 147 deaths with 10 or more years of latency. There were 51 incident cases of cancer observed *versus* 42 expected with a latency of 10 years or more, i.e. a rate ratio of 1.2 (95% CI, 0.9–1.6). Lung cancer was the only type of tumour in clear excess, with 10 observed and 4.9 expected with a latency of 10 years or more (rate ratio, 2.0; 95% CI, 1.0–3.8). There were slight excesses of some other cancers with a latency of 10 years or more, namely three brain tumours *versus* 1.1 expected (RR, 2.7; 95% CI, 0.5–7.7), three kidney cancers *versus* 1.9 expected (1.6; 0.3–4.7), five urinary bladder cancers *versus* 2.9 expected (1.7; 0.6–4.1) and 10 prostatic cancers *versus* 8.6 expected (1.2; 0.6–2.1). The excess of lung cancer was thought to be due to exposure to asbestos; one case of mesothelioma was observed. Smoking was considered to explain 10% of the excess of lung cancer, although information on smoking habits was available for only a 7% random sample of the cohort. The authors noted that chloralkali workers have five to 10 times the mercury exposure of dental personnel.

In a cohort study of 674 male Norwegian chloralkali workers exposed to inorganic mercury for more than one year prior to 1980, who had a mean cumulative urinary concentration of 740 µg/L, there were 204 deaths *versus* 210.7 expected (SMR, 0.97; 95% CI, 0.84–1.11) and 89 incident cases of cancer *versus* 85.0 expected (SIR, 1.05; 95% CI, 0.84–1.29) (Ellingsen *et al.*, 1993). During the follow-up period (1953–89 for incidence and 1953–88 for mortality), there were 19 incident cases of lung cancer, with 11.5 expected (SIR, 1.66; 95% CI, 1.00–2.59) on the basis of national rates. There was no correlation with cumulative mercury dose, employment or latency; a somewhat increased frequency of smoking and exposure to asbestos (one mesothelioma was found) were considered to explain the excess of lung cancer. Three kidney cancers and two brain tumours were observed *versus* 3.2 and 2.45 expected, respectively. These two sites were considered by the authors to be of primary interest with regard to exposure to mercury.

(d) *Mercury miners*

In a cohort study of the relationship between silicosis and mortality from lung cancer in US metal miners, the difference in risk for silicotic miners compared with nonsilicotic white metal miners was greater for mercury miners than for other miners (Amandus & Costello,

Table 12. Cohort studies of populations exposed to inorganic mercury compounds

Study population Period of follow-up	End-point		Site	No. of cases	SMR	95% CI	Reference
<i>Nuclear weapons industry workers</i>							
2133 Mercury exposed, 3260 unexposed male workers, USA, 1953–79	Mortality	Exposed	Lung	42	1.34	[1.0–1.8]	Cragle <i>et al.</i> (1984)
			Kidney	4	1.65	[0.4–4.2]	
			Brain	4	1.22	[0.3–3.1]	
	Unexposed	Lung	71	1.34	[1.0–1.7]		
		Kidney	3	0.72	[0.1–2.1]		
		Brain	13	2.30	[1.2–3.9]		
<i>Dentists</i>							
9201 Dentists and dental nurses, Sweden, 1961–79	Incidence		Glioblastoma	18	2.1	1.3–3.4	Ahlbom <i>et al.</i> (1986)
			Glioma	4	1.8	0.5–4.7	
			Meningioma	6	1.3	0.5–2.8	
2498 Dentists, US veterans, 1954–80	Mortality		Pancreas	27	1.4	0.96–1.86	Hrubec <i>et al.</i> (1992)
			Brain	6	0.9	0.45–1.74	
			Kidney	6	0.8	0.39–1.50	
267 Medical and dental assistants, US veterans, 1954–80	Mortality		Colon	7	1.9	1.01–3.53	Hrubec <i>et al.</i> (1992)
			Brain	1	1.5	NR	
			Kidney	2	2.8	NR	
<i>Chloralkali workers</i>							
1190 Males, Sweden, 1946–82	Incidence		Lung	13	[1.8]	[0.9–3.0]	Barregård <i>et al.</i> (1990)
			Kidney	4	[1.3]	[0.4–3.4]	
			Brain	4	[1.8]	[0.5–4.7]	
674 Males, Norway, 1953–89	Incidence		Lung	19	1.66	1.00–2.59	Ellingsen <i>et al.</i> (1993)
			Kidney	3	0.95	0.2–2.8	
			Brain	2	0.8	0.1–3.0	
<i>Mercury miners</i>							
274 Males, USA, 1959/61–75	Mortality	11 Silicotics	Lung	3	14.0	2.89–41.0	Amandus & Costello (1991)
		263 Nonsilicotics	Lung	8	2.66	1.15–5.24	

NR, not reported

1991). The follow-up was from date of examination in 1959–61 to 31 December 1975. For the 11 silicotic mercury miners, the SMR was 14.0 (95% CI, 2.89–41.0) based on three lung cancer deaths, whereas the SMR for the 263 nonsilicotic mercury miners was 2.66 (95% CI, 1.15–5.24) based on eight cases. For other miners (copper, lead–zinc, iron and others), the corresponding figures were 1.39 [95% CI, 0.70–2.49] based on 11 silicotic lung cancer deaths and [1.14; 95% CI, 0.93–1.37] based on 110 deaths from nonsilicotic lung cancer. The reference for calculating the SMRs was death rates in white US males. No explanation was offered for the differences seen between mercury and other miners. [The Working Group noted that the small numbers of silicotic mercury miners may make the estimate unstable.]

2.1.3 Case-control studies (see Table 13, p. 274)

In a case-control study of incident cases of lung cancer admitted during 1981–83, 340 male and 36 female cases and 817 male and 75 female hospital controls, all residents of metropolitan Florence, Italy, were drawn from the regional general hospital for the analyses (Buiatti *et al.*, 1985). Occupational histories were collected from each subject directly; six female cases but no control had ever worked as felt-hat makers ($p = 0.01$). Heavy exposure to mercury but also to arsenic and other chemicals was reported to have occurred in the Italian hat-making industry.

In a study described in detail in the monograph on beryllium (pp. 73–74; Carpenter *et al.*, 1988), based on 29 cases identified from information on death certificates as ever exposed to mercury, the odds ratio for cancer of the central nervous system was 1.77 [95% CI, 0.5–5.8] when compared with unexposed cases. The matched analysis by highest rank ever held *versus* rank 0 yielded odds ratios of 2.01, 1.33 and 1.19 for ranks 1, 2 and 3, respectively (all odds ratios had a p value of 0.26 or greater). When risk estimates were calculated with a 10-year latency, the odds ratios were 1.58, 0.77 and 1.57 for ranks 1, 2 and 3, respectively, with a p value of 0.47 or greater. A further analysis based on time spent in ranks 2 and 3, assuming a 10-year latency, yielded odds ratios of 0.00, 0.96, 0.00 and 1.86 for workers with > 1 year and < 3 years, 3–10 years, 11–20 years and 21 years or more in ranks 2 and 3 compared with ranks 0 and 1. The authors concluded that their study does not support the hypothesis that occupational exposures to any of the 26 chemicals studied increase appreciably the risk for cancers of the central nervous system.

The effects of a great number of exposures were considered in a case-control study from Montréal, Canada, involving all major cancer forms and population controls as well as two hospital control series, i.e. cancer cases and other cases (Siemiatycki, 1991). In total, 4576 incident cancer cases were recruited through local informants at the hospitals. Completed questionnaires and interviews on occupational exposures (293 agents were considered) were obtained for 3730 of these (response rate, 81.5%). A total of 740 population controls were drawn from electoral lists or obtained by random-digit dialling. Of these, exposure was successfully assessed for 533 (72.0%). The prevalence of exposure to metallic mercury was 0.6% and that to any mercury compound (including metallic mercury), 2%. For prostatic cancer, 14 of 449 cases were exposed to mercury compounds, resulting in an odds ratio of 1.7 (90% CI, 1.0–3.0); five cases had been exposed to metallic mercury, giving an odds ratio of 6.2 (90% CI, 1.2–33.2). For lung cancer, four of the 857 cases had been exposed to metallic mercury (odds ratio, 4.0; 90% CI, 1.2–13.0). For bladder cancer, 14 of the 484 cases had been

Table 13. Case-control studies of populations exposed to inorganic mercury compounds

Study population	End-point	Exposure	Sex	No. of exposed cases	Odds ratio	95% CI	Reference
<i>Lung cancer</i>							
Hospital-based, Italy	Incidence	Hat makers	F	6		<i>p</i> = 0.01	Buiatti <i>et al.</i> (1985)
Population-based, Canada	Incidence	Mercury, metallic	M	4	4.0	1.2–13.0 ^a	Siemiatycki (1991)
<i>Prostatic cancer</i>							
Population-based, Canada	Incidence	Mercury, metallic	M	5	6.2	1.2–33.2 ^a	Siemiatycki (1991)
		Mercury and mercury compounds ^b	M	14	1.7	1.0–3.0	
<i>Bladder cancer</i>							
Population-based, Canada	Incidence	Mercury and mercury compounds ^b	M	14	1.5	0.9–2.6 ^a	Siemiatycki (1991)
<i>Brain tumours</i>							
Population-based, USA	Mortality	Nuclear facilities	Central nervous system	29	1.77	[0.5–5.8]	Carpenter <i>et al.</i> (1988)
Population-based, Australia	Incidence	Amalgam fillings	Glioma Meningioma		0.47 1.04	0.25–0.91 0.43–2.47	Ryan <i>et al.</i> (1992)

^a90% CI^bIncluding organomercury compounds

exposed to mercury compounds (odds ratio, 1.5; 90% CI, 0.9–2.6). Significant results were not obtained for cancers at other sites. [The Working Group noted that although several potential confounding factors were considered not all possible occupational confounders were addressed.]

A case-control study from Adelaide, Australia, considered incident brain tumours and exposure to amalgam fillings and diagnostic dental X-rays (Ryan *et al.*, 1992). Cases aged 25–74 were notified by neurosurgeons in Adelaide, and there was a further check for cases in cancer and brain tumour registries. Controls were selected from the Australian electoral roll, covering 95% of the adult population. In total, 190 cases of brain tumours were identified, together with 662 controls; of these, 110 glioma cases, 60 meningioma cases and 417 controls were included in the analyses. There was a decreased odds ratio (0.47; 95% CI, 0.25–0.91) for glioma in association with self-reported amalgam fillings (at least one filling) and with diagnostic X-rays (at least one X-ray) (odds ratio, 0.42; 95% CI, 0.24–0.76); the corresponding results for meningioma were 1.04 (95% CI, 0.43–2.47) in relation to fillings, whereas the risk associated with diagnostic X-rays was slightly increased (odds ratio, 1.37; 0.68–2.73). No dose-response pattern was seen for either glioma or meningioma with regard to amalgam fillings. The authors considered a biological protective mechanism unlikely.

2.2 Organomercury compounds

2.2.1 Descriptive studies

Direct SMRs for biliary tract cancer in the Japanese prefectures in 1975 were correlated with an environmental pollution index related to use of agricultural chemical products for the years 1962–66 (Yamamoto *et al.*, 1986). In both men and women, only weak, non-significant correlations were found for exposure to mercuric compounds (such as phenylmercury acetate, used as a fungicide in Japan until 1971) converted to the dose of inorganic mercury, whereas positive and significant correlations were obtained, especially for DDT and some phenoxy herbicides.

The mortality pattern was studied in the population of a small area of the city of Minamata, Kumamoto Prefecture, Japan, which consisted mainly of fishermen and their families (Tamashiro *et al.*, 1986) and where 70% of the 1612 confirmed cases (including 527 deaths) of Minamata disease (see pp. 291–292) in the Prefecture through 1983 were known to have occurred. SMRs were computed for different causes of death in 1970–81 by using age-specific rates for the entire city for 1972–78. The total population of the study area in 1975 was 3887 *versus* 36 782 in the city. Some migration took place during the study period, and, in particular, young adults moved out of the area and former residents returned. The SMR for all causes of death was 1.05 (95% CI, 0.95–1.15, based on 412 deaths) and that for all cancers was 1.18 (95% CI, 0.96–1.46, based on 84 deaths). For the various cancers reported, the corresponding figures were: oesophagus, 2.05 (95% CI, 0.67–4.78; 5 cases); stomach, 0.77 (95% CI, 0.42–1.29; 14 cases); liver, 2.07 (95% CI, 1.16–3.42; 15 cases); pancreas, 0.99 (95% CI, 0.20–2.88; 3 cases); trachea-bronchus-lung, 1.52 (95% CI, 0.79–2.65; 12 cases); breast, 2.64 (95% CI, 0.54–7.71; 3 cases); uterus, 0.89 (95% CI, 0.24–2.28; 4 cases); leukaemia, 1.82 (95% CI, 0.50–4.66; 4 cases); and other cancers, 0.98 (95% CI, 0.63–1.46; 24 cases). An elevated SMR was also seen for chronic liver disease and cirrhosis

(2.16; 95% CI, 1.41–3.17; based on 26 cases). There was some evidence that alcohol consumption in the area was above the Japanese average. [The Working Group noted that the increased risk for liver cancer seems consistent with the increased occurrence of chronic liver disease and cirrhosis and with a higher than average alcohol consumption; the latter might also have affected the risk for oesophageal cancer.]

The effects on life expectancy of elevated exposure to methylmercury compounds were studied in five coastal towns of southern Japan in comparison with a surrounding control area (Tamashiro *et al.*, 1987). The average hair concentrations of mercury were reported to be three to six times higher in the exposed area than in the control area. The study period was from 1969 through to 1982. The crude RR for death from malignant neoplasms was [1.05].

2.2.2 Cohort study

It was reported in letter to the Editor that 1657 people with a licence for seed disinfection using organomercury compounds and other agents, issued between 1965 and 1976, were followed through the Swedish Cancer Registry from the date of licencing until death or December 1982 (Wiklund *et al.*, 1988). The mean follow-up time was 14.7 years, resulting in 24 429 person-years of observation. Five tumours of the nervous system were observed *versus* 4.98 expected (SIR, 1.0; 95% CI, 0.33–2.34); rates of tumours at other sites were not reported. The authors noted that the use of alkylmercury compounds was banned in Sweden in the mid-1960s, and limitations were placed on mercury disinfection.

2.2.3 Case-control studies (see Table 14, p. 277)

Three similarly designed studies on soft-tissue sarcomas in different parts of Sweden, mainly focusing on exposure to phenoxyacetic acid herbicides and chlorophenols, also provide data on exposure to organomercury seed dressings and other pesticides (Eriksson *et al.*, 1981; Hardell & Eriksson, 1988; Eriksson *et al.*, 1990). The first study encompassed 110 cases and 219 population controls in the five southernmost counties. The second study involved 55 cases and 220 living and 110 dead controls and a third group of 190 other cancer controls in the three northernmost counties. In the third study, there were 237 cases and 237 controls from the seven central counties, matched on vital status. Information on exposure was obtained from questionnaires to the subjects or their next-of-kin, supplemented with telephone interviews. Exposure to mercury seed dressings was reported for 8.2% of cases and 4.6% of controls in the first study; for 1.9% of cases and 3.5% of living and 2.8% of dead controls in the second study; and for 4.6% of cases and 5.2% of controls in the third study. The resulting odds ratio in the first study [not given] was said to have a 90% CI that included unity. [A calculation results in a crude odds ratio of 1.9 (95% CI, 0.65–5.3) for the first study and, for the second study, 0.52 (95% CI, 0.01–4.3) with regard to living controls and 0.66 (95% CI, 0.08–5.74) using dead controls.] The odds ratio in the third study was given as 0.89 (95% CI, 0.40–1.96).

In a study from northern Sweden on malignant lymphomas, which mainly considered exposure to organic solvents, chlorophenols and phenoxyacetic acid herbicides, exposure frequencies to organomercury seed dressings were also reported (Hardell *et al.*, 1981). The study included 169 cases (60 Hodgkin's lymphomas, 109 non-Hodgkin lymphomas) and 338

(335 used in the calculation) population controls. Information on exposure was obtained through questionnaires. For the cases and controls, 5.3 and 3.0%, respectively, exposure to mercury seed dressings co-varied with exposure to phenoxyacetic acid herbicides, whereas asbestos and glass fibre exposure co-varied with chlorophenol exposure. Exposure to phenoxyacetic acid herbicides as well as to chlorophenols appeared to be strong risk factors for lymphomas, but after exclusion of subjects with exposure to phenoxy herbicides, 128 cases and 311 controls remained, with exposure frequencies to mercury seed dressings of 4.7 and 2.9%, respectively; for DDT, the corresponding figures were 5.5 and 3.5%. [For the restricted material, a calculation results in a crude odds ratio of 1.78 (95% CI, 0.62–5.11) for mercury seed dressings and 1.6 (95% CI, 0.51–4.6) for DDT.]

Table 14. Population-based case-control studies of populations of men exposed to organomercury seed dressings in Sweden

No. of exposed cases	Odds ratio	95% CI	Reference
<i>Soft-tissue sarcomas</i>			
[9]	[1.9]	[0.65–5.3]	Eriksson <i>et al.</i> (1981)
[1]	[0.52] ^a [0.66] ^b	[0.01–4.3] [0.08–5.7]	Hardell & Eriksson (1988)
[10]	0.89	0.40–1.96	Eriksson <i>et al.</i> (1990)
<i>Lymphomas</i>			
[6]	1.78	[0.62–5.1]	Hardell <i>et al.</i> (1981)

^a Living controls

^b Dead controls

3. Studies of Cancer in Experimental Animals

3.1 Metallic mercury

Intraperitoneal administration

Rat: A group of 39 male and female BDIII and BDIV rats, three months old, received two intraperitoneal injections of 0.05 ml **metallic mercury** [purity unspecified] over 14 days (total dose, 0.1 ml); mean survival was 580 days in treated rats and 780 days in controls. Only gross lesions were investigated histopathologically. At 22 months, when 12/39 animals were still alive, one female rat had a spindle-cell sarcoma in the abdominal cavity. Two females and two males of the 11 remaining rats developed similar tumours (Druckrey *et al.*, 1957). [The Working Group noted the incomplete reporting of the study and the possibility that the lesions seen were the result of a solid-state effect.]

3.2 Mercuric chloride

3.2.1 Oral administration

(a) Mouse

A group of 54 male and 54 female Swiss mice (Charles River CD strain), 20 days old, were given drinking-water containing **mercuric chloride** (5 ppm [mg/L] mercury) [purity unspecified] for life. A control group of 54 male and 54 female mice was given the drinking-water alone. Of the controls, 50% of the males were still alive at 602 days and 10% at 789 days, and 50% of females were still alive at 539 days and 10% at 691 days. Of the treated mice, 50% of males were still alive at 540 days and 10% at 697 days, and 50% of females at 575 days and 10% at 736 days. The numbers of mice autopsied were 38 control males and 47 control females and 48 male and 41 female treated mice. The authors reported that 11/41 treated female mice and 3/47 control females developed lymphoma or leukaemia [$p = 0.09$, Fisher exact test] (Schroeder & Mitchener, 1975). [The Working Group noted the incomplete reporting of the study and that only some of the animals were autopsied.]

Groups of 60 male and 60 female B6C3F1 mice, six weeks old, received 0, 5 or 10 mg/kg bw **mercuric chloride** (purity > 99%) in deionized water by gavage (10 ml/kg bw) on five days a week for 103–104 weeks. Ten animals from each group were killed at 15 months for evaluation. Survival at the end of the two-year study was 36/50, 36/50 and 31/50 in the control, low-dose and high-dose male groups and 41/50, 35/50 and 31/50 in the corresponding female groups. Body weights of both female and male treated mice were similar to those of controls throughout. Of the high-dose male mice, 2/49 developed renal tubular adenomas and 1/49 a renal tubular adenocarcinoma. No such tumour was seen in either the control or low-dose groups. No increase in the incidence of tumours was seen in the treated female mice (US National Toxicology Program, 1993).

(b) Rat

Groups of 60 male and 60 female Fischer 344/N rats, six weeks old, received 0, 2.5 or 5 mg/kg bw **mercuric chloride** (purity, > 99%) in deionized water by gavage (5 ml/kg bw) on five days a week for 103–104 weeks. Ten animals from each group were killed at 15 months for evaluation. Body weights of low- and high-dose males and high-dose females were lower than those of controls. Survival at two years was 26/50 male controls, 10/50 low-dose and 5/50 high-dose rats and 35/50, 28/49 and 30/50 in the females. The decrease in survival in male rats was due, in part, to an increased incidence of treatment-related renal disease. High-dose males had a greater incidence of renal tubular hyperplasia than control males (12/50 versus 3/50; $p = 0.005$), but the incidence of renal tubular adenomas was similar (control, 4/50; high-dose, 5/50). In female rats, renal tubular hyperplasia occurred in 5/50 high-dose rats and 2/50 controls; two high-dose female rats had renal tubular adenomas, but none was seen in controls. Treated male rats had a dose-related increase in the incidence of forestomach hyperplasia compared to controls (control, 3/49; low-dose, 16/50; high-dose, 35/50), as did high-dose female rats (control, 5/50; low-dose, 5/49; high-dose, 20/50). In addition, there was a dose-related increase in the incidence of squamous-cell papilloma of the forestomach in treated males (control, 0/50; low-dose, 3/50; high-dose, 12/50); such tumours also occurred in 2/50 high-dose female rats. High-dose males also had an increased

incidence of thyroid follicular-cell carcinoma (control, 1/50; low-dose, 2/50; high-dose, 6/50), but not of hyperplasia (control, 2/50; low-dose, 4/50; high dose, 2/50) or adenoma (control, 1/50; low-dose, 4/50; high-dose, 0/50) (US National Toxicology Program, 1993). [The Working Group noted the low survival rate of male animals.]

3.2.2 Administration with known carcinogens

As the purpose of the investigations described below was to study interactions with known carcinogens, the studies were limited to specific target sites, were often of short duration and were not intended to address the carcinogenicity of mercury *per se*.

(a) Mouse

Twenty female Sencar mice [age unspecified] received single topical applications of 0.2 ml of 10 nmol [2.6 µg] 7,12-dimethylbenz[*a*]anthracene (DMBA) followed by twice weekly topical applications of 200 µg mercuric chloride in 0.2 ml of a 90% acetone solution for 26 weeks. A positive control group of 20 mice, initiated with DMBA, received promotion with 12-*O*-tetradecanoylphorbol 13-acetate [dose and dosing regime unspecified]. All mice in the positive control group developed skin papillomas, and two developed carcinomas. No skin tumour occurred in the mercuric chloride-treated mice (Kurokawa *et al.*, 1989). [The Working Group noted the incomplete reporting of the study.]

(b) Rat

A group of 15 male Fischer 344 rats, seven weeks old, was administered *N*-nitroso-*N*-hydroxydiethylamine (NHDEA) at 500 ppm [mg/L] in the drinking-water for two weeks followed by drinking-water containing 40 ppm [mg/L] mercuric chloride (99.5% pure) for 25 weeks. A further group of 15 rats received only mercuric chloride at 40 ppm for 25 weeks; a control group of 15 rats received drinking-water for the 27-week experimental period; and a further group of 15 rats was given NHDEA for two weeks followed by 25 weeks of drinking-water alone. There was no significant difference in the number of renal-cell tumours in rats receiving NHDEA and mercuric chloride (5/15) and those receiving NHDEA alone (2/15), but there was a significant ($p < 0.01$, Student's *t* test) increase in the mean number of dysplastic foci/cm² in the NHDEA- plus mercuric chloride-treated group (1.09) over that in the group treated with NHDEA alone (0.23). No renal-cell tumour or dysplastic focus was reported in the group receiving mercuric chloride alone (Kurokawa *et al.*, 1985).

Groups of 20 male Fischer 344 rats [age unspecified] were given drinking-water containing 50 ppm [mg/L] *N*-nitrosodiethylamine (NDEA) for four weeks to initiate liver carcinogenesis, followed by 30 weeks of treatment with drinking-water containing 40 ppm [mg/L] mercuric chloride [purity unspecified], water alone or 1000 ppm [1 g/L] phenobarbital (positive control). All animals were killed at week 34. Mercuric chloride treatment did not increase the number of hepatocellular carcinomas, adenomas or hyperplastic nodules over that in rats treated with NDEA alone (Kurokawa *et al.*, 1989). [The Working Group noted the incomplete reporting of the study.]

Groups of 20 male Wistar rats [age unspecified] were given *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine in the drinking-water at 100 ppm [mg/L], together with a diet supplemented with 10% sodium chloride for eight weeks to initiate gastroduodenal carcinogenesis,

followed by 32 weeks of treatment with drinking-water containing 40 ppm [mg/L] **mercuric chloride** and basal diet without the 10% sodium chloride; controls were given the nitrosamine and basal diet containing sodium chloride for eight weeks then basal diet and standard drinking-water. The incidences of carcinoma and hyperplasia of the fundic and pyloric regions of the glandular stomach and of carcinoma of the duodenum were not increased by treatment with mercuric chloride over those caused by the nitrosamine alone (Kurokawa *et al.*, 1989). [The Working Group noted the incomplete reporting of the study.]

(c) *Hamster*

A group of 20 female Syrian golden hamsters [age unspecified] received three weekly injections [site unspecified] of *N*-nitrosobis(2-oxopropyl)amine (NBOPA) at a dose of 10 mg/kg bw to initiate pancreatic carcinogenesis, followed by treatment with drinking-water containing 40 ppm [mg/L] **mercuric chloride** for a further period [presumed to be 30 weeks]. A further group of 32 hamsters received NBOPA followed by drinking-water alone for 30 weeks. At the end of the study [duration unspecified], there was no difference in the multiplicity of either pancreatic adenocarcinomas or dysplastic lesions between the two groups (Kurokawa *et al.*, 1989). [The Working Group noted the incomplete reporting of the study.]

3.3 Methylmercury chloride

3.3.1 Oral administration

(a) *Mouse*

Groups of 60 male and 60 female ICR mice, five weeks of age, were fed a diet containing 0, 15 or 30 ppm [mg/kg] **methylmercury chloride** (99.3% purity) for 78 weeks. All animals were examined macroscopically, but histopathological examination was carried out only on kidneys of animals that died after week 53 and on lungs of mice with renal masses. The first renal tumour was detected in a male treated with 15 ppm and necropsied at week 58. Most mice given 30 ppm had severe neurotoxic effects and died or became moribund by week 26; similar, but less marked toxic effects occurred in the group treated with 15 ppm. At 78 weeks, survival among male mice was 24/60 given 0 ppm, 8/60 given 15 ppm and 0/60 given 30 ppm; survival among female mice was 33/60 given 0 ppm, 18/60 given 15 ppm and 0/60 given 30 ppm. The numbers of male mice that died after 53 weeks with renal tumours were: 1/37 (an adenoma) in the group given 0 ppm, 13/16 (total numbers of tumours: 11 adenocarcinomas [$p < 0.001$] and five adenomas [$p < 0.01$]) in the group given 15 ppm and none in the one surviving animal treated with 30 ppm. No renal tumour was reported in the female mice (Mitsumori *et al.*, 1981). [The Working Group noted the poor survival in the groups exposed to high doses of methylmercury chloride and the limited number of tissues subjected to histopathological evaluation.]

Groups of 60 male and 60 female ICR mice, five weeks of age, were administered diets containing 0, 0.4, 2 or 10 ppm (mg/kg) **methylmercury chloride** (99.3% pure) for 104 weeks. Six males and six females from each group were killed at 26-week intervals and subjected to histological examination, as were all other animals. No neurotoxic effect was observed in the

treated animals, and, although all male mice given 10 ppm were dead by week 98, there was no difference in survival rates between the control and treated groups. The first renal tumour occurred in a male treated with 10 ppm at 58 weeks. Epithelial degeneration of the renal proximal tubules was seen in both males (40/59) and females (19/60) given 10 ppm, and similar but milder degeneration was seen in males given 2 ppm (12/58). The incidence of renal tumours in male mice was 1/58 (an adenoma) at 0 ppm, 0/59 at 0.4 ppm, 0/58 at 2.0 ppm and 13/59 (10 adenocarcinomas and three adenomas) at 10 ppm. [The effective numbers of animals at risk for renal tumours could not be determined.] No such tumour was seen in treated female mice (Hirano *et al.*, 1986).

Groups of 60 male and 60 female specific-pathogen-free (SPF) B6C3F1 mice, five weeks of age, were fed diets containing 0, 0.4, 2.0 or 10 ppm [mg/kg] **methylmercury chloride** (99.3% pure) for 104 weeks. All animals were subjected to histopathological examination. In the group treated with 10 ppm, neurotoxicity was recorded in male mice at week 59 and in females at week 80; at termination, neurological signs were seen in 33/60 males and 3/60 females. Survival was similar to that of controls (48%) in all groups except males treated with 10 ppm, which had 17% survival. The incidence of chronic nephropathy was increased in male mice treated with 2 ppm (27/60) or 10 ppm (59/60) and in females given 10 ppm (56/60). The first renal tumour was seen in a male given 10 ppm and killed at week 70. Renal epithelial tumours occurred in 0/60 control males, 0/60 given 0.4 ppm, 1/60 (an adenoma) given 2 ppm and 16/60 (13 adenocarcinomas and five adenomas) given 10 ppm; among female mice, a single adenoma (1/60) was found in those given 10 ppm (Mitsumori *et al.*, 1990). [The Working Group noted the lower survival of high-dose males after 60 weeks.]

(b) *Rat*

Groups of 25 male and 25 female weanling SPF Wistar rats were administered diets containing 0, 0.1, 0.5 or 2.5 ppm [mg/kg] **methylmercury chloride** (100% pure) for two years. Apart from a slight reduction in growth of females treated with 2.5 ppm, there was no effect of treatment on growth. No clinical or neurological sign of methylmercury chloride toxicity was reported during the study; mortality at 104 weeks was: 6/25 female and 7/25 male controls, 10/25 females and 8/25 males at 0.1 ppm, 9/25 females and 13/25 males at 0.5 ppm and 11/25 females and 13/25 males at 2.5 ppm. Histopathological examination was carried out on the control and 2.5 ppm-treated animals and on all animals that died. The authors reported no difference in tumour incidence or latency among the groups [no further detail reported] (Verschuuren *et al.*, 1976a,b). [The Working Group noted the limited nature of the study.]

Groups of 56 male and 56 female SPF Sprague-Dawley rats, five weeks of age, were administered diets containing 0, 0.4, 2 or 10 ppm [mg/kg] **methylmercury chloride** (99.3% purity) for 130 weeks. Ten animals of either sex were killed at 13 and 26 weeks and 10 at 52 and 78 weeks. Neurological signs of methylmercury chloride toxicity were apparent in the 10 ppm-treated group from week 22 in males and from week 46 in females. All animals were subjected to necropsy and histopathological examination. Survival in the groups given 10 ppm was lower than in controls or in the other two treated groups; the cause of death was related to nephrotoxicity. The incidence of tumours did not differ significantly among the

treated and control groups. A single renal adenoma was found in a high-dose female (Mitsumori *et al.*, 1983, 1984).

3.3.2 Administration with known carcinogens

As the purpose of the investigations described below was to study interactions with known carcinogens, the studies were limited to specific target sites, were often of short duration and were not intended to address the carcinogenicity of mercury *per se*.

Mouse: Groups of 16–20 female Swiss-cross mice, 21–24 days old, were given 0, 0.2, 0.5, or 2.0 µg/ml (mg/L) **methylmercury chloride** [purity unspecified] in deionized drinking-water for 15 weeks and then killed. After the first three weeks of the exposure, mice received intraperitoneal injections of 1.5 mg/g [g/kg bw] urethane in normal saline [volume unspecified] or saline alone. The lung tumour incidence in the mice injected with saline was reported to be less than one tumour per mouse in all test groups [no further detail reported]. The number of pulmonary adenomas induced by urethane alone (21.5 ± 3.0) was exceeded only in the group that received the high dose of methylmercury chloride (33.1 ± 3.8) (Blakley, 1984).

Groups of 20 female W rats were maintained either on basal diet or on basal diet containing 10 ppm [mg/kg] **methylmercury chloride** [purity unspecified] dissolved in corn oil, from weaning until they delivered pups. They were also given either 0.159, 0.318 or 0.636% ethylurea in the diet from day 14 of the breeding period to parturition or 50 or 100 mg/kg bw by gavage on days 17, 18 and 19 of gestation; at the same time, they received 0.5, 1.0 or 2.0 g/L sodium nitrite in drinking-water or 25 or 50 mg/kg bw by gavage. Control groups received either the basal diet alone or the methylmercury chloride diet alone. All dams were returned to the basal diet at parturition, and progeny (generally about 25: 13 males and 12 females) were maintained on the basal diet for their lifespan. Survival was poor in some treatment groups. The incidence of neurogenic tumours was nearly 100% in some ethylurea/sodium nitrite-treated groups; there were 0/25 neurogenic tumours in the methylmercury chloride control group. Methylmercury chloride did not increase the incidence of neurogenic tumours in the groups receiving ethylurea/sodium nitrite, but schwannomas of the central nervous system tended to appear earlier than in the group given ethylurea/sodium nitrite alone (Nixon *et al.*, 1979). [The Working Group noted the reduced sensitivity of the study, due to the very high incidence of neurogenic tumours in ethylurea/sodium nitrite-treated groups, and the poor survival.]

3.3.3 Hormonal influences

Mouse: Groups of 50 intact male and 50 intact female SPF ICR mice, seven weeks of age, were fed basal diet or basal diet containing 10 ppm [mg/kg] **methylmercury chloride** (purity, 99.3%) for 80 weeks. Groups of 50 orchietomized male and 50 ovariectomized female mice, operated at five weeks of age, were fed basal diet containing 10 ppm methylmercury chloride only or also received weekly subcutaneous injections of 0.2 mg/mouse testosterone propionate in a 0.2% suspension (w/v) of sesame oil for 80 weeks. All groups receiving methylmercury chloride had nephrotoxic changes and caecal ulceration. No renal tumour was seen in intact males receiving basal diet alone, but one renal adenoma was seen in an

intact female mouse; renal adenocarcinomas (14/50) and an adenoma (1/50) were seen in intact male mice given the basal diet with methylmercury chloride but not in intact female mice. In addition, 6/50 intact male mice given methylmercury chloride in the diet had tubular-cell hyperplasia, a lesion that the authors considered to be preneoplastic. No renal tumour was seen in orchiectomized or ovariectomized mice receiving methylmercury chloride only, but two adenocarcinomas occurred in males and three in females that received methylmercury chloride together with testosterone propionate (Hirano *et al.*, 1988).

4. Other Relevant Data

4.1 Absorption, distribution, metabolism and excretion

The absorption, distribution, metabolism and excretion of inorganic mercury (Nordberg & Skerfving, 1972; WHO, 1976; Berlin, 1986; Clarkson *et al.*, 1988a; WHO, 1991; Clarkson, 1992), methylmercury compounds (Nordberg & Skerfving, 1972; WHO, 1976; Berlin, 1986; Magos, 1987; Clarkson *et al.*, 1988a; WHO, 1990) and phenylmercury acetate (Nordberg & Skerfving, 1972; WHO, 1976; Berlin, 1986; Clarkson *et al.*, 1988a) have been reviewed.

4.1.1 Humans

(a) Metallic mercury and inorganic mercury compounds

In five human volunteers who inhaled radioactive metallic mercury-197 or mercury-203 vapour for 14–24 min, an average of 74% was absorbed in the respiratory tract (Hursh *et al.*, 1976). The half-time for whole-body elimination averaged 58 days; however, elimination rates varied for different parts of the body: lung, 1.7 days; head, 21 days; kidney region, 64 days; chest, 43 days.

Absorbed metallic mercury is dissolved in the blood. Addition of metallic mercury-203 vapour to blood *in vitro* resulted in oxidation to mercuric mercury, but rather slowly (Hursh *et al.*, 1988). The authors concluded that metallic mercury may pass the blood–brain barrier. A man who accidentally ingested 135 g of liquid metallic mercury had raised mercury concentrations in blood, but to an extent indicating only minimal absorption (Suzuki & Tanaka, 1971). The average ratio of mercury in erythrocytes:plasma was about 2 during the first few days after a 14–24-min exposure of five volunteers by inhalation of metallic mercury-197 and mercury-203 vapour (Cherian *et al.*, 1978).

Studies in five volunteers who exposed their forearms to metallic mercury-203 vapour for 27–43 min indicated absorption of mercury through the skin of 0.01–0.04 ng/cm² per min per ng Hg/cm³ air (Hursh *et al.*, 1989).

In five human volunteers who inhaled metallic mercury-197 vapour for 11–21 min, the kidney region accumulated the highest levels of mercury (Hursh *et al.*, 1980). In autopsy samples from seven dentists and one dental assistant, particularly high levels of mercury were found in the renal cortex (average, 8.6 μ mol [1.7 mg]/kg wet weight) and pituitary glands (average, 9.8 μ mol [2 g]/kg wet weight). In 24 controls, the values were 1.4 μ mol [280 μ g]/kg wet weight in renal cortex and 0.12 μ mol [24 μ g]/kg wet weight in pituitary (Nylander & Weiner, 1991). High levels have also been recorded in the thyroid glands of deceased mercury miners (average, 35 mg/kg fresh weight) (Kosta *et al.*, 1975).

Equimolar ratios of mercury:selenium were found in pituitary and thyroid glands, kidney and brain in subjects with occupational exposure to metallic mercury vapour (Kosta *et al.*, 1975; Nylander & Weiner, 1991). Renal biopsy samples from two patients with inorganic mercury poisoning had inclusion bodies which contained mercury and selenium (Aoi *et al.*, 1985).

The blood mercury concentration of nine men who had been exposed to high levels ($> 100 \mu\text{g}/\text{m}^3$) of metallic mercury vapour for three days decreased with a half-time of three days for a fast phase and 18 days for a slow phase; the half-times in the urine were 28 and 141 days, respectively (Barregård *et al.*, 1992).

Analysis of brain samples from a deceased subject who had been exposed to metallic mercury vapour for 18 months 16 years before death showed high levels of mercury, indicating that the brain has a compartment with very slow turnover of mercury. Most of the deposited mercury was in colloidal form (Hargreaves *et al.*, 1988).

The concentration of mercury in the blood of the infants of two women who had been exposed accidentally to metallic mercury vapour during pregnancy was similar to that in maternal blood at the time of delivery, indicating transplacental passage (WHO, 1991).

An average urinary mercury concentration of about $50 \mu\text{g}/\text{g}$ creatinine was seen in 10 workers exposed to $40 \mu\text{g}/\text{m}^3$ of air in a dry alkaline battery factory; the concentration in blood was about $18 \mu\text{g}/\text{L}$ (Roels *et al.*, 1987).

In 10 volunteers who received single oral doses of either ^{203}Hg -mercuric nitrate as such or added to calf-liver protein, 75–92% of the dose was excreted in the faeces during the first four to five days. The average whole-body half-time for mercury (slow component) was 42 days. No difference was seen between the two forms of administration. The ratio of mercury in red blood cells to that in plasma was 0.4 over at least the first 50 days of the experiment. At that time, approximately equal amounts of mercury were excreted in faeces and urine (Rahola *et al.*, 1973).

In a study of two men who had accidentally inhaled aerosols of neutron-activated ^{203}Hg -mercuric oxide, the lung clearance pattern displayed two phases, with biological half-times of two and 24 days, respectively, in one man; in the second, lung clearance appears to have been more rapid. The authors stated that absorption may have occurred from the lung, gastrointestinal tract or both. The major site of systemic deposition was the kidney, the content of which decreased with half-times of 60 and 37 days, respectively, in the two subjects. After 40 days, excretion was mainly urinary (Newton & Fry, 1978).

In five human volunteers who inhaled metallic mercury-197 vapour for 11–21 min, mercury was excreted by exhalation of metallic mercury and excretion of mercury in faeces and urine (Hursh *et al.*, 1980).

(b) Methylmercury compounds

After a single oral dose of ^{203}Hg -methylmercury nitrate was given to three volunteers, methylmercury was almost completely absorbed. A maximum of 10% of the dose was deposited in the head region, presumably in the brain. Whole-body radiolabel decline followed a first-order process, with half-times of 70–74 days. The decline in radiolabel in the head was less rapid than in the rest of the body. In two of the subjects, faecal excretion accounted for about 87 and 90% of the total elimination during the 49 days that followed

administration (Åberg *et al.*, 1969). Gastrointestinal absorption was similarly high, whether methylmercury was given as the nitrate or bound to protein (Åberg *et al.*, 1969; Miettinen, 1973).

In six volunteers who ate a single meal of fish containing methylmercury, the ratio of the concentration of mercury in erythrocytes and plasma was 21. Incorporation of methylmercury into hair was proportional to the concentration in blood at the time of formation of the hair strand; the ratio hair:blood was 292. The average half-times in blood were 7.6 h and 52 days (Kershaw *et al.*, 1980).

In a study of 162 subjects who had been exposed to methylmercury through consumption of contaminated fish in Sweden in 1967–72, intake was associated with concentrations of mercury in blood and hair. After cessation of eating the contaminated fish, the concentration of mercury in the blood cells of four subjects decreased with a half-time of 58–87 days; in one subject, the half-time was 164 days (Skerfving, 1974). The ratio of mercury in blood cells and in plasma was 2–12 (Skerfving, 1988).

Individuals with long-term intake of around 200 µg methylmercury per day were estimated to have blood mercury concentrations of about 200 µg/L and hair concentrations of about 50 µg/g (WHO, 1990).

After consumption of bread contaminated with methylmercury for two months in Iraq, the molar fraction of total mercury as inorganic mercury in several people was 7% in whole blood, 22% in plasma, 39% in breast milk, 73% in urine and 16–40% in liver (WHO, 1990).

The average ratio of methylmercury in cord blood and in maternal blood was 1.66 (Suzuki *et al.*, 1984). The infants of 10 fishermen's wives who were exposed to methylmercury through consumption of fish in Sweden had about 47% higher mercury levels in erythrocytes and similar levels in plasma in comparison with their mothers. The concentration of total mercury in breast milk from 15 women was similar to that in plasma; only about 20% of the total mercury in the milk was methylmercury (Skerfving, 1988).

(c) *Phenylmercury compounds*

In 509 infants in Buenos Aires, Argentina, who were exposed to phenylmercury fungicide through contaminated diapers, the average urinary excretion of total mercury was about 20 times higher than in 166 matched controls; over 90% of the mercury was inorganic (Gotelli *et al.*, 1985).

4.1.2 *Experimental systems*

(a) *Metallic mercury and inorganic mercury compounds*

Kostial *et al.* (1983) observed that the retention of orally administered ^{203}Hg -mercuric chloride in the carcass, gut and whole body was higher in newborn rats (60–70%) than in weaned rats (14–15%).

Absorption of an aqueous solution of ^{203}Hg -mercuric chloride applied under occlusion onto about 3 cm² of the shaved skin of guinea-pigs was dependent on the mercury concentration. A maximal rate of about 0.02% per min was recorded during 5 h after application of 16 mg/ml (as mercury) (Friberg *et al.*, 1961).

In rats, rabbits and monkeys exposed for 4 h to 1 mg/m³ of metallic mercury vapour or injected intravenously with an equivalent dose of mercuric nitrate, the main accumulation

was in the kidney, but 10 times more mercury entered the brain after exposure to mercury vapour than after injection of mercuric nitrate (Berlin *et al.*, 1969).

In rats exposed to metallic mercury vapour, mercury deposits were found by a histochemical technique in the nerve cells in the cerebellum and hypothalamus (Møller-Madsen, 1992). In frog nerve-muscle preparations treated with mercuric chloride ($3\ \mu\text{M}$ [600 μg]), mercuric ions penetrated the nerve-cell membrane through sodium and calcium channels (Miyamoto, 1983).

Khayat and Dencker (1982) found four-fold higher fetal mercury concentrations in mice after exposure to metallic mercury vapour by inhalation than after intravenous injection of mercuric chloride. The passage of metallic mercury through the blood-brain barrier is usually ascribed to its lipophilicity.

In studies of cell suspensions of erythrocytes from humans, ducks and mice exposed *in vitro* to mercury vapour, uptake was proportional to catalase activity, which shows that this enzyme is involved in oxidation of mercury vapour in the erythrocyte (Halbach & Clarkson, 1978). Catalase-mediated oxidation of the vapour has also been demonstrated in other tissues, e.g. liver (Magos *et al.*, 1978).

Intravenous injection of rats with mercuric chloride at 0.7 mg/kg bw induced metallothionein in kidney tissue, which resulted in the binding of mercury (Nishiyama *et al.*, 1987).

After administration of 12 or 25 daily doses of mercury at 1 mg/kg bw as ^{203}Hg -mercuric chloride, the mitochondria in the proximal convoluted tubules were found to be enlarged and there were many very fine, dense, small particles. After fragmentation of the renal tissue and centrifugation at high speed, the radiolabel was found in two fractions, corresponding to mitochondria and microsomes (Bergstrand *et al.*, 1959).

Mice given parenteral doses of mercuric chloride exhaled metallic mercury vapour; exhalation was proportional to the body burden of mercury (Dunn *et al.*, 1978). Following intravenous treatment of rats with mercuric chloride, mercury was excreted into bile as a low-molecular-weight complex which had gel filtration properties similar to those of a mercury-glutathione complex (Ballatori & Clarkson, 1984).

In guinea-pigs exposed for a short time to metallic mercury vapour after parturition, the mercury concentration in milk was slightly lower than that in plasma. Neonates had increased concentrations of mercury in tissues and particularly in the kidney (Yoshida *et al.*, 1992). In rats given mercuric acetate orally, a linear relationship was observed between mercury concentrations in plasma and in milk (Sundberg *et al.*, 1991).

Selenium affects the tissue distribution and excretion of mercuric mercury. For example, three weeks' administration of sodium selenite or seleno-L-methionine (7.5, 37.5 or 75 $\mu\text{mol/L}$ in drinking water) to BOM:NMRI mice increased the whole-body retention of a single oral dose (5 or 25 μmol [1 or 5 mg]/kg bw of ^{203}Hg -mercuric chloride. The effect on organ distribution varied with the dose of mercury and the type and dose of selenium compound (Nielsen & Andersen, 1991).

Human oral bacteria caused some methylation of mercuric chloride *in vitro* (Heintze *et al.*, 1983).

(b) *Methylmercury compounds*

Exposure of rats to ^{203}Hg -methylmercury chloride vapour at 10–28 mg/m³ for 6–24 h was followed by efficient uptake of methylmercury through the lungs (0.6–7 nmol/g fresh tissue) [no data on absorbed fraction given]. Rats given a single oral dose of 0.75–2.3 mg/kg bw had several times higher mercury concentrations in organs (18.6–107.6 nmol/g fresh tissue). In liver and kidney, 42–50% of the mercury was in the soluble fraction, 32–43% in the crude nuclear fraction, 6–9% in the mitochondria and 9–11% in the microsomal fraction. In brain, 29% was in the soluble and 27% in the nuclear fraction, 31% was in mitochondria and 10% in microsomes (Fang, 1980).

Absorption of an aqueous solution of ^{203}Hg -methylmercury dicyandiamide applied under occlusion onto about 3 cm² of the shaved skin of guinea-pigs was dependent on concentration. A maximal disappearance of 5.9% was recorded during 5 h after application of 16 mg/ml (as mercury) (Friberg *et al.*, 1961).

Significant species differences have been observed in the distribution of methylmercury compounds in the body: The ratio between mercury concentrations in erythrocytes and plasma is about 20 in monkeys (17 in squirrel, 25 in rhesus), 25 in guinea-pigs, 7 in mice and more than 100 in rats (for review, see Magos, 1987). After prolonged administration of methylmercury compounds, the brain:blood ratios are 3–6 in squirrel monkeys (for review, see Berlin, 1986), 3.3 in pigs, 1.2 in guinea-pigs, 1.2 in mice and 0.06 in rats (for review, see Magos, 1987).

Following intraperitoneal injection of 1 mg/kg bw methylmercury chloride into four strains of mice, a significant difference in mercury concentrations was observed among strains, particularly in the blood. The rate of elimination from organs also differed: the biological half-time in blood (days) was 5.03 in BALB/c, 5.52 in C3H, 7.79 in C57Bl and 3.81 in CD-1 mice; that in kidneys was 8.73, 7.73, 7.47 and 4.54, respectively (Doi & Kobayashi, 1982). Eight days after intraperitoneal administration of ^{203}Hg -methylmercury chloride (0.4 mg/kg bw as Hg) to two strains of mice, males had significantly higher mercury concentrations in kidney than had females (C129F₁ strain: 5.33 and 3.34%; 129 strain: 7.47 and 3.57% of the dose in males and females, respectively). There was no sex difference in whole-body mercury retention (Doherty *et al.*, 1978).

The percentage of inorganic mercury in total mercury in tissues of squirrel monkeys that received single or repeated weekly doses of methylmercury nitrate by stomach tube at about 0.8 mg/kg bw Hg, was about 20% in liver, about 50% in kidney, 30–85% in bile and < 5% in brain, showing that methylmercury is demethylated (Berlin *et al.*, 1975). Similarly, inorganic mercury was demonstrated in the kidney and to a lesser extent in the liver of rats given daily doses of methylmercury dicyandiamide (Magos & Butler, 1972).

The relative concentration of inorganic mercury in mice increased gradually after a single intravenous injection of 25 µg methylmercury chloride and was about 30% after 22 days; the author concluded that mice obtain a lower fraction of inorganic mercury in the kidney than rats (Norseth, 1971). Cats fed either methylmercury-contaminated fish or methylmercury hydroxide added to fish accumulated inorganic mercury in the liver and kidney; 62% was recovered as methylmercury in kidney and 80% in liver. The metabolism of the methylmercury in the contaminated fish and of the added hydroxide was similar (Albanus

et al., 1972). Similar results were found in cats fed methylmercury-contaminated fish or methylmercury chloride (Charbonneau *et al.*, 1976), and no difference in metabolism was seen in rats given four different salts of methylmercury orally or subcutaneously (Ulfvarson, 1962).

Methylmercury added as the chloride *in vitro* to erythrocytes from humans, rabbits and mice was complexed to a low-molecular-weight compound—probably glutathione. In rats, such binding was minimal (Naganuma *et al.*, 1980). Following the addition of methylmercury chloride to erythrocytes from mice, rats and humans in another study, mercury was found to be bound to haemoglobin—probably cysteinyl residues (Doi & Tagawa, 1983). In rats, L-cysteine enhanced the uptake of mercury by the brain after administration of methylmercury chloride by intracarotid injection. There were indications of a transport system carrying methylmercury over the brain capillary endothelial cell membrane (Aschner & Clarkson, 1988).

In rats injected intravenously with methylmercury chloride, methylmercury was present in the bile as a low-molecular-weight compound complex, which was identified as methylmercury glutathione on the basis of thin-layer chromatography, gel filtration and ionic exchange (Refsvik & Norseth, 1975).

After intravenous injection into rats, methylmercury was excreted into the bile, predominantly as methylmercury cysteine, which is largely reabsorbed from the intestine. There is thus enterohepatic circulation of methylmercury (Norseth & Clarkson, 1971). In rat gut, however, a fraction of methylmercury is converted to inorganic mercury, which is then excreted mainly in the faeces (Rowland *et al.*, 1980).

Hamsters administered a single oral dose of 10 mg/kg bw methylmercury chloride excreted about 50% of the mercury (only about 10% of which was inorganic mercury) in the urine within one week. In rabbits given 0.4 mg/kg bw intravenously, < 2% was excreted in the urine (Petersson *et al.*, 1989).

After addition of 250 ng methylmercury chloride to three hydroxyl radical producing systems, copper ascorbate, xanthine oxidase hypoxanthine–ferric monosodium ethylenediaminetetraacetate and hydrogen peroxide–ultraviolet B light, analysis of inorganic mercury revealed significant dealkylation, which appeared to be unrelated to either superoxide or hydrogen peroxide production alone (Suda *et al.*, 1991). In rat liver microsomes treated with 500 ng methylmercury chloride, both inorganic mercury and hydroxy radical contents increased after addition of NADPH and were further increased by KCN (Suda & Hirayama, 1992).

Selenium affects the tissue distribution and excretion of methylmercury. For example, selenite increased the brain levels of mercury in rats treated with methylmercury (Magos & Webb, 1977).

(c) *Phenylmercury and methoxyethylmercury compounds*

Faecal excretion of 0.120 mg/kg bw Hg as phenylmercury acetate in rats was 65% during 48 h after a single oral dose and 30% after intravenous administration of the same dose, indicating that more than half of the phenylmercury salt was absorbed (Prickett *et al.*, 1950).

In rats given an intraperitoneal injection of phenylmercury acetate, the compound was metabolized rapidly to mercuric mercury (Magos *et al.*, 1982).

Daniel *et al.* (1971) administered a single subcutaneous dose of methoxy- ^{14}C -ethylmercury chloride to rats. Within three days, about half of the radiolabel appeared in exhaled air, with 44% in ethylene and 5% in carbon dioxide (44% after pyrolysis of air). Mercury was accumulated in kidney: A few hours after dosing, inorganic mercury constituted about one-half of the total mercury in that organ; after one day, all of the mercury was inorganic. About 25% of the radiolabel was excreted in urine over 4 days and about 10% after 8 days.

4.2 Toxic effects

The toxic effects of inorganic mercury (WHO, 1976; Kark, 1979; Berlin, 1986; Clarkson *et al.*, 1988a; Dayan *et al.*, 1990; WHO, 1991; Clarkson, 1992), methylmercury compounds (WHO, 1976; Berlin, 1986; Clarkson *et al.*, 1988a; Dayan *et al.*, 1990; WHO, 1990) and phenylmercuric acetate (Skerfving & Vostal, 1972; WHO, 1976; Berlin, 1986; Clarkson *et al.*, 1988a) have been reviewed.

4.2.1 Humans

(a) Inorganic mercury

Workers accidentally exposed for 4–8 h to metallic mercury at levels estimated to have ranged from 1 to 44 mg/m³ developed chest pain, dyspnoeic cough, haemoptysis, impairment of pulmonary function and interstitial pneumonitis (McFarland & Reigel, 1978). Acute massive exposure to metallic mercury vapour can result in psychotic reactions with delirium (for review, see Kark, 1979).

Troen *et al.* (1951) reported 18 cases of human poisoning by ingestion of single doses of mercuric chloride. In nine fatal cases, the lowest estimated dose was 2 g. Gastrointestinal and renal lesions were observed at autopsy.

Roels *et al.* (1985) examined 131 male and 54 female workers exposed to metallic mercury vapour in several factories in Belgium and 114 and 48 unexposed control male and female workers. In responses to a questionnaire, several symptoms of central nervous system disorder (memory disturbances, depressive feelings, fatigue and irritability) were more prevalent among exposed subjects than controls. A significantly increased prevalence of hand tremor was recorded in the group of exposed men, as compared to male controls (15 *versus* 5%). The average concentrations of mercury in urine were 52 µg/g creatinine in exposed men and 37 µg/g creatinine in women; the corresponding levels in controls were 0.9 and 1.7 µg/g creatinine.

In a study of 89 chloralkali workers with a median urinary mercury concentration of 25 µg/g creatinine (range up to 83) and a control group of 75 workers from other industries (median concentration, 2 µg/g creatinine), an association was observed between urinary mercury concentration, self-reported symptoms—tiredness, confusion and degree of neuroticism (Langworth *et al.*, 1992a)—and urinary excretion of *N*-acetyl-β-glucosaminidase, a lysosomal enzyme originating from tubular epithelial cells. No significant effect on serum titres of autoantibodies (including antiglomerular basement membrane and antilaminin) was observed (Langworth *et al.*, 1992b). Elevated excretion of *N*-acetyl-β-glucosaminidase was also reported by Barregård *et al.* (1988) in chloralkali workers.

Of 44 African women with nephrotic syndrome, 70% used or had used mercury-containing skin-lightening creams; the corresponding fraction among other general medical female in-patients was 11% (Barr *et al.*, 1972). In eight other cases of nephrotic syndrome, IgG and C3 complement deposits were observed in glomeruli (Lindqvist *et al.*, 1974). Proteinuria and the nephrotic syndrome have also been described in workers exposed to mercury compounds (Kazantzis *et al.*, 1962).

Lauwerys *et al.* (1983) studied 62 workers in a chloralkali plant and a zinc-mercury amalgam factory with a mean urinary mercury concentration of 56 µg/g creatinine. Eight exposed workers, but none of 60 control workers who were not occupationally exposed to heavy metals but were matched to the exposed group with respect to age and socioeconomic status, had serum antibodies towards laminin, a non-collagen glycoprotein found *inter alia* in the glomerular basal membrane. No alterations were seen in a large battery of renal function tests.

In studies of dentists and chloralkali workers exposed to metallic mercury vapour (mean urinary mercury concentration, 1.3 nmol/mmol [2.3 µg/g] creatinine in dentists and 26 nmol/mmol [46 µg/g] creatinine in chloralkali workers), no significant effect on endocrine function (pituitary, thyroid and adrenal glands, testis) was observed as compared to controls (0.4–0.6 nmol/mmol [0.7–1.06 mg/g] creatinine) without occupational exposure (Erfurth *et al.*, 1990). Similar results were reported by Langworth *et al.* (1990) in dental personnel.

In a study reported in detail on p. 271, Barregård *et al.* (1990) studied mortality among 1190 chloralkali workers who had been monitored biologically for exposure to metallic mercury vapour for at least one year in 1946–84. For workers with > 10 years of latency, mortality from all causes was not significantly increased (SMR, 1.1; 95% CI, 0.9–1.3), but mortality from circulatory disease was slightly increased (SMR, 1.3; 95% CI, 1.0–1.5). No such elevation was reported in another study of workers exposed to metallic mercury (Cragle *et al.*, 1984; see p. 269).

Contact dermatitis with sensitization against metallic mercury has been reported. For example, Ancona *et al.* (1982) reported such a case in a dentist who had a positive epicutaneous patch test. Finne *et al.* (1982) performed patch tests on 29 patients with amalgam fillings and oral lichen planus. Positive reactions to mercury were found in 62% as compared with 3% of controls (2300 eczema cases). After the amalgam fillings had been removed from four patients, an improvement in the oral changes was recorded.

In the 1940s, 'pink disease' (acrodynia), presenting as irritation, insomnia, sweating, photophobia and general rash in children, was reported to be associated with exposure mainly to calomel (mercurous chloride) in, e.g. teething powder and ointments (Warkany, 1966). Cases have also been associated with exposure to other chemical forms of mercury, e.g. metallic mercury vapour from broken fluorescent tubes (Tunnessen *et al.*, 1987). The mechanism by which the condition occurs has not been elucidated. Three of six children with mucocutaneous lymph node syndrome (Kawasaki disease), including increased serum IgE and eosinophilia, had urinary concentrations of mercury (16–25 µg/24 h) higher than established normal levels (< 10 µg/24 h). The syndrome may represent a hypersensitivity reaction to environmental pollution with mercury (Orlowski & Mercer, 1980).

(b) *Methylmercury compounds*

The first case of 'methylmercury poisoning' was described in a worker exposed to methylmercury phosphate and nitrate for a period of four months (Hunter & Russell, 1954). Since then, numerous descriptions have been published, mainly in connection with outbreaks of poisoning in subjects consuming contaminated fish in Japan (Minamata disease) (Igata, 1991) or seeds treated with methylmercury dicyandiamide, e.g. in Iraq (Bakir *et al.*, 1973). Its main features are that: (i) the target organ is the central nervous system; (ii) there is a latent period between exposure and onset of clinical disease; (iii) the symptoms and signs include paraesthesia in the hands, feet and lips, concentric constriction of visual fields and ataxia; and (iv) morphological changes occur in the visual and precentral cortical areas as well as in the cerebellum. There is also evidence of peripheral neuropathy (Rustam *et al.*, 1975).

In the cohort study in two administrative subunits in the vicinity of Minamata City, Japan (Tamashiro *et al.*, 1986; see pp. 275–276), significantly elevated SMRs were observed for cerebral haemorrhage (1.67, 95% CI; 1.24–2.24), liver disease (2.00; 1.33–2.89), senility (2.34; 1.67–3.26) and violent death (accident, poisoning, suicide) (1.48; 1.12–1.97).

(c) *Phenylmercury, ethylmercury and methoxyethylmercury compounds*

A study of 509 infants exposed to phenylmercury acetate from contaminated diapers showed a clear dose–response relationship between the concentration of organomercury compounds in urine and urinary excretion of γ -glutamyl transpeptidase, an enzyme in the brush borders of renal tubular cells. Children with the highest mercury excretion also had increased 24-h urine volumes. Some of the children also had 'pink disease' (Gotelli *et al.*, 1985).

A few cases of systemic poisoning by ethylmercury and methoxyethyl compounds have been reported (for review, see Skerfving & Vostal, 1972). Most patients showed symptoms and signs of disorders in the gastrointestinal tract and kidneys (albumin, red cells and casts in urine).

4.2.2 *Experimental systems*

(a) *Metallic mercury and inorganic mercury compounds*

Application of 2 ml of a solution containing 0.24 mol [65 g] mercuric chloride resulted in the death of 3/20 guinea-pigs after two days (Wahlberg, 1965).

Ashe *et al.* (1953) reported damage to brain, liver, kidney, heart and lungs of rabbits exposed to mercury vapour at a concentration of 29 mg/m³ air. Damage was seen after exposure as short as 1 h. Microscopic changes were observed in mitochondria of the renal proximal tubule after 12 or 25 daily doses of 1 mg/kg bw Hg as mercuric chloride to rats (Bergstrand *et al.*, 1959).

In a susceptible strain of rats (Brown–Norway), subcutaneous injections of mercuric chloride caused a systemic autoimmune nephritis characterized by the production of various antibodies to self and non-self antigens and an increase in total serum IgE concentrations. A biphasic autoimmune glomerulonephritis occurred: initially, anti-glomerular basement membrane antibodies were produced, resulting in linear IgG deposition along the glomerular capillary walls. Later, granular IgG deposits appeared which are responsible for

an immune-complex type glomerulonephritis (Druet *et al.*, 1978). Mercuric chloride appears to induce a T cell-dependent polyclonal activation of B cells in Brown-Norway rats (Pelletier *et al.*, 1986); most animals develop proteinuria, which in some animals progresses to a nephrotic syndrome that is sometimes lethal (Druet *et al.*, 1978), while in other animals the condition is transient. There is a striking strain difference. By crossing susceptible rats with unsusceptible Lewis rats, susceptibility was shown to depend on three or four genes, one of which is located within the major histocompatibility complex (Druet *et al.*, 1982). Certain strains of mice may develop similar glomerular conditions after injection with mercuric chloride (Hultman & Eneström, 1987).

In Lewis rats injected subcutaneously with mercuric chloride (1 mg/kg bw three times a week for up to 4 weeks), no autoimmune disorder was observed. Instead, animals showed proliferation of suppressor/cytotoxic T cells in the spleen and lymph nodes. As a consequence, they developed a non-antigen-specific immunosuppression and responded to neither classical mitogens nor alloantigens (Pelletier *et al.*, 1987a). Mercuric chloride could also inhibit the development of an organ-specific autoimmune disorder, Heymann's nephritis (Pelletier *et al.*, 1987b).

Micromolar concentrations of mercury have been shown to increase the release of acetylcholine in frog neuromuscular preparations (Manalis & Cooper, 1975) and that of dopamine in adult mouse brain homogenates (Bondy *et al.*, 1979).

Significant decreases in the activities of several enzymes of the glutathione (GSH) metabolic pathway in kidney—GSH disulfide reductase, GSH-peroxidase, γ -glutamyl-cysteine synthetase and γ -glutamyl transpeptidase—were seen 24 h after subcutaneous administration of 10 μ mol[2.5 ml]/kg bw mercuric chloride to Sprague-Dawley rats; in the liver, only the activity of GSH disulfide reductase was decreased. After administration of 30 μ mol [7.5 mg]/kg bw, the decreases in specific enzyme activities were accompanied by large losses of cellular protein and decreased GSH concentrations in both kidney and liver. The effects could be blocked by sodium selenite (Chung *et al.*, 1982). The mercuric ion binds to reduced sulfhydryl groups in proteins and inhibits a wide range of enzymes (for review, see Kark, 1979).

In Holtzman rats given a lethal intravenous dose of 3 mg/kg bw Hg as mercuric chloride and sacrificed after 4 h, there was extensive renal haemorrhage. Kidney mitochondria contained mercury at 4–5 nmol[0.8–1 μ g]/mg protein and showed uncoupling of oxidative phosphorylation.

In mitochondrial preparations of kidney cortex from Sprague-Dawley rats, mercury at concentrations of 2 nmol/mg protein and above affected mitochondrial respiration: clear stimulation of state 4, mild stimulation of state 3 and inhibition of the 2,4-dinitrophenol-induced uncoupled respiration rate. These effects were both preventable and reversible by addition of albumin or dithioerythritol to the in-vitro system (Weinberg *et al.*, 1982a), but not in mitochondria isolated 3 h after subcutaneous administration of mercuric chloride at 5 mg/kg bw, when the concentration of mercury in mitochondrial protein was 0.72 ± 0.10 nmol/mg (Weinberg *et al.*, 1982b).

Addition of mercuric chloride at concentrations of 1–6 μ m [0.2–1.2 mg] mercury to preparations of mitochondria from rat kidney cortex and heart in the presence of antimycin

A decreased the production of superoxide but increased hydrogen peroxide production. The authors concluded that mercuric ion caused dismutation of the superoxide, leading to increased hydrogen peroxide formation, which could lead to oxidative tissue damage. Addition of mercurous ions did not affect superoxide production (Miller *et al.*, 1991). [The Working Group noted that the mercury concentrations employed were high.]

Mercuric ions from mercuric chloride, added at 1 mM (270 mg), reacted *in vitro* with isolated DNA (Eichhorn & Clark, 1963). [The Working Group noted the very high concentration used.] No study has shown covalent binding to DNA, e.g. by isolating such an adduct from DNA after complete hydrolysis to nucleosides.

Inhibition of protein synthesis was observed in cell-free systems prepared from mouse glioma after addition of mercuric chloride at a concentration of 2×10^{-5} M [5 mg] (Nakada *et al.*, 1980). [The Working Group noted the high concentration used.] Mercuric chloride at a concentration of 10 μ M [2.3 mg] reduced lipid synthesis in isolated mouse sciatic nerve (Clözé *et al.*, 1987).

Sodium selenite dramatically decreased the acute nephrotoxicity of mercuric chloride in rats, when given simultaneously or even 1 h after mercury (Pařízek & Ošťádalová, 1967).

(b) Methylmercury compounds

There are clear species differences in symptoms and signs of poisoning by methylmercury compounds. Blindness has been reported in man, rats, monkeys and pigs, but not in cats (for reviews, see WHO, 1976, 1990). Man, monkeys and cats develop ataxia; but in rats dosed orally with methylmercury chloride reduced conduction velocities and histopathological changes occurred in peripheral nerves, while the central nervous system was not affected (Fehling *et al.*, 1975).

Renal damage is a typical finding in rats. Male Wistar rats fed mercury at 0.250 mg/kg bw per day as methylmercury chloride for up to 26 months developed severe renal tubular damage. The estimated mercury level in kidney was 30.2 mg/kg in males and 60 mg/kg in females (Munro *et al.*, 1980). Nuclear swelling and vacuolar degeneration of the cytoplasm were seen in SPF ICR mice fed a diet containing 10 ppm methylmercury chloride for 26 weeks (Hirano *et al.*, 1986). Treatment of monkeys with daily oral doses of 80–125 μ g/kg bw methylmercury hydroxide for 3–12 months did not appear to affect the general well-being of the animals, but ultrastructural changes occurred in the kidneys, with intracytoplasmic vacuoles and electron-dense inclusion bodies in the proximal tubuli (Chen *et al.*, 1983).

In mice fed mercury at 3.9 mg/kg diet as methylmercury chloride for 12 weeks, thymus weight and cell number were decreased, the lymphoproliferative response to T and B mitogens was increased in thymus and spleen, and natural killer cell activity was decreased in the spleen and blood (Ilbäck, 1991). Mice fed methylmercury chloride at doses of 1–10 mg/kg diet for 84 days had significantly higher mortality rates when inoculated with encephalomyelitis virus (nononcogenic) than did animals not given methylmercury (Koller, 1975).

Impairment of adrenal and testicular function occurred in rats given 23 intraperitoneal injections of 0.26 mg methylmercury chloride over six weeks (Burton & Meikle, 1980); thyroid function was impaired in mice given two intraperitoneal doses of 5 mg/kg bw (Kawada *et al.*, 1980).

Female Charles River CD rats were given 3–10 mg/L methylmercury hydroxide in drinking-water four weeks prior to mating and through day 19 of pregnancy. With concentrations of 3–5 mg/L, there was decreased synthesis of mitochondrial structural proteins in the livers of the fetuses and inhibition of several mitochondrial enzymes (Fowler & Woods, 1977a). In male rats of the same strain treated similarly for six weeks, electron microscopy revealed swelling of the renal proximal tubule cell mitochondria at a dose of 5 mg/L. The respiratory control ratios were decreased (mitochondrial respiratory dysfunction), and effects were seen on enzyme activities, including decreased monoamine oxidase and cytochrome oxidase and increased δ -aminolaevulinic acid synthetase. The rats had increased urinary excretion of porphyrins but no deterioration in standard renal function tests (Fowler & Woods, 1977b).

Mouse glioma cell cultures treated with methylmercury chloride (5×10^{-6} M for 4 h) showed inhibition of cell mitosis, by blockage of the polymerization of tubulin to microtubuli, with accumulation of cells during mitosis. Electron microscopy showed an absence of microtubuli as mitotic spindle fibres and disorganization of chromosomes (Miura *et al.*, 1978).

Sodium selenite, and possibly also the chemically unknown form of selenium found in marine foods, delayed the onset of the toxic effects of methylmercury chloride in rats and reduced the severity of its effects (Chang & Suber, 1982).

Methylmercury hydroxide added to fish homogenate and methylmercury-contaminated fish were equally neurotoxic to cats (Albanus *et al.*, 1972). Similar results (ataxia, loss of balance or motor incoordination, loss of nerve cells) were found in cats fed either methylmercury chloride or methylmercury-contaminated fish (Charbonneau *et al.*, 1976).

(c) *Phenylmercury, ethylmercury and methoxyethylmercury*

Renal damage was observed in mice, rats and rabbits given phenylmercury nitrate and chloride intraperitoneally or intravenously (Weed & Ecker, 1933). Ethylmercury poisoning has been described in rats, rabbits, cats, sheep, swine and calves. The symptoms are similar to those of methylmercury poisoning (Skerfving & Vostal, 1972). In rats administered the fungicide methoxyethyl mercury chloride (2 mg/kg bw for 50 days or 0.2 mg/kg bw for 80 days) intraperitoneally, impaired weight gain, renal damage and signs of nervous system damage (e.g. tremor, ataxia) were seen (Lehotzy & Bordas, 1968).

4.3 Reproductive and prenatal effects

4.3.1 Humans

The effects of inorganic and organomercury compounds on human reproduction and development have been reviewed (Khera, 1979; Inskip & Piotrowski, 1985; Schardein, 1985; Burbacher *et al.*, 1990; Roeleveld *et al.*, 1990; Shepard, 1992).

(a) *Metallic mercury and inorganic mercury compounds*

(i) *Exposure of women*

Adverse pregnancy outcomes have been reported following exposure to mercuric chloride tablets (Afonso & de Alvarez, 1960), to mercuric iodide-containing soap (Lauwerys

et al., 1987) and in a dental surgery unit where the concentration of mercury exceeded the threshold limit value of 0.05 mg/m^3 (Gelbier & Ingram, 1989). After exposure of a woman prior to week 17 of pregnancy to metallic mercury in a contaminated carpet (24-h urinary concentration of mercury, $230 \text{ } \mu\text{g/L}$), no adverse effect was seen on birth weight, growth or on acquisition of developmental milestones in the child at the age of two (Thorp *et al.*, 1992).

Heidam (1984) conducted a historical prospective study of pregnancy outcomes in women in 12 selected occupations in the Danish county of Funen. Controls were employed in occupations with less exposure to chemicals. Dental assistants returned 94% of the 772 mailed questionnaires on pregnancy history. The incidence of spontaneous abortions in dental assistants in private clinics was 11.2% in 259 pregnancies, yielding a crude odds ratio of 1.1 (95% CI, 0.7–1.8). After control for confounding variables, including age at gravidity, pregnancy order and maternal age at pregnancy, the odds ratio was 1.0 (0.6–1.6). Dichotomization of dental assistants according to whether they reported exposure to inorganic mercury compounds also showed no increase in the spontaneous abortion rate in the exposed subgroup.

Brodsky *et al.* (1985) conducted a postal survey of 30 272 female dental assistants in California (USA) regarding the use of anaesthetic agents and mercury amalgams and health and pregnancy histories for the years 1968–78. The response rate was 70%. Exposure was categorized on the basis of the number of amalgam restorations prepared per week into no, low (0–40) or high (> 40). Outcomes were adjusted for maternal age and cigarette smoking. No relationship was observed between exposure and spontaneous abortion or congenital abnormalities.

Sikorski *et al.* (1987) evaluated reproductive function and outcome in 81 women (45 dentists, 36 dental assistants) exposed occupationally to metallic mercury and in 34 unexposed women [occupational details not given] recruited at random in the Lublin region of Poland. Exposure was ascertained by determination of mercury in samples of scalp and pubic hair; the mercury content in hair was related to duration of employment and to the number of amalgams used per week. A total of 57 exposed women had 117 pregnancies, 24% of which ended in spontaneous abortion, stillbirth or congenital malformations (including five cases of spina bifida). Thirty unexposed women had 63 pregnancies, 11% of which ended in an adverse outcome. Reproductive failure was associated with the mercury content of the hair. The frequency of menstrual disorders was also high in exposed women and was related to the number of years employed and to the mercury content of scalp hair. [The Working Group noted that temporal matching of exposure and pregnancy was not carried out, that no mention of potential confounders was made and that hair mercury levels poorly reflect exposure to metallic mercury.]

Ericson and Källén (1989) evaluated 8157 infants born to dentists, dental assistants and dental technicians in Sweden between 1976 and 1986. Outcomes were standardized for maternal age and parity, year of birth and sex of the infant. There was no suggestion of an increased rate of stillbirths or congenital malformations. The risk ratio for low birth weight ($< 2500 \text{ g}$) was 0.9 (95% CI, 0.7–1.2) for dentists, 1.2 (1.0–1.3) for dental assistants and 0.8 (0.5–1.4) for dental technicians. Data on spontaneous abortions were available only for 1980–81, and the rates for dentists, dental assistants and dental technicians corresponded to expected figures. The authors also reported no increase in the rates of spontaneous abortion

or neural tube defects among women working in dentistry, as ascertained in a small prospective study in Malmö in 1964–65. [The Working Group noted that no marker of exposure to mercury was used.]

De Rosi *et al.* (1985) studied the possible effects on reproductive function and outcome in women of exposure to mercury vapour in two mercury vapour lamp factories in Italy. Workers were exposed to mercury in only one plant, where time-weighted averages exceeded 0.05 mg/m^3 in 1972–76; they were subsequently reduced to $< 0.01 \text{ mg/m}^3$. Workers in a second plant were used as the reference group. Participation was 79% (153 women) in the exposed plant and 88% (293) in the reference plant. Past health events were ascertained by interview. The prevalence and incidence of menstrual cycle disorders were higher in the exposed group, with an age-standardized ratio of abnormal cycles of 1.4. Exposed married women also had a higher prevalence of primary subfecundity. No difference in the rates of spontaneous abortion was found, but the malformation rate, particularly of dislocations of the hip, was higher in the exposed group (6/106 births) than in the unexposed group (0/218 births); however, the authors noted that the prevalence of the condition differed between northern and southern Italy.

(ii) *Exposure of men*

A questionnaire on fertility was distributed to the total male work force of three factories in which workers were exposed to mercury vapour and of two control plants with comparable work characteristics in Belgium (Lauwerys *et al.*, 1985). Blood and urine mercury concentrations were used as indices of exposure. The mercury-exposed group consisted of 17 workers in a zinc–mercury amalgam factory, 35 workers in a chloralkali plant and 51 workers in plants for the manufacture of electrical equipment. The 50th and 95th percentiles of mercury in the urine were 36.9 and $147.1 \text{ } \mu\text{g/g}$ creatinine. No difference was noted between the observed and expected numbers of children in the mercury-exposed group.

Alcser *et al.* (1989) conducted a retrospective study of reproductive function in 247 white male workers who had been employed for at least four months between 1953 and 1966 at a US Department of Energy plant where large quantities of metallic mercury were used in 1953–1963. Intermittent periods of potentially high exposure occurred, especially between 1955 and 1956, and a quarterly programme of urine analysis charted worker exposure from 1953 onwards. A control group was selected from unexposed workers at the same plant. Most measures of reproductive health (fertility rates, incidence of major malformations and childhood illnesses) did not differ between the two groups. The wives of the exposed men had a higher rate of miscarriages; however, this effect was also present prior to exposure to mercury.

Cordier *et al.* (1991) studied the rate of spontaneous abortions in wives of workers exposed to mercury vapour at a chloralkali plant in France and compared it with that of the wives of controls from the same plant. Reproductive history was ascertained by questionnaire in 1984, and exposure history was provided by a plant physician. Urinary mercury levels were measured in most potentially exposed workers at least once a year from 1968. The response to the questionnaire was about 75%, resulting in the inclusion of 118 exposed and 283 unexposed workers. Results were adjusted for maternal age, gravidity, tobacco use and

alcohol consumption. The risk for spontaneous abortion increased significantly with increasing urinary mercury concentration in the three months preceding pregnancy. For example, for urinary mercury concentrations in excess of 50 µg/ml in the three-month period prior to the initiation of pregnancy, the spontaneous abortion rate was 18.4/100 pregnancies, as compared with 8.6/100 in the wives of unexposed men [RR, 2.1; 95% CI, 1.1–4.1]. No relationship between exposure to mercury and birth weight or the frequency of malformations was found.

(b) *Methylmercury compounds*

In a review of the literature, Inskip and Piotrowski (1985) found no evidence that miscarriage, stillbirth, major deficits in birthweight, chromosomal damage or hormonal imbalances in infants were associated with exposure to methylmercury compounds; microcephaly appeared to be the only congenital malformation associated with exposure. Most effects were expressed as clinical symptoms, such as delay in disappearance of primitive reflexes, mental disturbances, retardation of physical development, retardation in emergence of behaviour patterns, disturbances in chewing and swallowing, motility disturbances, impairment of voluntary movements and coordination (ataxia) and constriction of the visual field. Manifestations of toxicity were not always evident at birth but sometimes developed later in childhood.

In a review of autopsy reports on children exposed *in utero* to methylmercury compounds, Burbacher *et al.* (1990) concluded that high concentrations in the brain (12–20 ppm [mg/kg]) decreased brain size, damaged the cortex, basal ganglia and cerebellum and resulted in ventricular dilatation, ectopic cells, gliosis, disorganized layers, misorientated cells and loss of cells. At those tissue concentrations, the neurobehavioural effects included blindness, deafness, cerebral palsy, spasticity, mental deficiency and seizures. At concentrations of 3–11 ppm (mg/kg), mental deficiency, abnormal reflexes and muscle tone and retarded motor development occurred [no data were presented on neuropathological effects]. At low levels (< 3 ppm), delayed psychomotor development was reported.

Foldspang and Hansen (1990) studied reproductive outcomes in Godthaab (365 infants; 45.9% of total births in the period) and Thule (11 infants; 100% of total births), Greenland, between January 1983 and December 1986. Women were invited to participate in the study when they entered maternity clinics at the beginning of labour; nonparticipation was attributed to the difficulty of collecting data in the Arctic. Socioeconomic data, cigarette consumption, consumption of traditional Greenlandic foods (eating whale and seal meat was widespread) and other data were obtained by interview or from hospital records. Maternal and umbilical blood samples were collected at delivery and assayed for total mercury. The average blood mercury concentration of the infants was 21 µg/ml (range, 2–136 µg/L), while maternal levels averaged 14.9 µg/ml (range, 2–128 µg/L). Birth weights were inversely proportional to maternal and offspring blood mercury concentrations. [The Working Group noted that maternal height was not taken into account although birthplace was.]

Grandjean and Weihe (1993) studied birthweight in relation to fish consumption in residents of the Faroe Islands. A total of 1024 births that occurred between March 1986 and December 1987 were included. The average birthweights of the infants of nonsmoking

mothers were 3400 g in 13 infants whose mothers consumed no fish and 3600 g ($n = 83$), 3850 ($n = 220$), 3800 ($n = 183$) and 3750 g ($n = 114$) in women who consumed 1, 2, 3 and > 4 fish dinners per week, respectively. The average total mercury concentrations in cord blood were 20, 118, 105, 133 and 138 nmol/L [4, 23.6, 21, 26.6 and 27.6 $\mu\text{g/L}$] in the same groups. Thus, elevated cord blood mercury concentrations were associated with increased birth-weight, but the authors attributed this correlation to the content of (*n*-3)-polyunsaturated fatty acids in fish.

4.3.2 Experimental systems

(a) Metallic mercury and inorganic mercury compounds

The reproductive and developmental effects of metallic mercury and its salts in laboratory animals have been reviewed (Khera, 1979; Barlow & Sullivan, 1982; Léonard *et al.*, 1983; Schardein, 1985; Shepard, 1992). In a review, Barlow and Sullivan (1982) concluded that exposure to metallic mercury (e.g. inhalation of 0.3 ppm [2.5 mg/m³] by rats for 6 h per day for three weeks prior to pregnancy and again on gestation days 7–20) and to inorganic mercury (e.g. intravenous injections of 2–4 mg/kg bw mercury into hamsters on gestation day 8) can cause fetal growth retardation and prenatal and postnatal mortality.

Altered oocyte maturation was reported after exposure of hamsters to mercuric chloride on day 1 of the oestrous cycle. The effective dose levels were as low as 1 mg/kg bw per day (Lamperti & Printz, 1973; Watanabe *et al.*, 1982).

When mercuric chloride (at 5–80 μM [1.2–18.8 mg]) was added to freshly prepared human semen samples *in vitro*, a dose- and time-dependent decrease in sperm motility was observed. In addition, morphological changes and silver-enhanced mercury deposits in the sperm were noted (Ernst & Lauritsen, 1991).

Exposure of mice to mercuric chloride as 1.5 or 2.0 mg/kg bw Hg by intravenous injection on day 0 resulted in a high incidence of abnormal blastocysts when cells were examined on day 3.5 of gestation (Kajiwarra & Inouye, 1986). The minimal effective doses of mercuric acetate that reduced embryonic viability and increased the incidence of malformations and growth retardation in hamsters treated on day 1 of gestation were 35, 25 and 8 mg/kg by oral administration, 8 mg/kg by subcutaneous injection, 4 mg/kg by intravenous injection and 2 mg/kg by intraperitoneal injection (Gale, 1974). Subcutaneous injection of 15 mg/kg bw mercuric acetate to six strains of hamsters on gestation day 8 caused increased resorptions and abnormal and growth retarded fetuses, the incidence of which varied slightly from strain to strain (Gale, 1981).

Exposure of mice to doses of 7.5–25 mg/kg mercuric chloride by subcutaneous injection on day 16 of gestation resulted in a 40% reduction in fetal accumulation of vitamin B₁₂ and α -aminobutyric acid within 4 h; no overt fetal toxicity was observed with doses up to 15 mg/kg, although some fetal deaths occurred with 20 mg/kg and maternal lethality was seen at 25 mg/kg (Danielsson *et al.*, 1984). Reduced levels of fetal zinc, copper and iron were reported 4–24 h after exposure of pregnant rats to 0.79 mg/kg mercury by intravenous injection on gestation day 12 (Holt & Webb, 1986a). Intravenous injections of 0.5–0.6 mg/kg mercuric chloride on gestation day 7 were reported to cause fetal malformations. A slightly higher dose (0.79 mg/kg) caused resorptions when given on day 12 and growth retardation

when given on days 8, 10, 12, 14 or 16. The authors attributed the fetal effects to alterations in maternal renal function resulting from exposure to mercury (Holt & Webb, 1986b).

Subcutaneous administration of 1 mg/kg bw mercuric chloride to Sprague-Dawley rats on the last eight days of gestation induced a transient increase in urinary excretion of β_2 -microglobulin and albumin in both mothers and offspring. In male offspring, these effects reappeared at 180 days of age. A follow-up experiment in which females were dosed throughout pregnancy showed an effect on male offspring renal function at 3–4 months of age, but not at 10 months (Bernard *et al.*, 1992).

Decreased embryonic growth was observed after exposure *in vitro* of day-10 rat embryos to 4 μ M mercuric chloride for 48 h or to 20 μ M for 24 h; the concentrations required to affect morphogenesis were 1 μ M and 20 μ M, respectively (Kitchin *et al.*, 1984; Saillenfait *et al.*, 1990).

(b) *Methylmercury compounds*

The literature on the effects of exposure to methylmercury compounds on prenatal development in experimental animals, including effects on the function of several organ systems in postnatal animals following exposure *in utero*, is extensive (for reviews, see Khera, 1979; Reuhl & Chang, 1979; Léonard *et al.*, 1983; Inskip & Piotrowski, 1985; Mottet *et al.*, 1985; Schardein, 1985; Burbacher *et al.*, 1990; Shepard, 1992).

Subcutaneous injection of hamsters with 6.4 or 12.8 mg/kg bw mercury as methylmercury chloride on day 1 of the oestrous cycle did not affect the number of oocytes released (Watanabe *et al.*, 1982).

Albino male rats received 0, 5 or 10 μ g/kg bw per day methylmercury chloride by intraperitoneal injection for 15–90 days. Time- and dose-dependent decrease in seminiferous tubular diameter, numbers of Sertoli cells per tubular cross section, and numbers of spermatogonia, preleptotene spermatocytes, pachytene spermatocytes and step-7 spermatids were found. Zygotenes at stages XII through XIII and pachytenes at stages XII through early XIV of seminiferous tubules were most affected. The Sertoli cell was suggested as the target of toxicity (Vachhrajani *et al.*, 1992). [The Working Group noted that the testes were immersed, fixed and embedded in paraffin.] In freshly prepared human semen samples to which methylmercury chloride was added *in vitro*, similar, but less rapid and pronounced changes than those seen with mercuric chloride (see above) were present; however, no silver-enhanced mercury deposition was seen in sperm (Ernst & Lauritsen, 1991).

No effect on the ability to inseminate females or produce viable young was noted in male mice exposed by oral intubation for five to seven days to up to 5 mg/kg mercury as methylmercury chloride in seven consecutive five-day breeding trials. When male Wistar rats were exposed by oral intubation at the same dose regimen for seven days and followed over 14 consecutive five-day breeding trials, a reduced incidence of pregnancy was seen at 5 mg/kg on days 0–15 after treatment and reduced numbers of viable implants were seen on days 5–20 after 2.5 or 5 mg/kg. With longer-term exposures (up to 125 days), reduced numbers of viable implants were seen 25–30 days after exposure to 1 mg/kg and 85–90 days after exposure to 0.5 mg/kg (Khera, 1973a).

A three-generation study showed a lower viability index in F₁ and F₂ generations of rats following dietary exposure to 2.5 but not to 0.5 ppm (mg/kg) methylmercury chloride.

Growth retardation was observed in F_{2a} females at 0.1, 0.5 and 2.5 ppm and in F_{2a} males at 2.5 ppm; and increased relative kidney weights were observed in P males and females, F_{1a} males and F_{2a} females and males at 2.5 ppm, in F_{2a} males and females at 0.5 ppm and in F_{2a} females and F_{1a} males at 0.1 ppm (Verschuuren *et al.*, 1976c).

The development of mouse blastocysts *in vitro* was affected by exposure to methylmercury chloride at 1 μ M (Matsumoto & Spindle, 1982) and 0.3 μ M (Müller *et al.*, 1990). Intravenous injections of 2 mg/kg bw mercury or more as methylmercury chloride to mice on day 0 of gestation (Kajiwara & Inouye, 1986) or intraperitoneal injection of 5 mg/kg bw to rats during the pre-implantation period (Giavini *et al.*, 1985) affected embryonic development and/or viability.

Prenatal effects on offspring were reported following exposure of mice *in vivo* to methylmercuric chloride during pregnancy at doses as low as 5 mg/kg (fetal weight, Fuyuta *et al.*, 1978; embryonic death, Curle *et al.*, 1983, 1987); malformations commonly seen at this dose or at slightly higher doses included cleft palate and hydronephrosis. The effective dose levels in the rat fetus appear to be similar to those that cause effects in mice (Fuyuta *et al.*, 1979); however, the manifestations vary somewhat (e.g. cleft palate is observed less frequently at 7.5 mg/kg). Reductions in embryonic growth and viability were seen in rat embryos taken on gestation day 10 and exposed *in vitro* to 30 μ M methylmercury chloride for 48 h; effects on morphogenesis were reported at the lowest dose tested (3 μ M) (Kitchin *et al.*, 1984). Histological damage to the developing brain was observed following exposure *in utero* to methylmercury chloride of hamsters (10 mg/kg on gestation day 10; 2 mg/kg on gestation days 10–15; Reuhl *et al.*, 1981), guinea-pigs (7.5 mg/kg on day 21, 28, 35, 42 or 49; Inouye & Kajiwara, 1988) and cats (0.25 mg/kg on gestation days 10–58; Khera, 1973b). Monkeys (*Macacca fascicularis*) exposed to 50 or 90 μ g/kg bw methylmercury hydroxide by oral intubation for 124 days before mating appeared to have lower conception rates and smaller offspring, but small sample sizes precluded statistical significance (Burbacher *et al.*, 1984).

Exposure of mice *in utero* to doses as low as 8 mg/kg of methylmercury compounds on single days of gestation resulted in neonatal mortality and growth impairment (Gates *et al.*, 1986, using the chloride), hydrocephaly (Choi *et al.*, 1988, chloride), changes in activity in the open field (Spyker *et al.*, 1972, dicyandiamide [CH3HgNHC(NH)NHCN]; Su & Okita, 1976, hydroxide) and altered swimming behaviour (Spyker *et al.*, 1972, dicyandiamide). Snell *et al.* (1977, chloride) reported lower concentrations of liver glycogen in fetal (two-day prenatal) rats but higher concentrations in six-day-old rats exposed to 4 or 8 mg/kg on gestation day 9. Robbins *et al.* (1978, chloride) found decreased levels of cytochrome P450 and certain xenobiotic metabolizing enzymes in 26–36-week-old male but not female offspring exposed by oral intubation to 5 mg/kg on gestation day 0 or to 2.75 mg/kg on gestation day 7. Chang and Sprecher (1976a,b) reported morphological evidence of renal tubular damage (degenerative changes in epithelial cells of the proximal tubule, hyperplastic changes in distal convoluted tubules and thickening of the tubular epithelial linings) in neonatal rats after injection of dams with 1 or 4 mg/kg methylmercury chloride on day 8 of gestation. Smith *et al.* (1983) exposed rats to 4 mg/kg methylmercury chloride on days 8, 10 and 12 of gestation and observed reduced uptake of an organic anion (*para*-aminohippurate) by renal slices on postnatal day 42, but not on days 1 or 7, and reduced ability to eliminate sodium and water in volume-loaded rats when measured on postnatal day 42. Slotkin *et al.* (1986) reported slightly

reduced growth rates after weaning, alteration of renal function, an increased level of liver ornithine decarboxylase and altered renal ornithine decarboxylase response to isoproterenol and vasopressin in rats injected subcutaneously with 0.5 or 1.0 mg/kg methylmercury hydroxide on days 8–21 of gestation.

Bornhausen *et al.* (1980) reported impaired operant behaviour performance in the offspring of rats exposed to daily doses as low as 0.01 mg/kg methylmercury chloride on days 6–9 of gestation. A large, multilaboratory evaluation of behavioural effects in rat offspring exposed to 2 or 6 mg/kg methylmercury chloride on days 6–9 of gestation found increased auditory startle response at the high dose in young offspring and dose-related effects on figure-8 maze exploratory activity and in the pharmacological response to d-amphetamine challenge in older animals (Buelke-Sam *et al.*, 1985). In rats exposed to mercury at 3.9 mg/kg diet as methylmercury chloride *via* their dams during gestation and lactation and *via* the diet up to the age of 50 days, no histological change occurred in the brain, but increased noradrenaline levels were observed in the cerebellum (Lindström *et al.*, 1991). Rice (1992) found deficits in fixed interval performance, but not in discrimination reversal or activity patterns, in offspring of *Macaca fascicularis* exposed to steady-state levels during gestation and subsequently of 0, 10, 25 or 50 µg/kg per day mercury as methylmercury chloride. One infant in the high-dose group showed overt signs of methylmercury toxicity.

4.4 Genetic and related effects

4.4.1 Humans (see also Table 15, pp. 304–305 and Appendices 1 and 2)

(a) Dietary exposures

Skerfving *et al.* (1970) examined nine Swedish subjects who ate methylmercury-contaminated fish (containing 1–7 ppm [mg/kg] mercury) at least three times a week for more than five years and four controls who ate uncontaminated fish (containing ≤ 0.05 ppm [mg/kg] mercury) less than once a week. There was no significant difference in the frequency of chromosomal aberrations between exposed subjects and controls nor any correlation between aneuploidy or polyploidy rates and concentrations of mercury in red blood cells, which were in the range of 5–17 ng/g in controls and 21–370 ng/g in exposed subjects. Mercury concentrations were, however, significantly correlated with the frequency of structural rearrangements. The study was expanded to include a total of 23 exposed subjects (including the nine subjects already reported in 1970) and 16 controls (Skерfving *et al.*, 1974). Small differences were observed in the frequency of chromosomal aberrations in the exposed groups, and a significant correlation was seen between chromatid-type aberrations, 'unstable' chromosome-type aberrations or aneuploidy and mercury concentrations in red blood cells, which were in the range of 3–17 ng/g in controls and 12–1100 ng/g in exposed subjects. There was no correlation between the frequency of chromosomal aberrations and variations in mercury concentrations in repeated samplings.

Wulf *et al.* (1986) investigated the frequency of sister chromatid exchange in the lymphocytes of 147 Eskimos living in Greenland or Denmark, who were divided into three groups according to their intake of seal meat: subjects who ate seal meat at least six times a week and had an average concentration of mercury in the blood of 62.5 µg/L (range, 41.9–

65.4); subjects who ate seal meat two to five times per week and had mercury concentrations of 23.2–51.0 µg/L; subjects who ate seal meat once a week or less and had an average mercury concentration in the blood of 22.2 µg/L (range, 5.4–26.4). The mean frequency of sister chromatid exchange was 1.7-fold higher in the group that ate seal meat at least six times a week than in those who ate it less than once a week and 10.7-fold higher in the intermediary group. An increase of 10 µg/L in blood mercury corresponded to an increase of 0.2–0.3 sister chromatid exchanges per cell. [The Working Group noted that the reported results are difficult to interpret because only a series of statistical analyses is provided in the article, with limited original cytogenetic data.]

No increase in the frequency of sister chromatid exchange or numerical chromosomal alterations was detected in 16 subjects who ate fish caught from a methylmercury-contaminated area in Colombia as compared to 14 controls who ate fish from an uncontaminated area. The blood mercury ranges were 2.2–25.8 µg/L in unexposed and 10.2–97.3 µg/L in the exposed people. The frequency of structural chromosomal aberrations was increased only when achromatic lesions (chromatid and chromosome gaps) were included (Monsalve & Chiappe, 1987).

(b) *Occupational exposures*

Verschaeve *et al.* (1976) examined seven control subjects (blood mercury concentration, 2.5–8.4 µg/L) and 28 mercury-exposed subjects (blood mercury concentration, < 0.1–13 µg/L; urinary mercury concentration, < 0.1–114.12 µg/L) in Belgium, 10 of whom were under medical supervision for mercury intoxication, and 18 subjects working with mercury at Brussels University. An increased frequency of aneuploidy was seen in those subjects exposed to metallic mercury (14), amalgams (3), phenylmercury (8) and ethylmercury (3), while the incidence of structural chromosomal aberrations was increased only in the last (small) group. An increased rate of hyperploidy was also reported by Verschaeve *et al.* (1978) in 16 workers exposed to phenylmercury acetate (blood mercury concentration, 0–5.6 µg/L), compared to 12 unexposed controls (0–3.5 µg/L). In an abstract, Verschaeve and Susanne (1979) reported an increased rate of aneuploidy, with no increase in the frequency of structural chromosomal aberrations in 10 subjects exposed to mercury-containing amalgams in a dental practice and compared with 10 controls, but they could not rule out other factors such as X-rays. Verschaeve *et al.* (1979) failed to detect any chromosomal effect in 28 workers exposed to metallic mercury (urinary mercury concentration, 7–175 µg/L) in a chloralkali plant, as compared with 20 unexposed controls (eight from the same plant [< 5 –15 µg/L] and 12 from the general population). In the discussion of the report, the authors questioned the positive findings in their previous three studies, stating that they might have been due to lack of information on exposures to agents other than mercury in the subjects. Negative results were also reported in a more recent Belgian study (Mabille *et al.*, 1984) involving cytogenetic analyses of 25 unexposed subjects (urinary mercury, < 5 µg/g creatinine, and blood mercury, < 6 µg/L) and of 22 workers exposed to metallic mercury in a chloralkali plant and in a plant in which mercury is amalgamated with zinc (urinary mercury, 8.2–286 µg/g creatinine, and blood mercury, 7.5–105.2 µg/L).

Popescu *et al.* (1979) examined peripheral blood lymphocytes from 22 workers in two departments of a chemical plant in Romania, four of whom were exposed to vapours of

metallic mercury and 18 to a mixture of mercuric chloride, methylmercury chloride and ethylmercury chloride. During the year before the study, atmospheric mercury concentrations ranged from 0.15 to 0.44 mg/m³. Urinary analysis demonstrated high concentrations of mercury (100–896 µg/L). When compared with 10 unexposed controls, neither exposed group had an increased rate of aneuploidy or of total structural chromosomal aberrations; however, both had a significant increase in the frequency of acentric fragments.

In an abstract, Mottironi *et al.* (1986) reported an increased rate of sister chromatid exchange in somatic cells [presumably lymphocytes] from 29 workers exposed to metallic mercury and inorganic mercury in two plants [presumably in the USA] (blood mercury, 6.7–103.9 µg/L) over that in 26 unexposed controls (blood mercury, 1.5–6.1 µg/L). The increase was significant in 'exposed workers with high mercury levels' in the blood [no detail given] and was enhanced by cigarette smoking. An increased rate of sister chromatid exchange, related to time since exposure, was detected in Argentina in the lymphocytes of 38 children, aged one month to five years, who had been intoxicated by the use of phenylmercury acetate to disinfect diapers. Nineteen unexposed children served as controls. The increased rate of sister chromatid exchange disappeared nine months after exposure ceased (Mudry de Pargament *et al.*, 1987).

Barregård *et al.* (1991) compared the incidence of lymphocytic micronuclei in 26 chloralkali workers (air mercury concentration, 25–50 µg/m³) and 26 unexposed controls in Sweden. No difference was found between the two groups, and no correlation was found with current mercury concentrations in erythrocytes, plasma or urine (exposed, 4.8–64.6 µg/L, 2.8–40 µg/L and 5–186 µg/L; controls, 2.4–20.2 µg/L, 0.8–2.6 µg/L and 0.2–9.6 µg/L, respectively). A significant association was found between the number of micronuclei in phytohaemagglutinin-stimulated blood and previous exposure to mercury (measured either by a cumulative exposure index, i.e. integrated yearly mean blood mercury over employment time, or number of occasions when blood mercury peaks were > 150 nmol/L [30 µg/L]), suggesting an accumulation of cytogenetic effects in T lymphocytes. No such association was seen in pokeweed mitogen-stimulated blood. Anwar and Gabal (1991) examined 29 workers in an explosives factory in Egypt (mean urinary mercury concentration, 123 µg/L) who were exposed to mercury fulminate [Hg(CNO)₂] (which results from the reaction between mercuric nitrate, ethanol and nitric acid) and 29 controls (mean urinary mercury concentration, 39 µg/L). The frequencies of both micronuclei and chromosomal aberrations (gaps, breaks and fragments) were increased in the exposed group. The authors reported, however, that the increases were not correlated with urinary concentration of mercury or duration of exposure, putting in question the role of mercury in the observed clastogenic effect. In addition, there was no increased incidence of either aneuploidy or polyploidy.

4.4.2 *Experimental systems*

The genetic and related effects of mercury compounds have been reviewed (Ramel, 1972; Léonard *et al.*, 1983; Kazantzis & Lilly, 1986).

- (a) *Inorganic mercury compounds* (see also Table 16, pp. 307–309 and Appendices 1 and 2)

Almost all of the studies reported were carried out with mercuric chloride.

Table 15. Genetic and related effects of mercury in humans

Test system	Result without exogenous metabolic system	Dose ^a (LED/HID)	Reference
Food contaminated with organomercury compounds^b			
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	(+) ^c	0.042	Wulf <i>et al.</i> (1986)
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	-	0.040	Monsalve & Chiappe (1987)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	(+)	0.126	Skerfving <i>et al.</i> (1970)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	(+)	0.12	Skerfving <i>et al.</i> (1974)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	- ^d	0.040	Monsalve & Chiappe (1987)
AVH, Aneuploidy, human lymphocytes <i>in vivo</i>	-	0.126	Skerfving <i>et al.</i> (1970)
AVH, Aneuploidy, human lymphocytes <i>in vivo</i>	(+)	0.12	Skerfving <i>et al.</i> (1974)
Occupational and environmental exposures to mercury			
Metallic mercury			
SLH, Sister chromatid exchange, human lymphocytes <i>in vivo</i> ^e	+	0.027	Mottironi <i>et al.</i> (1986) abstract
MVH, Micronucleus induction, phytohaemagglutinin-stimulated human (T) lymphocytes <i>in vivo</i>	(+)	0.025	Barregård <i>et al.</i> (1991)
MVH, Micronucleus induction, pokeweed mitogen-stimulated human (T/B) lymphocytes <i>in vivo</i>	-	0.025	Barregård <i>et al.</i> (1991)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-	NR	Verschaeve <i>et al.</i> (1976)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	+	NR	Popescu <i>et al.</i> (1979)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-	NR	Verschaeve <i>et al.</i> (1979)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-	0.031	Mabille <i>et al.</i> (1984)
AVH, Aneuploidy, human lymphocytes <i>in vivo</i>	?	NR	Verschaeve <i>et al.</i> (1976)
AVH, Aneuploidy, human lymphocytes <i>in vivo</i>	-	NR	Popescu <i>et al.</i> (1979)
AVH, Aneuploidy, human lymphocytes <i>in vivo</i>	-	NR	Verschaeve <i>et al.</i> (1979)
Amalgams			
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-	NR	Verschaeve <i>et al.</i> (1976)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-	NR	Verschaeve & Susanne (1979) abstract
AVH, Aneuploidy, human lymphocytes <i>in vivo</i>	?	NR	Verschaeve <i>et al.</i> (1976)
AVH, Aneuploidy, human lymphocytes <i>in vivo</i>	?	NR	Verschaeve & Susanne (1979) abstract

Table 15 (contd)

Test system	Result without exogenous metabolic system	Dose ^a (LED/HID)	Reference
Ethylmercury compounds [unspecified]			
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	?	NR	Verschaeve <i>et al.</i> (1976)
AVH, Aneuploidy, human lymphocytes <i>in vivo</i>	?	NR	Verschaeve <i>et al.</i> (1976)
Mercury fulminate [Hg(CNO) ₂]			
MVH, Micronuclei, human lymphocytes <i>in vivo</i>	?	NR	Anwar & Gabal (1991)
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	?	NR	Anwar & Gabal (1991)
AVH, Aneuploidy, human lymphocytes <i>in vivo</i>	-	NR	Anwar & Gabal (1991)
Methylmercury chloride/ethylmercury chloride/mercuric chloride mixture			
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	+	NR	Popescu <i>et al.</i> (1979)
AVH, Aneuploidy, human lymphocytes <i>in vivo</i>	-	NR	Popescu <i>et al.</i> (1979)
Phenylmercury compounds [unspecified]			
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-	0.008	Verschaeve <i>et al.</i> (1976)
AVH, Aneuploidy, human lymphocytes <i>in vivo</i>	(+)	0.008	Verschaeve <i>et al.</i> (1976)
Phenylmercury acetate			
CLH, Chromosomal aberrations, human lymphocytes <i>in vivo</i>	-	0.002	Verschaeve <i>et al.</i> (1978)
AVH, Aneuploidy, human lymphocytes <i>in vivo</i>	?	0.002	Verschaeve <i>et al.</i> (1978)
SCE, Sister chromatid exchange, human lymphocytes <i>in vivo</i>	+	NR	Mudry de Pargament <i>et al.</i> (1987)

+, positive; (+), weakly positive; +?, positively questioned by the authors themselves; -, negative; ?, inconclusive

^aMean concentration of Hg in blood; µg/ml. NR, not reported

^bOrganic mercury of alimentary source (seal or fish meat)

^cAlso contaminated with lead, cadmium and selenium

^dExcluding gaps; positive if gaps are included

^eExposure to metallic mercury mixed with organic mercury

In bacteria, assays for differential toxicity suggest that mercuric chloride induces DNA damage very weakly in *Bacillus subtilis* and not in *Escherichia coli*. No studies of bacterial mutation were available to the Working Group.

Mercuric chloride weakly induced mitotic recombination and induced mitochondrial mutations in *Saccharomyces cerevisiae*. It induced various types of mutations in the plant, *Anacharis canadensis*.

Mercuric chloride did not increase the frequency of micronuclei in cultured fish cells, even when tested at doses 10 times higher than those that were effective for some organo-mercury compounds.

In cultured mammalian cells, mercuric chloride inhibited DNA repair induced by X-rays but not that induced by ultraviolet radiation. Gene mutations at the *tk* locus were induced in a single study with mouse lymphoma L5178Y cells, but only in the presence of an exogenous metabolic system from rat liver. Sister chromatid exchange was induced in cultured Chinese hamster ovary cells and in human lymphocytes. Spindle disturbance and chromosomal aberrations were induced by mercuric chloride in most studies with cultured mammalian cells, including human lymphocytes, and by mercuric acetate in mouse oocytes *in vitro*.

Mercuric chloride enhanced cell transformation produced by simian adenovirus SA7 in Syrian hamster embryo primary cell cultures. Mercuric acetate did not induce anchorage-dependent growth of human fibroblasts.

In larvae of the newt, *Pleurodeles waltl*, mercuric chloride induced chromosomal aberrations and micronuclei in erythrocytes. Studies of mammals exposed to mercuric chloride *in vivo* have given negative or conflicting results. Neither chromosomal aberrations nor aneuploidy were observed in Syrian hamster bone marrow or oocytes following a single subcutaneous dose of 12.8 mg/kg mercuric chloride. In mice, the frequency of chromosomal aberrations in bone-marrow cells was increased in one study after a single oral dose of 3 mg/kg but not in another study by intraperitoneal injection of a dose of 6 mg/kg. In a second study in mice, chromosomal aberrations were not induced in spermatogonia. Weak dominant lethal effects have been described in rats and mice. Mercuric acetate did not induce chromosomal aberrations in mouse oocytes after subcutaneous or intravenous dosing.

(b) *Organomercury compounds* (see also Table 17, pp. 312–320 and Appendices 1 and 2)

Very weak differential toxicity was induced in *B. subtilis* by methylmercury chloride, but negative results were obtained with phenylmercury chloride and bis(ethylmercury)hydrogen phosphate. Mutations were not induced in *Salmonella typhimurium* by methylmercury chloride or methylmercury acetate.

Methylmercury chloride did not induce gene conversion or mitotic recombination in *S. cerevisiae*, but conflicting results were obtained for induction of mutations, mitochondrial mutations and aneuploidy.

Phenylmercury nitrate induced mutations in seedlings of *Zea mays*, and phenylmercury hydroxide induced mutations in seedlings of a number of different plants. Chromosomal aberrations and/or spindle disturbances were induced in *Allium cepa* roots by all of eight compounds tested.

Table 16. Genetic and related effects of inorganic mercury compounds in experimental systems

Test system	Result		Dose ^a (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>Mercuric chloride</i> (74% Hg)				
PRB, Lambda-prophage induction, <i>E. coli</i>	-	0	0.036	Rossman <i>et al.</i> (1991)
BSD, <i>Bacillus subtilis</i> rec strains, differential toxicity	(+)	0	10 000	Kanematsu <i>et al.</i> (1980)
ERD, <i>Escherichia coli</i> rec strains, differential toxicity	-	0	0.5	Brandi <i>et al.</i> (1990)
SCH, <i>Saccharomyces cerevisiae</i> D7, mitotic crossing-over	(+)	0	60	Fukunaga <i>et al.</i> (1981)
SCR, <i>Saccharomyces cerevisiae</i> N123, petite mutation	(+)	0	20	Fukunaga <i>et al.</i> (1981)
PLM, <i>Anacharis canadensis</i> , mutation	+	0	740	MacFarlane & Messing (1953)
***, Micronuclei, fish (<i>Lepomis macrochirus</i>) ^b cells <i>in vitro</i>	-	0	0.8	Babich <i>et al.</i> (1990)
***, Inhibition of X-ray-induced DNA repair, Chinese hamster ovary cells <i>in vitro</i>	+	0	0.2	Christie <i>et al.</i> (1986)
***, Inhibition of UV-induced DNA repair, Chinese hamster ovary cells <i>in vitro</i>	-	0	10	Christie <i>et al.</i> (1986)
DIA, DNA strand breaks, Chinese hamster ovary cells <i>in vitro</i>	+	0	5.0	Cantoni <i>et al.</i> (1982)
DIA, DNA strand breaks, Chinese hamster ovary cells <i>in vitro</i>	+	0	2.0	Robison <i>et al.</i> (1982)
DIA, DNA strand breaks, Chinese hamster ovary cells <i>in vitro</i>	+	0	5.0	Cantoni & Costa (1983)
DIA, DNA strand breaks, Chinese hamster ovary cells <i>in vitro</i>	+	0	5.0	Cantoni <i>et al.</i> (1984a)
DIA, DNA strand breaks, Chinese hamster ovary cells <i>in vitro</i>	+	0	1.0	Cantoni <i>et al.</i> (1984b)
DIA, DNA strand breaks, Chinese hamster ovary cells <i>in vitro</i>	+	0	10	Robison <i>et al.</i> (1984)
DIA, DNA strand breaks, mouse embryo fibroblasts <i>in vitro</i>	+	0	0.02	Zasukhina <i>et al.</i> (1983)
DIA, DNA strand breaks, rat embryo fibroblasts <i>in vitro</i>	+	0	0.02	Zasukhina <i>et al.</i> (1983)
RIA, DNA repair, Syrian hamster cells <i>in vitro</i>	(+)	0	2.0	Robison <i>et al.</i> (1984)
G5T, Gene mutation, mouse lymphoma L5178Y cells, <i>tk</i> locus, <i>in vitro</i>	-	(+)	4.44	Oberly <i>et al.</i> (1982)
SIC, Sister chromatid exchange, Chinese hamster ovary cells <i>in vitro</i>	(+)	0	2.0	Howard <i>et al.</i> (1991)
CIC, Chromosomal aberrations, Chinese hamster ovary cells <i>in vitro</i>	+	0	0.2	Howard <i>et al.</i> (1991)

Table 16 (contd)

Test system	Result		Dose ^a (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Mercuric chloride (contd)				
***, Spindle disturbances, Indian muntjac fibroblasts <i>in vitro</i>	+	0	0.1	Verschaeve <i>et al.</i> (1984)
***, Spindle disturbances, human lymphocytes <i>in vitro</i>	+	0	0.2	Verschaeve <i>et al.</i> (1984)
T7S, Cell transformation, SA7/Syrian hamster embryo cells <i>in vitro</i>	+	0	10	Casto <i>et al.</i> (1979)
SHL, Sister chromatid exchange, human lymphocytes <i>in vitro</i>	+	0	0.08	Morimoto <i>et al.</i> (1982)
CHL, Chromosomal aberrations, human lymphocytes <i>in vitro</i>	+	0	4.0	Verschaeve <i>et al.</i> (1985)
CHT, Chromosomal aberrations, human HeLa cells <i>in vitro</i>	-	0	7.4	Umeda <i>et al.</i> (1969)
***, Chromosomal condensation, human lymphocytes <i>in vitro</i>	+	0	2.0	Andersen <i>et al.</i> (1983)
***, Micronuclei in red blood cells, newt larvae <i>in vivo</i>	+		0.012	Zoll <i>et al.</i> (1988)
CBA, Chromosomal aberrations, mouse bone-marrow cells <i>in vivo</i>	-		4.44, ip × 1	Poma <i>et al.</i> (1981)
CBA, Chromosomal aberrations, Syrian hamster bone-marrow cells <i>in vivo</i>	(+)		4.74, sc × 1	Watanabe <i>et al.</i> (1982)
CBA, Chromosomal aberrations, mouse bone-marrow cells <i>in vivo</i>	+		2.22, po × 1	Ghosh <i>et al.</i> (1991)
CGG, Chromosomal aberrations, mouse spermatogonia <i>in vivo</i>	-		4.44, ip × 1	Poma <i>et al.</i> (1981)
COE, Chromosomal aberrations, Syrian hamster oocytes <i>in vivo</i>	-		9.47, sc × 1	Watanabe <i>et al.</i> (1982)
***, Chromosomal aberrations, newt larvae and embryos <i>in vivo</i>	+		0.06, water × 4 days	Zoll <i>et al.</i> (1988)
AVA, Aneuploidy, Syrian hamster bone-marrow cells <i>in vivo</i>	-		9.47, sc × 1	Watanabe <i>et al.</i> (1982)
AVA, Aneuploidy, Syrian hamster oocytes <i>in vivo</i>	-		9.47, sc × 1	Watanabe <i>et al.</i> (1982)
DLM, Dominant lethal mutation, mice	(+)		1.48, ip × 1	Suter (1975)
DLR, Dominant lethal mutation, rats	(+)		0.0003, po/day × 12 months	Zasukhina <i>et al.</i> (1983)
Mercurous chloride				
BSD, <i>Bacillus subtilis</i> rec strains, differential toxicity	+	0	10 000	Kanematsu <i>et al.</i> (1980)

Table 16 (contd)

Test system	Result		Dose ^a (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Mercuric acetate (63% Hg)				
CIA, Chromosomal aberrations, mouse oocytes <i>in vitro</i>	+	0	35	Jagiello & Lin (1973)
TIH, Anchorage-independent growth, human foreskin fibroblasts <i>in vitro</i>	-	0	2.0	Biedermann & Landolph (1987)
COE, Chromosomal aberrations, mouse oocytes <i>in vivo</i>	-		2, iv × 1	Jagiello & Lin (1973)
COE, Chromosomal aberrations, mouse oocytes <i>in vivo</i>	-		10, sc × 1	Jagiello & Lin (1973)

+, considered to be positive; (+), considered to be weakly positive in an inadequate study; -, considered to be negative; ?, considered to be inconclusive (variable responses in several experiments within an adequate study); 0, not tested

^aLED, lowest effective dose; HID, highest ineffective dose. In-vitro tests, µg/ml; in-vivo tests, mg/kg bw. Doses given as concentration of element, not concentration of compound; ip, intraperitoneally; sc, subcutaneously; iv, intravenously; po, orally by gavage

^bBluegill sunfish

***Not displayed on profile

Methylmercury hydroxide induced sex-linked recessive lethal mutations, but not chromosomal aberrations or meiotic crossing-over, in *Drosophila melanogaster*. It induced chromosomal aberrations in *Stethophyma grossum*. Methylmercury chloride, methylmercury hydroxide, phenylmercury hydroxide and phenylmercury acetate consistently induce aneuploidy in *D. melanogaster*. Methylmercury chloride did not induce aneuploidy in silkworms.

Studies of gene mutations in cultured mammalian cells have yielded varying responses. In one study, both methylmercury chloride and methoxyethylmercury chloride induced ouabain-resistance and *hprt* locus mutations in Chinese hamster V79 cells, whereas in another study with the same cells methylmercury hydroxide did not induce mutations at the *hprt* locus.

The induction of spindle disturbances and chromosomal aberrations has been studied extensively in cultured mammalian cells, including human lymphocytes. Significant responses were obtained consistently with methylmercury chloride in a number of studies of both end-points. In addition, chromosomal aberrations and/or spindle disturbances have been induced by methylmercury hydroxide, methoxyethylmercury chloride, dimethylmercury, ethylmercury chloride and phenylmercury chloride in independent studies using Chinese hamster V79 cells, human HeLa cells and lymphocytes *in vitro*. The frequency of micronuclei, which may be an expression of either spindle disturbances or chromosomal breakage, was increased in cultured cells from *Lepomis macrochirus* (bluegill sunfish) after treatment with methylmercury chloride, ethylmercury chloride or phenylmercury chloride.

Methylmercury chloride induced chromosomal aberrations in larvae and embryos of the newt, *Pleurodeles waltl*, and micronuclei in peripheral erythrocytes of the larvae. Treatment of pregnant rats with methylmercury chloride induced chromosomal aberrations in the livers of the fetuses, but it did not induce chromosomal aberrations in the bone marrow of Syrian hamsters and rats or in oocytes of Syrian hamsters. Intraperitoneal injection of methylmercury acetate did not induce micronuclei in mouse bone-marrow cells. Spindle disturbances were induced by methylmercury chloride in fetal lung and liver cells after treatment of mice *in vivo* in two studies and in killifish (*Fundulus heteroclitus*) embryo cells. Aneuploidy was seen in bone-marrow cells and oocytes of Syrian hamsters in one study but not in another. Methylmercury chloride appears therefore to be more active as a clastogen in fetal than in adult tissues of mice and more active as a spindle poison in Syrian hamster bone marrow than in Syrian hamster oocytes.

Dominant lethal mutation has been demonstrated in male rats and female mice, but not in male mice, treated with methylmercury chloride. Methylmercury hydroxide induced either weak or no dominant lethal effect in male mice. Methylmercury acetate did not induce sperm-head abnormalities in mice.

Few studies have been conducted on nonionized organomercury compounds; those that have been performed reflect the properties described above. Thus, dimethylmercury induced DNA fragmentation in the slime mould *Physarum polycephalum* and chromosomal aberrations and aneuploidy in cultured human lymphocytes; it induced chromosomal aberrations in mouse oocytes *in vitro* but not *in vivo*.

Several fungicides containing organomercury compounds have been tested for genotoxic activity in various plant systems. Spindle disturbances were induced by Panogen 5, 8 and 15, while chromosomal aberrations were induced by Agrimax M, Granosan, Ceresan M, Betoxin and New Improved Ceresan. [The Working Group noted that the different results may not reflect different properties, as various authors were involved.] In *D. melanogaster*, sex-linked recessive lethal mutation was induced by Ceresan and Ceresan M, but not by Agallol 3, and neither Ceresan nor Agallol 3 induced dominant lethal effects.

The azo dye, mercury orange, was not mutagenic to strains of *S. typhimurium*.

Considerations with regard to genotoxic mechanisms

Mercury has not only a direct effect on chromosomes, resulting in clastogenic effects in eukaryotes, but also causes disturbance of the spindle mechanism, owing to its high affinity for the sulfhydryl groups contained in spindle fibre proteins. Organomercury compounds inhibit the spindle mechanism even more strongly than colchicine, but, in contrast to colchicine, produce a gradual transition to c-mitosis at sub-lethal doses, which may result in aneuploidy and/or polyploidy.

In general, inorganic mercury compounds are less effective than ionizable organomercury compounds in inducing genetic effects *in vitro*. Similarities in the effects of different mercury compounds may suggest similar modes of action, while differences may be due to variations in solubility and bioavailability and in the rate of formation of a common toxic entity.

The possible contribution of reactive oxygen species to the genotoxicity of inorganic mercury compounds has also been addressed. ^{203}Hg -Mercuric chloride is taken up by Chinese hamster ovary cells and was reported to bind to the DNA in these cells in a temperature-dependent manner; however, DNA strand breaks were induced at 37 °C, but not at 4 °C, even though there was uptake and DNA binding of mercury at the lower temperature. It therefore appears that DNA strand breaks induced by Hg^{2+} require cellular metabolic processes. Mercuric chloride induced single-strand breaks in DNA of Chinese hamster ovary cells, and the effect was related linearly to leakage of superoxide into the medium. DNA damage induced by mercuric chloride can be increased by the addition of diethyldithiocarbamate, which inhibits superoxide dismutase, as well as by diethyl maleate, which depletes glutathione. Single strand breakage was inhibited by superoxide dismutase, catalase, glycerol and ascorbate (Cantoni *et al.*, 1984b).

5. Summary of Data Reported and Evaluation

5.1 Exposure data

Mercury occurs at low concentrations in the Earth's crust, mainly in sulfide ores (cinnabar), from which it has been extracted for a variety of uses for many centuries. Common applications of metallic mercury are as a cathode in the electrolytic production of chlorine, in dental amalgams, in the extraction of gold from ore concentrates, in electrical equipment and in devices for measuring temperature and pressure. Mercury compounds have been used as fungicides in paints and on seeds and grains, as antiseptics, in electrical applications, and as catalysts and intermediates.

Table 17. Genetic and related effects of organomercury compounds in experimental systems

Test system	Result		Dose ^a (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Methylmercury chloride (80% Hg)				
BSD, <i>Bacillus subtilis</i> rec strains, differential toxicity	+	0	1000	Kanematsu <i>et al.</i> (1980)
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation (spot test)	-	0	NR	Kanematsu <i>et al.</i> (1980)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation (spot test)	-	0	NR	Kanematsu <i>et al.</i> (1980)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation (spot test)	-	0	NR	Kanematsu <i>et al.</i> (1980)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation (spot test)	-	0	NR	Kanematsu <i>et al.</i> (1980)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation (spot test)	-	0	NR	Kanematsu <i>et al.</i> (1980)
EC2, <i>Escherichia coli</i> WP2, reverse mutation (spot test)	-	0	NR	Kanematsu <i>et al.</i> (1980)
ECR, <i>Escherichia coli</i> WP2 B/r, reverse mutation (spot test)	-	0	NR	Kanematsu <i>et al.</i> (1980)
SCG, <i>Saccharomyces cerevisiae</i> , gene conversion	-	0	NR	Nakai & Machida (1973) abstract
SCH, <i>Saccharomyces cerevisiae</i> , mitotic recombination	-	0	NR	Nakai & Machida (1973) abstract
SCH, <i>Saccharomyces cerevisiae</i> D7, mitotic recombination	-	0	10.0	Phipps & Miller (1982)
SCF, <i>Saccharomyces cerevisiae</i> , forward mutation	+	0	NR	Phipps & Miller (1983)
SCR, <i>Saccharomyces cerevisiae</i> , reverse mutation	-	0	NR	Nakai & Machida (1973) abstract
SCR, <i>Saccharomyces cerevisiae</i> , petite mutation	+	0	NR	Nakai & Machida (1973) abstract
SCR, <i>Saccharomyces cerevisiae</i> , petite mutation	-	0	10.0	Phipps & Miller (1983)
SCN, <i>Saccharomyces cerevisiae</i> , aneuploidy	?	0	NR	Nakai & Machida (1973) abstract
PLI, Water hyacinth (<i>Eichhornia crassipes</i>), micronuclei	+	0	0.1 (acute exposure)	Panda <i>et al.</i> (1988)
***, <i>Allium cepa</i> , spindle disturbances	+	0	1.0	Fiskesjö (1969)
***, Silkworm, aneuploidy	-		NR	Tazima (1974) abstract
DMN, <i>Drosophila melanogaster</i> aneuploidy (FIX system)	+		5.0	Osgood <i>et al.</i> (1991)
DMN, <i>Drosophila melanogaster</i> aneuploidy (ZESTE system)	-		100	Osgood <i>et al.</i> (1991)

Table 17 (contd)

Test system	Result		Dose ^a (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Methylmercury chloride (contd)				
G9O, Gene mutation, Chinese hamster lung V79 cells, ouabain resistance, <i>in vitro</i>	+	0	0.32	Fiskesjö (1979)
G9H, Gene mutation, Chinese hamster lung V79 cells, 8-azaguanine resistance, <i>in vitro</i>	+	0	0.16	Fiskesjö (1979)
***, Micronucleus test, fish (<i>Lepomis macrochirus</i>) cells <i>in vitro</i>	+	0	0.08	Babich <i>et al.</i> (1990)
CIC, Chromosomal aberrations, Chinese hamster brain cells <i>in vitro</i>	+	0	NR	Kato (1976) abst.
***, Spindle disturbances, Indian muntjac fibroblasts <i>in vitro</i>	+	0	1.0	Verschaeve <i>et al.</i> (1984)
SHL, Sister chromatid exchange, human lymphocytes <i>in vitro</i>	+	0	0.016	Morimoto <i>et al.</i> (1982)
***, Spindle disturbances, human lymphocytes <i>in vitro</i>	+	0	0.4	Fiskesjö (1970)
CHL, Chromosomal aberrations, human lymphocytes <i>in vitro</i>	+	0	0.04	Kato (1976) abstract; Kato <i>et al.</i> (1976) abstract
***, Spindle disturbances, human lymphocytes <i>in vitro</i>	+	0	0.08	Tournamille <i>et al.</i> (1982)
CHL, Chromosomal aberrations, human lymphocytes <i>in vitro</i>	+	0	1.0	Verschaeve <i>et al.</i> (1985)
CHL, Chromosomal aberrations, human lymphocytes <i>in vitro</i>	+	0	0.12	Betti <i>et al.</i> (1992)
AIH, Aneuploidy, human lymphocytes <i>in vitro</i>	+	0	0.12	Betti <i>et al.</i> (1992)
SVA, Sister chromatid exchange, mouse fetal lung and liver cells <i>in vivo</i>	+		8.0, po × 1	Curle <i>et al.</i> (1987)
***, Micronuclei, newt (<i>Pleurodeles waltl</i>) larvae red blood cells, <i>in vivo</i>	+		0.012, in water × 12 days	Zoll <i>et al.</i> (1988)
***, Nuclear abnormalities, cat bone-marrow cells <i>in vivo</i>	+		0.008, po × 39 months	Miller <i>et al.</i> (1979)
***, Spindle disturbances, mouse fetal lung and liver cells <i>in vivo</i>	+		4.0, po × 1	Curle <i>et al.</i> (1983)
AVA, Aneuploidy, Syrian hamster bone-marrow cells <i>in vivo</i>	+		4.74, sc × 1	Watanabe <i>et al.</i> (1982)
***, Spindle disturbances, mouse fetal lung and liver cells <i>in vivo</i>	+		4.0, po × 1	Curle <i>et al.</i> (1987)
***, Spindle disturbances, killifish (<i>Fundulus heteroclitus</i>) embryos <i>in vivo</i>	+		0.04, in water	Perry <i>et al.</i> (1988)
CBA, Chromosomal aberrations, Syrian hamster bone-marrow cells <i>in vivo</i>	(+)		9.47, sc × 1	Watanabe <i>et al.</i> (1982)

Table 17 (contd)

Test system	Result		Dose ^a (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Methylmercury chloride (contd)				
CBA, Chromosomal aberrations, rat bone-marrow cells <i>in vivo</i>	+		4.64, ip × 1	Li & Lin (1991)
AVA, Aneuploidy, Syrian hamster oocytes <i>in vivo</i>	-		9.47, sc × 1	Watanabe <i>et al.</i> (1982)
AVA, Aneuploidy, Syrian hamster bone-marrow cells <i>in vivo</i>	+		4.47, sc × 1	Watanabe <i>et al.</i> (1982)
COE, Chromosomal aberrations, Syrian hamster oocytes <i>in vivo</i>	-		9.47, sc × 1	Watanabe <i>et al.</i> (1982)
COE, Chromosomal aberrations, Syrian hamster oocytes <i>in vivo</i>	-		8.0, ip × 1	Mailhes (1983)
***, Chromosomal aberrations, newt (<i>Pleurodeles waltl</i>) larvae or embryos <i>in vivo</i>	+		0.04, in water × 4 days	Zoll <i>et al.</i> (1988)
COE, Chromosomal aberrations, rat embryo liver cells <i>in vivo</i>	+		1.52, ip × 1	Li & Lin (1991)
DLM, Dominant lethal mutation, male mice <i>in vivo</i>	-		5, po × 7 days	Khera (1973a)
DLM, Dominant lethal mutation, female mice <i>in vivo</i>	+		2.0, ip × 1	Verschaeve & Léonard (1984)
DLR, Dominant lethal mutation, male rats <i>in vivo</i>	+		2.5, po × 7 days	Khera (1973a)
DLR, Dominant lethal mutation, male rats <i>in vivo</i>	+		0.5, po × 90 days	Khera (1973a)
AVA, Aneuploidy, Syrian hamster oocytes <i>in vivo</i>	+		8.0, ip × 1	Mailhes (1983)
Methylmercury hydroxide [CH ₃ HgOH] (86% Hg)				
ACC, <i>Allium cepa</i> , chromosomal aberrations	+	0	0.05	Ramel (1969)
***, <i>Allium cepa</i> , spindle disturbances	+	0	0.05	Ramel (1969)
***, <i>Vicia faba</i> , spindle disturbances	+	0	0.02	Ramel (1972)
DMG, <i>Drosophila melanogaster</i> , meiotic crossing-over	-		4.3	Ramel (1972)
DMX, <i>Drosophila melanogaster</i> , sex-linked recessive lethal mutations	(+)		4.3	Ramel (1972)
DMX, <i>Drosophila melanogaster</i> , sex-linked recessive lethal mutations	+		8.6, diet	Magnusson & Ramel (1986)
***, <i>Drosophila melanogaster</i> , effect on radiation-induced chromosomal aberrations	-		4.3	Ramel (1972)
***, <i>Stethophyma grossum</i> , chromosomal aberrations	+		8 ng/animal	Klásterská & Ramel (1978)

Table 17 (contd)

Test system	Result		Dose ^a (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Methylmercury hydroxide (contd)				
DMN, <i>Drosophila melanogaster</i> , aneuploidy	+		0.25, diet	Ramel & Magnusson (1969)
DMN, <i>Drosophila melanogaster</i> , aneuploidy	+		0.25, diet	Ramel & Magnusson (1979)
DMN, <i>Drosophila melanogaster</i> , aneuploidy	+		0.43, diet	Magnusson & Ramel (1986)
***, Spindle disturbances, Chinese hamster lung V79 cells <i>in vitro</i>	+	0	0.42	Önfelt (1983)
G9H, Gene mutation, Chinese hamster lung V79 cells, <i>hprt</i> locus, <i>in vitro</i>	-	0	0.16	Önfelt & Jenssen (1982)
DLM, Dominant lethal mutation, (SEC×C57Bl)F ₁ male mice <i>in vivo</i>	(+)		7.4, ip × 1	Suter (1975)
DLM, Dominant lethal mutation, (101×C3H)F ₁ male mice <i>in vivo</i>	-		7.4, ip × 1	Suter (1975)
Methylmercury acetate [CH ₃ HgCO ₂ CH ₃] (73% Hg)				
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	-	-	183	Bruce & Heddle (1979)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	-	-	183	Bruce & Heddle (1979)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	-	-	183	Bruce & Heddle (1979)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	-	-	183	Bruce & Heddle (1979)
MVM, Micronuclei, B6C3F1 mouse bone-marrow cells <i>in vivo</i>	-		11.0, ip × 5	Bruce & Heddle (1979)
SPM, Sperm-head abnormalities, B6C3F1 mice <i>in vivo</i>	-		11.0, ip × 5	Bruce & Heddle (1979)
Methylmercury dicyandiamide [CH ₃ HgNHC(NH)NHCN] (67% Hg)				
***, <i>Allium cepa</i> , spindle disturbances	+	0	0.5	Ramel (1969)
DLM, Dominant lethal mutation, mice <i>in vivo</i>	-		2.0	Ramel (1972)
Ethylmercury chloride [CH ₃ CH ₂ HgCl]				
***, <i>Allium cepa</i> , spindle disturbances	+	0	0.4	Fiskesjö (1969)
***, Micronuclei, fish (<i>Lepomis macrochirus</i>) cells <i>in vitro</i>	+	0	0.08	Babich <i>et al.</i> (1990)
***, Spindle disturbances, human HeLa cells <i>in vitro</i>	+	0	1	Umeda <i>et al.</i> (1969)

Table 17 (contd)

Test system	Result		Dose ^a (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
<i>Bis(ethylmercury)hydrogen phosphate</i> [(CH ₃ CH ₂ Hg) ₂ HPO ₄]				
BSD, <i>Bacillus subtilis</i> rec strains, differential toxicity	-	0	14.4	Shirasu <i>et al.</i> (1976)
<i>Butylmercury bromide</i> [(CH ₃ (CH ₂) ₃ HgBr]				
***, <i>Allium cepa</i> , spindle disturbances	+	0	0.2	Fiskesjö (1969)
<i>Methoxyethylmercury chloride</i> [(CH ₃ O)CH ₂ CH ₂ HgCl]				
***, <i>Allium cepa</i> , spindle disturbance	+	0	0.63	Ramel (1969)
***, <i>Allium cepa</i> , spindle disturbances	+	0	2.0	Fiskesjö (1969)
DMN, <i>Drosophila melanogaster</i> , aneuploidy	-		20, diet	Ramel & Magnusson (1969)
DMN, <i>Drosophila melanogaster</i> , aneuploidy	-		2.5, larval diet	Ramel & Magnusson (1979)
G9O, Gene mutation, Chinese hamster lung V79 cells, ouabain resistance, <i>in vitro</i>	+	0	0.07	Fiskesjö (1979)
G9A, Gene mutation, Chinese hamster lung V79 cells, 8-azaguanine resistance, <i>in vitro</i>	+	0	0.07	Fiskesjö (1979)
***, Spindle disturbances, human lymphocytes <i>in vitro</i>	+	0	2.0	Fiskesjö (1970)
<i>Phenylmercury chloride</i> [ -HgCl]				
BSD, <i>Bacillus subtilis</i> rec strains, differential toxicity	-	0	12.8	Shirasu <i>et al.</i> (1976)
***, Micronucleus test, fish (<i>Lepomis macrochirus</i>) cells <i>in vitro</i>	+	0	0.03	Babich <i>et al.</i> (1990)
***, Spindle disturbances, human HeLa cells <i>in vitro</i>	+	0	1.0	Umeda <i>et al.</i> (1969)
<i>Phenylmercury hydroxide</i> [ -HgOH]				
PLM, <i>Anacharis canadensis</i> , mutation	+	0	6.8	MacFarlane & Messing (1953)

Table 17 (contd)

Test system	Result		Dose ^a (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Phenylmercury hydroxide (contd)				
PLM, <i>Coleus blumei</i> , mutation	+	0	0.5	MacFarlane & Messing (1953)
PLM, <i>Raphanus sativus</i> , mutation	+	0	68	MacFarlane & Messing (1953)
PLM, <i>Ruppia maritima</i> , mutation	+	0	6.8	MacFarlane & Messing (1953)
PLM, <i>Zea mays</i> , mutation	+	0	6.8	MacFarlane & Messing (1953)
ACC, <i>Allium cepa</i> , chromosomal aberrations	+	0	6.8	MacFarlane (1956)
ACC, <i>Allium cepa</i> , chromosomal aberrations	+	0	0.24	Ramel (1969)
***, <i>Allium cepa</i> , spindle disturbances	+	0	0.16	Ramel (1969)
Phenylmercury nitrate [C ₆ H ₅ -HgNO ₃]				
PLM, <i>Zea mays</i> , mutation	+	0	2.4	MacFarlane & Messing (1953)
ACC, <i>Allium cepa</i> , chromosomal aberrations	+	0	5.9	MacFarlane (1956)
Phenylmercury acetate [C ₆ H ₅ -HgCO ₂ CH ₃]				
BSD, <i>Bacillus subtilis</i> rec strains, differential toxicity	-	0	12	Shirasu <i>et al.</i> (1976)
BSD, <i>Bacillus subtilis</i> rec strains, differential toxicity	+	0	200	Kanematsu <i>et al.</i> (1980)
DMN, <i>Drosophila melanogaster</i> , aneuploidy	(+) ^b		0.32	Ramel & Magnusson (1969)
Dimethylmercury [CH ₃ HgCH ₃]				
***, <i>Physarum polycephalum</i> (slime mould), DNA fragments	+	0	500	Yatscoff & Cummins (1975)

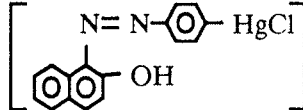
Table 17 (contd)

Test system	Result		Dose ^a (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Dimethylmercury (contd)				
CIM, Chromosomal aberrations, mouse oocytes <i>in vitro</i>	+	0	10	Jagiello & Lin (1973)
CHL, Chromosomal aberrations, human lymphocytes <i>in vitro</i>	+	0	8.7	Betti <i>et al.</i> (1992)
AIH, Aneuploidy, lymphocytes <i>in vitro</i>	+	0	0.34	Betti <i>et al.</i> (1992)
COE, Chromosomal aberrations, mouse oocytes <i>in vivo</i>	–		140, iv × 1	Jagiello & Lin (1973)
Mercury-containing fungicides (denomination and composition as reported by authors)				
Panogen 5 (containing methylmercury dicyandiamide; Hg, 5 g/L)				
***, <i>Allium cepa</i> , spindle disturbances	+	0	0.05	Ramel (1969)
Panogen 8 (containing methylmercury dicyandiamide; Hg, 6.4 g/L)				
***, <i>Allium cepa</i> , spindle disturbances	+	0	0.16	Ramel (1969)
Panogen 15 (containing 2.3% methylmercury dicyandiamide; 1.54% Hg)				
***, <i>Vicia faba</i> , spindle disturbances	+	0	0.015	Ahmed & Grant (1972)
***, <i>Tradescantia</i> species, spindle disturbances	+	0	0.015	Ahmed & Grant (1972)
Ceresan (containing phenylmercury acetate; 1% Hg)				
DMX, <i>Drosophila melanogaster</i> , sex-linked recessive lethal mutations	+		200, diet, adult	Gayathri & Krishnamurthy (1985)
DML, <i>Drosophila melanogaster</i> , dominant lethal mutations	–		200, diet, adult	Gayathri & Krishnamurthy (1985)
Agrimax M (containing phenylmercury dinaphthylmethanedisulfonate; % Hg not known)				
***, <i>Avena sativa</i> , polyploidy	+	0	NR	Bruhin (1955)
***, <i>Crepis capillaris</i> , polyploidy	+	0	NR	Bruhin (1955)

Table 17 (contd)

Test system	Result		Dose ^a (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Granosan (2% ethylmercury chloride [EMC] + 98% talc; 1.5% Hg)				
***, <i>Crepis capillaris</i> , nuclear abnormalities	-	0	15	Bruhin (1955)
***, <i>Linum usitatissimum</i> , nuclear abnormalities	-	0	75	Kostoff (1940)
***, <i>Pisum sativum</i> , nuclear abnormalities	+	0	75	Kostoff (1940)
***, <i>Secale cereale</i> , nuclear abnormalities	+	0	15	Kostoff (1939, 1940)
***, <i>Triticum aegilopodes</i> , nuclear abnormalities	+	0	15	Kostoff (1939, 1940)
***, <i>Triticum durum</i> , nuclear abnormalities	+	0	15	Kostoff (1939, 1940)
***, <i>Triticum persicum</i> , nuclear abnormalities	+	0	15	Kostoff (1939, 1940)
***, <i>Triticum polonicum</i> , nuclear abnormalities	+	0	15	Kostoff (1939, 1940)
***, <i>Triticum vulgare</i> , nuclear abnormalities	+	0	15	Kostoff (1939, 1940)
Ceresan M^c (containing ethylmercury <i>para</i> -toluenesulfonanilide)				
DMX, <i>Drosophila melanogaster</i> , sex-linked recessive lethal mutations	+		14.3, diet	Mathew & Al-Doori (1976)
Agallol 3 (containing methoxyethylmercury chloride; 3% Hg)				
DMX, <i>Drosophila melanogaster</i> , sex-linked recessive lethal mutations	-		600, diet, adult	Gayathri & Krishnamurthy (1985)
DML, <i>Drosophila melanogaster</i> , dominant lethal mutations	-		600, diet, adult	Gayathri & Krishnamurthy (1985)
Betoxin (containing 90% ethylmercury halogenide; % Hg not known)				
ACC, <i>Allium cepa</i> , chromosomal aberrations	+	0	NR	Fiskesjö (1969)
***, <i>Allium cepa</i> , spindle disturbances	+	0	NR	Fiskesjö (1969)
New improved Ceresan (containing ethylmercury phosphate)				
PLC, <i>Zea mays</i> , chromosomal aberrations	+	0	NR	Sass (1937)

Table 17 (contd)

Test system	Result		Dose ^a (LED/HID)	Reference
	Without exogenous metabolic system	With exogenous metabolic system		
Azo dye				
Mercury orange (41% Hg) 				
SA0, <i>Salmonella typhimurium</i> TA100, reverse mutation	–	–	103	Brown <i>et al.</i> (1978)
SA5, <i>Salmonella typhimurium</i> TA1535, reverse mutation	–	–	103	Brown <i>et al.</i> (1978)
SA7, <i>Salmonella typhimurium</i> TA1537, reverse mutation	–	–	103	Brown <i>et al.</i> (1978)
SA8, <i>Salmonella typhimurium</i> TA1538, reverse mutation	–	–	103	Brown <i>et al.</i> (1978)
SA9, <i>Salmonella typhimurium</i> TA98, reverse mutation	–	–	103	Brown <i>et al.</i> (1978)

+, considered to be positive; (+), considered to be weakly positive in an inadequate study; -, considered to be negative; ?, considered to be inconclusive (variable responses in several experiments within an adequate study); 0, not tested

^aLED, lowest effective dose; HID, highest ineffective dose. In-vitro tests, µg/ml; in-vivo tests, mg/kg bw. Doses given as concentration of element, not concentration of compound; NR, not reported; po, orally, by gavage; sc, subcutaneously; ip, intraperitoneally; iv, intravenously

^bStatistically significant, but may be due to control values lower than those in other experiments

^cClaimed to be responsible for two outbreaks of poisoning in 1956 and 1960 in Iraq

***Not displayed on profile

Workers are exposed to mercury by inhalation, principally to metallic mercury but also to inorganic and organic mercury compounds. Occupations in which the highest exposures occur include mercury mining, work in chloralkali and alkaline battery plants and production of devices for measuring temperature and pressure. Lower exposures have been measured for people employed in hospital laboratories and dental clinics. Exposures have been measured by both ambient air monitoring and biological monitoring.

Nonoccupational sources of exposure to mercury include food (methylmercury compounds, mainly in aquatic organisms) and dental amalgam fillings (metallic mercury). These exposure levels are usually lower than those typically detected in occupational settings.

5.2 Human carcinogenicity data

Metallic mercury and inorganic mercury compounds

A cohort study in a nuclear weapons factory in the USA on exposure to metallic mercury showed no difference in risk for lung cancer in exposed and unexposed subcohorts from the same factory. In a nested case-control study at two nuclear facilities in the USA, the risk for cancers of the central nervous system was not associated with estimated levels of exposure to mercury.

A cohort study of chloralkali workers in Sweden identified a two-fold, significant excess risk for lung cancer and some nonsignificant excess risks for cancers of the brain and kidney. Lung cancers also occurred in an almost two-fold excess in Norwegian chloralkali workers, whereas the numbers of cases of cancer of the brain and kidney were close to those expected. In both studies, asbestos and smoking were judged to be the main determinants of the excess risk for lung cancer.

In a study of male and female dentists and female dental nurses in Sweden, a two-fold risk for brain tumours was found in each of the three cohorts. No such risk appeared among dentists or medical and dental technicians in a US study of military veterans; these groups had excess risks for pancreatic and colon cancer, respectively. In an Australian case-control study of brain tumours and amalgam fillings, there was a decreased risk for gliomas and no effect was seen with regard to meningiomas.

The risk for lung cancer was found to be higher among individuals with silicosis who had been working in US mercury mines than in subjects with silicosis who had worked elsewhere. This finding was based on small numbers, however, and the confidence limits overlapped.

A case-control study in Italy indicated an excess risk for lung cancer among women in the felt-hat industry who had heavy exposure to mercury but also to arsenic.

In a population-based case-control study from Canada, risk for prostatic cancer was associated with exposure to mercury compounds in general and the risk for lung cancer with exposure to metallic mercury.

Organomercury compounds

Studies in Minamata, Japan, on causes of death in populations with high exposure to mercury included areas with a high prevalence of methylmercury poisoning. The only clear indication of an increased cancer risk was in the most informative of these studies, in which

excess mortality from cancer of the liver and cancer of the oesophagus was found in the area with the highest exposure, together with an increased risk for chronic liver disease and cirrhosis. Consumption of alcoholic beverages was known to be higher than average in the area.

A cohort study of individuals in Sweden with a licence for seed disinfection with mercury compounds and other agents found no excess of brain cancer. Of the three Swedish case-control studies on exposure to mercury seed dressings and soft-tissue sarcomas, only one showed an odds ratio above unity; in all three studies, the confidence intervals included unity. For malignant lymphomas, there was a slightly but nonsignificantly elevated odds ratio for exposure to mercury seed dressings, but other exposures had higher odds ratios and, consequently, potential confounding.

5.3 Animal carcinogenicity data

Mercuric chloride was tested for carcinogenicity in two studies in mice, by oral gavage and by administration in the drinking-water; only the study by gavage was adequate for an evaluation of carcinogenicity. Mercuric chloride was also tested in one study in rats by oral gavage. In mice, a few renal adenomas and adenocarcinomas occurred in males only. In rats, a few renal adenomas occurred in females; there was a dose-related increase in the incidence of squamous-cell papilloma of the forestomach in males, and a few papillomas were seen in females. Dose-related hyperplasia of the forestomach was seen in both males and females.

Methylmercury chloride was tested for carcinogenicity in three studies in mice and two studies in rats by oral administration in the diet. In all three studies in mice, the incidence of renal adenomas and adenocarcinomas was increased in males. In the two studies in rats, no increase in tumour incidence was reported. In another study in mice given methylmercury chloride, a significant number of renal tumours was found in intact male mice and a few renal tumours were found in gonadectomized male and female mice that also received testosterone propionate; no renal tumour was found in male or female gonadectomized mice that did not receive testosterone propionate.

5.4 Other relevant data

After inhalation, about 70–80% of metallic mercury vapour is retained and absorbed. Little metallic mercury is taken up in the gastrointestinal tract, and less than 10% is absorbed. Metallic mercury passes into the brain and fetus. In the body, metallic mercury is oxidized to mercuric mercury, which binds to reduced sulfhydryl groups. The kidney is the main depository following exposure to both metallic and mercuric mercury. Mercuric mercury is eliminated mainly in urine and faeces; it is also excreted in milk. In humans, inorganic mercury compounds have two half-times: one lasts for days or weeks and the other much longer. Mercury concentrations in urine, blood and plasma are useful for biological monitoring.

Methylmercury compounds present in seafood are almost completely absorbed from the gastrointestinal tract and are distributed to most tissues. The methylmercury compounds bind to reduced sulfhydryl groups; a fraction is converted to mercuric mercury, the extent of

conversion differing among species. Methylmercury compounds are excreted mainly in the bile; in the intestine, some mercury is biotransformed into inorganic mercury compounds and excreted in the faeces. Methylmercury compounds pass into the fetus and are excreted in milk. In humans, methylmercury compounds have a single biological half-time of approximately two months. Concentrations in blood and hair are useful for monitoring exposure to methylmercury compounds.

Following intense exposure to metallic mercury vapour, lung damage occurs; gastro-intestinal and renal tubular necrosis occur after ingestion of mercuric mercury. Long-term exposure to metallic mercury causes encephalopathy and renal damage; chronic exposure to mercuric mercury causes renal tubular damage. Immunologically based glomerulonephritis can occur. In rats, mercuric chloride may cause immunosuppression. Effects on the immune system vary considerably among rodent strains. Inorganic mercury is a cause of allergic contact dermatitis. The nervous system is the main target organ for methylmercury compounds, but interspecies differences exist; in some species, there are also effects on the kidney. Some selenium compounds affect the kinetics of inorganic and methylmercury compounds and have a protective effect against their toxicity.

In several studies of female dental assistants, no increased risk for spontaneous abortion or birth defects was seen. Parenteral administration of mercury salts to pregnant rodents induces fetal growth retardation, malformations and death; altered placental transport of nutrients may be involved. Methylmercury compounds induce adverse effects on human development—most notably microcephaly and deficits in neurological development. Similar effects have been shown in many laboratory species. The conceptus appears to be more sensitive than the maternal organism. The dose levels of methylmercury compounds that affect reproduction and development are generally lower than those of inorganic mercury and affect a wider range of end-points.

The findings of 14 studies of cytogenetic effects, such as sister chromatid exchange, micronucleus formation, structural chromosomal aberrations, aneuploidy and polyploidy, in peripheral lymphocytes of individuals exposed to metallic mercury and various mercury compounds are controversial and uncertain. Thus, four studies involving subjects exposed to methylmercury compounds from contaminated seal or fish meat were either inconclusive or indicated slight chromosomal effects. Nine studies in individuals exposed from occupational sources to metallic mercury, amalgams, alkyl- and arylmercury compounds or mercury fulminate gave either negative or borderline results, or the exact role of mercury in any positive result was uncertain. A slight yet significant increase in the frequency of sister chromatid exchange was observed in only one subset of children intoxicated with phenylmercury acetate used for disinfecting diapers.

Several organomercury compounds and fungicides containing organomercury compounds were assayed in a variety of short-term tests. Tests for unscheduled DNA synthesis, sister chromatid exchange, chromosomal aberrations and dominant lethal mutations in mammals *in vivo* gave conflicting results. Tests for clastogenicity in fish and amphibians gave more convincingly positive results. All studies of induction of c-mitosis (spindle disturbances), sister chromatid exchange, structural chromosomal aberrations and aneuploidy in cultured human lymphocytes gave positive results. The results of the majority of studies of the induction of forward mutations, c-mitosis and polyploidy in cultured

mammalian (non-human) cells were positive, and those of one study on micronucleus induction in cultured fish cells were also positive. In *Drosophila melanogaster* and other insects, the majority of mercury compounds induced sex-linked recessive lethal mutation and nondisjunction (aneuploidy) but did not induce dominant lethal mutation. The assessment of nuclear or mitochondrial DNA mutations, mitotic recombination and gene conversion in the yeast *Saccharomyces cerevisiae* led to conflicting results. Most of the few studies available in bacteria (investigating differential killing in *rec⁻* *Bacillus subtilis* or reversion in *his⁻* *Salmonella typhimurium* or *trp⁻* *Escherichia coli*) gave negative results.

There were fewer studies of inorganic mercury compounds (mostly mercuric chloride), and a minority compared inorganic and organic compounds. No experimental study was available on metallic mercury. As in studies with organomercury compounds, studies in rodents treated *in vivo* with mercuric chloride gave weakly positive results for dominant lethal mutation. Studies on the induction of chromosomal aberrations in rodents yielded conflicting results. One study on chromosomal effects in amphibians gave positive results for mercuric chloride and methylmercury chloride at similar doses. Chromosomal alterations were reported in cultured human lymphocytes. The dose of mercuric chloride required to induce sister chromatid exchange in cultured human lymphocytes was 5–25 times higher than those needed of methylmercury chloride. Mercuric acetate did not induce anchorage-independent growth in human cells. Five to ten times higher doses of mercuric chloride than methylmercury chloride were required to induce polyploidy. DNA damage has been induced repeatedly in mammalian cells by mercuric chloride. Although the information comes from single studies, this compound also induced sister chromatid exchange, chromosomal aberrations, aneuploidy (spindle disturbances) and enhancement of virus-induced morphological transformation. Unlike organomercury compounds, mercuric chloride failed to enhance the frequency of micronuclei in cultured fish cells. Mercuric chloride failed to enhance lethality in a DNA repair-deficient strain of *E. coli*.

5.5 Evaluation¹

There is *inadequate evidence* in humans for the carcinogenicity of mercury and mercury compounds.

There is *inadequate evidence* in experimental animals for the carcinogenicity of metallic mercury.

There is *limited evidence* in experimental animals for the carcinogenicity of mercuric chloride.

There is *sufficient evidence* in experimental animals for the carcinogenicity of methylmercury chloride.

In making the overall evaluation, the Working Group took into account evidence that methylmercury compounds are similar with regard to absorption, distribution, metabolism, excretion, genotoxicity and other forms of toxicity.

¹For definition of the italicized terms, see Preamble, pp. 26-30.

Overall evaluation

Methylmercury compounds *are possibly carcinogenic to humans (Group 2B)*.

Metallic mercury and inorganic mercury compounds *are not classifiable as to their carcinogenicity to humans (Group 3)*.

6. References

- Åberg, B., Ekman, L., Falk, R., Greitz, U., Persson, G. & Snihs, J.-O. (1969) Metabolism of methylmercury (^{203}Hg) compounds in man. Excretion and distribution. *Arch. environ. Health*, **19**, 478–484
- Afonso, J.F. & de Alvarez, R.R. (1960) Effects of mercury on human gestation. *Am. J. Obstet. Gynecol.*, **80**, 145–154
- Agency for Toxic Substances and Disease Registry (1989) *Toxicological Profile for Mercury* (US NTIS PB90-181256), Atlanta, GA, US Public Health Service
- Agocs, M.M., Etzel, R.A., Parrish, R.G., Paschal, D.C., Campagna, P.R., Cohen, D.S., Kilbourne, E.M. & Hesse, J.L. (1990) Mercury exposure from interior latex paint. *New Engl. J. Med.*, **323**, 1096–1101
- Ahlbom, A., Norell, S., Rodvall, Y. & Nylander, M. (1986) Dentists, dental nurses, and brain tumours. *Br. med. J.*, **292**, 662
- Ahmed, M. & Grant, W.F. (1972) Cytological effects of the mercurial fungicide Panogen 15 on *Tradescantia* and *Vicia faba* root tips. *Mutat. Res.*, **14**, 391–396
- Akesson, I., Schutz, A., Attewell, R., Skerfving, S. & Glantz, P.-O. (1991) Status of mercury and selenium in dental personnel: impact of amalgam work and own fillings. *Arch. environ. Health*, **46**, 102–109
- Albanus, L., Frankenberg, L., Grant, C., von Haartman, U., Jernelöv, A., Nordberg, G., Rydälv, M., Schütz, A. & Skerfving, S. (1972) Toxicity for cats of methylmercury in contaminated fish from Swedish lakes and of methylmercury hydroxide added to fish. *Environ. Res.*, **5**, 425–442
- Alcser, K.H., Brix, K.A., Fine, L.J., Kallenbach, L.R. & Wolfe, R.A. (1989) Occupational mercury exposure and male reproductive health. *Am. J. ind. Med.*, **15**, 517–529
- Aldrich Chemical Co. (1992) *Aldrich Catalog/Handbook of Fine Chemicals 1992–1993*, Milwaukee, WI, pp. 507, 793–794, 1001
- Alfa Products (1990) *Alfa Catalog—Research Chemicals and Accessories*, Ward Hill, MA, pp. 250, 258, 294, 530
- Amandus, H. & Costello, J. (1991) Silicosis and lung cancer in US metal miners. *Arch. environ. Health*, **46**, 82–89
- American Conference of Governmental Industrial Hygienists (1992) *1992–1993 Threshold Limit Values for Chemical Substances and Physical Agents and Biological Exposure Indices*, Cincinnati, OH, p. 25
- Ancona, A., Ramos, M., Suarez, R. & Macotela, E. (1982) Mercury sensitivity in a dentist (Short communication). *Contact Derm.*, **8**, 218
- Andersen, O., Rønne, M. & Nordberg, G.F. (1983) Effects of inorganic metal salts on chromosome length in human lymphocytes. *Hereditas*, **98**, 65–70
- Angerer, J. & Schaller, K.H., eds (1988) *Analyses of Hazardous Substances in Biological Materials*, Vol. 2, *Methods for Biological Monitoring*, Weinheim, VCH Verlagsgesellschaft, pp. 195–207

- Angotzi, G., Cassitto, M.G., Camerino, D., Cioni, R., Desideri, E., Franzinelli, A., Gori, R., Loi, F. & Sartorelli, E. (1980) Relationship between mercury exposure and health in workers of a mercury distillation plant in the Province of Siena (Ital.). *Med. Lav.*, **6**, 463-480
- Anttila, A., Jaakkola, J., Tossavainen, A. & Vainio, H. (1992) *Occupational Exposure to Chemicals in Finland* (Altisteet Työssä 34), Helsinki, Institute of Occupational Health & Finnish Work Environmental Fund
- Anwar, W.A. & Gabal, M.S. (1991) Cytogenetic study in workers occupationally exposed to mercury fulminate. *Mutagenesis*, **6**, 189-192
- Aoi, T., Higuchi, T., Kidokoro, R., Fukumura, R., Yagi, A., Ohguchi, S., Sasa, A., Hayashi, H., Sakamoto, N. & Hanaichi, T. (1985) An association of mercury with selenium in inorganic mercury intoxication. *Hum. Toxicol.*, **4**, 637-642
- Arbeidsinspectie [Labour Inspection] (1986) *De Nationale MAC-Lijst 1986* [National MAC List 1986], Voorburg, p. 15
- Arbejdstilsynet [Labour Inspection] (1992) *Graensevaerdier for Stoffer og Materialer* [Limit Values for Compounds and Materials] (No. 3.1.0.2), Copenhagen, p. 22
- Aschner, M. & Clarkson, T.W. (1988) Uptake of methylmercury in the rat brain: effects of amino acids. *Brain Res.*, **462**, 31-39
- Ashe, W.F., Largent, E.J., Dutra, F.R., Hubbard, D.M. & Blackstone, M. (1953) Behavior of mercury in the animal organism following inhalation. *Arch. ind. Hyg. occup. Med.*, **7**, 19-43
- Assennato, G., Porro, A., Longo, G., Longo, F. & Ambrosi, L. (1989) Effects of low mercury concentrations on the nervous system among workers employed in the manufacture of fluorescent lamps (Ital.). *Med. Lav.*, **80**, 307-315
- Atomergic Chemetals Corp. (undated) *High Purity Metals Brochure*, Farmingdale, NY
- Aylett, B.J. (1973) Mercury. In: Bailar, J.C., Jr, Emeléus, H.J., Nyholm, R. & Trotman-Dickenson, A.F., eds, *Comprehensive Inorganic Chemistry*, Vol. 3, Oxford, Pergamon Press, pp. 275-328
- Babich, H., Goldstein, S.H. & Borenfreund, E. (1990) In vitro cyto- and genotoxicity of organomercurials to cells in culture. *Toxicol. Lett.*, **50**, 143-149
- Bakir, F., Damluji, S.F., Amin-Zaki, L., Murtadha, M., Khalidi, A., Al-Rawi, N.Y., Tikriti, S., Dhahir, H.I., Clarkson, T.W., Smith, J.C. & Doherty, R.A. (1973) Methylmercury poisoning in Iraq. An interuniversity report. *Science*, **181**, 230-241
- Baldi, G., Vigliani, E.C. & Zurlo, N. (1953) Chronic mercurialism in felt hat industries (Ital.). *Med. Lav.*, **44**, 161-198
- Ballatori, N. & Clarkson, T.W. (1984) Inorganic mercury secretion into bile as a low molecular weight complex. *Biochem. Pharmacol.*, **33**, 1087-1092
- Barlow, S.M. & Sullivan, F.M. (1982) *Reproductive Hazards of Industrial Chemicals. An Evaluation of Animal and Human Data*, London, Academic Press, pp. 386-406
- Barr, R.D., Rees, P.H., Cordy, P.E., Kungu, A., Woodger, B.A. & Cameron, H.M. (1972) Nephrotic syndrome in adult Africans in Nairobi. *Br. med. J.*, **ii**, 131-134
- Barregård, L., Hultberg, B., Schütz, A. & Sällsten, G. (1988) Enzymuria in workers exposed to inorganic mercury. *Int. Arch. occup. environ. Health*, **61**, 65-69
- Barregård, L., Sällsten, G. & Järholm, B. (1990) Mortality and cancer incidence in chloralkali workers exposed to inorganic mercury. *Br. J. ind. Med.*, **47**, 99-104
- Barregård, L., Högstedt, B., Schütz, A., Karlsson, A., Sällsten, G. & Thiringer, G. (1991) Effects of occupational exposure to mercury vapor on lymphocyte micronuclei. *Scand. J. Work Environ. Health*, **17**, 263-268

- Barregård, L., Sällsten, G., Schütz, A., Attewell, R., Skerfving, S. & Järnholm, B. (1992) Kinetics of mercury in blood and urine after brief occupational exposure. *Arch. environ. Health*, **47**, 176–184
- Bergstrand, A., Friberg, L., Mendel, L. & Odeblad, E. (1959) The localization of subcutaneously administered radioactive mercury in the rat kidney (Abstract No. 8). *J. ultrastruct. Res.*, **3**, 238
- Berlin, M. (1986) Mercury. In: Friberg, L., Nordberg, G.F. & Vouk, V.B., eds, *Handbook on Toxicology of Metals*, Vol. II, *Specific Metals*, 2nd ed., Amsterdam, Elsevier, pp. 387–445
- Berlin, M., Fazackerley, J. & Nordberg, G.F. (1969) The uptake of mercury in the brains of mammals exposed to mercury vapor and to mercuric salts. *Arch. environ. Health*, **18**, 719–729
- Berlin, M., Carlson, J. & Norseth, T. (1975) Dose-dependence of methylmercury metabolism. A study of distribution: biotransformation and excretion in the squirrel monkey. *Arch. environ. Health*, **30**, 307–313
- Bernard, A.M., Collette, C. & Lauwerys, R. (1992) Renal effects of in utero exposure to mercuric chloride in rats. *Arch. Toxicol.*, **66**, 508–513
- Betti, C., Davini, T. & Barale, R. (1992) Genotoxic activity of methyl mercury chloride and dimethyl mercury in human lymphocytes. *Mutat. Res.*, **281**, 255–260
- Biedermann, K.A. & Landolph, J.R. (1987) Induction of anchorage independence in human diploid foreskin fibroblasts by carcinogenic metal salts. *Cancer Res.*, **47**, 3815–3823
- Blakley, B.R. (1984) Enhancement of urethan-induced adenoma formation in Swiss mice exposed to methylmercury. *Can. J. comp. Med.*, **48**, 299–302
- Bondy, S.C., Anderson, C.L., Harrington, M.E. & Prasad, K.N. (1979) The effects of organic and inorganic lead and mercury on neurotransmitter high-affinity transport and release mechanisms. *Environ. Res.*, **19**, 102–111
- Bornhausen, M., Müsch, H.R. & Greim, H. (1980) Operant behavior performance changes in rats after prenatal methylmercury exposure. *Toxicol. appl. Pharmacol.*, **56**, 305–310
- Brandi, G., Schiavano, G.F., Albano, A., Cattabeni, F. & Cantoni, O. (1990) Growth delay and filamentation of *Escherichia coli* wild-type and *rec A* cells in response to hexavalent chromium and other metal compounds. *Mutat. Res.*, **245**, 201–204
- Brodsky, J.B., Cohen, E.N., Whitcher, C., Brown, B.W., Jr & Wu, M.L. (1985) Occupational exposure to mercury in dentistry and pregnancy outcome. *J. Am. dent. Assoc.*, **111**, 779–780
- Brown, J.P., Roehm, G.W. & Brown, R.J. (1978) Mutagenicity testing of certified food colors and related azo, xanthene and triphenylmethane dyes with the *Salmonella*/microsome system. *Mutat. Res.*, **56**, 249–271
- Bruce, W.R. & Heddle, J.A. (1979) The mutagenic activity of 61 agents as determined by the micro-nucleus, *Salmonella*, and sperm abnormality assays. *Can. J. genet. Cytol.*, **21**, 319–334
- Bruhin, A. (1955) Polyploidizing action of a seed corrosive (Ger.). *Phytopathol. Z.*, **23**, 381–394
- Brune, D., Nordberg, G.F., Vesterberg, O., Gerhardsson, L. & Wester, P.O. (1991) A review of normal concentrations of mercury in human blood. *Sci. total Environ.*, **100**, 235–282
- Budavari, S., ed. (1989) *The Merck Index*, 11th ed., Rahway, NJ, Merck & Co., pp. 512, 923–927
- Buelke-Sam, J., Kimmel, C.A., Adams, J., Nelson, C.J., Vorhees, C.V., Wright, D.C., St Omer, V., Korol, B.A., Butcher, R.E., Geyer, M.A., Holson, J.F., Kutscher, C.L. & Wayner, M.J. (1985) Collaborative behavioral teratology studies: results. *Neurobehav. Toxicol. Teratol.*, **7**, 591–624
- Buiatti, E., Kriebel, D., Geddes, M., Santucci, M. & Pucci, N. (1985) A case control study of lung cancer in Florence, Italy. I. Occupational risk factors. *J. Epidemiol. Community Health*, **39**, 244–250
- Burbacher, T.M., Monnett, C., Grant, K.S. & Mottet, N.K. (1984) Methylmercury exposure and reproductive dysfunction in the nonhuman primate. *Toxicol. appl. Pharmacol.*, **75**, 18–24

- Burbacher, T.M., Rodier, P.M. & Weiss, B. (1990) Methylmercury developmental neurotoxicity: a comparison of effects in humans and animals. *Neurotoxicol. Teratol.*, **12**, 191-202
- Burton, G.V. & Meikle, A.W. (1980) Acute and chronic methyl mercury poisoning impairs rat adrenal and testicular function. *J. Toxicol. environ. Health*, **6**, 597-606
- Campbell, D., Gonzales, M. & Sullivan, J.B., Jr (1992) Mercury. In: Sullivan, J.B., Jr & Krieger, G.R., eds, *Hazardous Materials Toxicology. Clinical Principles of Environmental Health*, Baltimore, Williams & Wilkins, pp. 824-833
- Cantoni, O. & Costa, M. (1983) Correlations of DNA strand breaks and their repair with cell survival following acute exposure to mercury(II) and X-rays. *Mol. Pharmacol.*, **24**, 84-89
- Cantoni, O., Evans, R.M. & Costa, M. (1982) Similarity in the acute cytotoxic response of mammalian cells to mercury(II) and X-rays: DNA damage and glutathione depletion. *Biochem. biophys. Res. Commun.*, **108**, 614-619
- Cantoni, O., Christie, N.T., Robison, S.H. & Costa, M. (1984a) Characterization of DNA lesions produced by HgCl_2 in cell culture systems. *Chem.-biol. Interactions*, **49**, 209-224
- Cantoni, O., Christie, N.T., Swann, A., Drath, D.B. & Costa, M. (1984b) Mechanisms of HgCl_2 cytotoxicity in cultured mammalian cells. *Mol. Pharmacol.*, **26**, 360-368
- Carpenter, A.V., Flanders, W.D., Frome, E.L., Tankersley, W.G. & Fry, S.A. (1988) Chemical exposures and central nervous system cancers: a case-control study among workers at two nuclear facilities. *Am. J. ind. Med.*, **13**, 351-362
- Carrico, L.C. (1985) Mercury. In: *Mineral Commodity Summaries 1985*, Washington DC, Bureau of Mines, US Department of the Interior, pp. 98-99
- Carrico, L.C. (1987) Mercury. In: *Mineral Commodity Summaries 1987*, Washington DC, Bureau of Mines, US Department of the Interior, pp. 100-101
- Casto, B.C., Meyers, J. & DiPaolo, J.A. (1979) Enhancement of viral transformation for evaluation of the carcinogenic or mutagenic potential of inorganic metal salts. *Cancer Res.*, **39**, 193-198
- CERAC, Inc. (1991) *Advanced Specialty Inorganics*, Milwaukee, WI, p. 149
- Chang, L.W. & Sprecher, J.A. (1976a) Hyperplastic changes in the rat distal tubular epithelial cells following in utero exposure to methylmercury. *Environ. Res.*, **12**, 218-223
- Chang, L.W. & Sprecher, J.A. (1976b) Degenerative changes in the neonatal kidney following in-utero exposure to methylmercury. *Environ. Res.*, **11**, 392-406
- Chang, L.W. & Suber, R. (1982) Protective effect of selenium on methylmercury toxicity: a possible mechanism. *Bull. environ. Contam. Toxicol.*, **29**, 285-289
- Charbonneau, S.M., Munro, I.C., Nera, E.A., Armstrong, F.A.J., Willes, R.F., Bryce, F. & Nelson, R.F. (1976) Chronic toxicity of methylmercury in the adult cat. Interim report. *Toxicology*, **5**, 337-349
- Chen, W.-J., Body, R.L. & Mottet, N.K. (1983) Biochemical and morphological studies of monkeys chronically exposed to methylmercury. *J. Toxicol. environ. Health*, **12**, 407-416
- Cherian, M.G., Hursh, J.B., Clarkson, T.W. & Allen, J. (1978) Radioactive mercury distribution in biological fluids and excretion in human subjects after inhalation of mercury vapor. *Arch. environ. Health*, **33**, 109-114
- Choi, B.H., Kim, R.C. & Peckham, N.H. (1988) Hydrocephalus following prenatal methylmercury poisoning. *Acta neuropathol.*, **75**, 325-330
- Christie, N.T., Cantoni, O., Sugiyama, M., Cattabeni, F. & Costa, M. (1986) Differences in the effects of Hg(II) on DNA repair induced in Chinese hamster ovary cells by ultraviolet or X-rays. *Mol. Pharmacol.*, **29**, 173-178

- Chung, A.-S., Maines, M.D. & Reynolds, W.A. (1982) Inhibition of the enzymes of glutathione metabolism by mercuric chloride in the rat kidney: reversal by selenium. *Biochem. Pharmacol.*, **31**, 3093-3100
- Cicchella, G., Focardi, L. & Rossaro, R. (1968) Urinary excretion of mercury in healthy people living in mercury mine regions and elsewhere (Ital.). *Lav. Um.*, **20**, 3-9
- Clarkson, T. (1992) The uptake and disposition of inhaled mercury vapor. In: *Potential Biological Consequences of Mercury Released from Dental Amalgam*, Stockholm, Swedish Medical Research Council, pp. 59-75
- Clarkson, T.W., Hursh, J.B., Sager, P.R. & Syversen, T.L.M. (1988a) Mercury. In: Clarkson, T.W., Friberg, L., Nordberg, G.F. & Sager, P.R., eds, *Biological Monitoring of Toxic Metals*, New York, Plenum Press, pp. 199-246
- Clarkson, T.W., Friberg, L., Hursh, J.B. & Nylander, M. (1988b) The prediction of intake of mercury vapor from amalgams. In: Clarkson, T.W., Friberg, L., Nordberg, G.F. & Sager, P.R., eds, *Biological Monitoring of Toxic Metals*, New York, Plenum Press, pp. 247-264
- Cloëz, I., Dumont, O., Piciotti, M. & Bourre, J.M. (1987) Alterations of lipid synthesis in the normal and dysmyelinating trembler mouse sciatic nerve by heavy metals (Hg, Pb, Mn, Cu, Ni). *Toxicology*, **46**, 65-71
- Commission of the European Communities (1981) Council Directive on the approximation of the rules of the Member States concerning the colouring matters authorised for use in foodstuffs intended for human consumption. *Off. J. Eur. Comm.*, **L43**, 11
- Commission of the European Communities (1990) Proposal for a Council Directive on the approximation of the laws of the Member States relating to cosmetic products. *Off. J. Eur. Comm.*, **C322**, 29-77
- Commission of the European Communities (1991) Thirteenth Commission Directive of 12 March 1991 (91/814/EEC) on the approximation of the laws of the Member States relating to cosmetic products. *Off. J. Eur. Commun.*, **L91**, 59-62
- Cook, W.A. (1987) *Occupational Exposure Limits—Worldwide*, Akron, OH, American Industrial Hygiene Association, pp. 123, 145, 197
- Cordier, S., Deplan, F., Mandereau, L. & Hemon, D. (1991) Prenatal exposure to mercury and spontaneous abortions. *Br. J. ind. Med.*, **48**, 375-381
- Cragle, D.L., Hollis, D.R., Qualters, J.R., Tankersley, W.G. & Fry, S.A. (1984) A mortality study of men exposed to elemental mercury. *J. occup. Med.*, **26**, 817-821
- Curle, D.C., Ray, M. & Persaud, T.V.N. (1983) Methylmercury toxicity: in vivo evaluation of teratogenesis and cytogenetic changes. *Anat. Anz. Jena*, **153**, 69-82
- Curle, D.C., Ray, M. & Persaud, T.V.N. (1987) In vivo evaluation of teratogenesis and cytogenetic changes following methylmercuric chloride treatment. *Anat. Rec.*, **219**, 286-295
- Daniel, J.W., Gage, J.C. & Lefevre, P.A. (1971) The metabolism of methoxyethylmercury salts. *Biochem. J.*, **121**, 411-415
- Danielsson, B.R.G., Dencker, L., Khayat, A. & Orsén, L. (1984) Fetotoxicity of inorganic mercury in the mouse: distribution and effects of nutrient uptake by placenta and fetus. *Biol. Res. Pregn.*, **5**, 102-109
- Dayan, A.D., Hertel, R.F., Heseltine, E., Kazantzis, G., Smith, E.M. & Van der Venne, M.-T., eds (1990) *Immunotoxicity of Metals and Immunotoxicology*, New York, Plenum Press
- De Rosis, F., Anastasio, S.P., Selvaggi, L., Beltrame, A. & Moriani, G. (1985) Female reproductive health in two lamp factories: effects of exposure to inorganic mercury vapour and stress factors. *Br. J. ind. Med.*, **42**, 488-494

- Deutsche Forschungsgemeinschaft (1992) *MAK and BAT Values 1992. Maximum Concentrations at the Workplace and Biological Tolerance Values for Working Materials* (Report No. 28), Weinheim, VCH Verlagsgesellschaft, pp. 47, 50, 103
- D.F. Goldsmith Chemical & Metal Corp. (undated) *High Purity Elements; Fine Inorganic Chemicals; Precious Metals; Mercury*, Evanston, IL, p. 18
- Doherty, R.A., Gates, A.H., Sewell, C.E. & Freer, C. (1978) Methylmercury sexual dimorphism in the mouse. *Experientia*, **34**, 871
- Doi, R. & Kobayashi, T. (1982) Organ distribution and biological half-time of methylmercury in four strains of mice. *Japan J. exp. Med.*, **52**, 307-314
- Doi, R. & Tagawa, M. (1983) A study on the biochemical and biological behavior of methylmercury. *Toxicol. appl. Pharmacol.*, **69**, 407-416
- Drake, H.J. (1981) Mercury. In: Mark, H.F., Othmer, D.F., Overberger, C.G., Seaborg, G.T. & Grayson, N., eds, *Kirk-Othmer Encyclopedia of Chemical Technology*, 3rd Ed., Vol. 15, New York, John Wiley & Sons, pp. 143-156
- Druckrey, H., Hamperl, H. & Schmähel, D. (1957) Carcinogenic action of metallic mercury after intraperitoneal administration in rats (Ger.). *Z. Krebsforsch.*, **61**, 511-519
- Druet, P., Druet, E., Potdevin, F. & Sapin, C. (1978) Immune type glomerulonephritis induced by HgCl₂ in the Brown-Norway rat. *Ann. Immunol.*, **129C**, 777-792
- Druet, E., Sapin, C., Fournie, G., Mandet, C., Günther, E. & Druet, P. (1982) Genetic control of susceptibility to mercury-induced immune nephritis in various strains of rat. *Clin. Immunol. Immunopathol.*, **25**, 203-212
- Dunn, J.D., Clarkson, T.W. & Magos, L. (1978) Ethanol-increased exhalation of mercury in mice. *Br. J. ind. Med.*, **35**, 241-244
- Ehrenberg, R.L., Vogt, R.L., Smith, A.B., Brondum, J., Brightwell, W.S., Hudson, P.J., McManus, K.P., Hannon, W.H. & Phipps, F.C. (1991) Effects of elemental mercury exposure at a thermometer plant. *Am. J. ind. Med.*, **19**, 495-507
- Eichhorn, G.L. & Clark, P. (1963) The reaction of mercury (II) with nucleosides. *Am. J. chem. Soc.*, **85**, 4020-4024
- Elinder, C.-G., Gerhardsson, L. & Oberdoerster, G. (1988) Biological monitoring of metals. In: Clarkson, T.W., Friberg, L., Nordberg, G.F. & Sager, P., eds, *Biological Monitoring of Toxic Metals*, New York, Plenum Press, pp. 1-71
- Eller, P.M., ed. (1989) *NIOSH Manual of Analytical Methods*, 3rd Ed., Suppl. 3, (DHHS (NIOSH) Publ. No. 84-100), Washington DC, US Government Printing Office, pp. 6009-1-6009-4
- Ellingsen, D.G., Andersen, A., Nordhagen, H.P., Efskind, J. & Kjuus, H. (1993) Incidence of cancer and mortality among workers exposed to mercury vapour in the Norwegian chloralkali industry. *Br. J. ind. Med.*, **50** (in press)
- Erfurth, E.M., Schütz, A., Nilsson, A., Barregård, L. & Skerfving, S. (1990) Normal pituitary hormone response to thyrotrophin and gonadotrophin releasing hormones in subjects exposed to elemental mercury vapour. *Br. J. ind. Med.*, **47**, 639-644
- Ericson, A. & Källén, B. (1989) Pregnancy outcome in women working as dentists, dental assistants or dental technicians. *Int. Arch. occup. environ. Health*, **61**, 329-333
- Eriksson, M., Hardell, L., Berg, N.O., Möller, T. & Axelson, O. (1981) Soft-tissue sarcomas and exposure to chemical substances: a case-referent study. *Br. J. ind. Med.*, **38**, 27-33
- Eriksson, M., Hardell, L. & Adami, H.-O. (1990) Exposure to dioxins as a risk factor for soft tissue sarcoma: a population-based case-control study. *J. natl Cancer Inst.*, **82**, 486-490

- Ernst, E. & Lauritsen, J.G. (1991) Effect of organic and inorganic mercury on human sperm motility. *Pharmacol. Toxicol.*, **69**, 440-444
- Fang, S.C. (1980) Comparative study of uptake and tissue distribution of methylmercury in female rats by inhalation and oral routes of administration. *Bull. environ. Contam. Toxicol.*, **24**, 65-72
- Farant, J.-P., Brissette, D., Moncion, L., Bigras, L. & Chartrand, A. (1981) Improved cold-vapor atomic absorption technique for the microdetermination of total and inorganic mercury in biological samples. *J. anal. Toxicol.*, **5**, 47-51
- Fehling, C., Abdulla, M., Brun, A., Dictor, M., Schütz, A. & Skerfving, S. (1975) Methylmercury poisoning in the rat: a combined neurological, chemical, and histopathological study. *Toxicol. appl. Pharmacol.*, **33**, 27-37
- Finne, K., Göransson, K. & Winckler, L. (1982) Oral lichen planus and contact allergy to mercury. *Int. J. oral Surg.*, **11**, 236-239
- Fiskesjö, G. (1969) Some results from *Allium* tests with organic mercury halogenides. *Hereditas*, **62**, 314-322
- Fiskesjö, G. (1970) The effect of two organic mercury compounds on human leukocytes *in vitro*. *Hereditas*, **64**, 142-146
- Fiskesjö, G. (1979) Two organic mercury compounds tested for mutagenicity in mammalian cells by use of the cell line V 79-4. *Hereditas*, **90**, 103-109
- Foà, V., Caimi, L., Amante, L., Antonini, C., Gattinoni, A., Tettamanti, G., Lombardo, A. & Giuliani, A. (1976) Patterns of some lysosomal enzymes in the plasma and of proteins in urine of workers exposed to inorganic mercury. *Int. Arch. occup. environ. Health*, **37**, 115-124
- Foldspang, A. & Hansen, J.C. (1990) Dietary intake of methylmercury as a correlate of gestational length and birth weight among newborns in Greenland. *Am. J. Epidemiol.*, **132**, 310-317
- Fowler, B.A. & Woods, J.S. (1977a) The transplacental toxicity of methyl mercury to fetal rat liver mitochondria. Morphometric and biochemical studies. *Lab. Invest.*, **36**, 122-130
- Fowler, B.A. & Woods, J.S. (1977b) Ultrastructural and biochemical changes in renal mitochondria during chronic oral methyl mercury exposure. The relationship to renal function. *Exp. mol. Pathol.*, **27**, 403-412
- Friberg, L., Skog, E. & Wahlberg, J.E. (1961) Resorption of mercuric chloride and methyl mercury dicyandiamide in guinea-pigs through normal skin and through skin pre-treated with acetone, alkylaryl-sulphonate and soap. *Acta dermatovener.*, **41**, 40-52
- Fukunaga, M., Kurachi, Y., Ogawa, M., Mizuguchi, Y., Kodama, Y. & Chihara, S. (1981) The genetic effects of environmental pollutants on eukaryotic cells. Mutagenicity on nuclear and mitochondrial genes of yeast by metals (Jpn.). *J. Univ. occup. environ. Health. Jpn.*, **3**, 245-254
- Fuyuta, M., Fujimoto, T. & Hirata, S. (1978) Embryotoxic effects of methylmercuric chloride administered to mice and rats during organogenesis. *Teratology*, **18**, 353-366
- Fuyuta, M., Fujimoto, T. & Kiyofuji, E. (1979) Teratogenic effects of a single oral administration of methylmercuric chloride in mice. *Acta anat.*, **104**, 356-362
- Gale, T. (1974) Embryopathic effects of different routes of administration of mercuric acetate in the hamster. *Environ. Res.*, **8**, 207-213
- Gale, T.F. (1981) The embryotoxic response produced by inorganic mercury in different strains of hamsters. *Environ. Res.*, **24**, 152-161
- Gallagher, R.P., Threlfall, W.J., Band, P.R. & Spinelli, J.J. (1985) *Occupational Mortality in British Columbia 1950-1984*, Vancouver, BC, Cancer Control Agency of British Columbia and Workers' Compensation Board of British Columbia

- Gates, A.H., Doherty, R.A. & Cox, C. (1986) Reproduction and growth following prenatal methylmercuric chloride exposure in mice. *Fundam. appl. Toxicol.*, **7**, 486-493
- Gayathri, M.V. & Krishnamurthy, N.B. (1985) Investigations on the mutagenicity of two organomercurial pesticides, Ceresan and Agallol 3, in *Drosophila melanogaster*. *Environ. Res.*, **36**, 218-229
- Gelbier, S. & Ingram, J. (1989) Possible foetotoxic effects of mercury vapour: a case report. *Public Health*, **103**, 35-40
- Ghosh, A.K., Sen, S., Sharma, A. & Talukder, G. (1991) Effect of chlorophyllin on mercuric chloride-induced clastogenicity in mice. *Food chem. Toxicol.*, **29**, 777-779
- Giavini, E., Vismara, C. & Broccia, M.L. (1985) Effects of methylmercuric chloride administered to pregnant rats during the preimplantation period. *Ecotoxicol. environ. Saf.*, **9**, 189-195
- Goldberg, M., Klitzman, S., Payne, J.L., Nadig, R.J., McGrane, J.-A. & Goodman, A.K. (1990) Mercury exposure from the repair of blood pressure machines in medical facilities. *Appl. occup. environ. Hyg.*, **5**, 604-610
- Gotelli, C., Astolfi, E., Cox, C., Cernichiari, E. & Clarkson, T.W. (1985) Early biochemical effects of an organic mercury fungicide on infants: 'dose makes the poison'. *Science*, **227**, 638-640
- Grandjean, P. & Weihe, P. (1993) Neurobehavioral effects of intrauterine mercury exposure: potential sources of bias. *Environ. Res.*, **61**, 176-183
- Greenwood, M.R. (1985) Methylmercury poisoning in Iraq. An epidemiological study of the 1971-1972 outbreak. *J. appl. Toxicol.*, **5**, 148-159
- Halbach, S. & Clarkson, T.W. (1978) Enzymatic oxidation of mercury vapor by erythrocytes. *Biochim. biophys. Acta*, **523**, 522-531
- Hardell, L. & Eriksson, M. (1988) The association between soft tissue sarcomas and exposure to phenoxyacetic acids. A new case-referent study. *Cancer*, **62**, 652-656
- Hardell, L., Eriksson, M., Lenner, P. & Lundgren, E. (1981) Malignant lymphoma and exposure to chemicals, especially organic solvents, chlorophenols and phenoxy acids: a case-control study. *Br. J. Cancer*, **43**, 169-176
- Hargreaves, R.J., Evans, J.G., Janota, I., Magos, L. & Cavanagh, J.B. (1988) Persistent mercury in nerve cells 16 years after metallic mercury poisoning. *Neuropathol. appl. Neurobiol.*, **14**, 443-452
- Health & Safety Executive (1992) *EH40/92 Occupational Exposure Limits 1992*, London, Her Majesty's Stationary Office, p. 21
- Heidam, L.Z. (1984) Spontaneous abortions among dental assistants, factory workers, painters, and gardening workers: a follow up study. *J. Epidemiol. Community Health*, **38**, 149-155
- Heintze, U., Edwardsson, S., Dérand, T. & Birkhed, D. (1983) Methylation of mercury from dental amalgam and mercuric chloride by oral streptococci *in vitro*. *Scand. J. dent. Res.*, **91**, 150-152
- Helrich, K., ed. (1990a) *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th Ed., Vol. 1, Arlington, VA, Association of Official Analytical Chemists, pp. 508-511
- Helrich, K., ed. (1990b) *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th Ed., Vol. 1, Arlington, VA, Association of Official Analytical Chemists, pp. 326-327
- Helrich, K., ed. (1990c) *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th Ed., Vol. 1, Arlington, VA, Association of Official Analytical Chemists, pp. 262-269
- Helrich, K., ed. (1990d) *Official Methods of Analysis of the Association of Official Analytical Chemists*, 15th Ed., Vol. 1, Arlington, VA, Association of Official Analytical Chemists, pp. 162-163
- Hirano, M., Mitsumori, K., Maita, K. & Shirasu, Y. (1986) Further carcinogenicity study on methylmercury chloride in ICR mice. *Jpn. J. vet. Sci.*, **48**, 127-135

- Hirano, M., Ueda, H., Mitsumori, K., Maita, K. & Shirasu, Y. (1988) Hormonal influence on carcinogenicity of methylmercury in mice. *Jpn. J. vet. Sci.*, **50**, 886–893
- Holt, D. & Webb, M. (1986a) Comparison of some biochemical effects of teratogenic doses of mercuric mercury and cadmium in the pregnant rat. *Arch. Toxicol.*, **58**, 249–254
- Holt, D. & Webb, M. (1986b) The toxicity and teratogenicity of mercuric mercury in the pregnant rat. *Arch. Toxicol.*, **58**, 243–248
- Howard, W., Léonard, B., Moody, W. & Kochhar, T.S. (1991) Induction of chromosome changes by metal compounds in cultured CHO cells. *Toxicol. Lett.*, **56**, 179–186
- Hrubec, Z., Blair, A.E., Rogot, E. & Vaught, J. (1992) *Mortality Risks by Occupation among US Veterans of Known Smoking Status 1954–1980*, Vol. 1 (NIH Publication No. 92-3407), Washington DC, National Cancer Institute
- Hultman, P. & Eneström, S. (1987) The induction of immune complex deposits in mice by peroral and parenteral administration of mercuric chloride: strain dependent susceptibility. *Clin. exp. Immunol.*, **67**, 283–292
- Hunter, D. & Russell, D.S. (1954) Focal cerebral and cerebellar atrophy in a human subject due to organic mercury compounds. *J. Neurol. Neurosurg. Psychiatr.*, **17**, 235–241
- Hursh, J.B. (1985) Partition coefficients of mercury (^{203}Hg) vapor between air and biological fluids. *J. appl. Toxicol.*, **5**, 327–332
- Hursh, J.B., Clarkson, T.W., Cherian, M.G., Vostal, J.J. & Mallie, R.V. (1976) Clearance of mercury (Hg-197, Hg-203) vapor inhaled by human subjects. *Arch. environ. Health*, **31**, 302–309
- Hursh, J.B., Greenwood, M.R., Clarkson, T.W., Allen, J. & Demuth, S. (1980) The effect of ethanol on the fate of mercury vapor inhaled by man. *J. Pharmacol. exp. Ther.*, **214**, 520–527
- Hursh, J.B., Sichak, S.P. & Clarkson, T.W. (1988) In vitro oxidation of mercury by the blood. *Pharmacol. Toxicol.*, **63**, 266–273
- Hursh J.B., Clarkson, T.W., Miles, E.F. & Goldsmith, L.A. (1989) Percutaneous absorption of mercury vapor by man. *Arch. environ. Health*, **44**, 120–127
- IARC (1987a) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42*, Lyon, pp. 230–232
- IARC (1987b) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Suppl. 7, *Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42*, Lyon, pp. 120–122
- IARC (1992) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Volume 54, *Occupational Exposures to Mists and Vapours from Strong Inorganic Acids; and Other Industrial Chemicals*, Lyon
- IARC (1993) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, Vol. 57, *Occupational Exposures of Hairdressers and Barbers and Personal Use of Hair Colourants; Some Hair Dyes, Cosmetic Colourants, Industrial Dyestuffs and Aromatic Amines*, Lyon, p. 55
- Igata, A. (1991) Epidemiological and clinical features of Minamata disease. In: Susuki, T., ed, *Advances in Mercury Toxicology*, New York, Plenum Press, pp. 439–457
- Ilbäck, N.-G. (1991) Effects of methyl mercury exposure on spleen and blood natural killer (NK) cell activity in the mouse. *Toxicology*, **67**, 117–124
- Inouye, M. & Kajiwara, Y. (1988) Developmental disturbances of the fetal brain in guinea-pigs caused by methylmercury. *Arch. Toxicol.*, **62**, 15–21
- Inskip, M.J. & Piotrowski, J.K. (1985) Review of the health effects of methylmercury. *J. appl. Toxicol.*, **5**, 113–133

- International Labour Office (1991) *Occupational Exposure Limits for Airborne Toxic Substances: Values of Selected Countries* (Occupational Safety and Health Series No. 37), 3rd Ed., Geneva, pp. 252-255, 270-271
- Jacobs, G. (1977) Total and organically bound mercury content in fish from German fishing grounds (Ger.). *Z. Lebensmittel. Untersuch.-Forsch.*, **164**, 71-76
- Jagiello, G. & Lin, J.S. (1973) An assessment of the effects of mercury on the meiosis of mouse ova. *Mutat. Res.*, **17**, 93-99
- Janicki, K., Dobrowolski, J. & Krásnicki, K. (1987) Correlation between contamination of the rural environment with mercury and occurrence of leukaemia in men and cattle. *Chemosphere*, **16**, 253-257
- Janssen Chimica (1990) *1991 Catalog Handbook of Fine Chemicals*, Beerse, pp. 754-755, 954
- Jokstad, A., Thomassen, Y., Bye, E., Clench-Aas, J. & Aaseth, J. (1992) Dental amalgam and mercury. *Pharmacol. Toxicol.*, **70**, 308-313
- Joselow, M.M., Goldwater, L.J., Alvarez, A. & Herndon, J. (1968) Absorption and excretion of mercury in man. XV. Occupational exposure among dentists. *Arch. environ. Health*, **17**, 39-43
- Kaiser, G. & Tölg, G. (1984) Mercury. In: Hutzinger, O., ed., *The Handbook of Environmental Chemistry*, Vol. 3, Part A, *Anthropogenic Compounds*, New York, Springer-Verlag, pp. 1-58
- Kajiwara, Y. & Inouye, M. (1986) Effects of methylmercury and mercuric chloride on preimplantation mouse embryos *in vivo*. *Teratology*, **33**, 231-237
- Kanematsu, N., Hara, M. & Kada, T. (1980) *rec* Assay and mutagenicity studies on metal compounds. *Mutat. Res.*, **77**, 109-116
- Kark, P. (1979) Clinical and neurochemical aspects of inorganic mercury intoxication. In: Vinken, P.J. & Bruyn, G.W., eds, *Handbook of Clinical Neurology*, Vol. 36, Amsterdam, Elsevier, pp. 147-197
- Karthikeyan, K.S., Parameswaran, A. & Rajan, B.P. (1986) Mercury toxicity in dental personnel. *J. Indian Dent. Assoc.*, **58**, 215-220
- Kato, R. (1976) Chromosome breakage associated with organic mercury in Chinese hamster cells *in vitro* (Abstract No. 10). *Mutat. Res.*, **58**, 340-341
- Kato, R., Nakamura, A. & Sawai, T. (1976) Chromosome breakage associated with organic mercury in human leukocytes *in vitro* and *in vivo* (Abstract). *Jpn. J. Hum. Genet.*, **20**, 256-257
- Kawada, J., Nishida, M., Yoshimura, Y. & Mitani, K. (1980) Effects of organic and inorganic mercurials on thyroidal functions. *J. Pharm. Dyn.*, **3**, 149-159
- Kazantzis, G. & Lilly, L.J. (1986) Mutagenic and carcinogenic effects of metals. In: Friberg, L., Nordberg, G.F. & Vouk, W.B., eds, *Handbook on the Toxicology of Metals*, Vol. 1, 2nd Ed, Amsterdam, Elsevier, pp. 319-390
- Kazantzis, G., Schiller, K.F.R., Asscher, A.W. & Drew, R.G. (1962) Albuminuria and the nephrotic syndrome following exposure to mercury and its compounds. *Q. J. Med. New Ser.*, **31**, 403-418
- Kazantzis, G., Al-Mufti, A.W., Al-Jawad, A., Al-Shahwani, Y., Majid, M.A., Mahmoud, R.M., Soufi, M., Tawfiq, K., Ibrahim, M.A. & Dabagh, H. (1976) Epidemiology of organomercury poisoning in Iraq. II. Relationship of mercury levels in blood and hair to exposure and to clinical findings. *Bull. WHO*, **53** (Suppl.), 37-48
- Kershaw, T.G., Clarkson, T.W. & Dhahir, P.H. (1980) The relationship between blood levels and dose of methylmercury in man. *Arch. environ. Health*, **35**, 28-36
- Khayat, A. & Dencker, L. (1982) Fetal uptake and distribution of metallic mercury vapor in the mouse: influence of ethanol and aminotriazole. *Biol. Res. Pregn.*, **3**, 38-46
- Khera, K.S. (1973a) Reproductive capability of male rats and mice treated with methyl mercury. *Toxicol. appl. Pharmacol.*, **24**, 167-177

- Khera, K.S. (1973b) Teratogenic effects of methylmercury in the cat: note on the use of this species as a model for teratogenicity studies. *Teratology*, **8**, 293–303
- Khera, K.S. (1979) Teratogenic and genetic effects of mercury toxicity. In: Nriagu, J.O., ed., *The Biogeochemistry of Mercury in the Environment*, Amsterdam, Elsevier, pp. 503–518
- Kitchin, K.T., Ebron, M.T. & Svendsgaard, D. (1984) In vitro study of embryotoxic and dysmorphogenic effects of mercuric chloride and methylmercury chloride in the rat. *Food Chem. Toxicol.*, **22**, 31–37
- Klásterská, I. & Ramel, C. (1978) The effect of methyl mercury hydroxide on meiotic chromosomes of the grasshopper *Stethophyma grossum*. *Hereditas*, **88**, 255–262
- Koller, L.D. (1975) Methylmercury: effect on oncogenic and nononcogenic viruses in mice. *Am. J. vet. Dis.*, **36**, 1501–1504
- Kosta, L., Byrne, A.R. & Zelenko, V. (1975) Correlation between selenium and mercury in man following exposure to inorganic mercury. *Nature*, **254**, 238–239
- Kostial, K., Šimonović, I., Rabar, I., Blanuša, M. & Landeka, M. (1983) Age and intestinal retention of mercury and cadmium in rats. *Environ. Res.*, **31**, 111–115
- Kostoff, D. (1939) Effect of the fungicide 'Granosan' on atypical growth and chromosome doubling in plants (Short communication). *Nature*, **144**, 334
- Kostoff, D. (1940) Atypical growth, abnormal mitosis and polyploidy induced by ethylmercury-chloride. *J. Phytopathol.*, **2**, 91–96
- Kurokawa, Y., Matsushima, M., Imazawa, T., Takamura, N., Takahashi, M. & Hayashi, Y. (1985) Promoting effect of metal compounds on rat renal tumorigenesis. *J. Am. Coll. Toxicol.*, **4**, 321–330
- Kurokawa, Y., Takahashi, M., Maekawa, A. & Hayashi, Y. (1989) Promoting effect of metal compounds on liver, stomach, kidney, pancreas and skin carcinogenesis. *J. Am. Coll. Toxicol.*, **8**, 1235–1239
- Ladd, A.C., Zuskin, E., Valic, F., Almonte, J.B. & Gonzales, T.V. (1966) Absorption and excretion of mercury in miners. *J. occup. Med.*, **8**, 127–131
- Lamperti, A.A. & Printz, R.H. (1973) Effects of mercuric chloride on the reproductive cycle of the female hamster. *Biol. Reprod.*, **8**, 378–387
- Langworth, S., Röjdmark, S. & Åkesson, A. (1990) Normal pituitary response to thyrotrophin releasing hormone in dental personnel exposed to mercury. *Swed. Dent. J.*, **14**, 101–103
- Langworth, S., Elinder, C.-G., Göthe, C.J. & Vesterberg, O. (1991) Biological monitoring of environmental and occupational exposure to mercury. *Int. Arch. occup. environ. Health*, **63**, 161–167
- Langworth, S., Almkvist, O., Söderman, E. & Wilkström, B.-O. (1992a) Effects of occupational exposure to mercury vapour on the central nervous system. *Br. J. ind. Med.*, **49**, 545–555
- Langworth, S., Elinder, C.-G., Sundquist, K.G. & Vesterberg, O. (1992b) Renal and immunological effects of occupational exposure to inorganic mercury. *Br. J. ind. Med.*, **49**, 394–401
- Lauwerys, R.R. (1983) Mercury. In: Parmeggiani, L., ed., *Encyclopedia of Occupational Health and Safety*, 3rd rev. Ed., Vol. 2, Geneva, International Labour Office, pp. 1332–1335
- Lauwerys, R.R. & Buchet, J.P. (1973) Occupational exposure to mercury vapors and biological action. *Arch. environ. Health*, **27**, 65–68
- Lauwerys, R., Bernard, A., Roels, H., Buchet, J.P., Gennart, J.P., Mahieu, P. & Foidart, J.M. (1983) Anti-laminin antibodies in workers exposed to mercury vapour. *Toxicol. Lett.*, **17**, 113–116
- Lauwerys, R., Roels, H., Genet, P., Toussaint, G., Bouckaert, A. & De Cooman, S. (1985) Fertility of male workers exposed to mercury vapor or to manganese dust: a questionnaire study. *Am. J. ind. Med.*, **7**, 171–176

- Lauwerys, R., Bonnier, C., Evrard, P., Gennart, J.P. & Bernard, A. (1987) Prenatal and early postnatal intoxication by inorganic mercury resulting from the maternal use of mercury containing soap. *Hum. Toxicol.*, **6**, 253-256
- Lehotzky, K. & Bordas, S. (1968) Study on the subacute neurotoxic effect of methoxy-ethyl mercury chloride (MEMC) in rats. *Med. Lav.*, **59**, 241-249
- Léonard, A., Jacquet, P. & Lauwerys, R.R. (1983) Mutagenicity and teratogenicity of mercury compounds. *Mutat. Res.*, **114**, 1-18
- Li, Y.-H. & Lin, X.-W. (1991) The transplacental effect of methylmercury on the chromosome of embryo liver cells in rat (Chin.). *Chin. J. prev. Med.*, **25**, 220-221
- Lide, D.R., ed. (1991) *CRC Handbook of Chemistry and Physics*, 72nd Ed., Boca Raton, FL, CRC Press, pp. 4-74-4-75
- Lindqvist, K.J., Makene, W.J., Shaba, J.K. & Nantulya, V. (1974) Immunofluorescence and electron microscopic studies of kidney biopsies from patients with nephrotic syndrome, possibly induced by skin lightening creams containing mercury. *East Afr. med. J.*, **51**, 168-169
- Lindstedt, G., Gottberg, I., Holmgren, B., Jonsson, T. & Karlsson, G. (1979) Individual mercury exposure of chloralkali workers and its relation to blood and urinary mercury levels. *Scand. J. Work Environ. Health*, **5**, 59-69
- Lindström, H., Luthman, J., Oskarsson, A., Sundberg, J. & Olson, L. (1991) Effects of long-term treatment with methyl mercury on the developing rat brain. *Environ. Res.*, **56**, 158-169
- Mabille, V., Roels, H., Jacquet, P., Léonard, A. & Lauwerys, R.R. (1984) Cytogenetic examination of leukocytes of workers exposed to mercury vapour. *Int. Arch. occup. environ. Health*, **53**, 257-260
- MacFarlane, E.W.E. (1956) Cytological conditions in root tip meristem after gross antagonism of phenylmercuric poisoning. *Exp. Cell Res.*, **5**, 375-385
- MacFarlane, E.W.E. & Messing, A.M. (1953) Shoot chimeras after exposure to mercurial compounds. *Bot. Gaz.*, **115**, 66-76
- Magnusson, J. & Ramel, C. (1986) Genetic variation in the susceptibility to mercury and other metal compounds in *Drosophila melanogaster*. *Teratog. Carcinog. Mutag.*, **6**, 289-305
- Magos, L. (1987) The absorption, distribution and excretion of methyl mercury. In: Eccles, C.U. & Annau, Z., eds, *The Toxicity of Methylmercury*, Baltimore, MD, Johns Hopkins University Press, pp. 24-44
- Magos, L. & Butler, W.H. (1972) Cumulative effects of methylmercury dicyandiamide given orally to rats. *Food Cosmet. Toxicol.*, **10**, 513-517
- Magos, L. & Clarkson, T.W. (1972) Atomic absorption determination of total, inorganic and organic mercury in blood. *J. Assoc. off. anal. Chem.*, **55**, 966-971
- Magos, L. & Webb, M. (1977) The effects of selenium on the brain uptake of methylmercury. *Arch. Toxicol.*, **38**, 201-207
- Magos, L., Halbach, S. & Clarkson, T.W. (1978) Role of catalase in the oxidation of mercury vapor. *Biochem. Pharmacol.*, **27**, 1373-1377
- Magos, L., Sparrow, S. & Snowden, R. (1982) The comparative renotoxicology of phenylmercury and mercuric chloride. *Arch. Toxicol.*, **50**, 133-139
- Mailhes, J.B. (1983) Methylmercury effects on Syrian hamster metaphase II oocyte chromosomes. *Environ. Mutag.*, **5**, 679-686
- Manalis, R.S. & Cooper, G.P. (1975) Evoked transmitter release increased by inorganic mercury at frog neuromuscular junction. *Nature*, **257**, 690-691
- Mathew, C. & Al-Doori, Z. (1976) The mutagenic effect of the mercury fungicide Ceresan M in *Drosophila melanogaster*. *Mutat. Res.*, **40**, 31-36

- Matsumoto, N. & Spindle, A. (1982) Sensitivity of early mouse embryos to methylmercury toxicity. *Toxicol. appl. Pharmacol.*, **64**, 108–117
- McFarland, R.B. & Reigel, H. (1978) Chronic mercury poisoning from a single brief exposure. *J. occup. Med.*, **20**, 532–534
- McLaughlin, J.K., Malker, H.S.R., Blot, W.J., Malker, B.K., Stone, B.J., Weiner, J.A., Ericsson, J.L.E. & Fraumeni, J.F., Jr (1987) Occupational risks for intracranial gliomas in Sweden. *J. natl Cancer Inst.*, **78**, 253–257
- McNerney, J.J., Buseck, P.R. & Hanson, R.C. (1972) Mercury detection by means of thin gold films. *Science*, **178**, 611–612
- Miettinen, J.K. (1973) Absorption and elimination of dietary mercury (Hg^{++}) and methyl mercury in man. In: Miller, M.W. & Clarkson, T.W., eds, *Mercury, Mercurials and Mercaptans*, Springfield, IL, C.C. Thomas, pp. 233–243
- Milham, S., Jr (1976) *Occupational Mortality in Washington State 1950–1971*, Vols I and II, Washington DC, US Department of Health, Education, and Welfare
- Miller, C.T., Zawadzka, Z., Nagy, E. & Charbonneau, S.M. (1979) Indicators of genetic toxicity in leucocytes and granulocytic precursors after chronic methylmercury ingestion by cats. *Bull. environ. Contam. Toxicol.*, **21**, 296–303
- Miller, D.M., Lund, B.-O. & Woods, J.S. (1991) Reactivity of Hg(II) with superoxide: evidence for the catalytic dismutation of superoxide by Hg(II) . *J. Biochem. Toxicol.*, **6**, 293–298
- Minoia, C., Sabbioni, E., Apostoli, P., Pietra, R., Pozzoli, L., Gallorini, M., Nicolaou, G., Alessio, L. & Capodaglio, E. (1990) Trace elements reference values in tissues from inhabitants of the European Community. I. A study of 46 elements in urine, blood and serum of Italian subjects. *Sci. total Environ.*, **95**, 89–105
- Mitsumori, K., Maita, K., Saito, T., Tsuda, S. & Shirasu, Y. (1981) Carcinogenicity of methylmercury chloride in ICR mice: preliminary note on renal carcinogenesis. *Cancer Lett.*, **12**, 305–310
- Mitsumori, K., Takahashi, K., Matano, O., Goto, S. & Shirasu, Y. (1983) Chronic toxicity of methylmercury chloride in rats: clinical study and chemical analysis. *Jpn. J. vet. Sci.*, **45**, 747–757
- Mitsumori, K., Maita, K. & Shirasu, Y. (1984) Chronic toxicity of methylmercury chloride in rats: pathological study. *Jpn. J. vet. Sci.*, **46**, 549–557
- Mitsumori, K., Hirano, M., Ueda, H., Maita, K. & Shirasu, Y. (1990) Chronic toxicity and carcinogenicity of methylmercury chloride in B6C3F₁ mice. *Fundam. appl. Toxicol.*, **14**, 179–190
- Miyamoto, M.D. (1983) Hg^{2+} causes neurotoxicity at an intracellular site following entry through Na and Ca channels. *Brain Res.*, **267**, 375–379
- Møller-Madsen, B. (1992) Localization of mercury in CNS of the rat. V. Inhalation exposure to metallic mercury. *Arch. Toxicol.*, **66**, 79–89
- Møller-Madsen, B., Hansen, J.C. & Kragstrup, J. (1988) Mercury concentrations in blood from Danish dentists. *Scand. J. dent. Res.*, **96**, 56–59
- Monsalve, M.V. & Chiappe, C. (1987) Genetic effects of methylmercury in human chromosomes: I. A cytogenetic study of people exposed through eating contaminated fish. *Environ. mol. Mutag.*, **10**, 367–376
- Morimoto, K., Iijima, S. & Koizumi, A. (1982) Selenite prevents the induction of sister-chromatid exchanges by methyl mercury and mercuric chloride in human whole-blood cultures. *Mutat. Res.*, **102**, 183–192

- Mottet, N.K., Shaw, C.-M. & Burbacher, T.M. (1985) Health risks from increases in methylmercury exposure. *Environ. Health Perspectives*, **63**, 133-140
- Mottironi, V.D., Harrison, B., Pollara, B., Gooding, R. & Banks, S. (1986) Possible synergistic effect of mercury and smoking on sister-chromatid exchange (SCE) rates in humans (Abstract No. 1669). *Fed. Proc.*, **45**, 441
- Mudry de Pargament, M.D., Larripa, I., Labal de Vinuesa, M., Barlotti, M., De Biase, P. & Brioux de Salum, S. (1987) Sister chromatid exchange and accidental exposure to phenylmercury acetate (Fr.). *Bol. Estud. méd. biol. Méx.*, **35**, 207-211
- Müller, W.-U., Streffer, C. & Joos, A.L. (1990) Toxicity of cadmium sulphate and methylmercuric chloride applied singly or in combination to early mouse embryos *in vitro*. *Toxicol. in vitro*, **4**, 57-61
- Munro, I.C., Nera, E.A., Charbonneau, S.M., Junkins, B. & Zawidzka, Z. (1980) Chronic toxicity of methylmercury in the rat. *J. environ. Pathol. Toxicol.*, **3**, 437-447
- Naganuma, A., Koyama, Y. & Imura, N. (1980) Behavior of methylmercury in mammalian erythrocytes. *Toxicol. appl. Pharmacol.*, **54**, 405-410
- Nakada, S., Nomoto, A. & Imura, N. (1980) Effect of methylmercury and inorganic mercury on protein synthesis in mammalian cells. *Ecotoxicol. environ. Saf.*, **4**, 184-190
- Nakai, S. & Machida, I. (1973) Genetic effect of organic mercury on yeast (Abstract No. 7). *Mutat. Res.*, **21**, 348
- Naleway, C., Sakaguchi, R., Mitchell, E., Muller, T., Ayer, W.A. & Hefferren, J.J. (1985) Urinary mercury levels in US dentists, 1975-1983: review of health assessment program. *J. Am. Dent. Assoc.*, **111**, 37-42
- Naleway, C., Chou, H.-N., Muller, T., Dabney, J., Roxe, D. & Siddiqui, F. (1991) On-site screening for urinary Hg concentrations and correlation with glomerular and renal tubular function. *J. public Health Dent.*, **51**, 12-17
- Newton, D. & Fry, F.A. (1978) The retention and distribution of radioactive mercuric oxide following accidental inhalation. *Ann. occup. Hyg.*, **21**, 21-32
- Nielsen, J.B. & Andersen, O. (1991) A comparison of the effects of sodium selenite and seleno-L-methionine on disposition of orally-administered mercuric chloride. *J. Trace Elem. Electrolytes Health Dis.*, **5**, 245-250
- Nilsson, B., Gerhardsson, L. & Nordberg, G.F. (1990) Urine mercury levels and associated symptoms in dental personnel. *Sci. total Environ.*, **94**, 179-185
- Nishiyama, S., Taguchi, T. & Onosaka, S. (1987) Induction of zinc-thionein by estradiol and protective effects on inorganic mercury-induced renal toxicity. *Biochem. Pharmacol.*, **36**, 3387-3391
- Nixon, J.E., Koller, L.D. & Exon, J.H. (1979) Effect of methylmercury chloride on transplacental tumors induced by sodium nitrite and ethylurea in rats. *J. natl Cancer Inst.*, **63**, 1057-1063
- Nordberg, G.F. & Skerfving, S. (1972) Metabolism. In: Friberg, L. & Vostal, J., eds, *Mercury in the Environment. An Epidemiological and Toxicological Appraisal*, Cleveland, OH, CRC Press, pp. 29-91
- Norseth, T. (1971) Biotransformation of methyl mercuric salts in the mouse studied by specific determination of inorganic mercury. *Acta pharmacol. toxicol.*, **29**, 375-384
- Norseth, T. & Clarkson, T.W. (1971) Intestinal transport of ²⁰³Hg-labeled methyl mercury chloride. Role of biotransformation in rats. *Arch. environ. Health*, **22**, 568-577
- Nriagu, J.O., Pfeiffer, W.C., Malm, O., de Souza, C.M.M. & Mierle, G. (1992) Mercury pollution in Brazil (Letter to the Editor). *Nature*, **356**, 389

- Nylander, M. & Weiner, J. (1991) Mercury and selenium concentrations and their interrelations in organs from dental staff and the general population. *Br. J. ind. Med.*, **48**, 729–734
- Oberly, T.J., Piper, C.E. & McDonald, D.S. (1982) Mutagenicity of metal salts in the L5178Y mouse lymphoma assay. *J. Toxicol. environ. Health*, **9**, 367–376
- Önfelt, A. (1983) Spindle disturbances in mammalian cells. I. Changes in the quantity of free sulfhydryl groups in relation to survival and c-mitosis in V79 Chinese hamster cells after treatment with colcemid, diamide, carbaryl and methyl mercury. *Chem.-biol. Interactions*, **46**, 201–217
- Önfelt, A. & Jenssen, D. (1982) Enhanced mutagenic response of MNU by post-treatment with methylmercury, caffeine or thymidine in V79 Chinese hamster cells. *Mutat. Res.*, **106**, 297–303
- Orlowski, J.P. & Mercer, R.D. (1980) Urine mercury levels in Kawasaki disease. *Pediatrics*, **66**, 633–636
- Osgood, C., Zimmering, S. & Mason, J.M. (1991) Aneuploidy in *Drosophila*. II. Further validation of the FIX and ZESTE genetic test systems employing female *Drosophila melanogaster*. *Mutat. Res.*, **259**, 147–163
- Panda, B.B., Das, B.L., Lenka, M. & Panda, K.K. (1988) Water hyacinth (*Eichhornia crassipes*) to biomonitor genotoxicity of low levels of mercury in aquatic environment. *Mutat. Res.*, **206**, 275–279
- Parizek, J. & Ostádalová, I. (1967) The protective effect of small amounts of selenite in sublimate intoxication. *Experientia*, **23**, 142–143
- Parkin, J.E. (1987) Assay of phenylmercury salts in pharmaceutical products by high-performance liquid chromatography of the morpholinedithiocarbamate derivative. *J. Chromatogr.*, **407**, 389–392
- Pelletier, L., Pasquier, R., Hirsch, F., Sapin, C. & Druet, P. (1986) Autoreactive T cells in mercury-induced autoimmune disease: in vitro demonstration. *J. Immunol.*, **137**, 2548–2554
- Pelletier, L., Pasquier, R., Rossert, J. & Druet, P. (1987a) HgCl₂ induced nonspecific immunosuppression in Lewis rats. *Eur. J. Immunol.*, **17**, 49–54
- Pelletier, L., Galceran, M., Pasquier, R., Ronco, P., Verroust, P., Bariety, J. & Druet, P. (1987b) Down modulation of Heymann's nephritis by mercuric chloride. *Kidney int.*, **32**, 227–232
- Perry, D.M., Weis, J.S. & Weis, P. (1988) Cytogenetic effects of methylmercury in embryos of the killifish, *Fundulus heteroclitus*. *Arch. environ. Contam. Toxicol.*, **17**, 569–574
- Petersson, K., Dock, L. & Vahter, M. (1989) Metabolism of methylmercury in rabbits and hamsters. *Biol. Trace Elem. Res.*, **21**, 219–226
- Pfeiffer, W.C., de Lacerda, L.D., Malm, O., Souza, C.M.M., da Silveira, E.G. & Bastos, W.R. (1989) Mercury concentrations in inland waters of gold-mining areas in Rondônia, Brazil. *Sci. total Environ.*, **87/88**, 233–240
- Phipps, J. & Miller, D.R. (1982) Some aspects of the genetic toxicity of methylmercury in yeasts (Fr.). *C.R. Acad. Sci. Paris Ser. III*, **295**, 683–686
- Phipps, J. & Miller, D.R. (1983) Genetic toxicity of methylmercury chloride (CH₃HgCl) on mitochondria of *Saccharomyces cerevisiae* (Fr.). *Can. J. Microbiol.*, **29**, 1149–1153
- Poma, K., Kirsch-Volders, M. & Susanne, C. (1981) Mutagenicity study on mice given mercuric chloride. *J. appl. Toxicol.*, **1**, 314–316
- Popescu, H.I., Negru, L. & Lancranjan, I. (1979) Chromosomal aberrations induced by occupational exposure to mercury. *Arch. environ. Health*, **34**, 461–463
- Prickett, C.S., Laug, E.P. & Kunze, F.M. (1950) Distribution of mercury in rats following oral and intravenous administration of mercuric acetate and phenylmercuric acetate. *Proc. Soc. exp. Biol. Med.*, **73**, 585–588

- Rahola, T., Hattula, T., Korolainen, A. & Miettinen, J.K. (1973) Elimination of free and protein-bound ionic mercury ($^{203}\text{Hg}^{2+}$) in man. *Ann. clin. Res.*, **5**, 214-219
- Ramel, C. (1969) Genetic effects of organic mercury compounds. I. Cytological investigations on *Allium* roots. *Hereditas*, **61**, 208-230
- Ramel, C. (1972) Genetic effects. In: Friberg, L. & Vostal, D., eds, *Mercury in the Environment: Toxicological Effects and Epidemiological and Toxicological Appraisal*, Cleveland, OH, CRC Press, pp. 169-181
- Ramel, C. & Magnusson, J. (1969) Genetic effects of organic mercury compounds. II. Chromosome segregation in *Drosophila melanogaster*. *Hereditas*, **61**, 231-254
- Ramel, C. & Magnusson, J. (1979) Chemical induction of nondisjunction in *Drosophila*. *Environ. Health Perspectives*, **31**, 59-66
- Rasmussen, G. (1984) *Kviksølv: Tunkonserves 1988* [Mercury in Tinned Tuna Fish 1983], Copenhagen, National Food Agency (in Danish)
- Reese, R.G., Jr (1990) Mercury. In: *Mineral Commodity Summaries 1990*, Washington DC, Bureau of Mines, US Department of the Interior, pp. 108-109
- Reese, R.G., Jr (1991) Mercury. In: *Mineral Commodity Summaries 1991*, Washington DC, Bureau of Mines, US Department of the Interior, pp. 102-103
- Reese, R.G., Jr (1992a) *Mineral Industry Surveys: Annual Review—Mercury in 1991*, Washington DC, Bureau of Mines, US Department of the Interior
- Reese, R.G., Jr (1992b) Mercury. In: *Mineral Commodity Summaries 1992*, Washington DC, Bureau of Mines, US Department of the Interior, pp. 112-113
- Refsvik, T. & Norseth, T. (1975) Methyl mercuric compounds in rat bile. *Acta pharmacol. toxicol.*, **36**, 67-78
- Reuhl, K.R. & Chang, L.W. (1979) Effects of methylmercury on the development of the nervous system: a review. *Neurotoxicology*, **1**, 21-55
- Reuhl, K.R., Chang, L.W. & Townsend, J.W. (1981) Pathological effects of in utero methylmercury exposure on the cerebellum of the golden hamster. I. Early effects upon the neonatal cerebellar cortex. *Environ. Res.*, **26**, 281-306
- Rice, D.C. (1992) Effects of pre- plus postnatal exposure to methylmercury in the monkey on fixed interval and discrimination reversal performance. *NeuroToxicology*, **13**, 443-452
- Richter, E.D., Peled, N. & Luria, M. (1982) Mercury exposure and effects at a thermometer factory. *Scand. J. Work Environ. Health*, **8** (Suppl. 1), 161-166
- Robbins, M.S., Hughes, J.A., Sparber, S.B. & Mannering, G.J. (1978) Delayed teratogenic effect of methylmercury on hepatic cytochrome P-450-dependent monooxygenase systems of rats. *Life Sci.*, **22**, 287-293
- Robinson, J.W. & Skelly, E.M. (1982) Speciation of mercury compounds by differential atomization-atomic absorption spectroscopy. *J. environ. Health Sci.*, **A17**, 391-425
- Robison, S.H., Cantoni, O. & Costa, M. (1982) Strand breakage and decreased molecular weight of DNA induced by specific metal compounds. *Carcinogenesis*, **3**, 657-662
- Robison, S.H., Cantoni, O. & Costa, M. (1984) Analysis of metal-induced DNA lesions and DNA-repair replication in mammalian cells. *Mutat. Res.*, **131**, 173-181
- Roeleveld, N., Zielhuis, G.A. & Gabreëls, F. (1990) Occupational exposure and defects of the central nervous system in offspring: review. *Br. J. ind. Med.*, **47**, 580-588

- Roels, H., Gennart, J.-P., Lauwerys, R., Buchet, J.-P., Malchaire, J. & Bernard, A. (1985) Surveillance of workers exposed to mercury vapour: validation of a previously proposed biological threshold limit value for mercury concentration in urine. *Am. J. ind. Med.*, **7**, 45-71
- Roels, H., Abdeladim, S., Ceulemans, E. & Lauwerys, R. (1987) Relationships between the concentrations of mercury in air and in blood or urine in workers exposed to mercury vapour. *Ann. occup. Hyg.*, **31**, 135-145
- Rossman, T.G., Molina, M., Meyer, L., Boone, P., Klein, C.B., Wang, Z., Li, F., Lin, W.C. & Kinney, P.L. (1991) Performance of 133 compounds in the lambda prophage induction endpoint of the Microscreen assay and a comparison with *S. typhimurium* mutagenicity and rodent carcinogenicity assays. *Mutat. Res.*, **260**, 349-367
- Rowland, I.R., Davies, M.J. & Evans, J.G. (1980) Tissue content of mercury in rats given methylmercuric chloride orally: influence of intestinal flora. *Arch. environ. Health*, **35**, 155-160
- Rustam, H., Von Burg, R., Amin-Zaki, L. & El Hassani, S. (1975) Evidence for a neuromuscular disorder in methylmercury poisoning. Clinical and electrophysiological findings in moderate to severe cases. *Arch. environ. Health*, **30**, 190-195
- Ryan, P., Lee, M.W., North, B. & McMichael, A.J. (1992) Amalgam fillings, diagnostic dental x-rays and tumours of the brain and meninges. *Oral Oncol. Eur. J. Cancer*, **28B**, 91-95
- Saillenfait, A.M., Languon, I., Sabate, J.P. & de Ceaurriz, J. (1990) Interaction between mercuric chloride and zinc in rat whole-embryo culture. *Toxicol. in vitro*, **4**, 129-136
- Sällsten, G., Barregård, L., Langworth, S. & Vesterberg, O. (1992) Exposure to mercury in industry and dentistry: a field comparison between diffusive and active samplers. *Appl. occup. environ. Hyg.*, **7**, 434-440
- Sass, J.E. (1937) Histological and cytological studies of ethyl mercury phosphate poisoning in corn seedlings. *Phytopathology*, **27**, 95-99
- Sax, N.I. & Lewis, R.J. (1987) *Hawley's Condensed Chemical Dictionary*, 11th Ed., New York, Van Nostrand Reinhold, pp. 741-742, 745-746, 904
- Schaller, K.-H., Triebig, G., Schiele, R. & Valentin, H. (1991) Biological monitoring and health surveillance of workers exposed to mercury. In: Dillon, H.K. & Ho, M.H., eds, *Biological Monitoring of Exposure to Chemical Metals*, New York, John Wiley & Sons, pp. 3-9
- Schardein, J.L. (1985) *Chemically Induced Birth Defects*, New York, Marcel Dekker, pp. 622-632
- Schroeder, H.A. & Mitchener, M. (1975) Life-term effects of mercury, methyl mercury and nine other trace metals on mice. *J. Nutr.*, **105**, 452-458
- Sequi, P. (1980) The behaviour of chromium and mercury in soil (Ital.). In: Frigerio A., ed., *Rischi e Tossicità dell'Inquinamento da Metalli: Cromo e Mercurio*, Milan, DiEsseTi Publications, pp. 27-50
- Shepard, T.H. (1992) *Catalog of Teratogenic Agents*, 7th Ed., Baltimore, MD, Johns Hopkins University Press, pp. 249-251
- Shirasu, Y., Moriya, M., Kato, K., Furuhashi, A. & Kada, T. (1976) Mutagenicity screening of pesticides in the microbial system. *Mutat. Res.*, **40**, 19-30
- Siemiątycki, J., ed. (1991) *Risk Factors for Cancer in the Workplace*, Boca Raton, FL, CRC Press
- Sikorski, R., Juszkiewicz, T., Paszkowski, T. & Szprengier-Juszkiewicz, T. (1987) Women in dental surgeries: reproductive hazards in occupational exposure to metallic mercury. *Int. Arch. occup. environ. Health*, **59**, 551-557

- Simon, M., Jönk, P., Wühl-Couturier, G. & Daunderer, M. (1990) Mercury, mercury alloys, and mercury compounds. In: Elvers, B., Hawkins, S. & Schulz, G., eds, *Ullmann's Encyclopedia of Industrial Chemistry*, Vol. A16, *Magnetic Materials to Mutagenic Agents*, New York, VCH Publishers, pp. 269–298
- Singer, W. & Nowak, M. (1981) Mercury compounds. In: Mark, H.F., Othmer, D.F., Overberger, C.G., Seaborg, G.T. & Grayson, N., eds, *Kirk-Othmer Encyclopedia of Chemical Technology*, 3rd Ed., Vol. 15, New York, John Wiley & Sons, pp. 157–171
- Skerfving, S. (1974) Methylmercury exposure, mercury levels in blood and hair, and health status in Swedes consuming contaminated fish. *Toxicology*, **2**, 3–23
- Skerfving, S. (1988) Mercury in women exposed to methylmercury through fish consumption, and in their newborn babies and breast milk. *Bull. environ. Contam. Toxicol.*, **41**, 475–482
- Skerfving, S. & Vostal, J. (1972) Symptoms and signs of intoxication. In: Friberg, L. & Vostal, J., eds, *Mercury in the Environment*, Cleveland, OH, CRC Press, pp. 93–107
- Skerfving, S., Hansson, K. & Lindsten, J. (1970) Chromosome breakage in humans exposed to methylmercury through fish consumption. Preliminary communication. *Arch. environ. Health*, **21**, 133–139
- Skerfving, S., Hansson, K., Mangs, C., Lindsten, J. & Ryman, N. (1974) Methylmercury-induced chromosome damage in man. *Environ. Res.*, **7**, 83–98
- Slotkin, T.A., Kavlock, R.J., Cowdery, T., Orband, L., Bartolome, M., Gray, J.A., Rehnberg, B.F. & Bartolome, J. (1986) Functional consequences of prenatal methylmercury exposure: effects on renal and hepatic responses to trophic stimuli and on renal excretory mechanisms. *Toxicol. Lett.*, **34**, 231–345
- Smith, R.G., Vorwald, A.J., Patil, L.S. & Mooney, T.F., Jr (1970) Effects of exposure to mercury in the manufacture of chlorine. *Am. ind. Hyg. Assoc. J.*, **31**, 687–700
- Smith, J.H., McCormack, K.M., Braselton, W.E., Jr & Hook, J.B. (1983) The effect of prenatal methylmercury administration on postnatal renal functional development. *Environ. Res.*, **30**, 63–71
- Snell, K., Ashby, S.L. & Barton, S.J. (1977) Disturbances of perinatal carbohydrate metabolism in rats exposed to methylmercury *in utero*. *Toxicology*, **8**, 277–283
- Southard, J.H. & Nitisewojo, P. (1973) Loss of oxidative phosphorylation in mitochondria isolated from kidneys of mercury poisoned rats. *Biochem. biophys. Res. Commun.*, **52**, 921–927
- Spyker, J.M., Sparber, S.B. & Goldberg, A.M. (1972) Subtle consequences of methylmercury exposure: behavioral deviations in offspring of treated mothers. *Science*, **177**, 621–623
- Stewart, W.K., Guirgis, H.A., Sanderson, J. & Taylor, W. (1977) Urinary mercury excretion and proteinuria in pathology laboratory staff. *Br. J. ind. Med.*, **34**, 26–31
- Stokinger, H.E. (1981) Mercury, Hg. In: Clayton, G.D. & Clayton, F.L., eds, *Patty's Industrial Hygiene and Toxicology*, 3rd rev. Ed., Vol. 2A, New York, John Wiley & Sons, pp. 1769–1792
- Strem Chemicals (1992) *Catalog No. 14—Metals, Inorganics and Organometallics for Research*, Newburyport, MA, pp. 70–72
- Su, M.-Q. & Okita, G.T. (1976a) Behavioral effects on the progeny of mice treated with methylmercury. *Toxicol. appl. Pharmacol.*, **38**, 195–205
- Suda, I. & Hirayama, K. (1992) Degradation of methyl and ethyl mercury into inorganic mercury by hydroxyl radical produced from rat liver microsomes. *Arch. Toxicol.*, **66**, 398–402
- Suda, I., Totoki, S. & Takahashi, H. (1991) Degradation of methyl and ethyl mercury into inorganic mercury by oxygen free radical-producing systems: involvement of hydroxyl radical. *Arch. Toxicol.*, **65**, 129–134

- Sundberg, J., Oskarsson, A. & Bergman, K. (1991) Milk transfer of inorganic mercury to suckling rats. Interaction with selenite. *Biol. Trace Elem. Res.*, **28**, 27–38
- Suter, K.E. (1975) Studies on the dominant-lethal and fertility effects of the heavy metal compounds methylmercuric hydroxide, mercuric chloride, and cadmium chloride in male and female mice. *Mutat. Res.*, **30**, 365–374
- Suzuki, T. & Tanaka, A. (1971) Absorption of metallic mercury from the intestine after rupture of Miller–Abbot balloon. *Jpn. J. ind. Health*, **13**, 222–223
- Suzuki, T., Yonemoto, J., Satoh, H., Naganuma, A., Imura, N. & Kigawa, T. (1984) Normal organic and inorganic mercury levels in the human feto-placental system. *J. appl. Toxicol.*, **4**, 249–252
- Swedish Expert Group (1970) Metallic mercury in fish. A toxicological–epidemiological risk evaluation. Report from an expert group. *Nord. Hyg. Tidskr.*, Suppl. 3
- Tamashiro, H., Arakaki, M., Futatsuka, M. & Lee, E.S. (1986) Methylmercury exposure and mortality in southern Japan: a close look at causes of death. *J. Epidemiol. Community Health*, **40**, 181–185
- Tamashiro, H., Fukutomi, K. & Lee, E.S. (1987) Methylmercury exposure and mortality in Japan: a life table analysis. *Arch. environ. Health*, **42**, 100–107
- Tazima, Y. (1974) Attempts to induce non-disjunction by means of irradiation and chemical treatment in the silkworm (Abstract No. E-15-8). *Radiat. Res.*, **59**, 267–268
- Thorp, J.M., Jr, Boyette, D.D., Watson, W.J. & Cefalo, R.C. (1992) Elemental mercury exposure in early pregnancy. *Obstet. Gynecol.*, **79**, 874–876
- Tournamille, J., Caporiccio, B., Michel, R. & Sentein, P. (1982) Action of methylmercury chloride on mitosis of human lymphocytes in culture: ultrastructural study (Fr.). *C.R. Soc. Biol.*, **176**, 194–203
- Troen, P., Kaufman, S.A. & Katz, K.H. (1951) Mercuric bichloride poisoning. *New Engl. J. Med.*, **244**, 459–463
- Tunnessen, W.W., Jr, McMahon, K.J. & Baser, M. (1987) Acrodynia: exposure to mercury from fluorescent light bulbs. *Pediatrics*, **79**, 786–789
- Ulfvarson, U. (1962) Distribution and excretion of some mercury compounds after long term exposure. *Int. Arch. Gewerbepathol. Gewerbehyg.*, **19**, 412–422
- Umeda, M., Saito, K., Hirose, K. & Saito, M. (1969) Cytotoxic effect of inorganic, phenyl, and alkyl mercuric compounds on HeLa cells. *Jpn. J. exp. Med.*, **39**, 47–58
- UNEP (1993) *IRPTC PC Database*, Geneva
- US Department of Health and Human Services (1992) *Cosmetics Handbook*, Washington DC, p. 16
- US Department of Health and Human Services (1993) *Dental Amalgam: A Scientific Review and Recommended Public Health Service Strategy for Research, Education and Regulation*, Washington DC, US Public Health Service
- US Environmental Protection Agency (1986a) Method 7470. Mercury in liquid waste (manual cold-vapor technique). In: *Test Methods for Evaluating Solid Waste—Physical/Chemical Methods*, 3rd Ed. (US EPA No. SW-846), Vol. 1A, Washington DC, Office of Solid Waste and Emergency Response, pp. 7470-1–7470-8
- US Environmental Protection Agency (1986b) Method 7471. Mercury in solid or semisolid waste (manual cold-vapor technique). In: *Test Methods for Evaluating Solid Waste—Physical/Chemical Methods*, 3rd Ed. (US EPA No. SW-846), Vol. 1A, Washington DC, Office of Solid Waste and Emergency Response, pp. 7471-1–74701-10
- US Environmental Protection Agency (1991) Maximum contaminant levels for inorganic chemicals. *US Code fed. Regul.*, Title **40**, pp. 585–586
- US Environmental Protection Agency (1992) National emission standard for mercury. *US Code fed. Regul.*, Title **40**, pp. 26–32

- US Food and Drug Administration (1992) Standards of quality—bottled water. *US Code fed. Regul.*, **Title 21**, pp. 61–64
- US National Institute for Occupational Safety and Health (1990) *NIOSH Pocket Guide to Chemical Hazards* (DHHS (NIOSH) Publication No. 90-117), Cincinnati, OH, pp. 140–141
- US National Toxicology Program (1993) *Toxicology and Carcinogenesis Studies of Mercuric Chloride (CAS No. 7487-14-7) in F344 Rats and B6C3F₁ Mice (Gavage Studies)* (NTP TR 408; NIH Publication No. 93-3139), Research Triangle Park, NC, US Department of Health and Human Services
- US Occupational Safety and Health Administration (1989) Air contaminants—permissible exposure limits. *US Code fed. Regul.*, **Title 29**, p. 601
- Vachhrajani, K.D., Chowdhury, A.R. & Dutta, K.K. (1992) Testicular toxicity of methylmercury: analysis of cellular distribution pattern at different stages of the seminiferous epithelium. *Reprod. Toxicol.*, **6**, 355–361
- Verschaeve, L. & Léonard, A. (1984) Dominant lethal test in female mice treated with methyl mercury chloride. *Mutat. Res.*, **136**, 131–136
- Verschaeve, L. & Susanne, C. (1979) Genetic hazards of mercury exposure in dental surgery (Abstract No. 81). *Mutat. Res.*, **64**, 149
- Verschaeve, L., Kirsch-Volders, M., Susanne, C., Groetenbriel, C., Haustermans, R., Lecomte, A. & Roossels, D. (1976) Genetic damage induced by occupationally low mercury exposure. *Environ. Res.*, **12**, 306–316
- Verschaeve, L., Kirsch-Volders, M., Hens, L. & Susanne, C. (1978) Chromosome distribution studies in phenylmercury acetate exposed subjects and in age-related controls. *Mutat. Res.*, **57**, 335–347
- Verschaeve, L., Tassignon, J.-P., Lefevre, M., De Stoop, P. & Susanne, C. (1979) Cytogenetic investigation of leukocytes of workers exposed to metallic mercury. *Environ. Mutag.*, **1**, 259–268
- Verschaeve, L., Kirsch-Volders, M. & Susanne, C. (1984) Mercury-induced segregational errors of chromosomes in human lymphocytes and in Indian muntjac cells. *Toxicol. Lett.*, **21**, 247–253
- Verschaeve, L., Kirsch-Volders, M., Hens, L. & Susanne, C. (1985) Comparative in vitro cytogenetic studies in mercury-exposed human lymphocytes. *Mutat. Res.* **157**, 221–226
- Verschuuren, H.G., Kroes, R., Den Tonkelaar, E.M., Berkvens, J.M., Helleman, P.W., Rauws, A.G., Schuller, P.L. & Van Esch, G.J. (1976a) Toxicity of methylmercury chloride in rats. I. Short-term study. *Toxicology*, **6**, 85–96
- Verschuuren, H.G., Kroes, R., Den Tonkelaar, E.M., Berkvens, J.M., Helleman, P.W., Rauws, A.G., Schuller, P.L. & Van Esch, G.J. (1976b) Toxicity of methylmercury chloride in rats. III. Long-term toxicity study. *Toxicologist*, **6**, 107–123
- Verschuuren, H.G., Kroes, R., Den Tonkelaar, E.M., Berkvens, J.M., Helleman, P.W., Rauws, A.G., Schuller, P.L. & Van Esch, G.J. (1976c) Toxicity of methylmercury chloride in rats. II. Reproduction study. *Toxicology*, **6**, 97–106
- Vouk, V.B., Fugaš, M. & Topolnik, Z. (1950) Environmental conditions in the mercury mine of Idria. *Br. J. Med.*, **7**, 168–176
- Wahlberg, J.E. (1965) Percutaneous toxicity of metal compounds. A comparative investigation in guinea pigs. *Arch. environ. Health*, **11**, 201–204
- Warkany, J. (1966) Acrodynia—postmortem of a disease. *Am. J. Dis. Child.*, **112**, 147–156
- Watanabe, T., Shimada, T. & Endo, A. (1982) Effects of mercury compounds on ovulation and meiotic and mitotic chromosomes in female golden hamsters. *Teratology*, **25**, 381–384
- Weed, L.A. & Ecker, E.E. (1933) Phenyl-mercuric compounds. Their action on animals and their preservative values. *J. infect. Dis.*, **52**, 354–362

- Weinberg, J.M., Harding, P.G. & Humes, H.D. (1982a) Mitochondrial bioenergetics during the initiation of mercuric chloride-induced renal injury. I. Direct effects of in-vitro mercuric chloride on renal cortical mitochondrial function. *J. biol. Chem.*, **257**, 60–67
- Weinberg, J.M., Harding, P.G. & Humes, H.D. (1982b) Mitochondrial bioenergetics during the initiation of mercuric chloride-induced renal injury. II. Functional alterations of renal cortical mitochondria isolated after mercuric chloride treatment. *J. biol. Chem.*, **257**, 68–74
- WHO (1976) *Mercury* (Environmental Health Criteria 1), Geneva
- WHO (1980) *Exposure to Heavy Metals* (Tech. Rep. Series 647), Geneva, p. 128
- WHO (1988) *Emission of Heavy Metal and PAH Compounds from Municipal Solid Waste Incinerators: Control Technology and Health Effects*, Copenhagen, pp. 23, 40
- WHO (1989a) *Mercury—Environmental Aspects* (Environmental Health Criteria 86), Geneva
- WHO (1989b) *Evaluation of Certain Food Additives and Contaminants* (Tech. Rep. Series 776), Geneva, pp. 33–34
- WHO (1990) *Methylmercury* (Environmental Health Criteria 101), Geneva
- WHO (1991) *Inorganic Mercury* (Environmental Health Criteria 118), Geneva
- WHO (1992) *Guidelines for Drinking-water Quality. Tables of Guideline Values*, Geneva, p. 2
- Wiklund, K., Dich, J., Holm, L.-E. & Eklund, G. (1988) Risk of tumors of the nervous system among mercury and other seed disinfectant applicators in Swedish agriculture (Letter to the Editor). *Acta oncol.*, **27**, 865
- Worthing, C.R., ed. (1987) *The Pesticide Manual. A World Compendium*, 8th Ed., Thornton Heath, British Crop Protection Council, pp. 658–659
- Wulf, H.C., Kromann, N., Kousgaard, N., Hansen, J.C., Niebuhr, E. & Albøge, K. (1986) Sister chromatid exchange (SCE) in Greenlandic Eskimos. Dose-response relationship between SCE and seal diet, smoking, and blood cadmium and mercury concentrations. *Sci. total Environ.*, **48**, 81–94
- Yamamoto, M., Endoh, K., Toyama, S., Sakai, H., Shibuya, N., Takagi, S., Magara, J. & Fujiguchi, K. (1986) Biliary tract cancers in Japan: a study from the point of view of environmental epidemiology. *Acta med. biol.*, **34**, 65–76
- Yatscoff, R.W. & Cummins, J.E. (1975) DNA breakage caused by dimethylmercury and its repair in a slime mould, *Physarum polycephalum*. *Nature*, **257**, 422–423
- Yoshida, M. (1985) Relation of mercury exposure to elemental mercury levels in the urine and blood. *Scand. J. Work Environ. Health*, **11**, 33–37
- Yoshida, M., Satoh, H., Kishimoto, T. & Yamamura, Y. (1992) Exposure to mercury via breast milk in suckling offspring of maternal guinea pigs exposed to mercury vapor after parturition. *J. Toxicol. environ. Health*, **35**, 135–139
- Zasukhina, G.D., Vasilyeva, I.M., Sdirkova, N.I., Krasovsky, G.N., Vasyukovich, L.Y., Kenesariyev, U.I. & Butenko, P.G. (1983) Mutagenic effect of thallium and mercury salts on rodent cells with different repair activities. *Mutat. Res.*, **124**, 163–173
- Zoll, C., Saouter, E., Boudou, A., Ribeyre, F. & Jaylet, A. (1988) Genotoxicity and bioaccumulation of methyl mercury and mercuric chloride *in vivo* in the newt *Pleurodeles waltl*. *Mutagenesis*, **3**, 337–343

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 $0.2 \mu\text{m}$ ([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 $910\text{--}920^\circ\text{C}$ 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'-Nitrosoornicotine (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](#)).

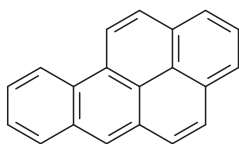
In summary, cigarette smoke is an exceedingly complex mixture which contains over 5300

compounds including multiple toxicants and carcinogens.

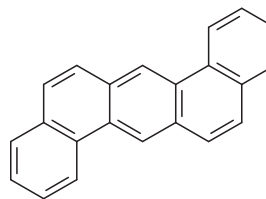
1.2.2 Smoke from other tobacco products

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](#)).

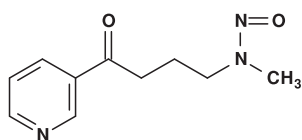
Fig. 1.1 Structures of some representative tobacco smoke carcinogens



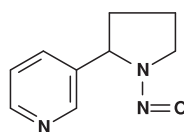
benzo[a]pyrene (BaP)



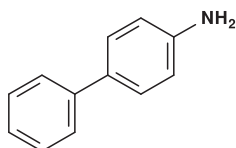
dibenz[a,h]anthracene



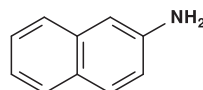
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)



N'-nitrososornicotine (NNN)



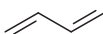
4-aminobiphenyl



2-naphtylamine



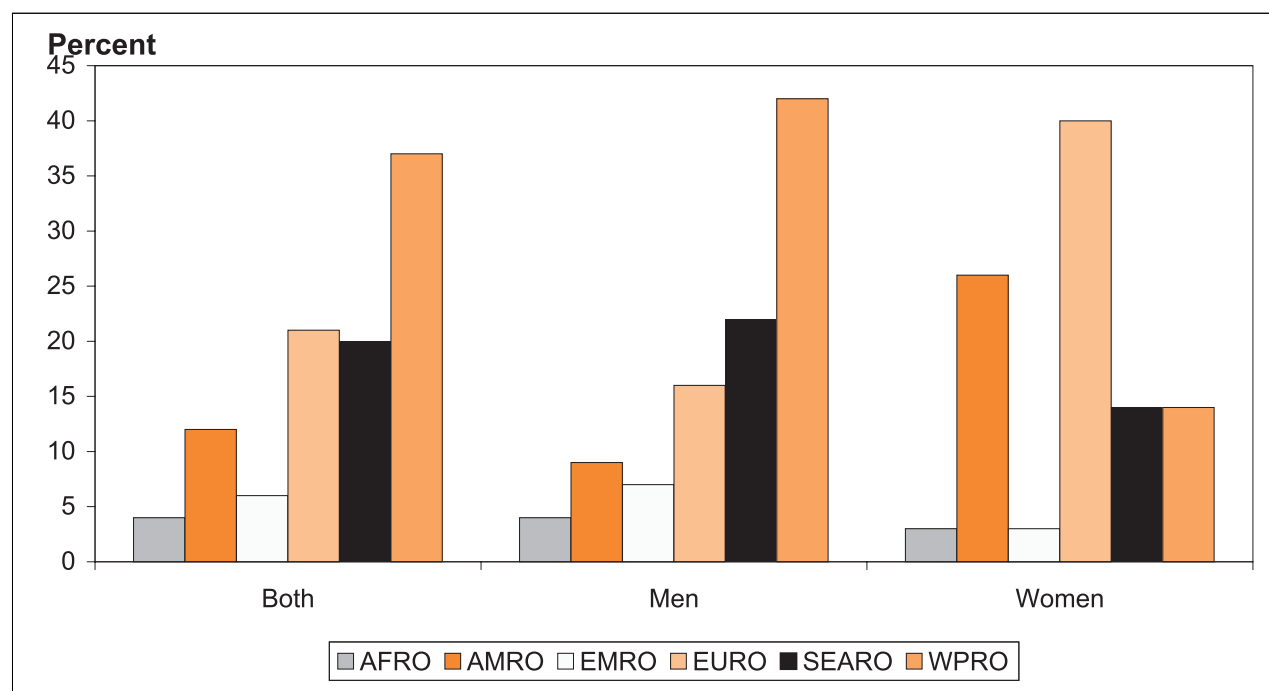
benzene



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](http://www.who.int/2011)). The six WHO regions are: EMRO, Eastern Mediterranean Region; EURO, European

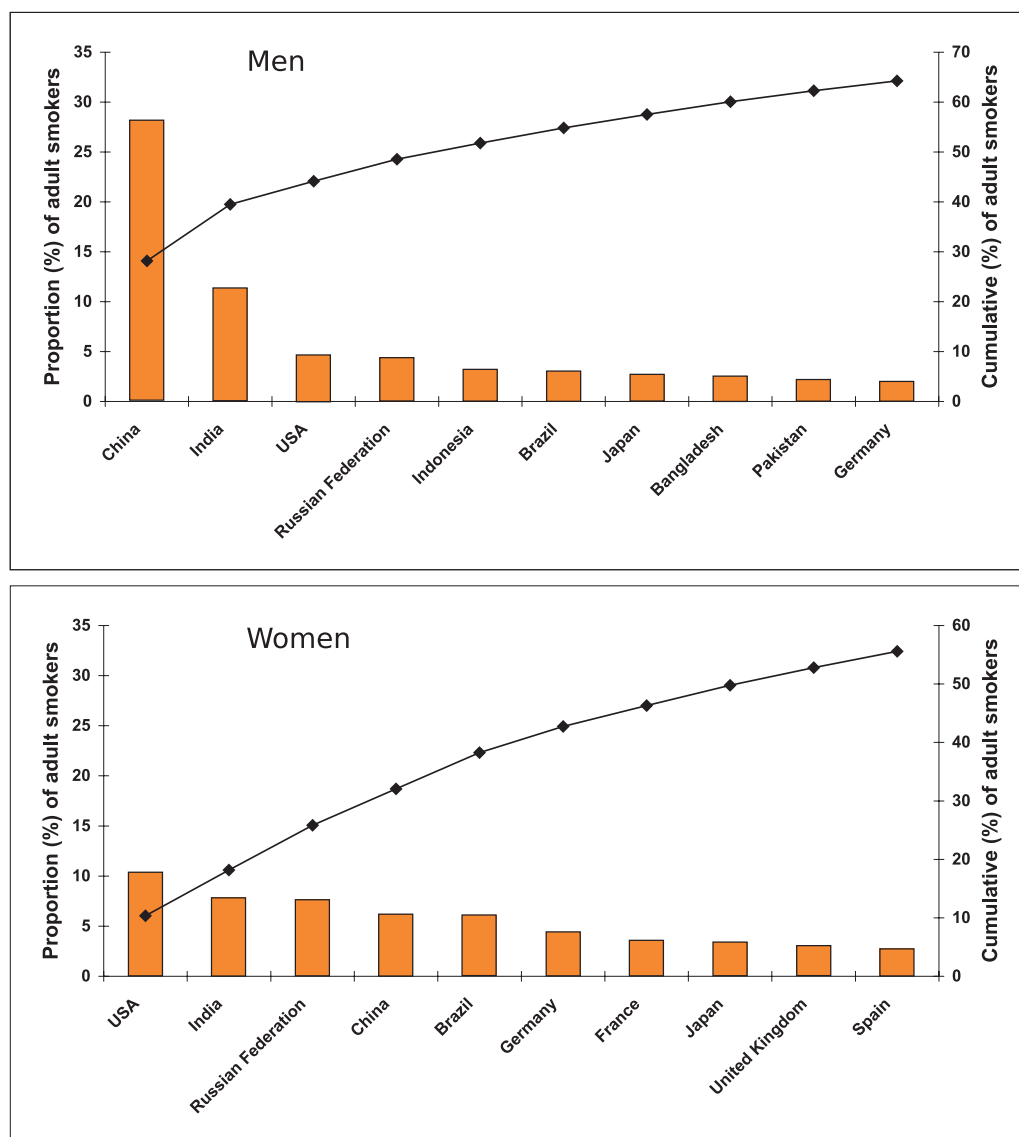
Region; AFRO, African Region; WPRO, Western Pacific Region; SEARO, South East Asian Region; AMRO, Region of the Americas. A listing of the countries in each region can be viewed at <http://www.who.int/about/structure/en/index.html>.

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).

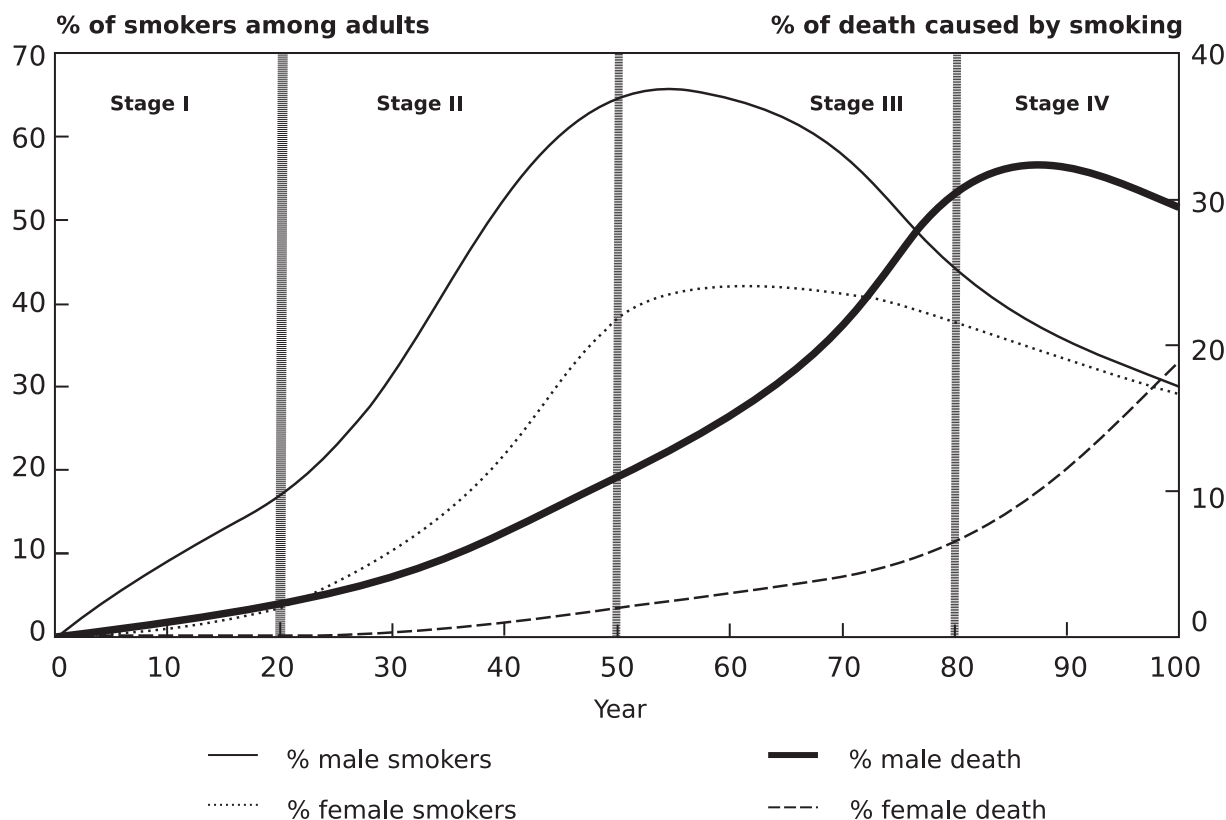
Stage 1 is characterized by low smoking prevalence in men (less than 15%) and very low in women (less than 10%). Death and disease from smoking are not apparent in this phase, as nearly all health effects from smoking are related to past smoking habits and their cumulative effects rather than current 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

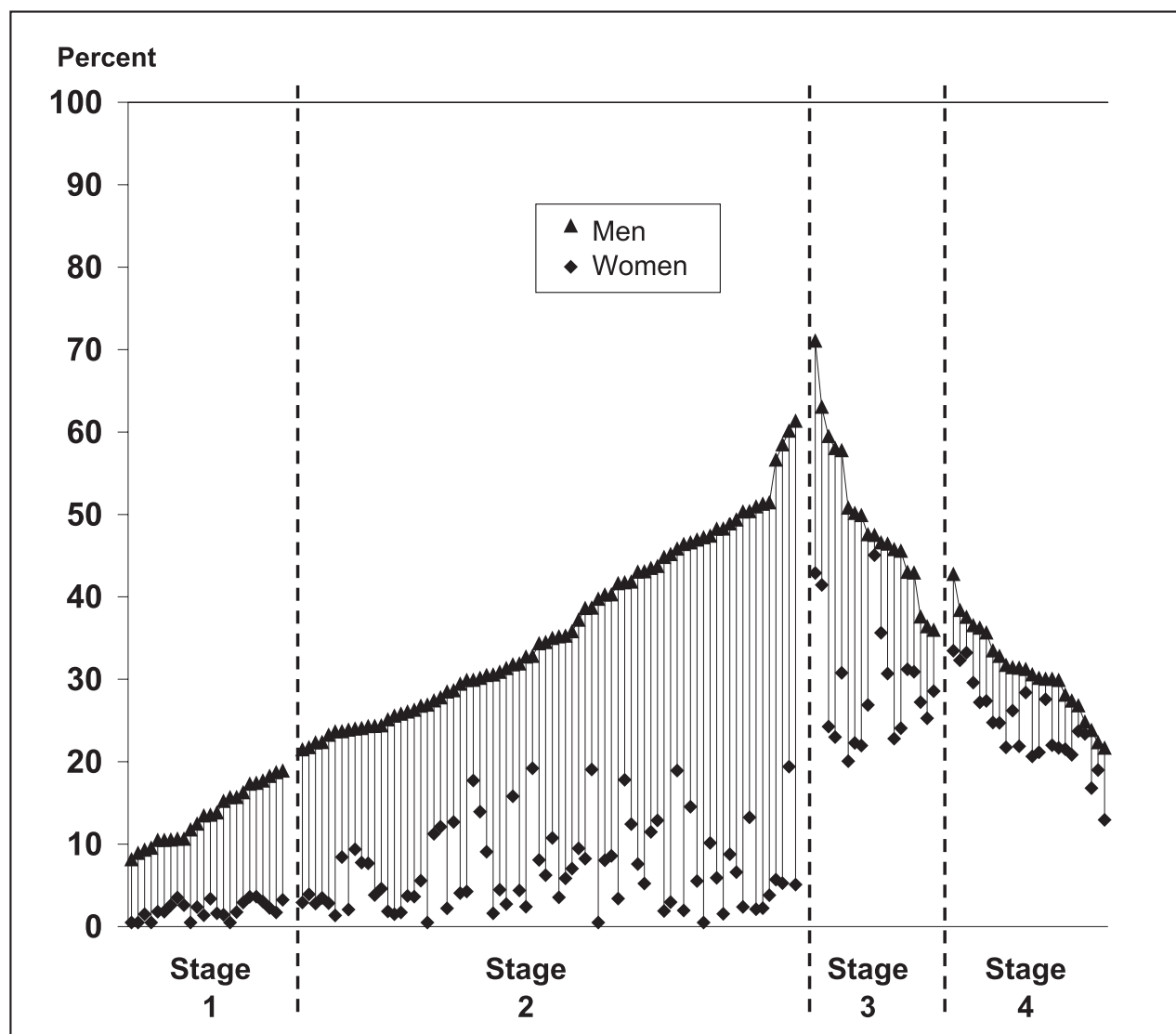
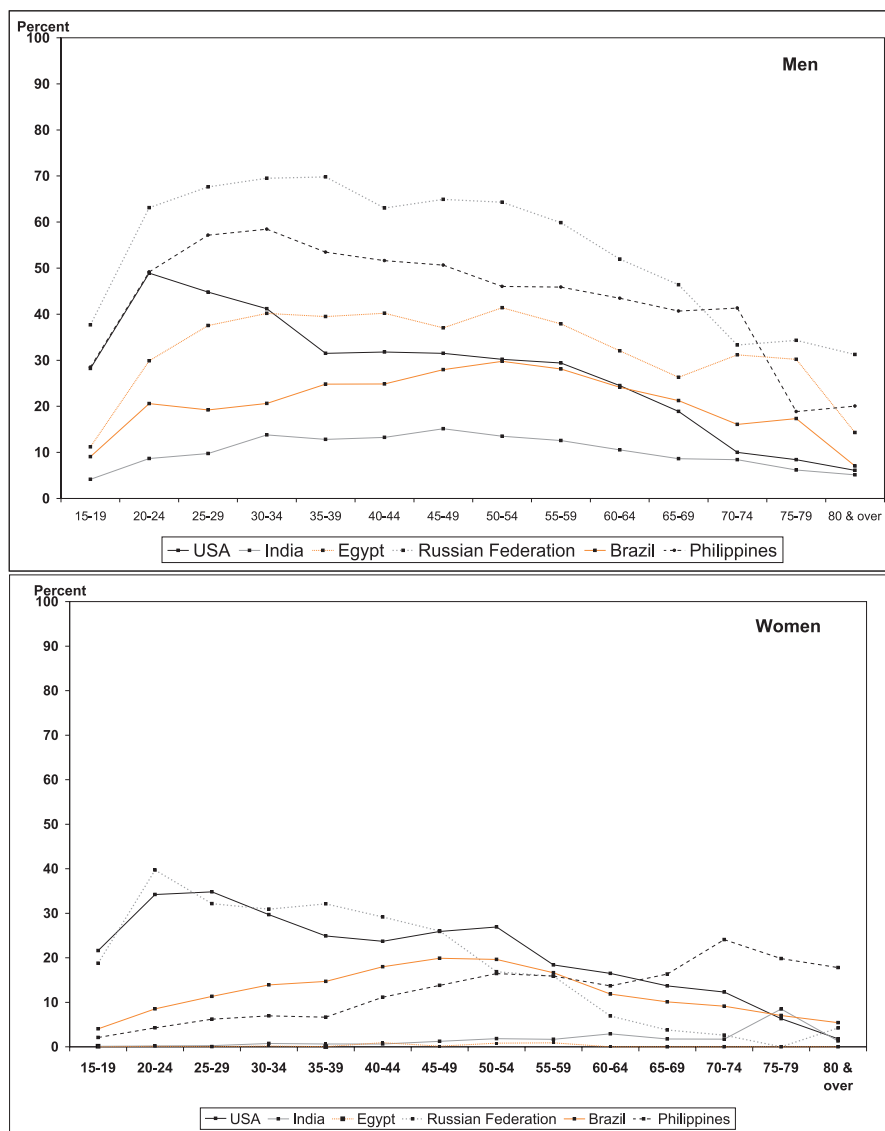


Fig. 1.6 Age-specific rates of smoking prevalence, in men and women in 2009

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 multi-lateral evidence-based treaty on tobacco control

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

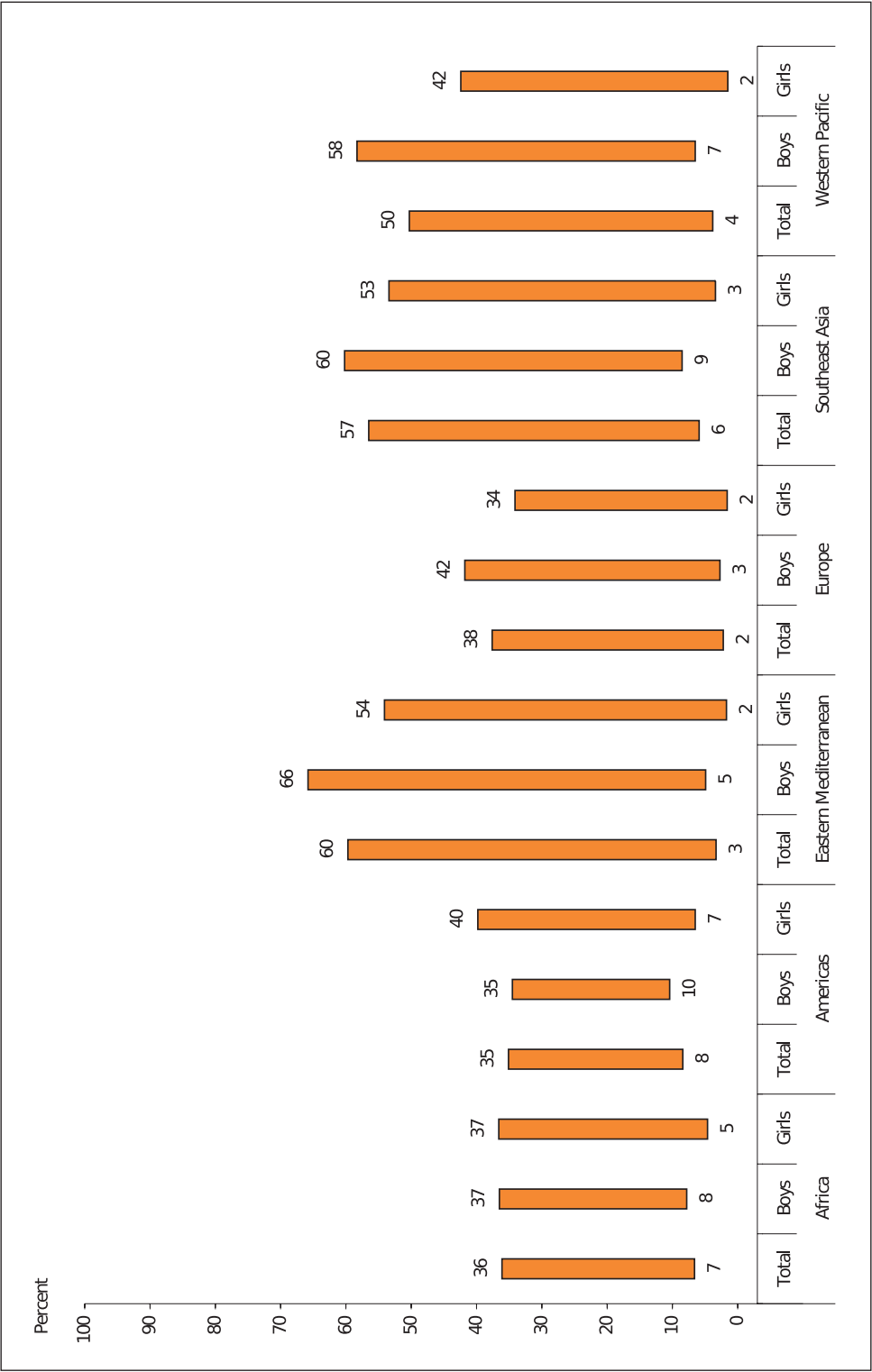
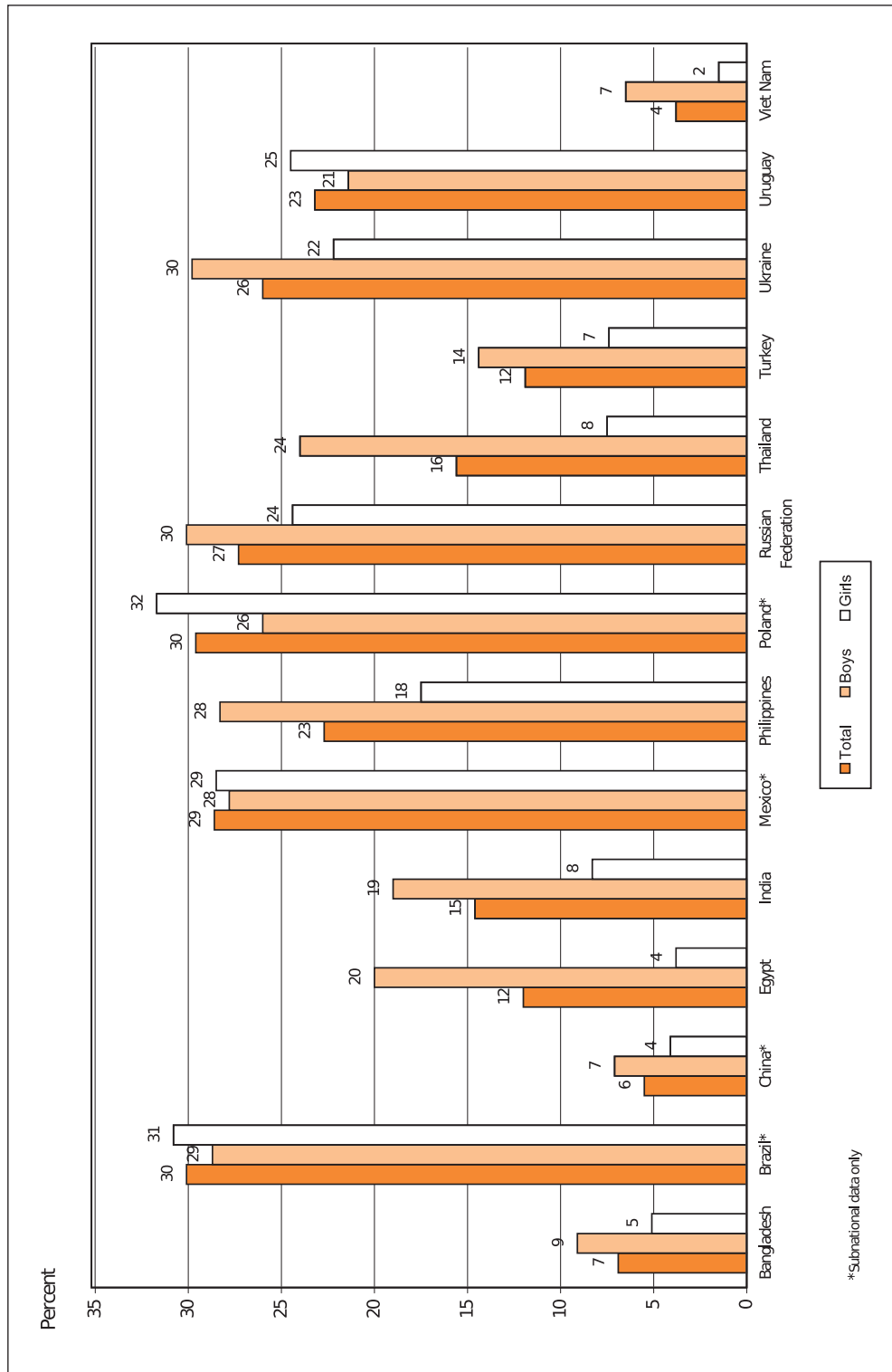


Fig. 1.8 Range of prevalence of current tobacco use in youth, 2005–2009, by WHO region



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 polycyclic 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

Ile⁴⁶²Val was associated with lung cancer susceptibility among Caucasians or African Americans. Among Caucasians, however, CYP1B1 Leu⁴³² 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 Leu⁴³² 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 (rs2909430), 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/m³. The absolute risks of lung cancer by age 75 years at radon concentrations of 0, 100, and 400 Bq/m³ 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 CYP1A1*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 ($\alpha(1)$ ATD) is one of the most common genetic disorders, especially among European descendants. Recent results suggest that $\alpha(1)$ 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/mm³), 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 sino-nasal 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. Friberg *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; Friberg *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 hepatitis 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 ([Kiemenev 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 ([Linnet 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 ([McDivitt 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 Malmö, 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 progesterone 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 or 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 (Ile¹⁴³Val) 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) reviewed 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 life-style 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 aerodigestive 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 B6C3F₁

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/m³ 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	Alveolar adenocarcinomas: M: 4/100 (alveolar adenocarcinoma) Controls–0/100 F: 4/100 (alveolar adenocarcinoma) 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) _{F₁} (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	
			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	
			Lung A: 93/330 (28%) Sham-exposed controls-22/326 (7%)	P < 0.001	
			Bronchiolar papillomas: 15/330 (4%) Controls-0/326	P < 0.001	
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 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 <i>et al.</i> (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 Lung AdC: 7/38 (18%) Controls–0/38 Kidney A: 6/16 (16%) (F only) Controls–0/21 Liver carcinomas: 2/16 (5%) (F only) Controls–0/21	$P < 0.001$ $P < 0.01$ $P < 0.01$	
Rats, Wistar (F) Davis <i>et al.</i> (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 <i>et al.</i> (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 alveoleogenic C) Controls–3/93 (3%) Subcutaneous sarcomas at forelimb ulceration sites: 21/80 (26%) Controls–0/93	$P < 0.05$ $P < 0.05$	
Rats, CDF (F344)/CrIBR (M, F) Finch <i>et al.</i> (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%) F: LCS–4/145 (3%) HCS–6/83 (7%) Filtered air–0/113	$P < 0.05$ $P < 0.05$	

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
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%) Lung AdC– LCS 1/178 (1%) HCS 5/82 (6%) Controls 3/118 (3%) All lung tumours– LCS 4/178 (2%) HCS 7/82 (8%) Controls 4/118 Nasal cavity (all tumour types)– LCS 1/178 (1%) HCS 5/82 (6%) Controls 1/118 (1%) F: Lung A– LCS 7/175 (6%) HCS 7/81 (9%) Sham-exposed controls 0/119 AdC– LCS 4/175 (2%) HCS 4/81 (5%) Controls 0/119 All lung tumours– LCS 10/175 (6%) HCS 11/81 (13%) Controls 0/119 Nasal cavity (all tumour types)– LCS 0/175 HCS 3/81 Controls 0/119	NS NS NS; trend, $P = 0.055$ $P = 0.032$ (HCS); trend, $P = 0.010$ NS (LCS); $P < 0.001$ (HCS) NS $P = 0.023$ (LCS); $P = 0.001$ (HCS) $P = 0.020$ (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. (1972)	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. (1972)	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%		
	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) Bernfield 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 ₂ O 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)/CrIBR (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 <i>et al.</i> (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 <i>et al.</i> (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)	<i>P</i> < 0.01 <i>P</i> < 0.01
Syrian golden (M) Harada <i>et al.</i> (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)	<i>P</i> < 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/m³ (LCS and HCS groups, respectively). At 12 weeks, rats were removed from smoke chambers and exposed nose-only to plutonium oxide (²³⁹PuO₂) 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 ²³⁹PuO₂ were higher than those in animals exposed to ²³⁹PuO₂, 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 IR4F 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: 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 IR4F 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

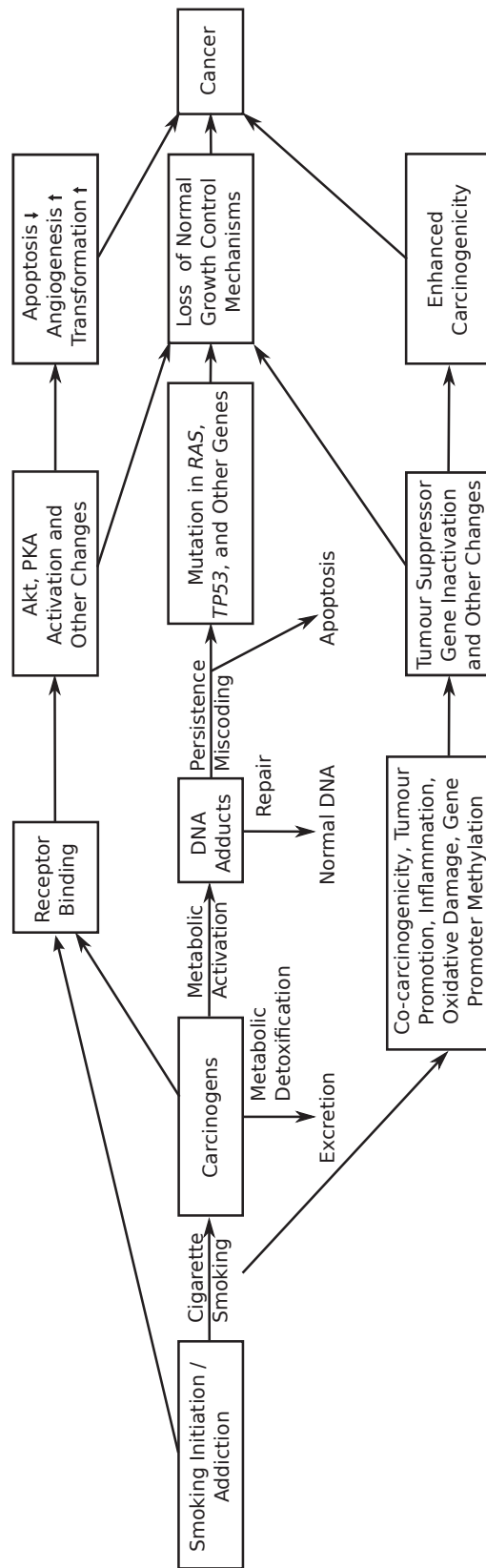
A conceptual model for understanding mechanisms by which tobacco smoke causes cancer is shown in Fig. 4.1 ([Hecht, 1999, 2003](#)). This model also applies to smokeless tobacco and other forms of smoked tobacco and, in theory, to second-hand tobacco smoke since it contains all of the same carcinogens 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.

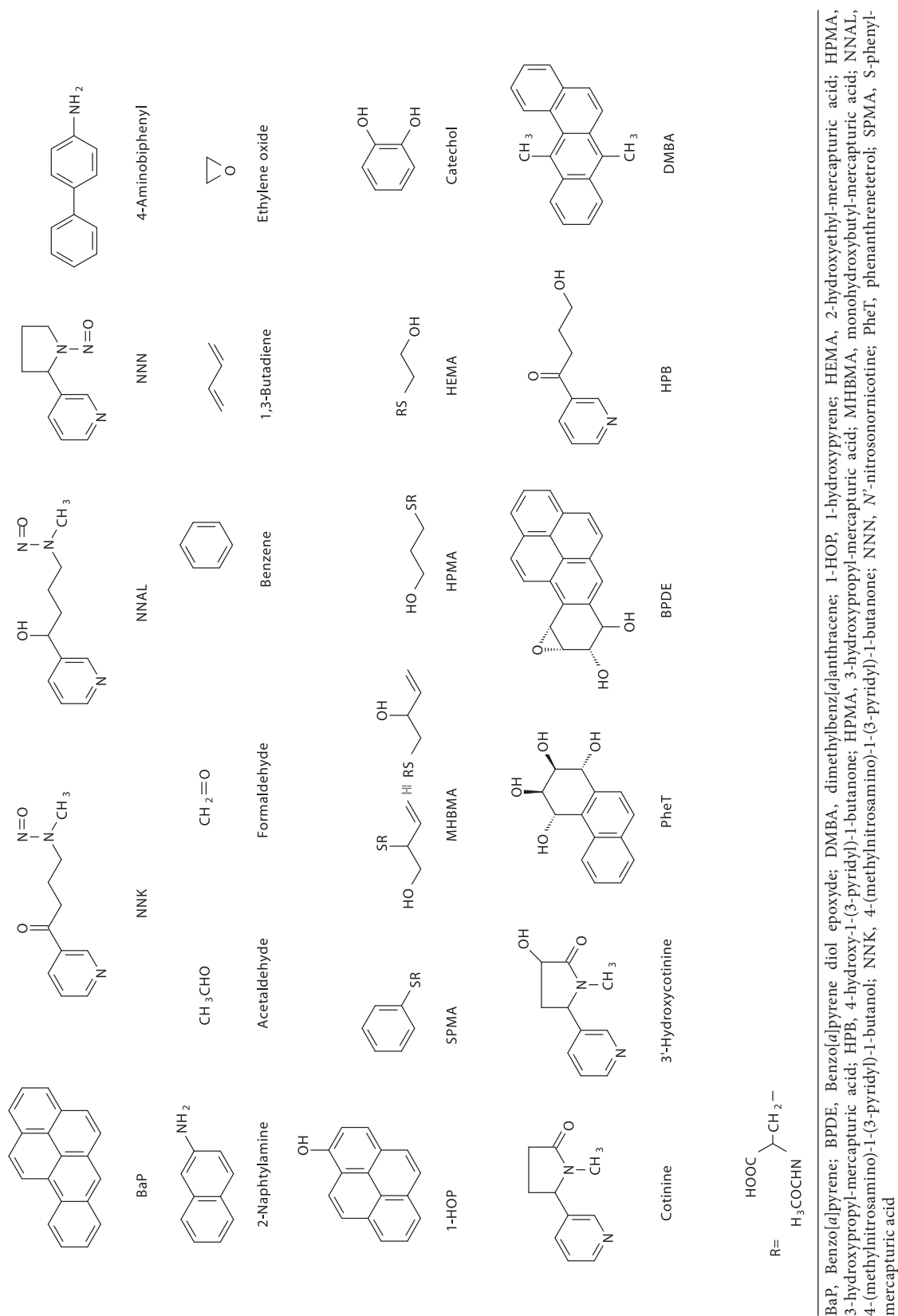
Numerous studies demonstrate the uptake of tobacco smoke carcinogens and toxicants by smokers, and showed higher levels of their metabolites in urine and blood of smokers than non-smokers (Sections 4.1.1 and 4.1.2). There are substantial differences in carcinogen exposure among people because of the 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



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'''- P¹, P³-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

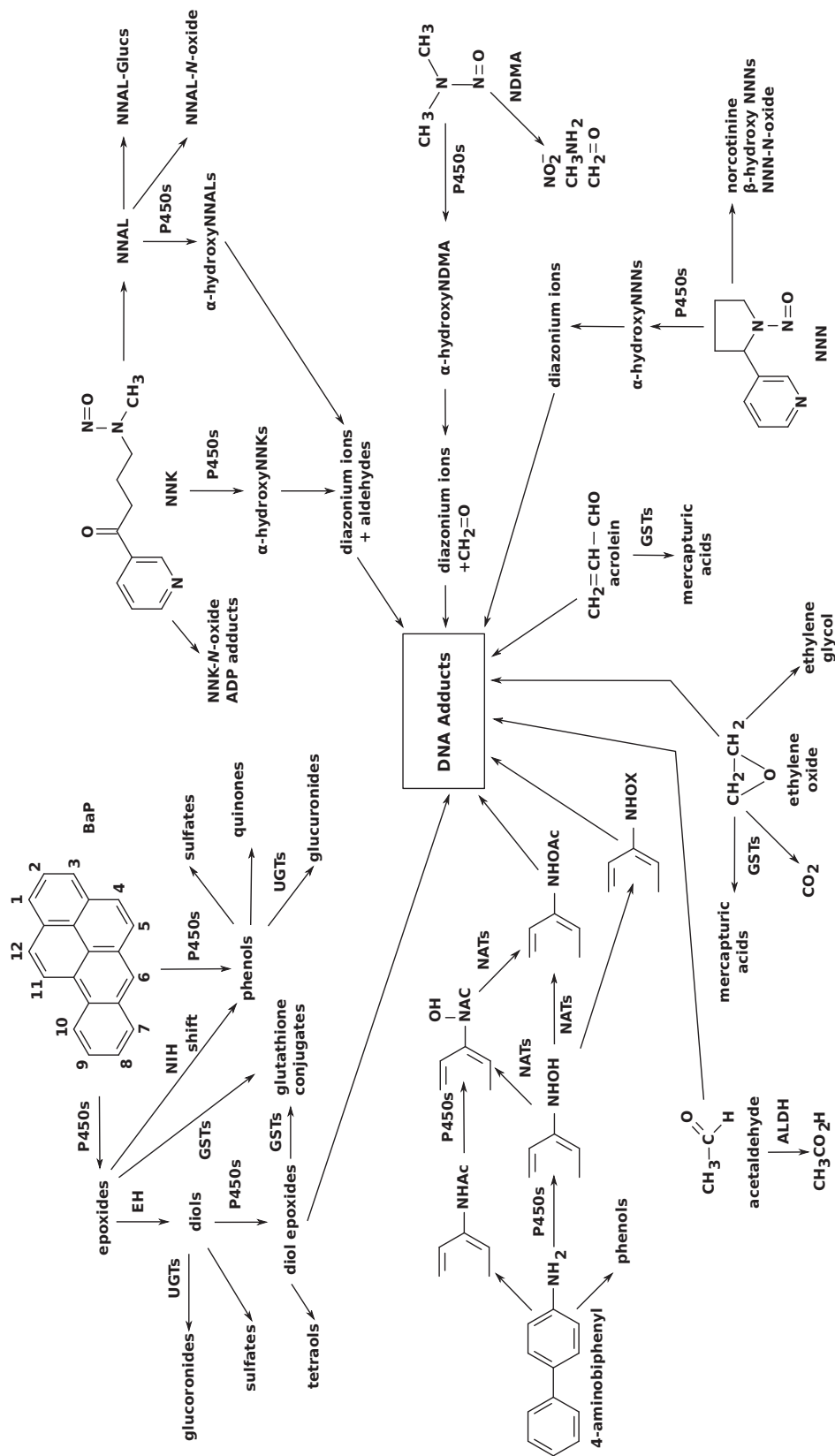
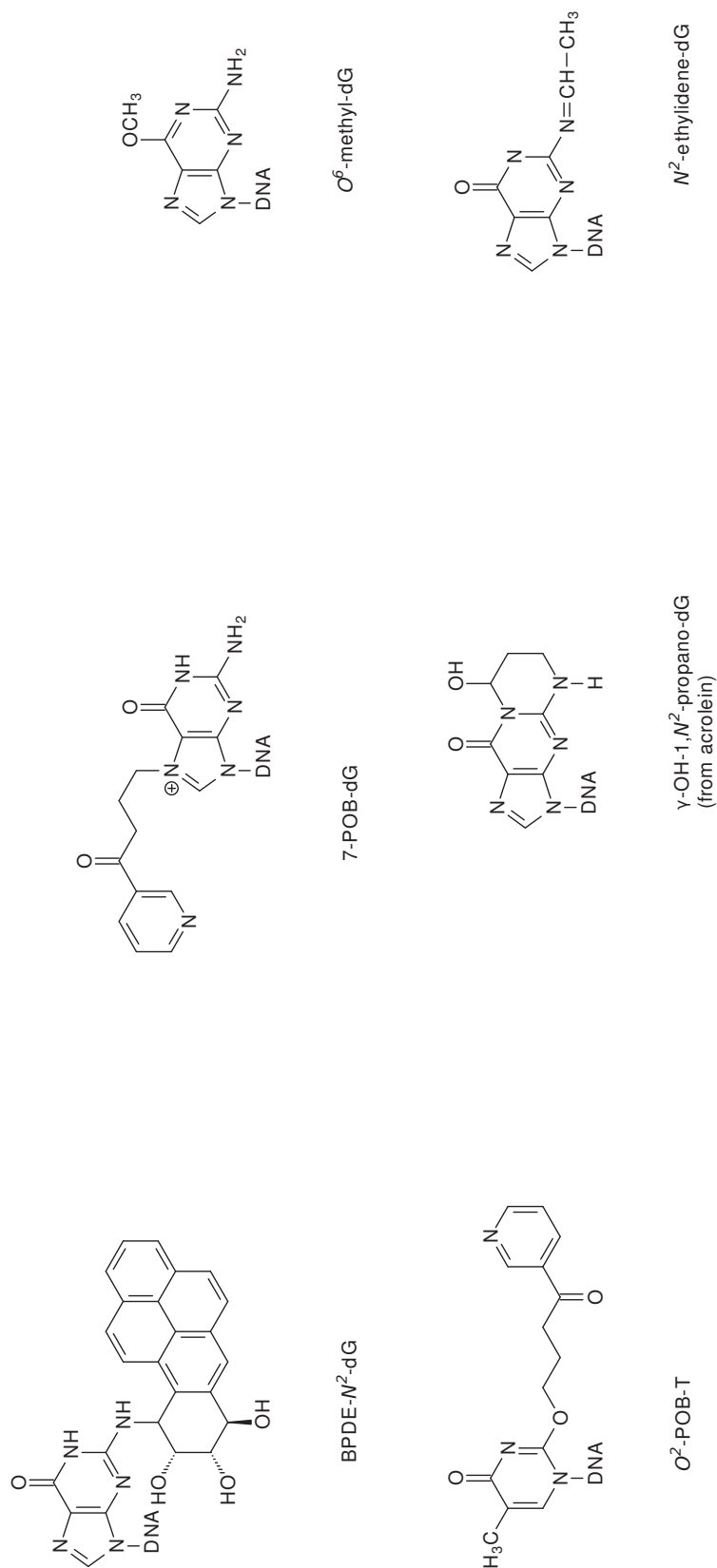


Fig. 4.4 Structures of some DNA adducts of tobacco smoke constituents



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 ofamol 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 immuno-sorbent 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

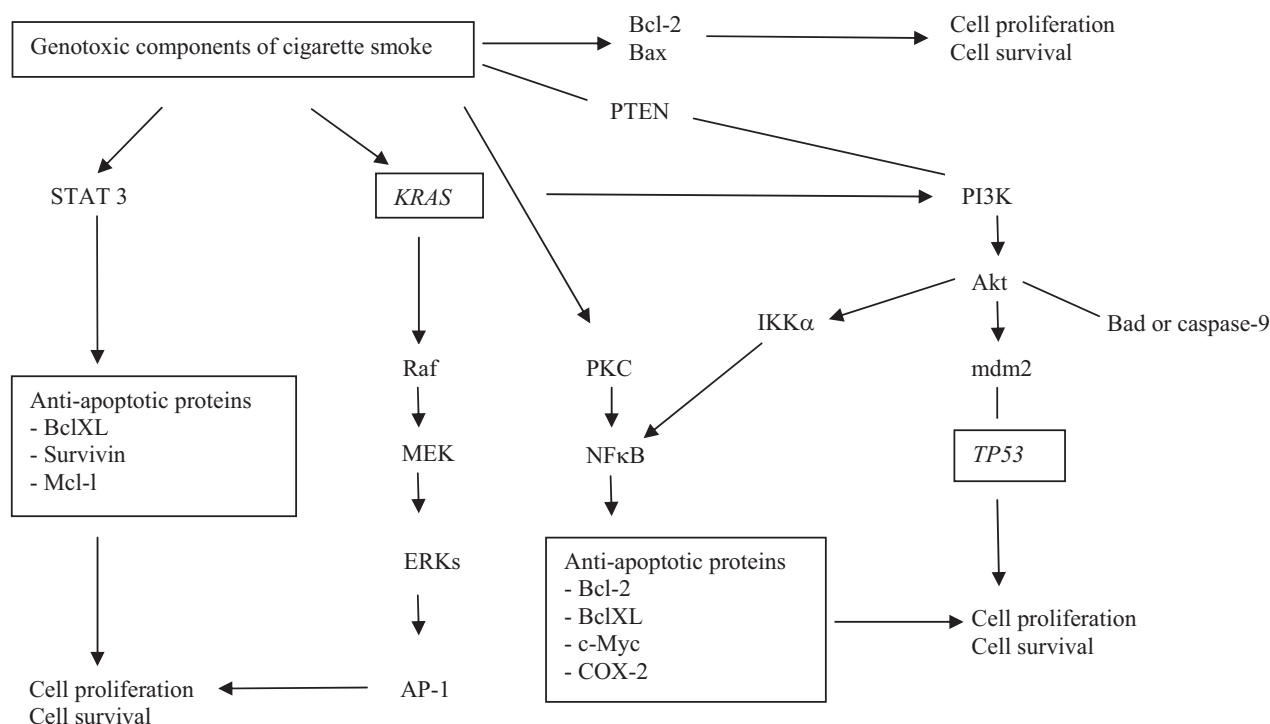
proteins, superoxide dismutase, glutathione transferase, heme oxygenase, *CYP* genes (*1A2*, *1A1* and *1B1*), interleukins and chemokines.

[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- κ B*, 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 (PGE₂) 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; Ile⁴⁶²Val). 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; Thr⁴⁶¹Asn) ([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](#); [Kamatani 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*; *Ile¹⁰⁵Val*), in exon 6 (*Ala¹¹⁴Val*), 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 Ile¹⁰⁵Val* polymorphism; but the pooled analysis suggested an overall statistically significant mild association between lung cancer and homozygosity or heterozygosity for the *Val¹⁰⁵* 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 polymorphisms 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 biomarkers 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](#); [Kuljucka-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](#); [Kuljukkka-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* Ile⁴⁶²Val 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 ([Kuljukkka-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 genotoxins 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 (Viezzler *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* (Ile⁴⁶²Val) 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 ³²P-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, genotoxins, 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 haemoglobin 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 etiological 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 (*Thr⁴⁶¹Asn, 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) Asp¹⁴⁸Glu, 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 *PI6^{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

haematopoietic 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 (16 α -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 16 α -OHE1 or to 2-OHE1 or to 4-OHE1. 16 α -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 16 α -hydroxylation pathway. However, the ratio of urinary 2-OHE1:16 α -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 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 (*K_m*) 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 ([Tuyens 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 O⁶-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 accessory 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 diol epoxide-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 O⁶-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 G Million 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 non-smoking 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
- Bjorge 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 cigarettes and cigarette 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: Suppl S259–S265. PMID:8943806
- Breast Cancer Family Registry; Kathleen Cuninghame 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 Assessment.
- 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 non-smokers. *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 O⁶-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-nitrosornicotine (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
- Person 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 L Iowa 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
- Friberg 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, Ravnkar 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, Lanás 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
- Gaspar 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 Prol2Ala 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 P450A2, 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, Torr  ng 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 Hazard 473–490. 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.0000022841.121719.25 PMID:17554200
- Hu J & Ugnat AM Canadian 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, Vuilleminot 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.html>.
- 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: 1–475. 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, pharmacoeconomic 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 Y Canadian 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, Teglbaerg 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
- Kiemeny 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 aerogastric 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 multi-centre 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
- Kuljucka-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'-nitrosomnicotine. *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
- Linnet 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
- Loerbroeks 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
- MacNicol 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 Invest*, 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'-nitrosornicotine in male Syrian golden hamsters. *Cancer Res*, 41: 2849–2854. PMID:7248945
- McDavit 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 2:16 α -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 & Rimmel 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 HuGE review. *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 life-style 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 microl 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 responses, 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, Bellocchio 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/internal-medicine.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 Multi-centre 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 life-style 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 Int.*, 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 S Japan 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 signaling 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 multi-ethnic 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, life-style 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, Samowitz 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). TGFβ1 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*'-nitrosornicotine 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*'-nitrosornicotine 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, Szymeja 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
- Viezzar 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 T³⁸⁰¹C 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 S Global 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, Ingle 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, Farewell 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. *Ophthalm 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'-nitrosomorpholine 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
- Zendejdel 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'-nitrosornicotine. *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 print PMID:19562492.

Ingredients and Emissions

Rodgman and Perfetti, The chemical components of tobacco and tobacco smoke, CRC Press, 2009

ISBN 978-1-4200-7883-1

For copyright reasons (see below) we are unable to provide a full copy of this reference.

THE CHEMICAL COMPONENTS OF TOBACCO AND TOBACCO SMOKE

Alan Rodgman
Thomas A. Perfetti



CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742

© 2009 by Taylor & Francis Group, LLC
CRC Press is an imprint of Taylor & Francis Group, an Informa business

No claim to original U.S. Government works
Printed in the United States of America on acid-free paper
10 9 8 7 6 5 4 3 2 1

International Standard Book Number-13: 978-1-4200-7883-1 (Hardcover)

This book contains information obtained from authentic and highly regarded sources. Reasonable efforts have been made to publish reliable data and information, but the author and publisher cannot assume responsibility for the validity of all materials or the consequences of their use. The authors and publishers have attempted to trace the copyright holders of all material reproduced in this publication and apologize to copyright holders if permission to publish in this form has not been obtained. If any copyright material has not been acknowledged please write and let us know so we may rectify in any future reprint.

Except as permitted under U.S. Copyright Law, no part of this book may be reprinted, reproduced, transmitted, or utilized in any form by any electronic, mechanical, or other means, now known or hereafter invented, including photocopying, microfilming, and recording, or in any information storage or retrieval system, without written permission from the publishers.

For permission to photocopy or use material electronically from this work, please access www.copyright.com (<http://www.copyright.com/>) or contact the Copyright Clearance Center, Inc. (CCC), 222 Rosewood Drive, Danvers, MA 01923, 978 750 8400. CCC is a not-for-profit organization that provides licenses and registration for a variety of users. For organizations that have been granted a photocopy license by the CCC, a separate system of payment has been arranged.

Trademark Notice: Product or corporate names may be trademarks or registered trademarks, and are used only for identification and explanation without intent to infringe.

Library of Congress Cataloging-in-Publication Data

Rodgman, Alan.
The chemical components of tobacco and tobacco smoke / Alan Rodgman and Thomas A. Perfetti.
p. : cm.
"A CRC title."
Includes bibliographical references and index.
ISBN 978-1-4200-7883-1 (hardcover : alk. paper) 1. Tobacco--Composition. 2. Tobacco smoke--Composition. I. Perfetti, Thomas Albert, 1952 II. Title.
[D]NLM: 1. Smoke--adverse effects. 2. Tobacco--chemistry. 3. Smoking--adverse effects. 4. Tobacco--adverse effects. WA 754 R691c 2009]
SB275.R63 2009
613.85--dc22

2008018913

Visit the Taylor & Francis Web site at
<http://www.taylorandfrancis.com>

and the CRC Press Web site at
<http://www.crcpress.com>